The Classification of Drugs and Drug Receptors in Isolated Tissues

TERRY P. KENAKIN

Department of Pharmacology, The Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, North Carolina

used to gain information about receptors. This bias is reflected in the still very timely statement made by Buchheim 135 years ago.

The information about drugs obtained from isolated tissues becomes useful for classification purposes which, it is hoped, leads to general statements about structure and activity. The essentially circular nature of the drug and drug receptor classification process should be kept in mind. New receptor types or subtypes are discovered only after the discovery of new selective drugs. Then new drugs are classified by their interactions with these new receptors. It will be another bias of this paper that drugs probably have more than one activity and are selective rather than specific. This is a parsimonious view in receptor terms since it seeks to explain selectivity or lack of it in terms of multiple properties of drugs rather than multiple subtypes of receptors.

The use of isolated tissues to classify drugs has its drawbacks in the dependence of this process on previous classifications. For example, in the early subclassification process for β-adrenoceptors, the guinea pig trachea was classified as containing β_2-adrenoceptors and tracheal-selective β-agonists were accorded the corresponding label of β_2-adrenoceptor selective. The advent of data which suggest that trachea contains both β_1- and β_2-adrenoceptors necessarily must bring into question the original β_2-selective classification of these agonists. Unfortunately, there is usually a time lag between the classification of the drugs and the reclassification of the tissue and the possibly erroneously classified agonists may be used to classify other tissues incorrectly. The potential for a baroque web of conflicting classification data for tissues and drugs in this process is obvious. Some of these problems may be avoided if tissue selectivity is not assumed to be receptor selectivity.

In this paper, an attempt will be made to review some of the null methods available to measure the strictly drug-receptor-related parameters of affinity and intrinsic efficacy, and more importantly, the internal checks in these methods to detect receptor heterogeneity. The major advantage of isolated tissue experimentation is the potential to directly measure relative efficacy of agonists (235). In this regard, this technique holds advantages over biochemical binding techniques which primarily yield estimates of affinity.

A. Isolated Tissue and Binding Studies

Currently there is controversy over the significance of the similarities and differences between binding and isolated tissue data. Unfortunately, there are all too few laboratories that do both techniques and critically compare the results (159). There are studies that show estimates of affinity of drugs in isolated tissue and binding studies to be equivalent for a variety of receptors including muscarinic receptors (52, 53, 235, 612, 712), β-adrenoceptors (458, 459, 699), and α-adrenoceptors (320, 606). However, differences between isolated tissue and binding studies have been noted as well. For example, the binding K_d values for some β_2-adrenoceptor agonists do not correlate well with the relative potencies of these drugs in isolated tissues (413). Similarly, although the selectivities of antagonists for β_1- and β_2-adrenoceptors as measured in binding and isolated tissue studies correlate well, the correlation for agonists is poor (71). This is most likely because pharmacological agonist activity depends upon affinity *and* intrinsic efficacy, the latter parameter being essentially inaccessible in binding studies. Agonist activity in broken cell preparations can be measured (for example, β-adrenoceptor agonists on adenylyl cyclase) but the lack of amplification which is present in tissues makes detection of all but quite powerful agonists not possible biochemically. The amplification processes inherent in the stimulus-response mechanisms of isolated tissues make isolated tissues much more suitable for prediction of agonist activity in vivo.

Ingenious methods have been applied in binding studies to differentiate between agonists and antagonists which theoretically could lead to quantification of relative efficacy biochemically. Thus the differential effects of sodium ion on opiate receptor binding (516), the differences in free energy of binding (691) or effects of GTP and GpNHpp (439, 699) on β-adrenoceptor binding and the differential effects of GABA on benzodiazepine binding (586) all offer unique approaches to this problem.

One apparent advantage binding studies have over isolated tissue studies is the ability to measure receptor density. Care must be taken, however, in interpreting these estimates of receptor density. The difficulty comes in predicting the relevance of the actual proportions of heterogeneous receptor populations to pharmacological responses; i.e., will a ratio of 80:20 β_1- to β_2-adrenoceptors in a tissue translate to a more β_1- than β_2-adrenoceptor profile in terms of pharmacological responses? Actually the probability of direct correspondence is low in view of the coupling processes involved between receptor activation and tissue response. For example, Homburger and coworkers (325) have noted that even though β_1- and β_2-adrenoceptors coexist on single C_6 cloned glioma cells, the coupling of the two receptors to adenyl cyclase is not of equal efficiency. The effects of receptors coupling on tissue responsiveness can be demonstrated in ontogenetic studies. For example, although β-adrenoceptors can be detected in fetal mouse hearts in binding studies, no responses to isoproterenol can be elicited until later in the cycle of tissue development (709). A study by Hoffmann and coworkers (318) has shown that although the state of oestrous in female rats greatly affected the relative number of α-adrenoceptor and β-adrenoceptor subtypes, the changes in pharmacological responses did not coincide with the receptor changes measured in the binding studies. In view of the influence of receptor coupling on drug responses, a theoretically more complete approach to the study of the mechanism of action

of drugs would incorporate both binding and isolated tissue studies.

II. Factors in the Choice of Isolated Tissues

There are many factors to consider when choosing an isolated tissue system for pharmacological experiments. Numerous techniques have been identified to detect and eliminate obstacles to the attainment of the primary requisites for isolated tissues, namely uniformity and stability.

A. Animals

The use of age-, weight-, and strain-matched animals should reduce the incidence of variation (246). For certain types of experiments, special animals such as the spontaneously hypertensive rat (479) or cardiomyopathic hamsters (619) can be utilized. For other procedures, surgically altered animals can yield tissues that approximate the effects of pathological conditions in various organs observed in man. Thus, cardiac tissues from animals in cardiac failure can be obtained from cats which chronically obstructed pulmonary arteries (248, 251) or rats with aortic insufficiency, aortocaval fistulae, or aortic stenosis (211). For certain tissues, such as myometrium, the hormonal state of the animal (oestrous, preoestrous, etc.) also is important (88, 91, 181, 542, 455).

B. Preservation of Tissue Viability

Historically, pioneering work by many researchers such as Tyrode (645) and Krebs (398, 399) has led to the definition of nutrient solutions capable of preserving isolated tissues in a viable state. Different tissues require a different milieu of ions and nutrients. Changes in ionic content and composition can affect tissue reactivity and base-line activity. For example, high osmotic pressure depresses cardiac pacemaker activity (393, 595). Changes in levels of potassium ion (252, 480, 584, 585) or magnesium ion (9) can greatly modify isolated blood vessel tone and reactivity to agonists. Specific changes in ionic composition can eliminate random spontaneous activity in some tissues and allow stable steady-state responses to agonists. For example, the reduction of calcium ion (30) or the elimination and graded readdition of calcium ion (375) to de Jalon solution bathing rat uterus produces a quiescent or regularly contracting tissue suitable for bioassay.

Adequate delivery of oxygen to the tissue is another prime consideration. The basal activity of smooth muscle (122, 180) and cardiac muscle (82) can be affected by changes in the partial pressure of oxygen in the organ bath (Po$_2$). For example, the contractile responsiveness of arterial smooth muscle decreases with decreasing Po$_2$, the effects being more pronounced for thick- rather than thin-walled vessels and also for high levels of contractile stimulus (122, 180). From A. V. Hill's classic equation describing diffusion of soluble substances into muscle (315), as modified by Chang and Detar (122), it can be

seen that complete delivery of dissolved oxygen to the cells of a given isolated tissue depends upon the partial pressure of oxygen in the organ bath medium (Po$_2$ bath), the thickness of the tissue, and the rate of oxygen consumption of the tissue (Vo$_2$). Thus, for a flat isolated tissue preparation (122):

$$\frac{Po_{2(wall)}}{Po_{2(bath)}} = 1 - \frac{Vo_2}{120 SD} \frac{(T \cdot X - X^2)}{Po_{2(bath)}} \qquad (1)$$

where $Po_{2(wall)}$ is the partial pressure of oxygen in the tissue wall (torr) at depth X (distance from surface toward center of the tissue, cm). Wall thickness is T (cm), S is the solubility coefficient of oxygen within the wall (ml$\cdot$cm$^{-3}\cdot$760 torr^{-1}), and D is the diffusion coefficient of oxygen within the wall (cm$^{-2}\cdot$min^{-1}). The oxygen consumption, Vo_2, is in ml$\cdot$cm$^{-3}\cdot$h^{-1}. With Hill's original equation, the so-called "critical" thickness or diameter of a tissue can be calculated beyond which a hypoxic core of cells would be expected (82, 122). However, calculations of this type assume homogeneous oxygen consumption and diffusion into the tissue, estimations which probably are oversimplifications making such predictions only rough guidelines. For example, the oxygen consumption of cardiac muscle has been shown to vary greatly with contractile state (82), or age of animal (157). The dependence of critical thickness upon contractile state makes it a possibility that an adequately oxygenated tissue in the resting state may become hypoxic during exposure to an agonist which increases contractile function. If, in turn, the functional contractility of the tissue is dependent upon viable cells (i.e., little contractile reserve) then the magnitude of the responses to the agonist may be dependent upon the Po$_2$ in the organ bath.

The temperature of the medium bathing the isolated tissue is another consideration. For example, cooling to 32°C reduces spontaneous activity in rat uterus (30). Cardiac tissue has been shown to provide more stable basal activity and responsiveness to agonists at temperatures below 37°C (81, 82).

The pH of the bathing solution can affect basal activity and responses of isolated tissues to drugs. For example, the spontaneous rate and force of rabbit atria (333, 671), dog atria (561), perfused hearts of guinea pigs (449), and rat atria (392) decrease with lowered bath pH. Alternatively, deviations in pH may influence ionization of drugs or charged chemical groups on receptors thereby changing the moieties which interact to produce response. The responses of rabbit atria to histamine are stable when the pH of the bathing medium is kept between 7 and 7.6. However, at pH <7, the responses to histamine become depressed (333). Similarly, the responses of smooth muscles to histamine are stable in a pH range of 7.0 to 8.3 but decline sharply at pH <7 (543, 544). The responses of rat atria to norepinephrine are increased during alkalosis and decreased by acidosis (116) while the re-

sponses of rat uterus to oxytocin are potentiated by acetic acid (57).

In general, there is much evidence to show that the ionic composition, PO_2, pH, and temperature of the bathing media for isolated tissues can affect viability and responsiveness.

C. Some Isolated Tissues from Animals and Man

The ideal experimental conditions for various experimental preparations have been reported in the literature and the subtle variations in experimental conditions that work best for particular isolated tissues can be obtained from papers describing pharmacological procedures with these tissues. A list of some of the isolated tissue preparations used for the study of receptors for neurotransmitters, autacoids, and hormones is given in table 1. This list excludes receptors for various biologically active substances such as leukotrienes, thromboxanes, prostaglandins, calcium ions (channels), and peptides which are discussed in specialized reviews and monographs. Also not shown in this table are receptor subclassifications (i.e. α_1- and α_2-adrenoceptors) since, for many tissues, the predominance of the receptor subtype was not clear at the time of writing. Also, for many of the putative receptor subclassifications, the pharmacological criteria for definition by selective agonists and antagonists have not yet been defined. Shown in table 1 are references to papers in which the conditions and method of preparation of the tissue for the specified receptor are described. Therefore, more than one reference may be given for the same tissue if different drug receptors are studied that require different experimental conditions. It should be noted that the references in table 1 are not necessarily historically accurate from the point of view of first describing the isolated tissue for pharmacological experimentation. This is because, in most cases, subsequent usage of the tissue preparation by other workers has led to modifications and improvements in the techniques. Therefore, examples of recent papers where authors have given methodological details and concentration-response curves to agonists are cited in the interest of enabling the reader to use the table as a reference to the isolated tissue preparations available.

There is a large body of experimental evidence to suggest that there is pharmacological correspondence between the drug receptors in animals and man. Obviously there are important ethical considerations in the testing of drugs in man but certain surgical procedures and the rapid post-mortem acquisition of biological tissue make possible the testing of drugs in human tissue in vitro. Some of the tissue preparations isolated from humans for the study of drugs are shown in table 2.

D. Methods of Tissue Preparation

The method of tissue preparation can be an important factor in pharmacological experiments. The wide range of sizes of vascular tissue requires a correspondingly wide

TABLE 1
Some commonly used isolated tissues from animals

Receptor	Species	Tissue	References
Adenosine	Rat	Bladder	(102)
		Ileum	(49)
		Portal vein	(583)
		Vas deferens	(130)
	Guinea pig	Atria	(372)
		Trachea	(140, 141)
	Rabbit	Ileum	(7, 225)
	Cat	Coronary artery	(489)
	Dog	Atria	(127)
		Basilar artery	(469, 630)
		Coronary artery	(630)
		Mesenteric artery	(630)
α-Adrenergic	Rat	Anococcygeus muscle	(258)
		Aorta	(503, 137)
		Carotid artery	(137)
		Papillary muscle	(587)
		Portal vein	(136)
		Portal vein (everted)	(443)
		Seminal vesicle	(505)
		Spleen	(5)
		Vas deferens	(505, 662)
	Guinea pig	Aorta	(503)
		Atria	(278)
		Bladder	(42)
		Jugular vein	(136)
		Taenia coli	(692)
		Trachea	(515)
		Vas deferens	(472, 42)
	Rabbit	Aorta	(237)
		Bladder	(42, 420)
		Duodenum	(230)
		Ear artery	(173)
		Facial vein	(510)
		Ileum	(42, 505)
		Inferior vena cava	(505)
		Jugular vein	(138)
		Main pulmonary artery	(319)
		Spleen	(42, 505, 578)
		Stomach fundus	(230)
		Vas deferens	(42)
	Cat	Aorta	(503)
		Nictitating membrane	(410)
		Spleen	(70, 282)
	Mouse	Anococcygeus muscle	(256)
		Spleen	(339)
		Vas deferens	(42)
	Dog	Basilar artery	(558)
		Coronary artery	(16, 464)
		Renal artery	(311)
		Saphenous vein	(163, 285)
	Pig	Coronary artery	(50)
Angiotensin	Rat	Aorta	(137)
		Carotid artery	(138)
		Colon	(531, 533)
		Jugular vein	(138)
		Mesenteric artery	(137)
		Stomach	(532)
		Vas deferens	(437)
	Rabbit	Aorta	(532)
	Cat	Coronary artery	(489)

TABLE 1—continued

TABLE 1—continued

Receptor	Species	Tissue	References
β-Adrenergic	Rat	Adipose tissue	(206)
		Atria	(505)
		Coronary artery	(50)
		Jugular vein	(138)
		Uterus	(205, 181)
	Guinea pig	Atria	(79, 230)
		Extensor digitorum longus	(233)
		Small intestine	(230)
		Soleus muscle	(679)
		Trachea	(104, 505)
		Vas deferens	(205)
	Rabbit	Aorta	(94, 194, 230, 505)
		Atria	(94, 505)
		Coronary artery	(171)
		Ear artery	(169)
		Facial vein	(510)
		Portal vein	(194)
		Small intestine	(230)
		Stomach fundus	(94)
		Trachea	(94)
		Vena cava	(194)
		Vesicourethral muscle	(390)
	Cat	Atria	(81)
		Coronary artery	(489)
		Middle cerebral artery	(193)
		Nictitating membrane	(670)
		Papillary muscle	(363)
		Trachea	(424)
	Mouse	Spleen	(86)
	Dog	Coronary artery	(46, 86)
		Skeletal muscle artery	(46)
	Pig	Coronary	(50, 186, 351)
	Bovine	Iris sphincter	(502)
		Trachea	(22)
Bradykinin	Rat	Uterus	(38)
		Stomach	(40, 666)
	Rabbit	Aorta	(237, 188, 530)
		Ear artery	(596)
		Jugular vein	(39)
	Cat	Cerebral artery	(697)
		Jejunum	(40)
		Terminal ileum	(38)
	Dog	Carotid artery	(150)
		Saphenous vein	(270)
Cholinergic (muscarinic)	Rat	Aorta	(137)
		Anococcygeus muscle	(258)
		Caecum	(622)
		Atria	(477)
		Fundus	(666)
		Jejunum	(664)
		Mesenteric artery	(137)
		Portal vein	(137)
	Guinea pig	Atria	(79)
		Bladder	(422)

Receptor	Species	Tissue	References
		Ileum	(527)
		Taenia caeci	(6)
		Trachea	(484)
	Rabbit	Aorta	(237)
		Ear artery	(169)
		Fundus	(238)
		Detrusor muscle	(184)
	Cat	Anococcygeus muscle	(259, 422)
	Mouse	Ileum	(168)
	Dog	Ventricular muscle	(199)
Dopamine	Rabbit	Mesenteric artery	(98)
		Middle cerebral artery	(492)
		Splenic artery	(313)
	Cat	Middle cerebral artery	(192)
	Dog	Cerebral artery	(631)
		Coronary artery	(633, 634)
		Mesenteric artery	(269)
		Renal artery	(269)
		Small femoral artery	(269)
Histamine (H₁)	Rat	Fundus	(666)
	Guinea pig	Gall bladder	(408)
		Duodenum	(89)
		Ileum	(30)
		Jugular vein	(138)
		Taenia caeci	(6)
	Rabbit	Aorta	(150, 194, 200)
		Portal vein	(194)
		Jugular vein	(138)
		Renal artery	(152)
		Trachea	(300)
		Vena cava	(194)
	Cat	Extracranial blood vessels	(194)
	Dog	Saphenous vein	(636)
Histamine (H₂)	Rat	Stomach fundus	(200)
		Uterus	(75)
	Guinea pig	Atria	(14)
		Gall bladder	(408)
		Papillary muscle	(14, 60)
		Parenchymal strips	(638)
		Ventricular strips	(677)
	Rabbit	Atria	(521)
		Aorta	(200)
		Trachea	(213, 380)
	Cat	Extracranial blood vessels	(194)
	Mouse	Stomach (secretion)	(13, 15)
		Vas deferens	(441)
5-Hydroxytryptamine	Rat	Aorta	(137, 139)
		Anococcygeus muscle	(258)
		Carotid artery	(138)
		Fundus	(488, 666)
		Jugular vein	(138, 139)
		Mesenteric artery	(137)
		Portal vein	(137)

TABLE 1—continued

Receptor	Species	Tissue	References
		Uterus	(247)
		Vas deferens	(488)
	Guinea pig	Ileum	(151)
		Taenia caecum	(6)
	Rabbit	Extracranial arteries	(407)
	Cat	Extracranial arteries	(407)
	Dog	Coronary artery	(16, 92)
		Extracranial arteries	(407)
		Saphenous vein	(163)
	Calf	Coronary artery	(361)
		Trachea	(488)
	Chicken	Oesophagus	(488)
Opioid	Rat	Vas deferens	(335)
	Guinea pig	Ileum	(291, 397)
		Vas deferens	(335)
	Mouse	Vas deferens	(331, 335)
Substance P	Rat	Everted portal vein	(443)
		Vas deferens	(155, 437)
	Guinea pig	Ileum	(155)
	Rabbit	Mesenteric vein	(63)
	Cat	Terminal ileum	(63, 530)
	Dog	Carotid artery	(156)

TABLE 2
Isolated tissues from humans

Tissue	Receptor*	References
Vascular-arterial		
Femoral	α	(266)
Basilar	5-HT	(220)
	Dopamine	(222)
Popliteal	α	(154)
Umbilical	ACh,5-HT,α,H_1	(268)
	α	(592)
	H_1,5-HT	(534)
Digital	β	(467, 602)
	α	(601)
Pilial	5-HT	(407)
Coronary	α, β	(12)
	H_1, H_2	(260, 267)
	ACh,α,β	(260)
Pulmonary	H_1	(454)
	α	(272)
	5-HT	(272, 454)
Crural	α	(453)
Mesenteric	α	(292)
Cerebral	5-HT,ACh,α,H_1	(579, 632)
Vascular-venous		
Saphenous	α	(154, 349)
	ACh	(546)
Femoral	α	(266)
Crural	α	(453)
Umbilical	α	(592)
Smooth muscle		
Bronchiole (lung)	β	(272, 306, 714)
	α	(62, 78)
	H_1	(185, 210, 272, 425, 568)
	ACh	(210, 272)
	5-HT	(62)
Stomach (duodenum)	α	(306)
Muscularis mucosae	ACh,α,β	(680)
Ileum	α	(154)
Colon	α	(306)
	β	(154)
Sphincter pupillae	ACh	(364)
Internal anal sphincter	ACh,α	(119)
Oesophagus	α	(154)
Vas deferens	α	(302)
Bladder detrusor	α	(154)
Ureter	H_1,H_2	(61)
Cardiac muscle		
	α	(571)
	β	(261, 290, 316, 365, 571)
	H_2	(262, 290, 283, 419)

* α, α-adrenergic; 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; H_1, H_2, histamine; β, β-adrenergic.

range of techniques to measure contractile function. Thus, very small vessels like the cat coronary artery can be perfused at a constant rate and the perfusion pressure used as a measure of vasoconstriction (489). Alternatively, spiral strips from vessels can be cut. The advantage of such a preparation is that enough muscle can be assayed to allow measurable responses to be obtained from very small vessels. Also, the effects of receptor heterogeneity along the length of the blood vessel can be eliminated. However, there are important geometrical considerations in the preparation of spiral strips (297, 309, 491, 635). For example, the helical and circular arrangement of smooth muscle cells in canine muscular arteries can cause contractile agonists to produce varying magnitudes of contraction, the variation being dependent upon the pitch of the angle of orientation of the helix from the transverse axis of the vessel (figure 1A). At the extreme, paradoxical contraction is observed with the normally relaxant drug papaverine and paradoxical relaxation from the normally contractile drug norepinephrine (β-adrenoceptors blocked) when these preparations are mounted longitudinally (figure 1B; 491). Similar effects were observed in aortae from normal and DOCA-hypertensive rats (297). A convenient alternative is the use of rings of vascular smooth muscle mounted on opposing stainless steel hooks (326). These preparations have the advantages of convenience and ease of preparation, minimal variation of differences in smooth muscle cell orientation, and minimal damage to the intimal surface of the vessel. In view of the importance of the intima to the tissue responses of blood vessels to some agonists this could be an important consideration (242). Perfused vessels, notably the rabbit ear artery, are useful in that they allow for the study of agonists exclusively applied to the intimal or advential side of the blood vessel (169).

There are numerous methods to prepare other smooth muscle preparations. In some tissues, attention must be paid to anatomical differentiation of the muscle as, for
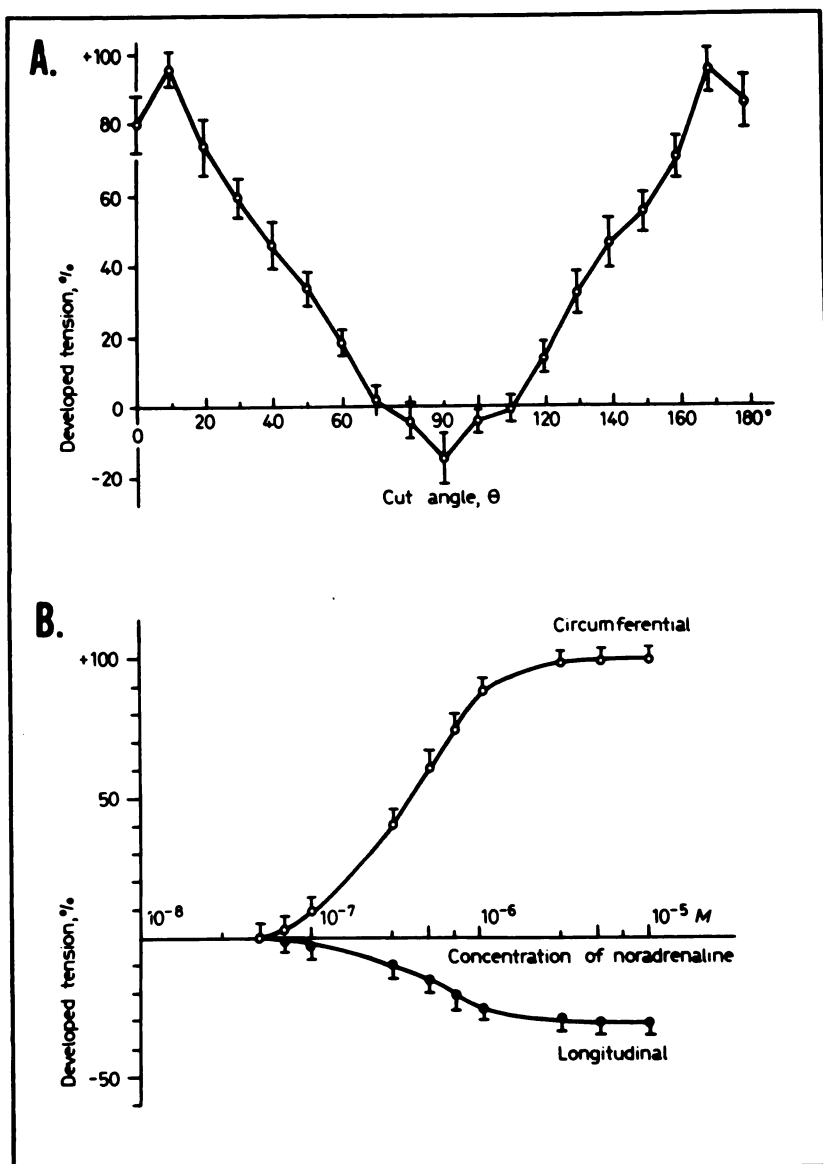
FIG. 1. Effect of angle of cut on responses of femoral artery to norepinephrine. A. Responses to 100 μM norepinephrine as a percent of the maximal contraction. Abscissae: Pitch of helical cut in degrees. Positive values indicate contraction; negative values indicate relaxation. B. Concentration-response curves to norepinephrine. Ordinates: Tension as a percent of maximal contraction. Abscissae: Molar concentrations of norepinephrine (log scale). Responses from rings [circumferential contraction (O)] and longitudinal preparations (●). For both figures $n = 10$; bars represent S.E.M. Reprinted with permission from Ohashi and Azuma (491).

example, in the rabbit urinary bladder base and body (421) or the longitudinal versus circular muscle of the rat fundus (666). The guinea pig ileum longitudinal muscle strip holds advantages over the whole ileum in isolated tissue work (527). There are a wide variety of ways to mount and monitor the contraction of airway smooth muscle (295).

Cardiac muscle function can be measured either by perfusion of the heart by the Langendorff technique (95, 409, 523) or measurement of twitch contraction from isolated atria, papillary muscle, or strips of ventricle (82, 393). Cardiac sinus nodal function can be obtained from isolated right atria. The electrical stimulation of cardiac tissue requires attention to the arrangement of the electrodes if release of neurotransmitters is to be avoided

(79–81). The use of a geometrically homogeneous tissue such as the papillary muscle is an advantage over preparations having irregular geometry for tension development such as strips of ventricular muscle (593, 594). Particular attention must be paid to the thickness of the cardiac muscle utilized in isolated tissue experiments since the high oxygen consumption of this tissue makes hypoxia a distinct possibility in the organ bath (82).

Certain physical procedures can alter existing isolated tissue preparations to suit special needs. For example, rabbit aorta can be effectively denervated by excision of the adventitia (68, 446). Similarly, stripping the serous coat and mesenteric attachments of vas deferentia effectively denervates this preparation (72).

E. Measurement of Tissue Responses

There are many methods of measuring the responses of various isolated tissue preparations to drugs. Responses can be measured in terms of magnitude as in isotonic shortening, isometric tonic or phasic contraction, isometric twitch contraction, or frequency (i.e., atrial rate, spontaneous motor activity). In some cases the method of recording responses does not influence the sensitivity of the preparation to agonists. Thus the sensitivity of guinea pig ileal longitudinal smooth muscle to histamine is not appreciably different when contractile responses are measured isotonically or isometrically (145). Similarly, the sensitivity of rat tail arteries to norepinephrine does not differ when isotonic and isometric responses are compared (660). In other tissues this does not appear to be true and the difference may depend upon the thickness of the preparation and the relative amount of muscle mass and receptor activation required for maximal response. Thus, in contrast to the guinea pig ileum, there is a significant difference between the concentration of carbachol required to produce half maximal isotonic contraction and that needed for half maximal isometric contraction in frog rectus abdominus and leech dorsal muscle (451). In these relatively thick muscles, more agonist is required for isometric tension than isotonic shortening. This differentiation carries over to partial agonists where it was observed that, relative to the maximal response produced by potassium depolarization, the intrinsic activity of partial agonists was greater in these muscles when measured isotonically as opposed to isometrically. In keeping with the concept of a greater receptor stimulation being needed for isometric contraction, the isometric responses to muscarinic agonists were more sensitive than isotonic responses to blockade by receptor alkylating agents (451).

When measuring responses isometrically, it is important to do so at L_{max}, the length of tissue which produces the maximal active response (597, 598). In tissues such as cardiac muscle (593, 594) and muscular, as opposed to elastic, arteries (693), it is especially important to work at the optimum resting tension.

F. Sources of Variation in Tissues

A great deal of pharmacological inference is derived from the relative sensitivity of tissues to agonists. Many factors including animal variation with respect to agonist uptake mechanisms, numbers of viable receptors, and differences in the efficiency of stimulus-response mechanisms can cause heterogeneity in sensitivity of tissues to agonists. To a large extent, increasing the number of experiments can accommodate these errors (246).

One method of reducing the between animal variation in experiments is to use paired tissue preparations from the same animal and make comparisons between pairs of treated and control tissues. Some tissues can be divided into equal portions such as atrial halves, rings of conduit vessels and airway smooth muscles, and segments of gastrointestinal tract. Other tissues such as uterus, vasa deferentia, seminal vesicles, and anococcygeus muscles conveniently come in matched pairs.

1. Heterogeneous Receptor Distribution. Another factor to consider in the choice of isolated tissue is the homogeneity and distribution of the drug receptors. The presence of a heterogeneous population of receptors subserving antagonistic responses with respect to each other can confound the quantification of drug effects on any one of the drug receptors. The most common setting for this situation is found in the study of adrenoceptors. For example, tissues with a predominant α-adrenoceptor population in the presence of pharmacologically antagonistic β-adrenoceptors include mouse spleen (339), rat mesenteric artery (90), and cat nictitating membrane (588). Tissues with a dominant β-adrenoceptor population in the presence of antagonistic α-adrenoceptors include guinea pig trachea (470, 515) and rat uterus (99). Opposing populations of histamine H_1 and H_2 receptors have been reported in rat stomach fundus and rabbit aorta (200). Synergistic α-adrenoceptors in the presence of dominant β-adrenoceptors are found in guinea pig and rabbit atria (278). The most comon method used to detect and eliminate this problem is by using selective antagonists of the interfering receptor population. For example, the α-adrenoceptor responses of rat veins are strikingly potentiated when the relaxant β-adrenoceptors are blocked by propranolol (136). The potentiation of the relaxant effects of histamine (H_2 receptors) by the antagonism of histamine H_1 receptors by chlorpheniramine in rabbit trachea illustrates another example of mixed receptors in an isolated tissue (381). The problem of heterogeneous receptors becomes more important when dealing with partial agonists. For example, little response can be elicited in the canine saphenous vein by dobutamine. However, blockade of the α-adrenoceptors with phentolamine reveals a relaxant β-adrenoceptor response and blockade of the β-adrenoceptors with propranolol reveals an α-adrenoceptor contractile response both of which cancel each other in the absence of the blocking drug (370).

There are gradients of responsiveness in tissues to agonists which coincide with anatomical orientation. For example, it has been shown that rabbit aorta is not uniformly sensitive to β-adrenoceptor agonists (8). There is a pronounced heterogeneity in rabbit trachea and bronchus in response to a variety of spasmogens and relaxants (213). In canine aorta, a gradient of phosphodiesterase activity has been observed (644). In the rabbit basilar artery a graded responsiveness to norepinephrine, increasing from the distal to the proximal portions and to serotonin and decreasing from the distal to the proximal segment of the tissue, has been reported (263). Some of the observed heterogeneity has been ascribed to the heterogeneous distribution of receptor types. For exam-

ple, the urinary bladder of the rabbit can be divided into the bladder body where α-adrenoceptors predominate over β-adrenoceptors and the bladder base where the opposite predominance occurs (421). In the rabbit aorta, Fleisch and coworkers (215) found that the thoracic aorta contained a greater predominance of β-adrenoceptors than the abdominal aorta. In the dog, there are mainly α-adrenoceptors in the common coronary artery, both α- and β-adrenoceptors in the proximal portion, and mainly β-adrenoceptors in the distal coronary artery (464). Bovine trachea has been shown to possess 37 times the number of muscarinic receptors and one eighth the number of β-adrenoceptors as bovine lung (126). Receptor subtypes also appear to be heterogeneously distributed within tissues. For example, responses of rat vasa deferentia to selective α-adrenoceptor agonists suggest a heterogeneity of pre- and postsynaptic α-adrenoceptors in the prostatic and epididymal portions of this tissue (427). In dog femoral and sphaneous veins α_1- and α_2-adrenoceptors appear to coexist while in the dog femoral and splenic artery, α_1-adrenoceptors predominate (177). In canine trachea α_2-adrenoceptors appear predominant while in the peripheral airways α_1-adrenoceptors dominate contraction (45). In guinea pig airways, the responses to various β-adrenoceptor agonists and antagonists suggest that the proportion of β_1- to β_2-adrenoceptors decreases from the trachea to the bronchus and intrapulmonary airways (713).

The heterogeneous distribution of drug receptors in various isolated tissues should be kept in mind when quantitative comparisons of drug effects, especially within tissues, are made. One previously mentioned method of reducing the consequences of such heterogeneity is the use of the complete tissue for assay by, for example, preparation of a spiral strip.

2. Animal Maturity. Age has been found to affect the receptor density and/or distribution and the reactivity of various isolated tissues. For example, the responsiveness of rat hearts to ouabain (255), the sensitivity of rat and guinea pig trachea to isoproterenol (2), the histamine H_2-receptor-mediated responses of rabbit aortae (321), and the effects of 5-HT agonists in rabbit aortae (301) have been shown to decrease with animal maturity. The sensitivity of responses in rat vasa deferentia postulated to be due to stimulation of presynaptic α-adrenoceptors also is inversely proportional to age (183). In contrast, age has been shown to increase the contractions of rat aortae to norepinephrine and serotonin (135). There is substantial evidence that β-adrenoceptor-mediated relaxation of rabbit and rat thoracic aortae decreases with increasing age (201, 212, 215, 288). This is in contrast to the lack of effects of age on the β-adrenoceptor-mediated responses of rat and rabbit portal vein (214). The responses of rabbit trachea to several agonists was found to change with age (300). The predominance of one receptor type over another can vary with animal maturity

as well. For example, the dominant positive inotropic responses of rat atria to phenylephrine shift from being predominantly β-adrenceptor-mediated in young rats to α-adrenoceptor-mediated in adult rats (299). This is in accordance to the measured decreased number of β-adrenoceptors (128) and increased number of α-adrenceptors (403) in rat hearts with age. While the effects of age would not be expected to produce serious variation in tissues from carefully matched animals, they could account for differences observed in different laboratories.

G. Comparisons between Isolated Tissues

Often it is desirable to compare the responses of isolated tissues from animals either between species or in the same species in different physiological or pathological states. For example, much research has been conducted on the relative contractile activity of blood vessels from normal and hypertensive rats. In these comparisons, the scale of responsiveness should be carefully normalized to eliminate differences due to muscle mass and thickness. Thus, simple comparisons of actual responses in Newtons may be misleading. It is important to compare the different tissues not at the same resting tension but rather at the optimal resting tension for each tissue. One way to normalize active tension is to use a scale that makes active muscle tension, as a function of resting or passive tension (or length), superimposable or parallel with the same maximum for the two types of tissue preparations. For example, Wyse found the force per cross sectional area of muscle a suitable scale for comparing muscular arteries and the force per unit of total volume for comparing elastic arteries of differing mass in rats (707). In comparing responses to agonists, the effects of muscle mass on diffusion coupled with differences in uptake, degradative metabolism, and innervation also should be considered as complicating factors.

III. Equilibrium Conditions in Isolated Tissues

The equations derived to describe drug and receptor interactions assume free diffusion and the establishment of thermodynamic equilibria. In well-mixed biochemical reactions these are reasonable assumptions but in many isolated tissue preparations the assumption that these conditions are attained enters more the realm of wishful thinking (698). Accurate knowledge of the magnitude of the independent variable is a prerequisite to the generation of meaningful dependent variables and in pharmacological experiments, the dependent variables that yield information about drugs and drug receptors are tissue responses and the critical independent variables are the concentrations of drug at the receptor. It is important to know the concentration of drug at the receptor, since it is from this parameter that all estimates of drug constants such as affinity and efficacy are made.

The need for the attainment of equilibrium conditions in isolated tissues has been stressed (230, 231, 552, 567, 663). A concise statement of the assumptions made

(sometimes tacitly) in isolated tissue experiments was presented by Furchgott (232) and is given in table 3 as a focus for discussion. Each of these points will be considered in some detail in this review.

The activation of receptors by drugs in isolated tissues can be divided into three processes: 1) the delivery of drug from the organ bath solution to the receptor compartment, 2) the interaction of the drug with the receptor, and 3) in the case of agonists, the transduction of receptor stimulus into tissue responses (27). These latter two processes have been termed the "pharmadynamic" phase of drug action by Ariens (23, 25, 271). The first step is the delivery of drug to the drug receptor, a bulk diffusion phenomenon that can be affected by chemical, physical, and biochemical processes.

A. Chemical Degradation of Drugs

Since the driving force of bulk diffusion is a concentration gradient, any process that changes concentration will affect the rate of diffusion. One way in which drug concentration can be changed in an organ bath is by chemical degradation. Perhaps the most well-known example of the chemical instability of drugs is the tendency of catecholamines to oxidize in the presence of trace amounts of divalent cations or in alkaline pH (293, 294). The addition of disodium EDTA (228) or ascorbate to the bathing medium prevents this chemical degradation. In the absence of these measures, the destruction of catecholamines can be quite rapid; for example, the half-time for degradation of 0.3 μM norepinephrine in physiological saline is 8 to 9 min at 37°C and 25 to 26 min at 32°C (330). The half-time at 37°C is within the time span required for the attainment of steady state responses in many isolated tissue preparations such as rabbit aorta, guinea pig trachea, and guinea pig extensor digitorum longus, thus inclusion of antioxidants to the bathing medium would be highly recommended. Another example of chemical instability in physiological saline is

the rapid inactivation of prostaglandins (219). The degradative process can be optically catalyzed as for example the photolysis of aqueous solutions of lysergic acid diethylamide (681) or the well-known instability of the calcium channel antagonist, nifedipine, in the presence of light.

The adsorption of drugs to the surface of the organ bath can serve as a physicochemical process of drug removal from the receptor compartment. This methodological problem has been encountered with basic antihistamines such as promethazine (442) where substantial dilution errors were introduced into experiments by the use of glass containers. Adsorption to glass surfaces has been encountered with peptides such as substance P. The addition of 0.1% bovine serum albumin (683), dithiothreitol (353), or the use of polypropylene organ baths (223) have been found to eliminate the problem. The adsorption of substances to surfaces and subsequent leeching into fresh physiological solution in future experiments also can be a significant methodological problem. This effect has been encountered with rubber surfaces [propranolol (234)], silicone surfaces [haloperidol (595A)], and glass surfaces [1-isoproterenol (234); guanabenz (427)].

B. Release of Endogenous Substances

Disparity between the concentration of drug in the organ bath and active drug at the receptor site can arise if the drug promotes release of an endogenous substance in the tissue. It would be expected that the magnitude of the response would be increased if the endogenous substance produced the same qualitative response or decreased if the substance produced a pharmacologically antagonistic response. The most common type of release encountered in pharmacological experiments is that of endogenous neurotransmitters such as norepinephrine and acetylcholine. Table 4 shows a number of agonists that release an endogenous substance (indirect agonist)

TABLE 3
*Optimal conditions in experiments for the pharmacological characterization of drug receptors in isolated tissues**

1. The response of the tissue preparation to an agonist should be due solely to the direct action of the agonist on one type of receptor. It should not be resultant of actions on more than one type of receptor, nor should it be due even partially to indirect action (e.g., release of endogenous noradrenaline).

2. The altered sensitivity to an agonist in the presence of a competitive antagonist should be due solely to competition between the antagonist and the agonist for the receptor. The altered sensitivity after treatment with an irreversible antagonist should be due solely to inactivation of the receptor.

3. The response following the addition of a given dose of agonist should be measured at the maximal level reached. In the most suitable tissues, this maximal level is maintained for a reasonable length of time.

4. In the case of either an agonist or competitive antagonist, the free concentration in the external solution should be maintained at a steady level at the time a response is measured, and should be known. In the case of an irreversible antagonist, the concentration in the solution should be essentially zero during the measurement of responses.

5. In the case of either an agonist or competitive antagonist, the concentration in the region of the receptors should be in diffusion-equilibrium with that in the external solution at the time a response is measured. To meet this condition, the rate of removal of drug from this region due to enzymic action, transport into cells, and binding should be negligible compared with the rate due to diffusion back to the outside solution. In the case of an irreversible antagonist, the fraction of receptors which is not inactivated should remain constant over the total period during which responses are measured.

6. The experimental design should include proper controls to permit measurements of, and corrections for, any changes in sensitivity of the tissue preparation to agonists during the course of an experiment that are not due to addition of an antagonist.

* Reprinted with permission from Furchgott (232).

TABLE 4
Some agonists that release endogenous substances in tissues

Agonist	Species	Tissue	Indirect Agonist	References
Dopamine	Guinea pig	Atria	Norepinephrine	(496)
		Trachea	Norepinephrine	(395)
	Rat	Gastric fundus	Norepinephrine	(414)
Histamine	Cat	Trachea	Norepinephrine	(204, 436)
	Rat	Atria	Norepinephrine	(3)
4-Methylhistamine	Rabbit	Atria	Norepinephrine	(521)
2-(2-Pyridyl)ethylamine	Guinea pig	Heart	Norepinephrine	(402)
	Rat	Atria	Norepinephrine	(3)
Tolazoline	Rabbit	Atria	Norepinephrine	(334)
			Histamine	(334)
	Guinea pig	Atria	Histamine	(334, 378, 423)
			Acetylcholine	(334)
Clonidine	Rabbit	Aorta	Histamine	(87)
	Guinea pig	Atria	Histamine	(378, 423)
Cimetidine	Rat	Uterus	Norepinephrine	(548)
Impromidine	Rabbit	Atria	Norepinephrine	(332)
5-Hydroxytryptamine (5-HT)	Guinea pig	Ileum	Acetylcholine	(151, 509)
Bradykinin	Rabbit	Pupillary sphincter	Substance P	(115)
Substance P	Rat	Mast cells	Histamine	(202)
Adenosine	Rat	Tail artery	5-HT	(100)

to produce responses in some isolated tissues. A comprehensive review of phenylethylamines and other drugs that release norepinephrine has been given by Trendelenburg (642).

Various methods are available to eliminate the release of indirect agonists in isolated tissues. If the indirect agonist (released substance) produces responses by activation of a receptor distinct from the receptor of interest, then selective antagonists can be used to eliminate the complicating effects. However, this sometimes is not possible if the indirect agonist activates the same receptor as the direct agonist; i.e., for obvious reasons, the direct effects of ephedrine on β-adrenoceptors cannot be separated from the effects of released norepinephrine by the addition of propranolol. The alternative here is to deplete the tissue stores of endogenous norepinephrine by 6-hydroxydopamine treatment in vivo (624, 642), or in vitro (17), by physical removal of the neural plexus (68, 446) or, most commonly, by pretreatment of the animal with a catecholamine-depleting drug such as reserpine (447, 642).

The effects of a competitive antagonist on concentration-response curves to an agonist may provide a clue as to whether the responses to the agonist are due to direct activation of a receptor or to release of an indirect endogenous agonist. Black and coworkers (76) have presented a theoretical model which predicts that under a variety of circumstances the concentration-response curve to an agonist that produces an effect by release of an indirect agonist will be shifted to the right by a competitive antagonist but also show a depressed maximal response. Experimentally, this was demonstrated by the shift and depression of concentration-response curves to tyramine by propranolol and sotalol in rat atria

(76). As shown in figure 2, the maximal responses to tyramine are progressively depressed with increasing dextral displacement of the concentration-response curves by propranolol. Similar effects have been reported for tyramine inhibition by propranolol (625) and phentolamine (207). The model predicts that the receptor
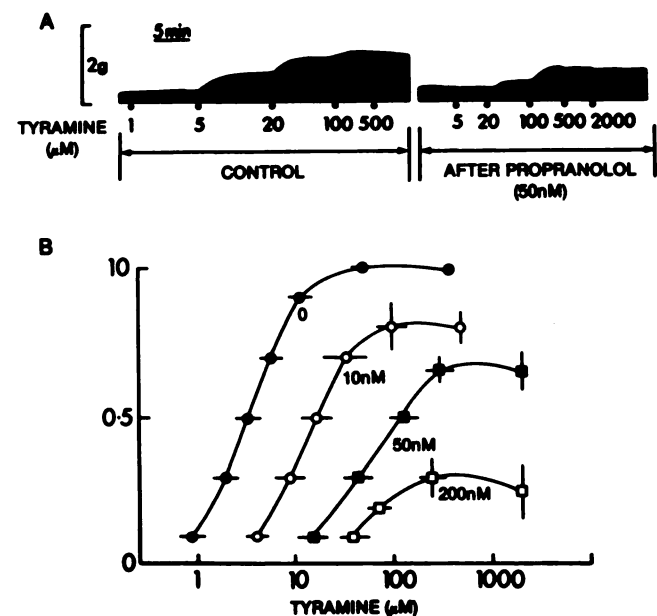

FIG. 2. Effects of propranolol on responses of rat left atria to tyramine. A. Dynograph tracing of electrically stimulated contractions of rat left atria; responses to tyramine before and after propranolol (50 nM). B. Concentration-response curves to tyramine. Ordinates: Responses to tyramine as fractions of the maximal control response. Abscissae: Logarithms of molar concentrations of tyramine. Responses in absence (●, *n* = 17) and presence of propranolol 10 nM (●, *n* = 5), 50 nM (□, *n* = 7), and 200 nM (□, *n* = 5). Bars represent S.E.M. Reprinted with permission from Black et al. (76).

occupancy (ρ) achieved by an agonist [A], which produces release of an indirect agonist [I], is given by (76):

$$\rho = \cfrac{1}{1 + \cfrac{K_I}{\theta}\left(1 + \cfrac{K_A}{[A]}\right)} \qquad (2)$$

where K_A and K_I refer to the equilibrium dissociation constant of agonist A for the site of release and I for the receptor, respectively, and θ a measure of the releasable pool of indirect agonist (e.g., the maximal concentration of I achieved by saturating concentrations of A) with dimensions of concentration. It can be seen from Eq. 2 that even in the absence of a competitive antagonist, the maximal receptor occupancy by the indirect agonist may not be achieved by any amount of agonist A if K_I/θ is large; i.e., if the pool is small (low θ) or K_I large (low affinity of I for the receptor). Depending upon the effective receptor reserve in the tissue, which is a function of the efficacy of I and the efficiency of the stimulus response mechanisms in the tissue, a high ratio of K_I/θ may preclude production of a maximal response by A. In the presence of a competitive antagonist B, the receptor occupancy becomes:

$$\rho' = \cfrac{1}{1 + \cfrac{K_I}{\theta}\left(1 + \cfrac{K_A}{[A]}\right)\left(1 + \cfrac{[B]}{K_B}\right)} \qquad (3)$$

where K_B is the equilibrium dissociation constant of the antagonist for the receptor. It can be seen intuitively from Eq. 3 that the tendency of values of K_I/θ to depress the maximal response to A is exacerbated by the addition of competitive antagonist; i.e., a competitive antagonist will tend to depress the maximal responses to agonist A. The amount of depression of the maximal response by B will depend upon the size of the releasable pool θ, the affinity and efficacy of the indirect agonist I, and the efficiency of the stimulus response mechanisms in the tissue. Therefore, in a tissue with a high receptor reserve and a large pool of releasable endogenous agonist, a competitive antagonist may produce parallel shifts of the control concentration-response curve (with no depression of maximum) to a releasing agent. This situation would closely resemble what would be expected of a direct agonist. However, if the concentration-response curves to an agonist are shifted to the right and depressed by a competitive antagonist in a pattern like that shown in figure 2, this would suggest that the agonist was releasing an endogenous agonist in the tissue.

Finally, the effects of drugs not mediated by drug receptors can obscure isolated tissue experiments. For example, the effects of high concentrations of histamine on organ bath pH have been noted in rabbit atria (178, 332), guinea pig pulmonary artery (296), and rabbit trachea (381). In this latter preparation, relaxant responses to histamine could be elicited which were insensitive to histamine H$_2$-receptor-blocking drugs but were eliminated by neutralization of the acidic stock solution of histamine.

C. The Removal of Drugs by Tissues

1. Diffusion into Isolated Tissues. A drug added to the solution bathing a tissue in an organ bath must diffuse into the receptor compartment to produce an effect. There are mathematical models to describe this process which are relevant to this discussion because they highlight some factors that can seriously affect parameters thought to reflect drug-receptor interactions in tissues. For the purposes of these discussions, the bathing solution is assumed to be an infinite reservoir of drug at constant concentration.

The dissolution of drug from the point of injection into a well-mixed organ bath occurs relatively rapidly. For example, Cuthbert and Dunant (164) found that conductance changes across electrodes in a 50-ml bath produced by injection of potassium chloride solution occurred within 0.05 sec of injection. However, there are reasons to suppose that the process of free diffusion which controls the access of drug *to* the surface of the tissue does not describe the entry of drug *into* the tissue.

It is known that the diffusion coefficient of drugs in tissues is slower than in free solution (101, 315, 401). For example, the diffusion of acetylcholine into rat diaphragm is 0.14 times that in free solution (400) while the diffusion of norepinephrine in the medial layer of rabbit aorta is 0.1 times the rate in free solution (67). One method of accommodating these phenomena is by defining a tortuosity factor (494, 495, 672) designated λ. Thus the diffusion coefficient of a drug in a tissue D' relates to the diffusion coefficient of the drug in free solution D by the following equation:

$$D' = \frac{D}{\lambda^2}. \qquad (4)$$

The rationale for such a factor is that while the diffusion coefficient measures the random rate of travel by a straight line, the path that a drug must take through a tissue is considerably longer since it must accommodate the numerous obstructions in the morphological organization of the muscle.

With diffusion equations derived by Crank (158), it is possible to calculate the theoretical rate of diffusion of a drug into tissues. Thus, for a tissue which can be approximated geometrically by a cylinder, the rate of change of drug concentration ($\partial C/\partial t$) across the radius of the muscle r is given by:

$$\frac{\partial C}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r} r \frac{D \partial C}{\partial r}. \qquad (5)$$

A graphic solution for Eq. 5 was provided by Venter (672) for a cat papillary muscle 1 mm in diameter with a tortuosity factor, estimated to be 1.44 for this tissue (495), and is shown in figure 3. It is interesting to note
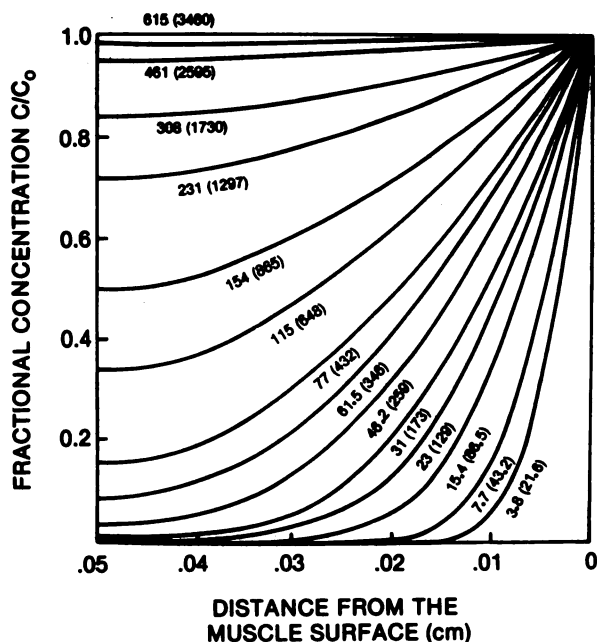
FIG. 3. Calculated distribution of isoproterenol and polymeric isoproterenol (12,800 mol. wt.) in a 1-mm diameter cat papillary muscle at 25°. Ordinates: Fraction of organ bath concentration within the muscle. Abscissae: Distance from muscle surface. Numbers refer to time in seconds for free isoproterenol and, in parentheses, polymeric isoproterenol. Reprinted with permission from Venter (672).

that complete diffusion of isoproterenol to the center core of the tissue requires 10 min.

The rate of equilibration of the extracellular space with [³H]inulin is known to vary with tissue type. Watson (682) has shown this time to be 30 sec in guinea pig ileal longitudinal muscle and 300 sec in rat vas deferens. Michelson and Shekovnikov (451) have shown a range from 10 sec in guinea pig ileum to 400 sec in the protractor pharynx of holothura. One factor thought to be responsible for these differences in diffusion rates for different tissues is tissue thickness. The diffusion time is related to tissue thickness (L) by the following equation (573):

$$t = \frac{L^2}{2D'}.$$ (6)

Considering the morphological architecture of tissues to be reflected by the tortuosity factor, this equation can be modified to:

$$t = \frac{L^2 \lambda^2}{2D}$$ (7)

where D is the diffusion coefficient of the drug in free solution. Factors which affect L will correspondingly alter diffusion time.

Another determinant of diffusion time is the conformation of the extracellular space. The extracellular space is a complex structure (274) and should be considered a dynamic compartment rather than a stiff box (650). The size of the extracellular space is affected by stretching

and relaxation (693) and this can affect diffusion. For example, the hydrolysis of acetylcholine by acetylcholinesterase is greater in stretched rather than contracted guinea pig ileum (462). The orientation of the tissue may be a factor as well in that diffusion paths for drugs may be longer in some physical configurations of tissues. For example, the $T_{1/2}$ for diffusion into thin but twisted longitudinal muscle strips of guinea pig ileum is longer than that for whole guinea pig ileum (164). Finally, changes in the effective extracellular space must be considered for drugs that have no visible effect on muscle tone but do nevertheless affect $T_{1/2}$ for diffusion (650).

The effects of diffusion on drug entry into tissues are not in themselves capable of seriously affecting the measurement of drug receptor parameters. However, these factors, when coupled to an active uptake process for drugs within the isolated tissue, take on a new dimension of relevance to in vitro experiments.

2. *Drug Removal Processes in Isolated Tissues.* When a tissue possesses an active removal mechanism for a drug then the response to that drug is governed by the steady-state concentration of the drug in the receptor compartment which in turn is controlled by the relative rate of drug entry (by diffusion) and removal (by tissue uptake). Depending upon these relative rates there could be a constant deficit of drug at the receptor when compared to the concentration in the organ bath. Thus, if the rate constant for tissue uptake of a drug exceeds the rate constant for diffusion into the tissue by a factor of 10, then a 10 μM drug concentration in the organ bath translates to a steady-state concentration at the receptor of 1 μM; i.e., tissue uptake shifts concentration-response curves of agonists to the right. Inhibition of tissue uptake corrects this deficit and allows more of the drug added to the organ bath to reach the receptor thus the concentration-response curves to agonists taken up by tissues shift to the left after inhibtion of the tissue uptake processes, i.e. tissue sensitization to the agonist. This type of sensitization, termed deviation supersensitivity by Fleming (216), should be distinguished from changes in tissue sensitivity brought about by changes in stimulus response mechanisms. The maximal deviational sensitization obtained in any one tissue reflects the effective importance of uptake in that tissue (the magnitude of drug deficit at the receptor) and is controlled by those factors which control the rate of drug entry (D, λ, L) and removal (the K_m and V_{max} of an uptake process described by Michaelis-Menten kinetics).

Differences in the factors reflecting tissue size and morphology (λ, L) can cause differences in the effects of uptake within a given tissue type. Ebner and Waud (191) have derived a useful model to describe certain effects of tissue geometry on the observed sensitization of tissues to agonists. Thus, variable tissue thickness (L) was expressed as an increased volume to surface area ratio (V/S). Eq. 8 describes the concentration of agonist in the

receptor compartment [A_i] as it relates to the concentration in the organ bath [A_0] for a tissue of volume V, surface area S, with an uptake process having a Michaelis-Menten constant designated K_m and maximal velocity of uptake J_m (191).

$$[A_i] = -\tfrac{1}{2}[-[A_0] + K_m + (J_m/k_{in})(V/S)]$$
$$+ \sqrt{\tfrac{1}{4}[-[A_0] + K_m + (J_m/k_{in})(V/S)]^2 + K_m[A_0]} \quad (8)$$

where k_{in} refers to a permeation constant encompassing diffusion and tortuosity. Note how the concentration of agonist in the receptor compartment [A_i] relates to the concentration in the organ bath [A_0], the avidity of uptake (J_m, K_m) and the diffusional (k_{in}) and geometrical (V/S) characteristics of the tissue. Ebner (189) used Eq. 8 to calculate the theoretical deficit of agonist at the receptor produced by an uptake process with given values of (J_m/k_{in}), (V/S), and K_m. Figure 4 shows the relationship between the concentration of an agonist in the organ bath and the concentration at the receptor (extracellular space). This figure illustrates the three general regions of deficit of agonist in the receptor compartment: 1) at concentrations of [A_0] below the effective K_m for agonist uptake, there is a constant ratio between the steady-state concentration at the receptor and that in the organ

bath; 2) as the concentration of agonist in the organ bath approaches, equals, and surpasses the effective K_m of uptake in the tissue, the concentration in the receptor approaches the concentration in the organ bath (nonlinear portions of the curves in figure 4); and 3) when the concentration of drug in the organ bath is well above the effective K_m of uptake in the tissue, then uptake is saturated and the concentration of agonist at the receptor equals the concentration in the organ bath. Ebner and Waud (191) used Eq. 8 to predict the effects of various ratios of V/S on concentration-response curves. Figure 5A shows that as V/S increases, concentration-response curves shift to the right and become steeper than the concentration-response curve to the agonist which would be obtained in the absence of uptake. In an elegant series of experiments, they then went on to correlate the sensitivity of guinea pig papillary muscles to norepinephrine with the thickness of the muscle (figure 5B), a positive correlation which graphically illustrated the tangible effect of V/S on uptake and the estimation of agonist potency.

When dealing with complex whole tissues, certain other geometrical factors become important such as whether the uptake process functions as an effective diffusion barrier (356) and the relative geometry of uptake and receptors (643). Ingenious experiments with techniques such as surface-selective perfusion of blood vessels (169) and inhibition of diffusion into strips (499) or rings (448) by application of selective diffusion barriers have demonstrated nonhomogeneously distributed removal mechanisms in vascular smooth muscle. For example, the greater sensitivity of perfused rabbit ear artery to norepinephrine when applied to the intimal rather than the adventitial surface (170, 172, 355) has been attributed to the nonhomogeneous distribution of adrenergic innervation in this tissue (170, 172). A nonhomogeneous distribution of neuronal and extraneuronal uptake mechanisms has been proposed for rabbit aorta (65, 66, 420, 500). Heterogeneity in the relative location of uptake processes within a tissue can be compounded by uneven sensitivity of muscle cells to agonists within the tissue. This latter factor has been proposed for rabbit aorta (501) and sheep carotid artery (279).

The aspects of heterogeneous distribution of uptake processes that are relevant to drug receptor responses relate to the relative geometry of the sites of uptake and the receptors. For example, considering the neuronal uptake of catecholamines as the site of loss, Trendelenburg (643) shows the poor correlation between the density of adrenergic innervation of tissues and the sensitivity to norepinephrine and highlights the correlation of sensitivity to neuromuscular interval (the distance from the nerve terminal to the muscle) given by Verity (676). It should be noted that neuromuscular distance may be superseded in importance in some tissues by asymmetry of innervation [i.e., rabbit ear artery (170, 174)]. Relative
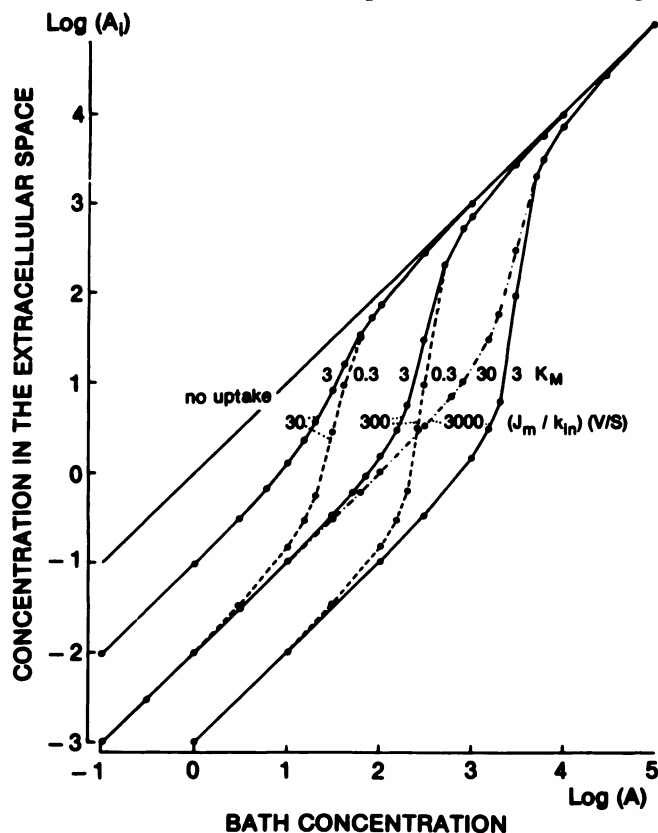

FIG. 4. The effect of agonist uptake on concentrations of agonist in the receptor compartment. Ordinates: Logarithms of molar concentrations of agonist in the extracellular space (receptor compartment). Abscissae: Logarithms of molar concentrations of agonist in the organ bath. Relationships shown for uptake processes with various rates and tissues with various geometrical shapes (V/S). Reprinted with permission from Ebner (189).
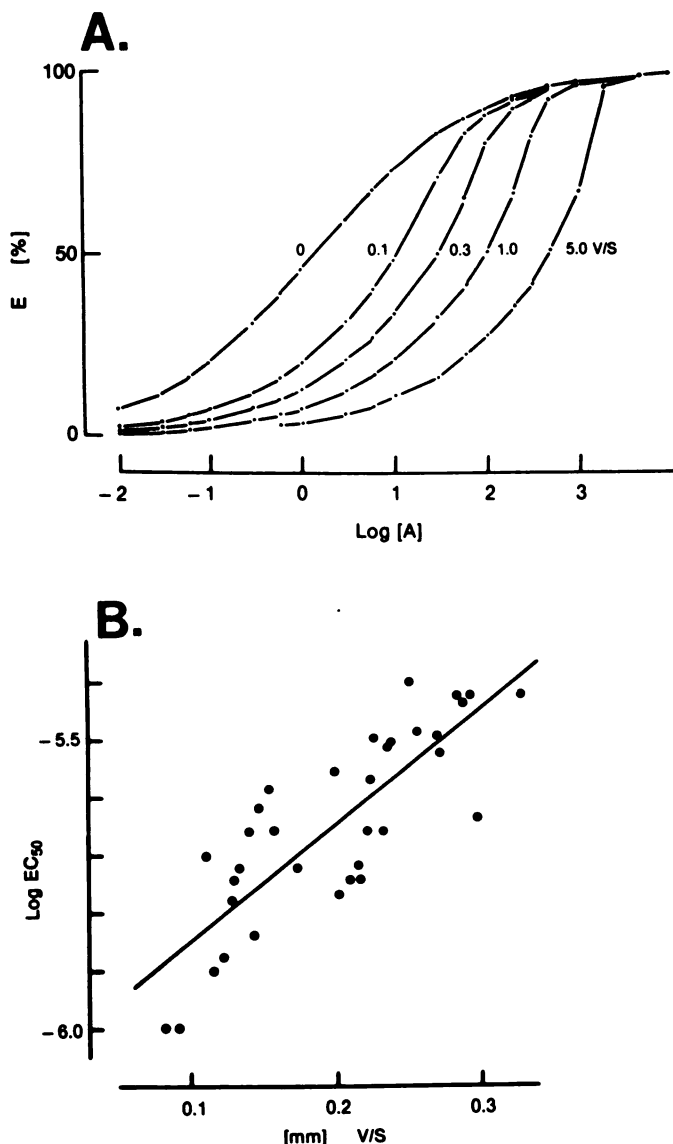
FIG. 5. Effect of geometry on sensitivity of tissues to agonists subject to uptake. A. Calculated effects of various geometrical shapes (V/S). Ordinates: Response calculated by a general logistic function E = 100 $(A_i^{0.5})/(A_i^{0.5} + 1.5^{0.5})$. Ordinates: Logarithms of agonist concentration. A_i calculated from Eq. 8 with $k_m = 3$, $J_{max}/k_m = 300$. Different curves show the effects of changes in V/S (Eq. 8). B. Correlation between geometry and sensitivity. Ordinates: Logarithms of molar concentrations of norepinephrine required for half maximal response in guinea pig papillary muscle. Abscissae: Volume to surface ratios assuming a cylindrical shape for muscles ($n = 34$). Reprinted with permission from Ebner and Waud (191).

geometry of uptake and receptors can lead to some extraordinary effects such as different effective receptor compartments for the one drug in one tissue acting on different receptors. For example, in the dog saphenous vein Guimares and Paiva (286) have postulated different biophases for α- and β-adrenoceptors, the α-adrenoceptors being closer to nerve terminals. A similar proposal describing the opposite situation has been described in rabbit facial vein where the β-adrenoceptors appear to be more under the influence of nerve terminals than α-adrenoceptors (703). In general, there is a considerable

body of evidence which suggests different receptor compartments for α- and β-adrenoceptors in vascular smooth muscle (287) and guinea pig cardiac muscle (190, 191).

3. Consequences of Uptake Inhibition in Isolated Tissues. The previous discussion has considered how an uptake mechanism for an agonist in a tissue coupled with various diffusion parameters can combine to produce a deficit of agonist at the receptor with respect to the concentration in the organ bath. Under these conditions, the potency of that agonist will be underestimated. To measure the true potency of an agonist for which the tissue possesses an uptake mechanism the uptake mechanism must be adequately inhibited or made otherwise inoperative. Inhibition of agonist uptake produces sinistral displacement of the concentration-response curve to the agonist if the conditions regarding the relative rate constants for uptake and diffusion are favorable and the concentrations of agonist in the organ bath do not saturate the uptake process (i.e., $[A]_i \ll K_m$). In a poorly coupled tissue where very high agonist concentrations are required to produce responses, intuitively it might be supposed that a saturable uptake process may not be an important determinant of tissue sensitivity since presumably the high agonist concentrations would saturate uptake. However, the concentrations required to saturate uptake in a structured isolated tissue may differ from those predicted from biochemical experiments. There are two possible reasons for this. Firstly, the true K_m for the uptake process in a tissue may be greater because of the stationary water layer surrounding all isolated tissues in an organ bath. This layer functions as a diffusion barrier and can cause low permeability coefficients for transport processes and a higher than normal K_m (702). The thickness of this unstirred layer, estimated in tissues to be from 70 to 220 μm, can be made larger by an increase in the viscosity of the bathing medium (164) and decreased by stirring (165). For example, the thickness of the unstirred layer for rat jejenum decreased from 198 μm to 141 μm and in rat ileum from 217 μm to 159 μm with stirring (702). The half-time for diffusion ($T_{1/2}$) is related to the thickness of the unstirred layer (d) by the following equation (702):

$$t_{1/2} = \frac{0.38d^2}{D} \tag{9}$$

where D is the coefficient of diffusion of the drug in free solution. Eq. 9 shows the effects that large unstirred layers, and therefore lack of stirring in the organ bath, can have on diffusion of drugs into the tissue.

The unstirred layer can grossly affect K_m of an uptake process in a tissue. The differences between the K_m observed in an isolated tissue and that obtained in a well-mixed biochemical experiment (difference = ΔK_m) is given by (589):

$$\Delta K_m = \frac{d \cdot V_{max}}{D}. \tag{10}$$

It can be seen from Eq. (10) that the greater the maximal velocity of the uptake process and the larger the thickness of the unstirred layer (d), the more disparate is the K_m in isolated tissues from the biochemical estimate. Henseling (308) has pointed out that in rabbit aorta, diffusional barriers falsify the kinetic constants for the uptake of [³H]norepinephrine and noted a steep concentration gradient of norepinephrine within this tissue if entry was restricted to one surface only.

A disparity between the effective K_m of the uptake process in the tissue and a biochemical estimate can occur also because of the concentration gradient of substrate created by the combination of ordered matrix of uptake sites in a tissue and slow diffusion. Thus, while a concentration of drug greater than the K_m may be present in the organ bath, the fact that the drug must pass through a matrix of uptake sites which depletes the concentration as it diffuses into the tissue may produce a large deficit between the concentration in the organ bath and that in the tissue (317). The importance of this effect is, as expected, dependent upon the relative rates of diffusion and uptake (281):

$$\frac{[A_i]}{[A_0]} = 1 - \left(\frac{V_{max}}{n \cdot D' \cdot [A_0]} (l^2 - r^2) \right) \qquad (11)$$

where $[A_i]$ is the concentration of substrate at point r in a tissue of radius 1, $[A_0]$ is the concentration of substrate in the bathing medium, V_{max} the maximal velocity of uptake and D' the diffusion coefficient of the drug *in the muscle* (i.e., $\lambda^2 \cdot D$). For this equation it is assumed that $[A_0] \ggg K_m$ and $n = 2, 4, 6$ depending upon whether the tissue can be approximated by a slice, cylinder, or a sphere, respectively. Thus, in the case of a *very high* $[A_0]$, $[A_i]/[A_0]$ will approach unity but under conditions where $[A_0]$ is already greater than K_m, and especially when V_{max} is high and diffusion is slow, a concentration gradient of substrate can still be formed in the tissue. There are examples of this effect in isolated tissues where it was found that the apparent K_m for the degradation of acetylcholine by acetylcholinesterase in rat diaphragm (460, 461) and guinea pig ileum (462) was 10 times greater than that in tissue homogenates. These disparities arise because the concentrations of acetylcholine in the extracellular space are considerably lower than those in the organ bath. Green (281) gives numerous other examples of this phenomenon.

The quantitative relationship between the degree of uptake inhibition by a competitive inhibitor of uptake I (with an equilibrium dissociation constant for the site of uptake of K_I) can be predicted from an equation based on models by Waud (685) and Furchgott (232). Thus, assuming $[A]_i \lll K_m$, the sensitization of a tissue to an agonist can be predicted by (367, 371):

$$x = \frac{[A]}{[A']} = \frac{y\left(1 + \frac{[I]}{K_I}\right)}{y + \frac{[I]}{K_I}} \qquad (12)$$

where $[A]$ and $[A']$ refer to equiactive molar concentrations of agonist before and after uptake inhibition, respectively, and the ratio $[A]/[A']$, designated x, refers to the sensitization of the tissue to the agonist produced by uptake inhibitor $[I]$. The maximal sensitization obtainable in any tissue (after complete inhibition of uptake, $[I] \ggg K_I$), designated y, is given by (232):

$$y = \frac{V_{max}}{k_t \cdot K_m} \cdot \frac{1}{\left(1 + \frac{[A]_i}{K_m}\right)} + 1 \qquad (13)$$

In this equation, V_{max} and K_m refer to the maximal rate of uptake and the equilibrium dissociation constant of $[A]$ for the uptake sites. The term k_t is a transfer rate constant [the reciprocal of the resistance term used by Waud (685)]. Before considering the quantitative relationship between tissue sensitization (x) and uptake inhibition some interesting aspects of the maximal sensitization (y) should be noted. From Eq. 13 it can be seen that if $[A]_i \ggg K_m$, i.e. if the concentration of agonist saturates uptake, then y will tend toward unity. In this case, the saturation of the uptake process allows no effective removal and therefore no deficit of $[A]$ at the receptor and no sensitization will be observed upon uptake inhibition. Large maximal sensitizations will occur if the tissue has a high maximal rate of uptake (large V_{max}) or poor diffusion characterisics (low k_t). The diffusion characteristics depend upon geometrical and morphological factors and since these can vary within the same tissue type (i.e., with age of animal, variations in the removal of access barriers such as fatty tissue or adventia), variance in y, the maximal sensitization obtainable, could be expected to occur. For example, the maximal sensitization of guinea pig trachea to isoproterenol after inhibition of extraneuronal uptake can be quite variable [5.0 (483); 8.5 (367); 30 (222)]. Figure 6A shows theoretical concentration-response curves for two tissues both with identical uptake processes (equal K_m and V_{max}). However, the diffusion characteristics differ in that k_t for tissue II is 0.1 × k_t for tissue I. The slower diffusion into tissue II produces a 10 times greater deficit of agonist at the receptor; i.e., the potency of A in tissue I is 10 times greater than in tissue II (curve A). This calculation illustrates that diffusional differences alone can produce varying effects of an uptake process with a given K_m and V_{max} in a variety of tissues. This is shown by the differences between the true concentration-response curves to the agonist and the observed potency as distorted by the uptake process.

The relative rates of uptake and diffusion also have relevance to the concentrations of uptake inhibitor required to completely inhibit the uptake process in a tissue. From Eq. 12 it can be seen that the larger is y, the greater must $[I]/K_I$ be to produce maximal sensiti-

A.

TISSUE I
$y = 5$

TISSUE II
$y = 50$

FRACTION OF MAXIMAL STIMULUS

1.0

0.5

0

CB A   C B A

Log ([A] /K$_A$)

B.

1.0

0.8

0.6

0.4

0.2

0

X/Y

$y=2$
5
10
20
50

[I]/K$_I$

20 40 60 80 100

C.

1.0

0.8

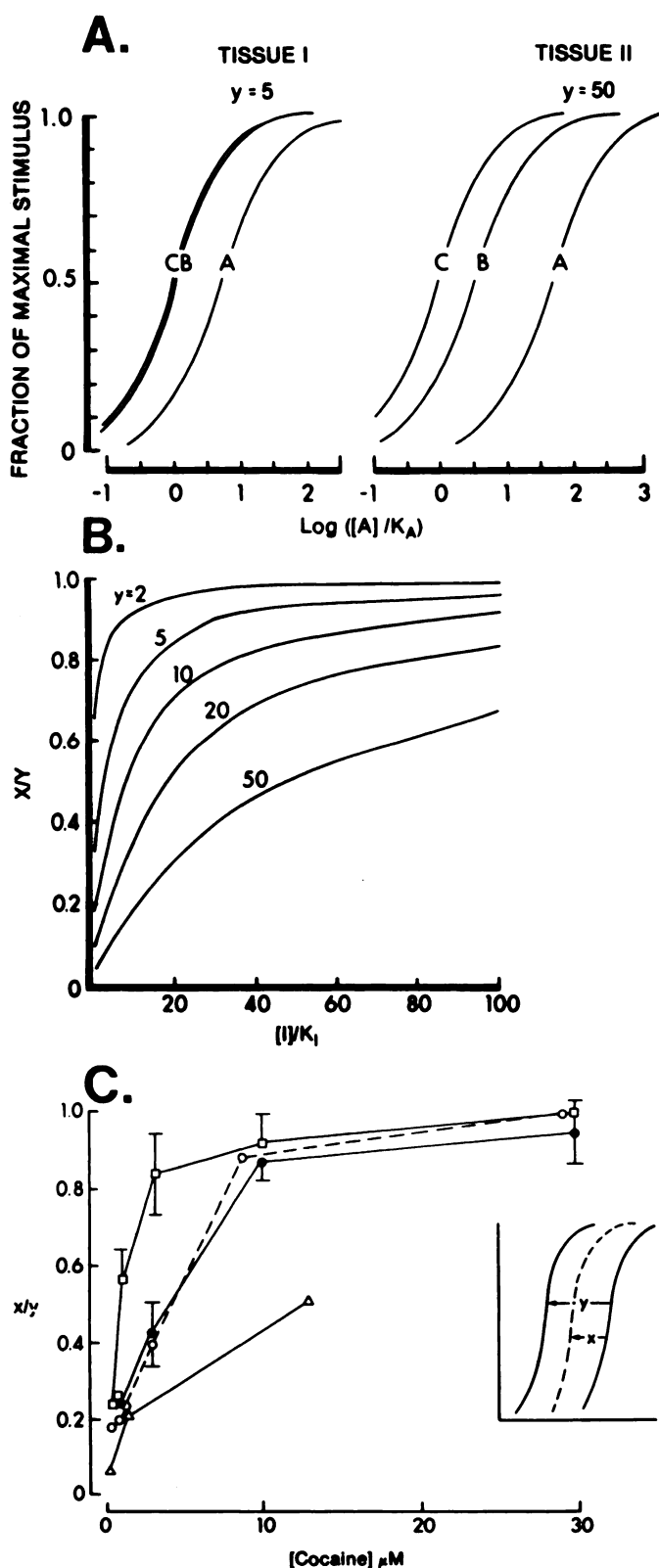0.6

0.4

0.2

0

x/y

[Cocaine] μM

0   10   20   30

FIG. 6. Relationship of tissue sensitization to uptake inhibition. A. Effects of an uptake inhibitor on concentration-response curve to an agonist taken up by the tissue. Ordinates: Fractions of maximal response. Abscissae: Logarithms of molar concentration of agonist expressed as a fraction of the equilibrium dissociation constant of the agonist. The maximal sensitization obtainable in tissue I is 5 and in tissue II is 50. Concentration-response curve in absence of uptake

zation. Figure 6A shows that a given concentration of uptake inhibitor ($[I]/K_I = 30$) produces very near maximal sensitization for tissue I but less than maximal sensitization in tissue II (compare curves B and C for each tissue). The dependence of tissue sensitization, as a fraction of the maximal sensitization possible, upon the concentration of uptake inhibitor is shown in figure 6B for a series of tissues with different maximal possible sensitizations (variable y). Note that for tissues that demonstrate a low maximal sensitization to agonists ($y = 2$), the concentration of uptake inhibitor required for near maximal sensitization (i.e., 90%) is 10 times the K_I for uptake inhibition. This concentration of uptake inhibitor would produce only 20% maximal sensitization in a tissue with a large maximal sensitization (i.e., $y = 50$) (367). In these terms it would be incorrect to extrapolate maximally effective concentrations of uptake inhibitors from one tissue to another. Experimental evidence for this effect was obtained by comparing the sensitization of various tissues from guinea pigs to norepinephrine produced by cocaine (see figure 6C).

A logarithmic metameter of Eq. ⬛ can be used to estimate the effective K_I for an uptake inhibitor in an isolated tissue (371),

$$\log\left[\frac{y(x-1)}{y-x}\right] = \log[I] - \log K_I \qquad (14)$$

where x is the sensitization of the tissue to the agonist produced by concentration (I) of the uptake inhibitor and y the maximal sensitization after complete inhibition of uptake (see insert figure 6C). This method was used to measure the K_I for inhibitors of neuronal and extraneuronal uptake of catecholamines (371) and the inhibition of adenosine uptake by benzodiazepines (372) in a variety of isolated tissues and yielded values comparable to those measured biochemically. On the surface, these results suggested that Eq. 14 could be used as a quantitative method to estimate uptake inhibitor potency in isolated tissues. However, it is difficult to assess the significance of these findings since there are theoretical reasons for the estimates obtained by this method and biochemical methods to be different. These theoret-

inhibitor (A), in the presence of a submaximal concentration of uptake inhibitor $[I]/K_I = 30$ (B), and when uptake completely blocked (C). Reprinted with permission from Kenakin and Leighton (389). B. Sensitization as a function of concentration of uptake inhibitor. Ordinates: Sensitization of tissues to agonist as a fraction of the maximal possible sensitization. Abscissae: Molar concentration of uptake inhibitor as a fraction of the equilibrium dissociation constant of the inhibitor for the site of uptake. Curves calculated for tissues with varying amounts of maximal sensitization ($y = 2$ to 50). C. Sensitization of guinea pig tissues to norepinephrine as a function of the concentration of cocaine. Ordinates: Sensitization to norepinephrine as a fraction of the maximal sensitization. Abscissae: Molar concentration concentrations of cocaine. Guinea pig left atria (□, $n = 20$), trachea (●, $y = 11$, $n = 15$) data from Kenakin (367); right atria (◉, $y = 13$) data from Trendelenburg (641); trachea (△, $y = 36$) data from Foster (222). Bars represent S.E.M. Figures B and C reprinted with permission from Kenakin (367).

ical aspects relate to the differences in the diffusional pathways of substrate (agonist) in pharmacological and biochemical experiments. Biochemical estimates of the potency of inhibitors of active removal processes can vary, the magnitude of the variation being dependent upon the importance of the formation of substrate concentration gradients within the tissue. If diffusion and substrate uptake and/or degradation limits the entry of substrate into the tissue, then inhibition of the removal process may increase the degree of penetration of substrate which in turn would distort the estimate of the extent of inhibition of the removal process (281). For example, in a tissue homogenate, where substrate concentration gradients would not be expected to occur, 80% to 90% inhibition of acetylcholinesterase by DFP is required before acetylcholine effects are potentiated (48). However, in a structured tissue, concentrations of DFP which inhibit acetylcholinesterase by 20% are sufficient to potentiate responses to acetylcholine (195, 303). This disparity may be due to the fact that inhibition of an outer core of acetylcholinesterase may enable acetylcholine to penetrate into and stimulate more muscle (281). Thus, the biochemical K_I and concentrations of uptake inhibitor required to potentiate responses may not be the same.

The selectivity of the uptake inhibitor is of paramount importance in isolated tissue experiments. Theoretical calculations show that an extraordinary degree of selectivity is required for an uptake inhibitor to fully potentiate the effects of an agonist in a tissue (367). For example, if the uptake inhibitor were a very weak receptor blocking agent (i.e., at concentrations 100 times those needed for uptake inhibition), complete sensitization would not be observed at any concentration. The receptor blocking properties would become manifest at the high concentrations of uptake inhibitor ($[I]/K_I > 300$) required to fully inhibit uptake processes in some tissues and produce shifts of the concentration-response curves to the right thereby cancelling deviational sensitization. Examples of uptake blockers which also antagonize receptors can be found in metanephrine [pK_I for extraneuronal uptake = 5.4, pK_B for β-adrenoceptors = 4.2 (367)] and amitriptyline [pK_I for neuronal uptake = 7.2, pK_B for α-adrenoceptors = 7.0 (380)]. In guinea pig trachea, metanephrine produces only 3% of the maximal possible sensitization to isoproterenol, a value consistent with the relatively low selectivity ratio (16 times) of potency for uptake over receptors (367). In rat anococcygeus muscle, virtually no sensitization to norepinephrine can be observed with amitriptyline probably because of the comparable potency of this drug for uptake inhibition and the pK_B for α-adrenoceptors (380, 415).

Most commonly, uptake inhibition produces sensitization of tissues to agonist substrates with no increase in maximal responses. This is to be expected if contraction coupling in the tissue is efficient and not all of the muscle mass needs to be activated by the agonist to produce the maximal response. There is experimental evidence in many isolated tissues that this is the case. For example, in the cat papillary muscle it has been shown that activation of the superficial muscle layers is sufficient to activate the complete muscle (51, 327, 672) and myogenic propagation has been proposed in blood vessels as well (69).

To date, there are two examples where inhibition of uptake processes in isolated tissues produce increases in the maximal responses of isolated tissues to agonists. One is in the dog saphenous vein where inhibition of catechol-O-methyl transferase increases the maximal relaxation obtained with isoproterenol, an effect more evident in thick rather than thin tissues (284). Another is the rat vas deferens where inhibition of neuronal uptake by either cocaine or desmethylimipramine increases the maximal responses to norepinephrine but not those to methoxamine (368). The mechanisms responsible for such increased maximal responses are not clear but a reasonable hypothesis could involve desensitization as a causative factor. In tissues with poor diffusion characteristics, severe concentration gradients for agonists could develop (as discussed previously) thereby causing the outer shell of muscle cells to be exposed to a much higher concentration of agonist than the inner core. This, in turn, could induce rapid receptor or muscle desensitization at the surface of the muscle. If cell-to-cell coupling within the muscle is poor and a large body of cells needs to be activated by agonist to produce the maximal response then the concentration gradient and desensitization process could combine to produce a condition whereby the mass of tissue needed to be activated for maximal response may never be achieved. Thus, the true tissue maximal response may not be realized until after the removal of the agonist concentration gradient by uptake inhibition. The rat vas deferens may be a tissue prone to such effects since cell-to-cell coupling is poor (277) and desensitization rapid (472, 695). A concentration gradient for catecholamines is further suggested by the 40 times greater estimate of the K_I for cocaine inhibition of neuronal uptake, when compared to other tissues, in rat vas deferens (187). A concentration differential for norepinephrine within this tissue is supported by two findings by Pennefather who observed that the maximal response to exogenously added but not neuronally released norepinephrine was increased by cocaine (513) and that, after desensitization of this tissue by incubation with high concentrations of exogenous added norepinephrine, the maximal responses to nerve stimulation were larger than the maximal responses to exogenous norepinephrine (512). Both results would be predicted if the muscle mass activated by exogenous norepinephrine and neurally released norepinephrine were different.

IV. Quantification of Responses to Agonists

A. Dose-Response Curves

All parameters of drug receptor interaction are derived from models which in one way or another rely upon dose-response curves. A dose-response curve can essentially be described by three parameters: a maximum ordinate, a location parameter, and slope. There are many factors not related to drug receptor interaction which can affect these parameters (410, 514), thus reliable information about drug receptor constants cannot be obtained directly from the dose-response curve. Instead, null methods have been devised which neutralize the unknown influences of stimulus-response mechanisms on tissue responses. Various non-receptor-related phenomena can still confound these methods by affecting the dose-response parameter from which most, if not all, drug receptor information is derived, namely the location parameter of the dose-response curve. This parameter defines the concentration at which a defined dependent variable, a certain level of response, is obtained. The most commonly used location parameter is the EC50 (effective concentration for 50% of maximal response) which serves as the measure of potency of an agonist.

Obviously, the success of a pharmacological procedure is contingent upon accurate location parameters for dose-response curves. One of the most common sources of potential error is a disparity between the agonist concentration added to the organ bath and that which is present at the receptor; this problem has been dealt with in previous sections of this paper. Other possible problems involve random or systematic alterations in stimulus-response characteristics or receptors to produce errors in location parameters. Systematic decreases in tissue sensitivity can occur with time and frequency of exposure to an agonist, a phenomenon often given the term "desensitization." This can be because of receptor events such as an agonist-induced conformational change of the receptor into a less sensitive form (179, 358) or a nonselective event [i.e., "fatigue," (684)]. For example, the ionic content of smooth muscle changes after exposure to acetylcholine (508), a factor which could contribute to altered muscle sensitivity. The change need not be a decrease in responsiveness. In some tissues, response can be increased, such as the three-fold increased maximal response to α-adrenoceptor stimulation observed over a 7.5-h equilibration period in the guinea pig oesophageal muscularis mucosa (646). Spontaneous muscle tone adds another dimension of complexity to isolated tissue experiments. For example, the sensitivity of guinea pig trachea to relaxants is greatly affected by the degree of contractile tone of this muscle (105, 106). If during the course of the experiment this were to change, a systematic error in the location parameters of the concentration-response curves to relaxants in this tissue would be introduced.

Dose-response curves can be obtained by addition of single concentrations of agonist to the organ bath (either in a random or ascending order) or by cumulative addition of concentrations (661). Different tissues are more suited to one or the other method. For example, the rat vas deferens is notorious for rapid desensitization to agonists (472, 695), thus a single-dose addition procedure with adequate quiescent periods between doses gives much more satisfactory results than does cumulative addition. Guinea pig parenchymal strip also demonstrates different sensitivity to agonists depending upon whether random single-dose or cumulative-dose procedures are used (93). Tissues with slowly developing responses such as guinea pig trachea or rabbit aorta are more suited to cumulative concentration-response curves.

Uncontrolled changes in tissue sensitivity can be devastating to pharmacological experiments designed for drug receptor analysis. Control experiments considering these factors should be carried out to determine procedures to minimize such changes. Furchgott (238) suggests a "bracketing" procedure of a concentration-response curve of a standard before and after the test agonist. Another possibility would be the use of matched preparations for comparisons of single concentration-response curves or correction for changed sensitivity in untreated tissues. These latter procedures involve tacit assumptions which subordinate them to the use of a stable tissue that can function as its own control.

Agonist potency is most often measured by the EC50 ($-\log EC50 = pD_2$). The statistical parametric procedures designed for determination of differences in potency are based upon normal distributions, thus it is important that, when dealing with log normal data from semi-logarithmic concentration-response curves, geometric means be used. These, unlike converted EC50 data, are normally distributed and give proper estimates of the means and errors (218, 245, 246).

There are numerous ways to analyze data from dose-response curves (687, 688, 690). A method used to minimize subjective errors is to fit the data to a general logistic function (175, 687, 689). The threshold concentrations at the lower end of the dose-response curve are often especially subject to error. Where accurate comparisons in this region of the dose-response curve are required, a linearizational method such as the logit function (59, 197) may be useful (675). On the other end of the dose-response curve, the maximum response at infinite dose can be estimated by a double reciprocal metameter (153, 508). This transformation relies on the goodness of fit of the data to a hyperbola and assumes this function describes the dose-response relationship to the maximum.

Once quantitative data is obtained from the dose-response curve which reliably reflects agonist receptor interaction, then a variety of statistical methods and procedures may be applied to the data (111, 114, 182,

246, 615). From this point onwards in this paper, unless stated otherwise, it will be assumed that 1) the responses of the isolated tissue emanate only from agonist added directly to the organ bath, 2) the concentration of drug at the receptor is equal to the concentration of drug added to the organ bath, 3) the responses of the tissue are a direct result of drug receptor interaction, and 4) these responses are not modified unpredictably by the stimulus response mechanism of the tissue or any other factor. Under these circumstances (exclusively) can reliable information about drug receptor interaction be obtained. As a preface to the discussion of the methods available to do this, a brief review of the rudiments of drug receptor theory is useful.

B. Drug Receptor Theory

The ideas and models, which on a molecular level serve to characterize the interactions of drugs with drug receptors, will collectively be referred to as drug receptor theory. There are numerous comprehensive reviews of this topic to which the reader is referred for more detailed information than will be given here (21, 228, 229, 232, 551, 654, 655, 684). Various models have been put forward to explain the complexities of drug receptor interaction, the most common one being occupation theory where it is assumed that the occupation of a receptor by a drug leads to stimulus and subsequent response. An alternative to this hypothesis is rate theory (506) which assumes efficacy to be a product of the rate of drug receptor interaction. These approaches are given continuity in the receptor inactivation theory proposed by Gosselin (275, 276). Other approaches include the conformational perturbation theory (54), the dynamic receptor hypothesis (83), the flux carrier hypothesis (428), the ion exchange model (620), and the mobile receptor hypothesis (56, 160, 347). Allosteric two-state models of receptors also have been proposed (125, 357, 627). A particularly useful comparison of the variants of allosteric models to each other and to occupation theory has been given by Colquhoun (143). Convenient comparisons of all of these models have been given by MacKay (431), Gosselin (276), and Ruffolo (551). The following analyses and procedures are derived from occupation theory but it should be noted that the resulting parameters calculated often correspond to similar constants in other receptor theories.

The Law of Mass Action is used to describe the binding of drugs to receptors (132, 133, 314, 411):

$$\frac{[A \cdot R]}{[R_t]} = \frac{[A]}{[A] + K_A} \tag{15}$$

where $[R_t]$ refers to the total concentration of receptors, $[A \cdot R]$ the concentration of drug receptor complex, and K_A the equilibrium dissociation constant of the drug for the receptor. Implicit in Eq. 15 is the assumption that a single drug molecule binds to a single site on the receptor.

Although this appears to be the case for many drug receptor interactions there are exceptions to this scheme. For example, two molecules of acetylcholine must bind to two apparently identical but cooperatively linked sites on a single nicotinic receptor in skeletal muscle to open the ion channel (580A). The concept of full and partial agonism was introduced by Ariens (20) in the form of a proportionality constant termed "intrinsic activity" (α):

$$\frac{E_A}{E_m} = \frac{\alpha[A \cdot R]}{[R_t]} = \frac{\alpha \cdot [A]}{[A] + K_A} \tag{16}$$

where E_A and E_m refer to the response to a given concentration of A and the tissue maximal response, respectively. On the basis of the idea that response was not necessarily directly proportional to receptor occupancy, Stephenson (599) introduced the concept of stimulus (S) which assumed that response was some undefined function of stimulus:

$$\frac{E_A}{E_m} = f(S) \tag{17}$$

the requirements for the function being that it be monotonic and continuous. Thus responses in a given tissue could be produced by concentrations of two agonists that produced equal stimuli regardless of their relative receptor occupancy. The parameter that related stimulus to occupancy was termed "efficacy" (e):

$$\frac{E_A}{E_m} = f\left(\frac{e \cdot [A]}{[A] + K_A}\right) \tag{18}$$

Implicit in this hypothesis was the fact that, given a nonlinear relationship between stimulus and response, not all of the receptors need be occupied to produce the tissue maximal response.

As defined by Stephenson (599), efficacy was a drug and tissue dependent term. Furchgott (229) modified this model to differentiate the drug and the tissue factors of efficacy by defining intrinsic efficacy(ϵ):

$$e = \epsilon \cdot [R_t]. \tag{19}$$

In these terms intrinsic efficacy was strictly a drug-related parameter which should be constant for given drug receptor pairs across species and tissues (373). The various tissue and receptor-related factors of agonist response are defined in Eq. 20:

$$\frac{E_A}{E_m} = f\left(\frac{\epsilon \cdot [R_t] \cdot [A]}{[A] + K_A}\right). \tag{20}$$

Thus, the tissue-related factors are 1) f, the function relating stimulus and response, and 2) the total receptor concentration (R_t). The receptor-related factors are 1) K_A, the equilibrium dissociation constant of the drug for the receptor, and 2) ϵ, the intrinsic efficacy. Insofar as the agonist may be removed from the receptor compartment by the tissue, $[A]$ may be influenced by tissue

factors but if meaningful drug receptor parameters are to be estimated, these influences must be minimized. The following analyses are all aimed at defining various constants describing solely drug receptor parameters by utilization of null methods which cancel the tissue factors. As a preface, a discussion of some of the factors relevant to the translation of receptor stimulus to tissue response is appropriate.

C. The Relationship between Stimulus and Response

The simplest relationship between receptor stimulus and tissue response is a linear one (direct relationship) and for some weak partial agonists this may be an accurate approximation (551). However, there is a wealth of evidence to suggest that in many isolated tissues the relationship between receptor occupancy (by the occupation model this is a direct function of stimulus) and tissue response is nonlinear. This has been proposed on theoretical grounds (599) and also is suggested by the fact that irreversible inactivation of a fraction of the available receptor pool can lead to dextral displacement of concentration-response curves to strong full agonists with no concomitant depression of the maximal response (27, 228, 229, 474, 673). This phenomenon is cited as evidence for "spare receptors" (receptor reserve, spare receptor capacity) in tissues, the rationale being that if an alkylating agent inactivates 99% of the viable receptors and the agonist still produces the tissue maximal response then 99% of the receptor population is "spare"; i.e., not required for the production of the maximal response. The term "receptor reserve" (spare receptors) is sometimes associated with tissues; e.g., the guinea pig ileum may be said to have a cholinergic receptor-reserve but, of course, this term cannot be applied generically to tissues but rather must be associated with a given agonist and tissue. Thus, the receptor reserve for two agonists in one tissue may be quite different.

Since the advent of pharmacological procedures to estimate the equilibrium dissociation constants of full agonists and the refinement of biochemical binding studies, quantitative evidence for nonlinear relationships between receptor occupancy and tissue response has accumulated. Table 5 shows examples of isolated tissues and drugs for which there is a disparity between the concentrations required for half maximal binding to receptors and those required for production of half maximal response. A plot of tissue response as a function of receptor occupancy for these full agonists (by the Langmuir adsorption isotherm) demonstrates a necessarily nonlinear function typically hyperbolic in shape [for example, see Besse and Furchgott (64)]. The steepness of this hyperbolic function reflects the efficiency of the stimulus-response mechanism in the tissue. It should not be assumed that for drugs that have no receptor reserve (partial agonists) the relationship between receptor occupancy and tissue response is linear. For some weak agonists a direct relationship is observed (387, 551) but

for other partial agonists, a hyperbolic relationship can be demonstrated. This has been shown for oxymetazoline in rat aorta (551), normorphine in ileum from morphine tolerant guinea pigs (522) and pirbuterol and prenalterol in rat atria (383). There are two factors that determine the efficiency of the stimulus response mechanism in a tissue: the number of receptors and the nature of the functions which translate receptor stimulus into tissue response. It is worth considering these separately.

1. Tissue Response as a Function of Stimulus. Since stimulus is a linear function of intrinsic efficacy, receptor occupancy and receptor number, the function relating stimulus and response must produce the nonlinearity between receptor occupancy and response if such is observed. Amplifier systems are common in biological systems (25, 271, 541) and if one step in the amplifier system reaches saturation, then a spare capacity results. Such systems hold advantages since they allow amplification and alternative regulatory input (271). A successive series of nonlinear functions where the product of one saturable process becomes a substrate or catalyst for the next saturable process provides for a much more skewed relationship between receptor occupancy and response than any one of the individual processes (541). A well-known cascade of this type is the formation of glucose by β-adrenergic drugs. In this system an amplification factor of eight orders of magnitude can be achieved (271), thus demonstrating a striking disparity between receptor occupancy and tissue response (figure 7).

There have been several mathematical models put forth to describe the nonlinear relationships between hormone binding and biological response which focus on the coupling between receptor and effector subunits (58, 85, 229, 347, 355). An interesting model that centers on the sequential nature of the second messenger theory has been put forward by Strickland and Loeb (605). This model is based on the interaction of the hormone with the receptor leading to the generation of an intracellular intermediate which interacts with an intracellular receptor to generate a response. The interesting aspect of this model is the mathematical consequence that the equilibrium dissociation constant for the overall process (K_{Total}) must be lower in magnitude than the equilibrium dissociation constant of the hormone binding to the extracellular receptor (K_d). Thus (605):

$$K_{Total} = \left(\frac{K}{K + a \cdot R_t} \right) \cdot K_d \qquad (21)$$

where K is the equilibrium dissociation constant for the binding reaction between the intracellular mediator and intracellular receptor, a is a proportionality constant reflecting the size of the pool of intracellular mediator and R_t is the number of extracellular hormone receptors. It can be seen from Eq. 21 that the concentration-response curve for the overall process must lie to the left of the receptor occupancy curve along the concentration

TABLE 5
Relationship between EC50 and equilibrium dissociation constants of agonists
A. K_A Measured Pharmacologically[*]

Receptor	Species	Tissue	Agonist	−Log (EC50)	pK_A	Reserve[†]	References
Muscarinic	Guinea pig	Ileum	Acetylcholine	7.23	5.6–5.1	190–398	(560)
			Carbachol	7.3–6.5	4.92	38–240	(235)
			Pilocarpine	6.3	5.35–5.48	6.6–9	(235)
		Left atrium	Carbachol	6.76	4.95	64	(229)
	Rabbit	Aorta	Carbachol	6.06	4.8	18	(229)
		Stomach fundus	Carbachol	7.26	4.8	288	(229)
α-Adrenergic	Rat	Anococcygeus muscle	Norepinephrine	7.5	6.3	16	(370)
			Oxymetazoline	7.5	6.7	6.3	(377)
	Rabbit	Aorta	l-Norepinephrine	7.9	6.47	21	(64)
				7.47	6.8	4.7	(578)
			l-Epinephrine	7.92	6.69	17.2	(64)
				7.49	6.53	9.1	(578)
			l-Phenylephrine	7.2	5.95	18.3	(64)
				6.74	5.9	7.0	(578)
		Papillary muscle	l-Phenylephrine	6.03	5.5	3.4	(580)
			Dopamine	5.63	4.2	27	(64)
				5.66	4.8	7.0	(578)
			l-Metaraminol	5.49	4.8	5.0	(578)
		Spleen	l-Norepinephrine	7.37	6.13	17.3	(578)
			l-Epinephrine	7.47	6.62	7.1	(578)
			l-Phenylephrine	5.62	4.87	5.6	(578)
			Dopamine	4.47	3.89	3.8	(578)
Opioid	Guinea pig	Ileum	Normorphine	6.6	5.8	6.2	(522)
Histamine	Guinea pig	Ileum	Histamine	6.8	5.0	63	(229)
β-Adrenergic	Rabbit	Papillary muscle	Isoproterenol	8.5	6.6	79	(580)

B. K_d Measured in Binding Studies[§]

Receptor	Species	Tissue	Agonist	−Log (EC50)	pK_d	Reserve[†]	References
Muscarinic	Guinea pig	Ileum	Acetylcholine	7.24	5.7–5.4	35–69	(235)
			Methacholine	7.15	5.77	24	(612)
			Carbachol	7.25–6.5	4.7–4.5	562–63	(235)
				7.4	4.89[‡]	323	(109)
			Oxotremorine	7.65	6.3	22	(612)
			Bethanechol	5.95	4.79	14.6	(612)
			Pilocarpine	6.3	6.15–6.04	1.4–2	(235)
				5.9	5.1	6.2	(612)
	Mouse	Ileum	Methacholine	6.65	6.04	4	(612)
			Oxotremorine	6.65	6.1	3	(612)
			Bethanechol	5.45	4.6	7	(612)
	Rabbit	Detrusor muscle	Carbachol	6.57	4.37	155	(11)
α-Adrenergic	Rat	Vas deferens	Norepinephrine	6.92	4.95	93	(456)
			Epinephrine	7.04	5.13	82	(456)
			Methoxamine	5.54	4.13	25	(612)
			Phenylephrine	5.85	4.88	9.2	(456)
β-Adrenergic	Rat	Cardiocytes	Isoproterenol	7.05[¶]	6.1	9	(535)
				9.43	6.96	295	(360)
			Dichloroisoproterenol	7.82	7.59	1.7	(360)
	Cat	Papillary muscle	Isoproterenol	9.1	6.7	251	(360)
			Dichloroisoproterenol	6.7	6.2	3.2	(360)
			Epinephrine	8.1	5.8	200	(360)
			Norepinephrine	8.0	5.7	200	(360)

[*] Method of Furchgott (229).

[†] Defined as antilog [−log EC50−pK_A (or pK_d)].

[§] The affinity of many agonists in radioligand binding studies is dependent upon experimental conditions and it is not yet clear which affinity state is relevant to the pharmacological K_A. Therefore, the reserves calculated may be substantially in error.

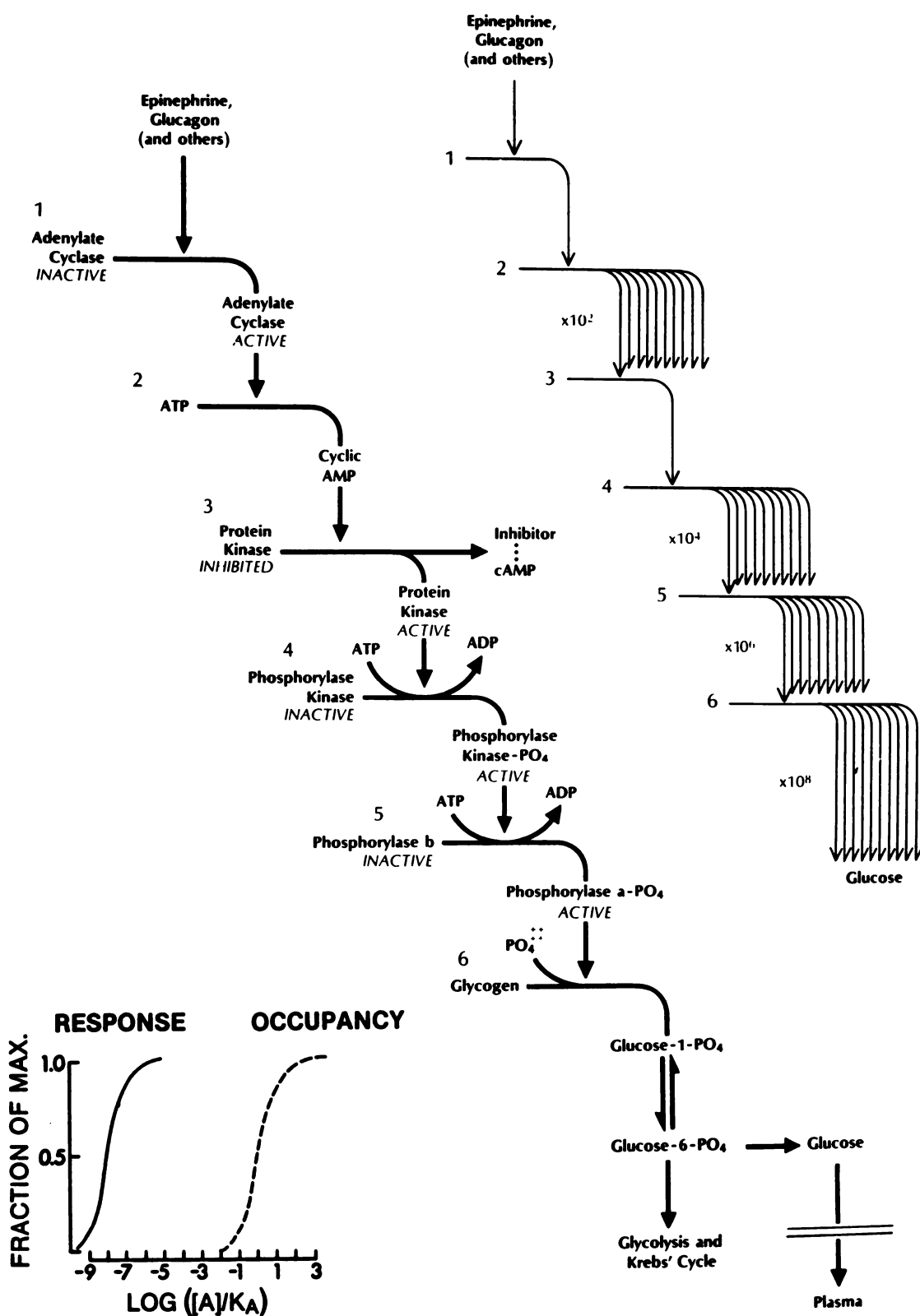[‡] Potassium and rubidium efflux.

[¶] Adenyl cyclase activity.

FIG. 7. The cascade of reactions involved in the production of glucose by β-adrenoceptor or glucagon stimulation. The amplification factor of 8 orders of magnitude can be expressed in the great difference between the binding curve for occupancy and response (inset). Reprinted with permission from Goldberg (271).

axis, the degree of shift depending upon the size of the pool of the intracellular messenger and number of hormone receptors.

In pharmacological systems, there are many examples of tissues that demonstrate hyperbolic functions between receptor occupancy and mechanisms which *precede* tissue response. For example, there is evidence to suggest nonlinear relationships between muscarinic receptor occupancy and calcium transport across cell membranes (11, 337, 338) and potassium efflux (109). A well-known example is the nonlinear relationship between β-adrenoceptor occupancy and adenylyl cyclase activation. Thus, an "enzyme reserve" has been noted in postnatal rat hearts (360, 535) and kitten papillary muscle (359). There also are examples of nonlinear relationships between intermediate steps in the stimulus-response chain and tissue response. Thus, in many tissues, submaximal cyclic AMP production is associated with maximal tissue responses (121, 360, 572, 605). Lastly, the degree of cell-to-cell coupling may determine how much of the tissue must be activated for syncytial responses (69, 327, 672, 674). For example, experimental evidence suggests that isoproterenol need activate only a small fraction of the total muscle mass of cat papillary muscle for complete activation of the tissue (51, 327, 672, 674). In general, there are numerous examples of saturable nonlinear functions relating receptor occupancy and processes which in turn initiate events leading to tissue response. Often these events are multiple which can increase the amplification factor. Cell-to-cell amplification factors may further skew the nonlinear relationship between receptor occupancy and tissue response.

2. Receptor Density. The other tissue factor that determines the magnitude of the agonist response is the number of viable receptors in the tissue. From Eq. 20 it can be seen that the larger the number of receptors, the more agonist response should be obtained. This also is evident in the second messenger model by Strickland and Loeb (605; Eq. 21). There are examples of correlations between receptor density and hormonal response (121, 250, 342, 396, 438, 450). For example, the relative potency of oxotremorine on guinea pig and mouse ileum (8:1) correlates well with the number of muscarinic receptors estimated in binding studies [7.6:1 (647)]. Takeyasu and coworkers (612) have shown a loss of sensitivity of mouse and guinea pig ileum to cholinergic stimulation with dibenamine treatment which parallels the reduction in cholinergic receptors as measured by ^{3}H-quinuclidinyl benzilate binding. On the other hand, desensitization of rat atria by implantation of mini-osmotic pumps delivering isoproterenol produces a reduction in β-adrenoceptors that is not directly proportional to the decrease in the sensitivity of atria to isoproterenol (388). Correlations between receptor density and tissue sensitivity can be misleading. For example, mouse thymus has 4.6 times more β-adrenoceptors than mouse spleen but the spleen is 20 times more sensitive to isoproterenol (519).

V. Methods of Drug Receptor Classification

Theoretically there are numerous methods to classify drug receptors (73, 74). For example, receptors can be classified in terms of the different stimuli imparted to the stimulus-response mechanism of tissues (24). There could be further classification on the basis of chemical messenger (26). Another approach is classification by anatomical location, a well-known example being pre- and postsynaptic receptors. Another case of this type of differentiation is the relative innervation of α- and β-adrenoceptors being associated with receptor subtypes (25, 26). As pointed out by Ariens and coworkers (24) these types of classification can lead to ambiguity; the recent discoveries of postsynaptic α_2-adrenoceptors being an example where classification by location is unsatisfactory (629).

A theoretically more sound and experimentally more fruitful approach has been classification by pharmacological criteria (73). The null methods which are used in these procedures hopefully yield parameters for drugs and receptors that transcend function and location and have relevance to studies in man. There are four commonly used pharmacological methods of drug and drug receptor classification: 1) agonist potency ratios (rank order of potency); 2) selective agonism; 3) comparison of agonist affinity and relative intrinsic efficacy; 4) quantification of competitive antagonist affinity (pK_B).

It is worth considering each of these separately.

A. Agonist Potency Ratios

The relative potency of agonists has long been (41) and is still often used for receptor classification but a number of caveats should be made to this method. Firstly, the effects of uptake processes must be eliminated since selective uptake can produce serious errors in agonist potency ratios. For example, the selective potency of salbutamol over norepinephrine (20:1) is eliminated (0.9:1) in guinea pig trachea by cocaine inhibition of neuronal uptake (367). Leighton (415) has shown that rank order of potency as well as potency ratios can change in rat anococcygeus muscle after cocaine inhibition of neuronal uptake. Thus, a potency ratio of 50:1 for methoxamine over norepinephrine in this tissue was changed to 0.2:1 after cocaine (415).

The relative potency of full and partial agonists can vary capriciously from tissue to tissue because of variances in receptor number and the relative efficiency of stimulus response relationships. This is because differences in receptor coupling cause differences in the location parameters of concentration-response curves to full agonists (shifts along the concentration axis) but not partial agonists. Instead, differences in receptor coupling produce changes in the maximal responses to partial agonists but little displacement of the concentration-

response curves. Thus, in two different tissues with identical receptors but different stimulus-response characteristics, a full and a partial agonist may have different potency ratios [see figure 1 of Furchgott (232)]. Therefore, it is theoretically more sound to compare potency ratios of full agonists.

Assuming that the K_A for full agonists significantly exceeds the concentrations required for response ($K_A \gg [A]$), then the stimulus to an agonist can be given by: $S = \epsilon \cdot [R_t] \cdot [A]/K_A$. Therefore, in any single tissue ($[R_t]$ constant) the respective stimuli to agonists A_1 and A_2 is:

$$S_1 = \frac{\epsilon_1[A_1]}{K_{A1}} \quad \text{and} \quad S_2 = \frac{\epsilon_2[A_2]}{K_{A2}}. \tag{22}$$

The potency ratio (pr) of these two full agonists in producing equal responses ($S_1 = S_2$) is:

$$pr = \frac{[A_1]}{[A_2]} = \frac{\epsilon_2 \cdot K_{A1}}{\epsilon_1 \cdot K_{A2}} \tag{23}$$

As can be seen from Eq. 23, the potency ratio for two full agonists in a tissue (assuming an adequate receptor reserve) reflects only the drug receptor parameters ϵ and K_A and therefore is tissue independent. Under these circumstances, the potency ratio is a powerful quantitative constant for drug receptor classification. The importance of pr as a quantity and not a qualitative statement of rank *order* of potency should be stressed. The rank order of potency is a crude and misleading parameter which has limited value in drug receptor classification. Agonists could have the same order of potency in different tissues but a different relative potency ratio. The quantitative data, namely the actual magnitudes of the potency ratios, would suggest that the receptors are different while the rank order would suggest identity of receptors. Assuming that pr reflects relative ϵ and K_A, the quantitative data would be correct.

A specialized approach related to agonist potency ratios is the use of optical isomers to classify receptors (517). Thus, the ratio of activities of two optical isomers of the same drug should be unique for a given receptor and yield an isomeric ratio for receptor classification purposes. The reader is referred to a comprehensive review by Patil and coworkers (504) on this method for further details.

B. Selective Agonism

Frequently, judgments are made about the presence or absence of a certain receptor in a tissue on the basis of the observation of the presence or absence of responses to a selective agonist (classified on some other tissue or tissues). There are two settings for these types of experiments. In one, the receptors of a new tissue, as yet unclassified, are subjected to trial by selective agonism. In the other, a given tissue, which responds to selective agonists, is modified either by receptor alkylation, receptor desensitization, or selective modification of stimulus-

response characteristics and the resulting effects on the responses to the selective agonists are used for classification purposes. There are criteria to be met for either of these procedures to be successful from the point of view of unequivocal receptor classification.

In the first type of experiment the tissue either responds or does not respond to the selective agonist. If the tissue does not respond it means either that the receptors for the particular selective agonist are not present in the tissue or that the stimulus-response mechanism of the tissue produced insufficient amplification of the receptor stimulus to generate a response. An example of the latter was observed with prenalterol, a relatively weak β-adrenoceptor partial agonist shown to have affinity and efficacy for β_1-adrenoceptors (379). An apparent paradox was observed when prenalterol produced no agonist responses in canine coronary artery, a β_1-adrenoceptor-containing tissue (46, 464, 382). However, it was found that prenalterol proved to be a competitive antagonist of isoproterenol, a more powerful agonist in this tissue (382), and yielded a pK_B consistent with β_1-adrenoceptor antagonism. These experiments showed that the canine coronary artery simply did not possess an adequately efficient stimulus-response mechanism to allow this weak β-adrenoceptor partial agonist to demonstrate a response.

If a selective agonist produces a response in a tissue then this constitutes circumstantial evidence that the receptor for which the agonist is selective is present in the tissue. However, a distinction should be made between selectivity and specificity. To assume that the selective agonist only has affinity and efficacy for one receptor is to confer upon it specificity. Pharmacological experience with drugs shows this to be the exception and not the rule. For example, the putative β_1-adrenoceptor selective agonists prenalterol, dobutamine, and tazolol all produce full agonist responses in rat uterus, a tissue generally thought to contain β_2-adrenoceptors. This observation leads to two possible conclusions: 1) the rat uterus contains previously undetected β_1-adrenoceptors, and 2) the putative β_1-adrenoceptor selective agonists have affinity and efficacy for β_2-adrenoceptors. Experiments with selective antagonists showed the second alternative to be true for rat uterus (375) and illustrates the importance of not assuming strict specificity for agonists.

Selective desensitization of responses to a certain agonist (47) or selective irreversible inhibition of responses by an alkylating agent have been techniques used to differentiate drug receptors in tissues. Coupled with the technique of selective alkylation is the selective protection of drug receptors by drugs during the alkylation process (227). However, these techniques have a major drawback is that they can give completely misleading information if the receptor reserves for the two agonists concerned are different. For example, if one agonist has

a 90% receptor reserve and another a 40% receptor reserve, then the responses to the latter agonist will be more sensitive to removal of portions of the receptor pool either by desensitization or alkylation. This point has been stressed for experiments with full and partial agonists (684) but can be extended to two full agonists as well. For example, consider the responses of rat anococcygeus muscle to oxymetazoline and norepinephrine (figure 8A). In control tissues, oxymetazoline is a slightly more potent agonist than norepinephrine (uptake blocked) but as the α-adrenoceptor population of the tissue is irreversibly inactivated by controlled exposure to the alkylating agent phenoxybenzamine, it can be seen from figure 8A that the responses to oxymetazoline, the more potent agonist, are disproportionately more depressed than those of norepinephrine (377). This is because the efficacy of norepinephrine is greater than that of oxymetazoline; this fact makes the maximal response to norepinephrine more resistant to decreases in receptor number than oxymetazoline. This latter agonist is more potent in untreated tissue because it has a higher affinity for α-adrenoceptors (377). In fact these agonists illustrate a general prediction from classical occupation theory namely that the maximal responses to agonists of high efficacy are less sensitive to decreases in receptor number or general efficiency of stimulus-response coupling than those of agonists of lower efficacy. Figure 8B shows a theoretical example of two agonists, A_1 and A_2; A_1 has 5 times the affinity but only 0.2 times the efficacy of A_2. The concentration-response curve to A_2 (broken line) is more easily depressed by serial decreases in receptor number.

This principle can be extended to the effects of agonists in two different tissues differing in stimulus-response characteristics. It can be seen from figure 8C that oxymetazoline, an agonist with a higher affinity but lower efficacy for α-adrenoceptors than norepinephrine, is a more potent full agonist than norepinephrine in rat anococcygeus muscle, a tissue with an efficient stimulus-response apparatus. However in rat vas deferens, a tissue with a less efficient stimulus response mechanism, oxymetazoline is a weak partial agonist when compared to norepinephrine by virtue of its low intrinsic efficacy relative to norepinephrine. The profile of reversed agonist activities for these two drugs in rat anococcygeus muscle and vas deferens resembles receptor selectivity but, in these experiments, no evidence of heterogeneous α-adrenoceptor populations was found (377) and the theoretical calculations (figure 8B) show that none is required to explain the experimental results.

The theory and the data illustrate that receptor alkylation or desensitization will preferentially block the responses to the agonist with the lower intrinsic efficacy. Therefore, if two agonists produce responses in a tissue and alkylation or desensitization selectively eliminates the responses to one of the agonists, this need not imply that the two agonists activate separate receptors in the tissue.

The same caveat should be made regarding selective modulation of stimulus-response mechanisms in tissues. For example, recent evidence suggests that external calcium ion is required for the production of responses subserved by α_2-adrenoceptors in contrast to α_1-adrenoceptors which appear to use intracellular calcium stores (628, 656, 658, 659, 667). The evidence for this hypothesis is the disparately greater degree of antagonism of responses to putatively selective agonists for α_2-adrenoceptors, compared to those of α_1-adrenoceptors, produced by calcium entry inhibitors. The rationale, therefore, is that the responses to α_2-adrenoceptors rely on the entry of extracellular calcium. It should be noted, however, that the selective α_2-adrenoceptor agonists are partial agonists in these preparations while the α_1-adrenoceptor agonists are full agonists leading to questions about sensitivity of these two types of agonist to differences in receptor reserves or receptor coupling. The hypotheses equating receptor type with calcium source rely on subjective assessments of antagonism of agonist; i.e., a given concentration of calcium antagonist may produce a small shift (1.5- to 2-fold) of the full α_1-agonist and a 40% decrease in the maximal response to the partial α_2-agonist. Unfortunately, the degree of maximal response depression of a partial agonist for a given shift to the right of a full agonist for a uniform modulation of receptor coupling is not known and, in fact, depends upon the nature of the stimulus response coupling. For example, a good estimation of experimental stimulus response curves can be obtained by the general logistic function of the form:

$$\frac{E_A}{E_m} = \frac{S^n}{S^n + \beta^n} \qquad (24)$$

where S is the stimulus, and β and n are fitting factors. With this model, a wide range of efficiencies of receptor coupling can be accommodated. Figure 9 shows the effects of a two-fold decrease in the efficiency of coupling in three tissues with different states of coupling ($n = 1$, 2, 3, Eq. 24). It can be seen from this figure that the degree of depression of maximal response to the partial agonist for a given shift to the right of the concentration-response curve to the full agonist is not constant. The important aspect of this calculation is the prediction that decreases in receptor number (i.e., receptor alkylation) should mimic the effects of calcium antagonists in these systems. However, the testing of the hypothesis with alkylating agents requires care as to the selective alkylation of α_1- or α_2-adrenoceptors (147, 148) and obvious problems with usage of alkylating agents in vivo (i.e., the pithed rat). A paper by Rimele and coworkers (539) suggests an alternative hypothesis, namely that the degree of dependence on activator calcium is not associated with receptor type as much as with the functional differ-
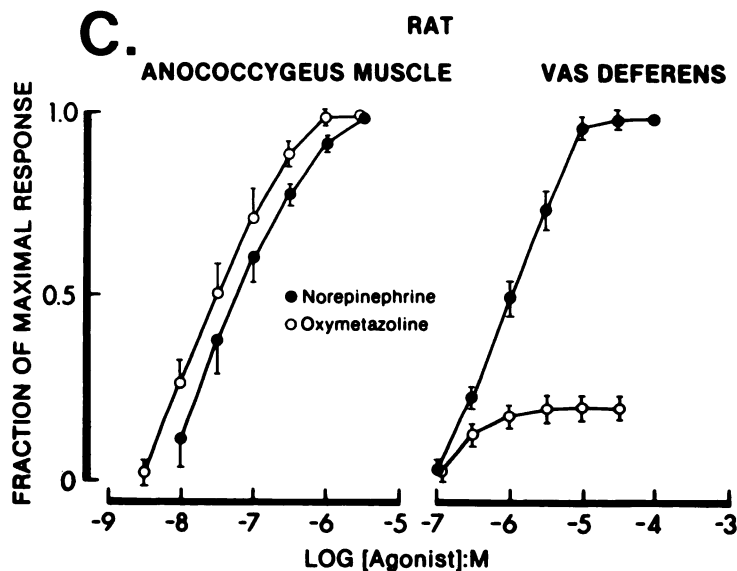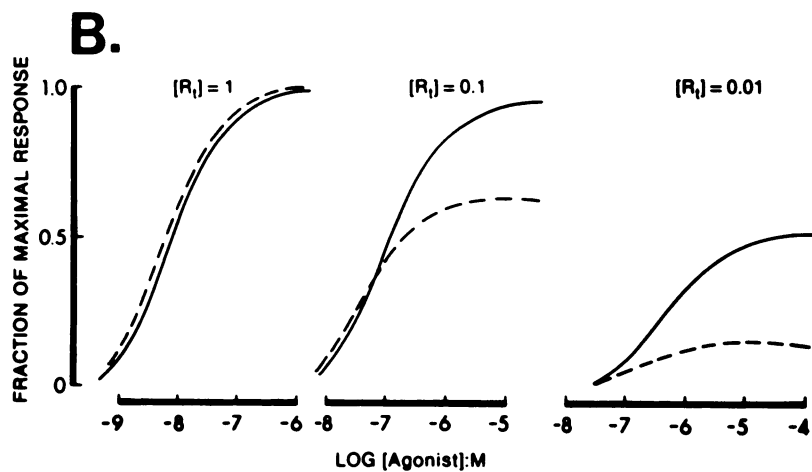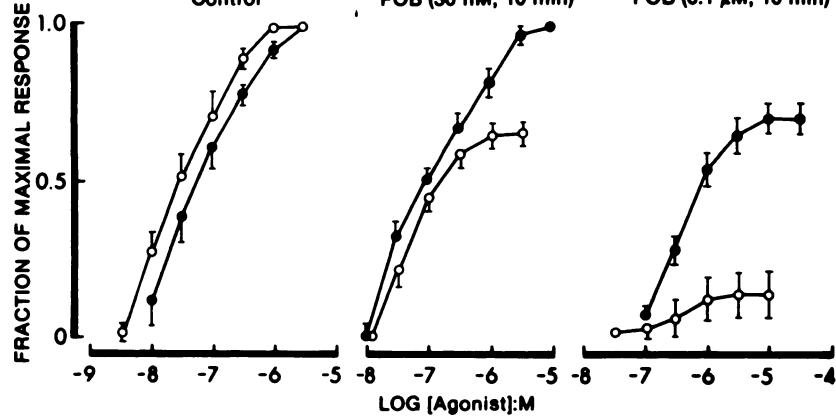
FIG. 8. The effects of changing receptor number on agonists with different ratios of efficacy and affinity. A. Experimental concentration-response curves in rat anococcygeus muscle. Ordinates: Response as a function of maximal control response. Abscissae: Logarithms of molar concentration of agonists. Responses to norepinephrine (●) and oxymetazoline (○) in untreated tissues (n = 6), treated with phenoxybenzamine 30 nM for 10 min (n = 6) and 0.1 μM for 10 min (n = 6). Bars represent S.E.M. B. Theoretical concentration-response curves to two agonists; broken line for agonist with 5 times the affinity but 0.2 times the efficacy of agonist designated by solid line. Ordinates: Responses calculated by Eq. 18 and 24 (β:0.1). Abscissae: Logarithms of molar concentrations of agonists. Effects of a 100-fold decrease in receptor number ([R$_t$] = 1 to 0.01). C. Experimental concentration-response curves. Ordinates and abscissae as for part B. Responses of norepinephrine (●) and oxymetazoline (○) in rat anococcygeus muscle (n = 6) and rat vasa deferentia (n = 6). Bars represent S.E.M. Reprinted with permission from Kenakin (377).
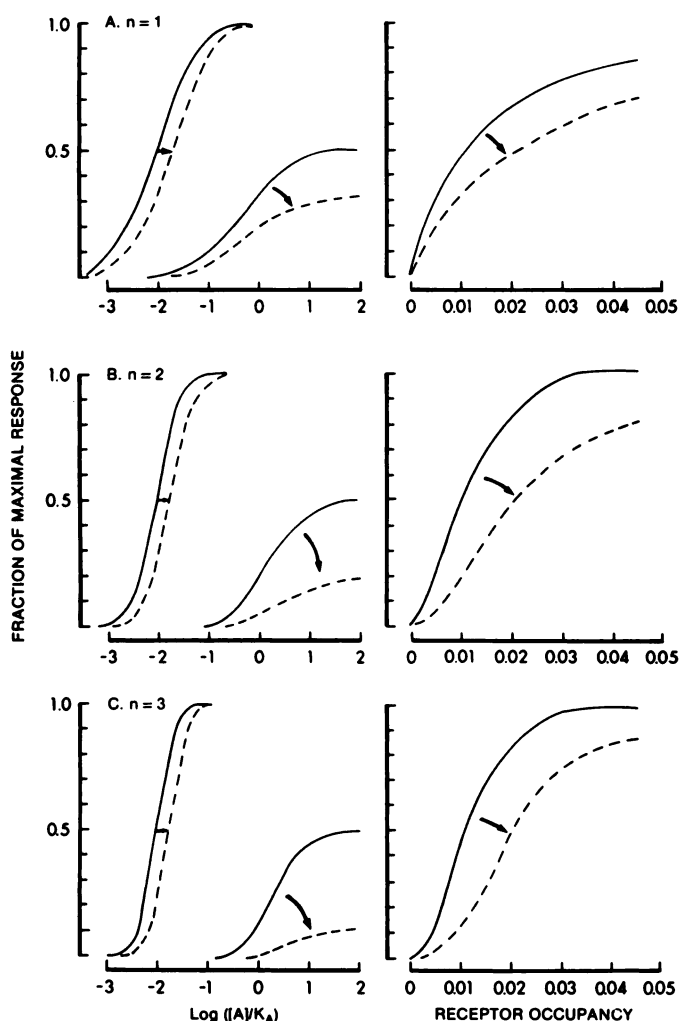
FIG. 9. The effects of receptor coupling on responses to full and partial agonists. Ordinates: Fractions of maximal response to the full agonist. Abscissae: Logarithms of molar concentrations of the agonist as a multiple of K_A for concentration-response curves and fractional receptor occupancy by the full agonist for occupancy-response curves. Solid and broken lines refer to response before and after a decrease in the efficiency of receptor coupling $\beta = 0.1$ to 0.2, Eq. 24). Stimuli calculated from Eq. 18 with the ratio of efficacy for the full and partial agonist of 100.

entiation of the vascular smooth muscle (see also 468, 668, 669). This alternative highlights the quantum jump involved in associating tissue differences with receptor differences.

C. Measurement of Agonist Affinity and Relative Efficacy

1. *Agonist Affinity.* Affinity is defined as the reciprocal of the equilibrium dissociation constant of the drug for the receptor (K_A, Eq. 15). This parameter determines what fraction of the free receptors will be occupied by a given concentration of drug and thus defines the "... signal-to-noise ratio in the chemical cacophony which surrounds every cell" (74). There are null methods available to estimate this important drug constant.

The method of partial alkylation of receptors (229,

428, 430) is widely used to measure the equilibrium dissociation constant of a full agonist for a receptor. The basic premise of the method is that equiactive concentrations of agonist before and after irreversible elimination of a fraction of the drug receptors can be equated to yield differences that depend upon receptor number and agonist affinity. The mathematical manipulation of the equation allows cancellation of the receptor number term to yield an estimate of K_A. The null nature of this method cancels effects of stimulus-response mechanisms but as originally described requires that the only difference in the tissue after treatment with alkylating agent is the number of viable drug receptors. Thus equiactive concentrations of a full agonist before ($[A]$) and after ($[A']$) partial alkylation of the receptor pool are compared in the following double reciprocal equation (229, 429, 430):

$$\frac{1}{[A]} = \frac{1}{[A']} \cdot \frac{1}{q} + \frac{1}{K_A} \cdot \frac{(1 - q)}{q} \qquad (25)$$

where q is the fraction of viable receptors left in the tissue after alkylation. The K_A then can be calculated by:

$$K_A = \frac{\text{Slope} - 1}{\text{Intercept}} \qquad (26)$$

There are methodological considerations to the effective use of this technique. For example, the concentration-response curve after receptor alkylation should have a depressed maximal response. Thron (626) has shown that values near to the top of the depressed concentration-response curve yield the best estimate of K_A with this method. While the linearization of the equations by a double reciprocal technique is convenient, Parker and Waud (498) have shown an improved fit of data points directly to the hyperbolic form of the relationship. Computer analysis has been applied to advantage with this technique as well (498, 714).

To use the method of partial receptor alkylation, as defined, an irreversible antagonist of the receptor is required. A useful group of drugs in this regard has been the β-haloalkylamines. Within this class are irreversible drugs for α-adrenoceptors (227, 578, 609), cholinoceptors (229, 257, 560), histamine H_1 receptors (227, 229, 386, 387), and serotonin receptors (227). These agents are convenient in that the noncyclized chemical species can be chemically removed from the bathing medium by addition of a large excess of thiosulphate ion (226, 386, 377, 475) thereby stopping the alkylation process and allowing fine control of the procedure.

There are a number of other irreversible drugs available for drug receptors. The extreme chemical reactivity of azides has led to the development of photoaffinity labels for receptors. This approach theoretically can produce very selective irreversible drugs since they alkylate only after transformation to a highly reactive species (i.e., nitrene) upon irradiation with light (289, 582).

Thus, photoaffinity labels have been reported for β-adrenoceptors (304, 412, 557), histamine H_1 receptors (208, 209, 481), muscarinic receptors (391), adenosine receptors (208, 482), angiotensin receptors (203, 249), and dopamine receptors (345). The incorporation of 4'-amino-3',5'-diiodophenylalanine into peptides and subsequent conversion in situ to 4'-azidiphenylalanine in theory makes possible the design of photoaffinity labels for many peptides (203). Other irreversible ligands include some toxins for muscarinic receptors (166) and halo-acetyl compounds for β-adrenoceptors [Ro 03-7894 (96, 473, 529, 701); bromoacetylated alprenolol (34); NHNP-NBE (31, 673)]. A thiadiazole, L-643,441, has been shown to irreversibly inhibit histamine H_2 receptors (43, 511, 639). Benextramine (BHC) is an irreversible inhibitor of α-adrenoceptors and muscarinic receptors (55) and α-chlornaltrexamine irreversibly binds to opiate receptors (120, 524, 562). Some amino derivatives of strong analgesics also have been shown to irreversibly bind to opiate receptors (537). A unique approach has been the incorporation of multiple pharmacophores into molecules to produce drugs with essentially irreversible kinetics (518).

Given the variety of irreversible antagonists the method of partial receptor alkylation has been applied to numerous receptor types including β-adrenoceptors (580, 701), α-adrenoceptors (64, 346, 370, 377, 578, 714), histamine H_1 receptors (43, 229), histamine H_2 receptors (43), muscarinic receptors (229, 238, 257, 497, 540, 560, 612), and opiate receptors (522).

Although this method was designed for use with irreversible receptor antagonists, other methods to alter the stimulus response characteristics of tissues have been applied. For example, functional antagonism of responses to full agonists has been utilized to estimate K_A values for full agonists (105, 106, 704). Functional antagonism can be achieved either by addition of a drug which by an action on some other receptor produces a stimulus that opposes the primary stimulus or by a drug which in some way modulates the primary stimulus as it is converted into tissue response. There are a number of caveats to be made to the use of functional antagonism with this method. The primary rationale is that functional antagonism produces effects on the concentration-response curves to full agonists which closely resemble the effects of alkylating agents. However, functional antagonism is a very complex phenomenon (198, 433, 652, 653) and there is no theoretical basis for its application to the partial receptor alkylation technique. Comparisons of equal tissue state as opposed to equal responses should be utilized (433). Thus, if a spasmogen is used to physiologically antagonize a relaxant in a tissue, the concentration-response curves to the relaxant should not first be "normalized" (i.e., percent maximum) and then compared. Rather, relaxant doses producing equiv-alent contractile states (i.e., actual tension) should be used.

There have been relatively few quantitative comparisons made of K_A estimates by partial alkylation and by functional antagonism. Those studies which have been done have yielded mixed results. For example, Siegl and McNeill (580) found in rabbit papillary muscle that the estimates of K_A for phenylephrine acting on α-adrenoceptors made by partial alkylation of receptors (with dibenamine) agreed with estimates made by functional antagonism with the calcium channel antagonist D-600. However, the estimates for the K_A of isoproterenol with the alkylating agent (Ro 3-7894) and D-600 were quite different. In rabbit aorta, Hurwitz and coworkers (337) estimated the K_A for norepinephrine in rabbit aorta by comparing concentration-response curves in the presence of varying concentrations of calcium and obtained an estimate very similar to one by Besse and Furchgott (64) who used dibenamine as an alkylating agent. However, the calcium technique yielded a K_A for acetylcholine in guinea pig ileum longitudinal smooth muscle which was much lower (337) than that obtained by alkylation of receptors (538, 560). Recently, Leighton and Su (416) have used functional antagonism to calculate K_A values for presynaptic α-adrenoceptors. By altering extracellular calcium concentration or transmural stimulus conditions, clonidine was converted from a full to a partial agonist. Estimates of K_A values for clonidine made by these methods were not significantly different from those obtained by the receptor alkylation method. Theoretical analyses (198, 433) indicate that functional antagonism possibly might be suitable for estimation of K_A for agonists of low efficacy but not those of high efficacy. Experimental support for this idea was obtained in guinea pig left atria where the K_A estimated by functional antagonism and by receptor alkylation agreed quite well for the low efficacy muscarinic agonist pentyltrimethylammonium but not so well for furmethide and oxotremorine, agonists of higher intrinsic efficacy (198).

A novel method of obtaining K_A for an agonist is by application of perturbation techniques. In this method, some variable is changed suddenly and the kinetics of return to or relaxation to (hence the term, relaxation method) equilibrium are observed. The perturbation can be a change in concentration, membrane potential, temperature, or irradiation. The disturbing influence should produce a sudden change in a steady-state response ($\Delta\epsilon$) to some agonist $[A]$. A time constant (τ), the time required for the response to recover to 0.37 times the original response, is recorded. This time constant is related to the kinetics of agonist binding by:

$$\frac{1}{\tau} = k_1 [A] + k_2 \qquad (27)$$

where k_1 and k_2 are the rates of onset and offset of the agonist. Thus, the repetition of this process at a number

of agonist concentrations furnishes data for a regression of $1/\tau$ upon $[A]$ and $K_A = k_2/k_1 =$ intercept ÷ slope. There are several caveats to be made for the use of this technique in pharmacological experiments (144). Diffusion must be shown not to be rate-limiting and the perturbation must only disturb the equilibrium between drug and receptor and not any of the processes in the stimulus-response chain for the method to yield valid estimates of K_A. An intriguing approach by Lester (419) utilized a light activated agonist and rapidly changed the concentration of the agonist with brief flashes of light. Ultraviolet radiation has been used as a perturbing influence in pharmacological experiments in rabbit aortae (346, 614, 616, 617).

When dealing with partial agonists, the preceding and other methods can be utilized to measure the K_A as well but there are certain specialized procedures available for the estimation of the equilibrium dissociation constant of these drugs. Two important methods utilize comparisons of concentration-response curves to a full and a partial agonist in the same tissue. Both methods assume that the concentrations of full agonist needed to produce submaximal responses are much less than the K_A ([A] ≪ K_A). Under these circumstances equiactive concentrations of full and partial agonist can be equated by (44, 686):

$$\frac{1}{[A]} = \frac{\epsilon_A}{\epsilon_P} \cdot \frac{K_P}{K_A} \cdot \frac{1}{[P]} + \frac{\epsilon_A}{\epsilon_P} \cdot \frac{1}{K_A}. \qquad (28)$$

The equilibrium dissociation constant of the partial agonist (K_P) can be calculated by $K_P =$ slope ÷ intercept. If, in fact, the tissue does not have a significant receptor reserve for the full agonist, an error term will be introduced into the calculation and the procedure will overestimate K_P (385):

$$K_P = \frac{\text{Slope}}{\text{Intercept}} \left(1 - \frac{\epsilon_P}{\epsilon_A}\right) \qquad (29)$$

The error diminishes to zero as the difference between the intrinsic efficacy of the full and partial agonist increases (429). Linear regressional analysis of $1/[A]$ upon $1/[P]$ makes this method convenient and accessible but a direct fit of the data points to a hyperbola by computer gives a more accurate value (498).

An estimate of K_P can be made by comparing equiactive doses of a full agonist (again assume $[A]$ ≪ K_A) in the absence $[A]$ and presence $[A']$ of a fixed concentration of partial agonist $[P]$. The equation relating these concentrations is (143, 599):

$$[A] = \frac{[A']}{\left(1 + \frac{[P]}{K_P}\right)} + \frac{\epsilon_P}{\epsilon_A} \cdot \frac{[P]}{K_P} \cdot \frac{K_A}{\left(1 + \frac{[P]}{K_P}\right)} \qquad (30)$$

to yield an estimate of K_P by:

$$K_P = \frac{[P] \cdot \text{Slope}}{1 - \text{Slope}}. \qquad (31)$$

If $[A]$ ≮ K_A, then an error term is introduced into the estimate (385, 429, 430):

$$K_P = \frac{[P] \cdot \text{Slope}}{(1 - \text{Slope})} \left(1 - \frac{\epsilon_P}{\epsilon_A}\right) \qquad (32)$$

which diminishes to zero if ϵ_A ≫ ϵ_P. The regression of $[A]$ upon $[A']$ can be greatly improved by weighting factors (440). With this method, one estimate of K_P may be made for every concentration of partial agonist at which the analysis is done. Kaumann and Marano (366) have derived an equation which utilizes data from a range of concentrations of partial agonist. Thus, the repeated analysis by Eq. 30 yields a range of slopes for a given range of concentrations of partial agonists which can all be used to generate an estimate of K_P by (366):

$$\log\left(\frac{1}{\text{Slope}} - 1\right) = \log[P] - \log K_P \qquad (33)$$

By its very nature, a partial agonist will produce competitive antagonism of responses to a full agonist. This antagonism may be analyzed by the Schild method (vide infra) to yield an estimate of the K_P but the intrinsic efficacy of the partial agonist complicates the analysis. Specifically, the relationship between stimulus and response in the tissue introduces an error factor of unknown magnitude into the calculation if the relationship is not one-to-one (350, 661). For example, if the tissue response is a rectangular hyperbolic function of stimulus, then the intercept of the Schild regression yields the logarithm of the K_P modified by a term which depends upon the relative efficacy of the full and partial agonist (350):

$$\text{Intercept} = \log K_P (1 - \alpha)^{-1} \qquad (34)$$

where α is the intrinsic activity (20) of the partial agonist. Clearly, if ϵ_A ≫ ϵ_P, this error will be negligible but not so if the partial agonist produces a sizeable response (high ϵ_P). In these cases, techniques have been developed to eliminate the responses to the partial agonist so that the K_P may be estimated unambiguously by the Schild method. Owing to the fact that partial agonists have no receptor reserve and that irreversible alkylation of a portion of the receptors depresses the maximal responses to partial agonists (27, 665), one method available is the controlled alkylation of a portion of the receptors such that the responses to the partial agonist are eliminated and those to the full agonist are not. Under these circumstances, the partial agonist may be utilized as a competitive antagonist and the K_P estimated by Schild analysis (238, 370, 377, 498, 685). A variant of this method is the use of physiological antagonism to eliminate the agonist responses to the partial agonist (105, 106, 379).

A method which will yield the K_P of a partial agonist by comparison of concentration-response curves of a full (it need not be assumed that $[A]$ ≪ K_A) and a partial agonist, providing that the K_A of the agonist is known,

has been proposed by Gero and Tallarida (254). If equiactive concentrations of full $[A_i]$ and partial $[P_i]$ agonists are determined along with the concentration of full agonist which is equiactive to the maximal response to the partial agonist $[A_P]$, then (254):

$$K_P = \frac{K_A \, ([A_P] - [A_i]) \, [P_i]}{[A_i] \, ([A_P] + K_A)}. \qquad (35)$$

2. Agonist Efficacy. Some drugs bind to receptors and produce a tissue response while others produce no agonist response; the differentiating factor between these two drugs is that the former is said to possess intrinsic efficacy while the latter does not. This property of drugs which enables some drugs to "sit at the piano and play ..." while others only sit (23A) has evoked much interest in pharmacology.

Because competitive antagonists produce no agonist responses, they are often said to possess no intrinsic efficacy. This is a theoretical concept, however, which disregards the limitations in the sensitivity of our systems to detect efficacy. For example, the well-known competitive antagonists propranolol and phentolamine both generate agonist responses in a primary cell culture of neonatal rat ventricle (310). These cells are 100 times more sensitive than intact neonatal rat hearts to α- and β-adrenoceptor agonists, a hypersensitivity presumably due to extraordinarily efficient receptor coupling. It may be that all drugs possess affinity *and* efficacy and that our tissue systems for detecting efficacy can be thought to possess windows with thresholds of detection for this property of drugs.

The mathematical functions to express efficacy have been given in a previous section of this review where it should be noted that, in terms of occupation theory, efficacy is an empirical proportionality constant the magnitude of which has no implications as to mechanism of response production. Nevertheless, there are drug receptor models that describe efficacy in molecular terms. Efficacy is most often thought of as being the ability of a drug to produce a protein conformational change by, for example, causing the drug receptor protein to take on a lower free energy ("conformational induction") (107). Drugs can be efficacious by completing the active site of an enzyme as in the case of certain polypeptide hormones (549) or by allosteric generation of an active site in an enzyme (24). The two-state model describes the efficacy of a drug as its relative affinity for the active and inactive state of the receptor ("conformational selection") (107, 125, 357).

An operational model of efficacy has recently been presented by Black and Leff (77) in which the receptor is considered to have cognitive and transitive properties. Thus, the binding of drug to the receptor is followed by the binding of the complex to an effector, a model similar in mathematical formulation to the floating receptor model (347, 610, 637) or the ternary complex model (176).

In these models the fluidity of the lipid matrix (84, 85, 341, 550, 581) and the hydrophobicity of the receptor complex (161, 280, 348) would affect intrinsic efficacy. In the Black and Leff (77) model, efficacy would be defined as $[R_t]/K_E$ where $[R_t]$ is the concentration of receptors and K_E the equilibrium dissociation constant of the drug receptor complex and the effector unit.

Mechanistic considerations need not be a hindrance to the use of quantitative estimates of relative efficacy for drug and drug receptor classification. Null methods have been described which enable estimates to be made of the relative intrinsic efficacy of two agonists. These measurements must be of the relative efficacy of two agonists since presently there is no absolute independent scale of efficacy. Attempts have been made to define the absolute efficacy of agonists in terms of Stephenson's (599) original formulation, namely that e equals unity at the reciprocal of the fractional receptor occupancy at 50% maximal response (253). However, this method cannot be used as a scale of intrinsic efficacy for the classification of agonists since it is tissue and not receptor-dependent. Therefore agonists will have a different efficacy for every tissue in keeping with Stephenson's (599) formulation of efficacy but no so in terms of Furchgott's (229) concept of intrinsic efficacy. The latter but not the former term is drug-receptor related and therefore of value in drug-receptor classification.

The relative efficacy of two agonists can be estimated by comparison of their respective concentration response curves (429, 430). A double reciprocal relationship between equiactive concentrations of agonists $[A_1]$ and $[A_2]$ yields a measure of the relative order of efficacy of the agonists:

$$\frac{1}{[A_1]} = \frac{1}{[A_2]} \cdot \left(\frac{K_2}{K_1} \cdot \frac{\epsilon_1}{\epsilon_2} \right) + \frac{\epsilon_1}{\epsilon_2 \cdot K_1} \left(1 - \frac{\epsilon_2}{\epsilon_1} \right). \qquad (36)$$

The arithmetic sign of the intercept indicates the relative order of the intrinsic efficacy of the agonists; if $\epsilon_2 > \epsilon_1$ the intercept will be positive and if $\epsilon_2 < \epsilon_1$, it will be negative (429, 430). A technical difficulty related to the effective use of this method occurs when the concentration-response curves are parallel causing the intercept to tend toward unity. Providing K_A for one of the agonists is known, a numerical estimate of the relative efficacy of the agonists can be calculated from (429, 430, 553):

$$\frac{\epsilon_2}{\epsilon_1} = (1 + \text{Intercept} \cdot K_1)^{-1}. \qquad (37)$$

Furchgott (229) has described a widely used method of estimating the relative efficacy of two agonists. Considering two agonists which give equal stimuli (S_1 and S_2) to a tissue such that $S_1 = \epsilon_1 \cdot \rho_1 \cdot [R_t]$ and $S_2 = \epsilon_2 \cdot \rho_2 \cdot [R_t]$ where ρ is the fractional receptor occupancy, then (229):

$$\frac{\rho_1}{\rho_2} = \frac{\epsilon_2}{\epsilon_1}. \qquad (38)$$

The responses to the two agonists are expressed as functions of log ρ and the displacement between the curves on the abscissal scale equals the logarithm of the relative efficacy of the two agonists [e.g., figure 4 of Furchgott and Bursztyn (238); figure 4 of Kenakin (370)].

3. Experimental Manipulation of Receptor Number and Efficiency of Stimulus-Response Coupling. Clearly, an estimate of the efficacy of an agonist cannot be made in an isolated tissue unless the agonist produces a response in that tissue. Also, with the methods currently available, an unambiguous estimate of the affinity of the agonist is required for the estimation of efficacy. Unfortunately, the production of an agonist response by a drug often hampers the estimation of the affinity of that drug for the receptor. Under these circumstances it is advantageous to control the magnitude of response to an agonist in a tissue so that estimates of affinity and efficacy can be made independently. This is most easily done with agonists of low intrinsic efficacy.

Before discussion of interventions that can control the mechanisms for response in given isolated tissues, a related approach will be considered. This is the analysis of drug receptor parameters in tissues differing in stimulus-response characteristics but shown by other methods to have a homogeneous receptor poulation with respect to each other. Basically, in this analysis the agonist with the lower intrinsic efficacy is utilized as an antagonist of the agonist with higher intrinsic efficacy in tissues with poorly coupled receptors, and the resulting estimate of K_A used to calculate relative efficacy in tissues with the more efficient receptor coupling. This approach is based on the fact that the maximal responses to agonists of lower efficacy are more subject to the efficiency of receptor coupling than agonists of higher efficacy (i.e., see figure 8A). Therefore, in a series of tissues with progressively less efficient receptor coupling, the potency of the full agonist should decrease (increasing EC50) and the maximal response to a partial agonist should decrease correspondingly. The exact relationship between the maximal respnse to the partial agonist in a tissue with a given sensitivity to a full agonist depends upon the actual nature of the coupling (e.g., figure 9) but some good correlations between sensitivity of tissues to full agonists and maximal responses to partial agonists can be found. For example, figure 10A shows the range of sensitivities of tissues, all containing β_1-adrenoceptors, to (−)-isoproterenol while figure 10B shows the corresponding concentration-response curves to the partial agonist prenalterol. Isoproterenol has 220 times the intrinsic efficacy of prenalterol (379) and, as predicted by classical receptor theory, the maximal responses to the drug with the lower intrinsic efficacy are depressed by reductions in the efficiency of coupling of receptors while the location parameters of the drug with the higher efficacy are displaced to the right. Figure 10C shows the

correlation between sensitivity of these tissues to isoproterenol and the maximal response to prenalterol (379, 381). Similar results have been reported by Mattsson and coworkers (444). Figure 10D shows the wide range of efficiencies of receptor coupling in some β_1-adrenoceptor-containing tissues and highlights the choices of tissues available for this type of approach. Such correlations would be predicted for full and partial agonists for all receptors. A similar relationship between the sensitivity of tissues to oxotremorine and the maximal responses to pilocarpine, full and partial agonists for cholinergic receptors, respectively, can be calculated (712).

The data in figures 10A and 10B shows the importance of receptor coupling for agonists of low intrinsic efficacy; even though both the rat right atria and canine coronary artery have β_1-adrenoceptors, the former tissue responds both to isoproterenol and prenalterol while the latter tissue produces responses only to isoproterenol. The tissue selectivity of prenalterol does not depend upon receptor selectivity in these tissues (379, 381). Assuming identity of receptors across the tissue types, an unambiguous estimate of the affinity of prenalterol can be made in canine coronary artery (by the Schild method) and applied to any of tissues, which respond to prenalterol, in measurements of relative efficacy.

Differences other than inherent stimulus-response relationships between tissues can be eploited to control the sensitivity of tissues to full agonists and the maximal responses to partial agonists. In previous sections of this review, it was shown how differences in tissue sensitivity to agonists could be determined by the age of the animal or the anatomical location of the tissue preparation. An example of the latter is the differences in sensitivity of various parts of the urinary bladder to β- and α-adrenoceptor agonists. Thus, Levin and Wein (421) have shown that methoxamine produces a 40% maximal response in the bladder body and 100% maximal response in the bladder base. Another determinant of sensitivity may be the method of measurement of responses. As noted earlier, the sensitivity of some tissues to cholinoceptor agonists is greater when the measurements are made isotonically rather than isometrically. Thus, in the frog rectus abdominus, carbachol is a full agonist for production of both isotonic and isometric responses, but with a larger contractile (receptor) reserve, as measured by receptor alkylation, under conditions of isotonic recording (451).

In general, these approaches introduce uncontrolled variables into the comparisons (i.e., species, tissue type) and theoretically are less sound than the modification of tissue stimulus-response characteristics to make the measurements of affinity and efficacy in the same tissue. The two tissue-related determinants of agonist response, namely receptor number and the efficiency of the mechanisms which convert receptor stimulus into response, are the primary targets for this type of experiment. As a
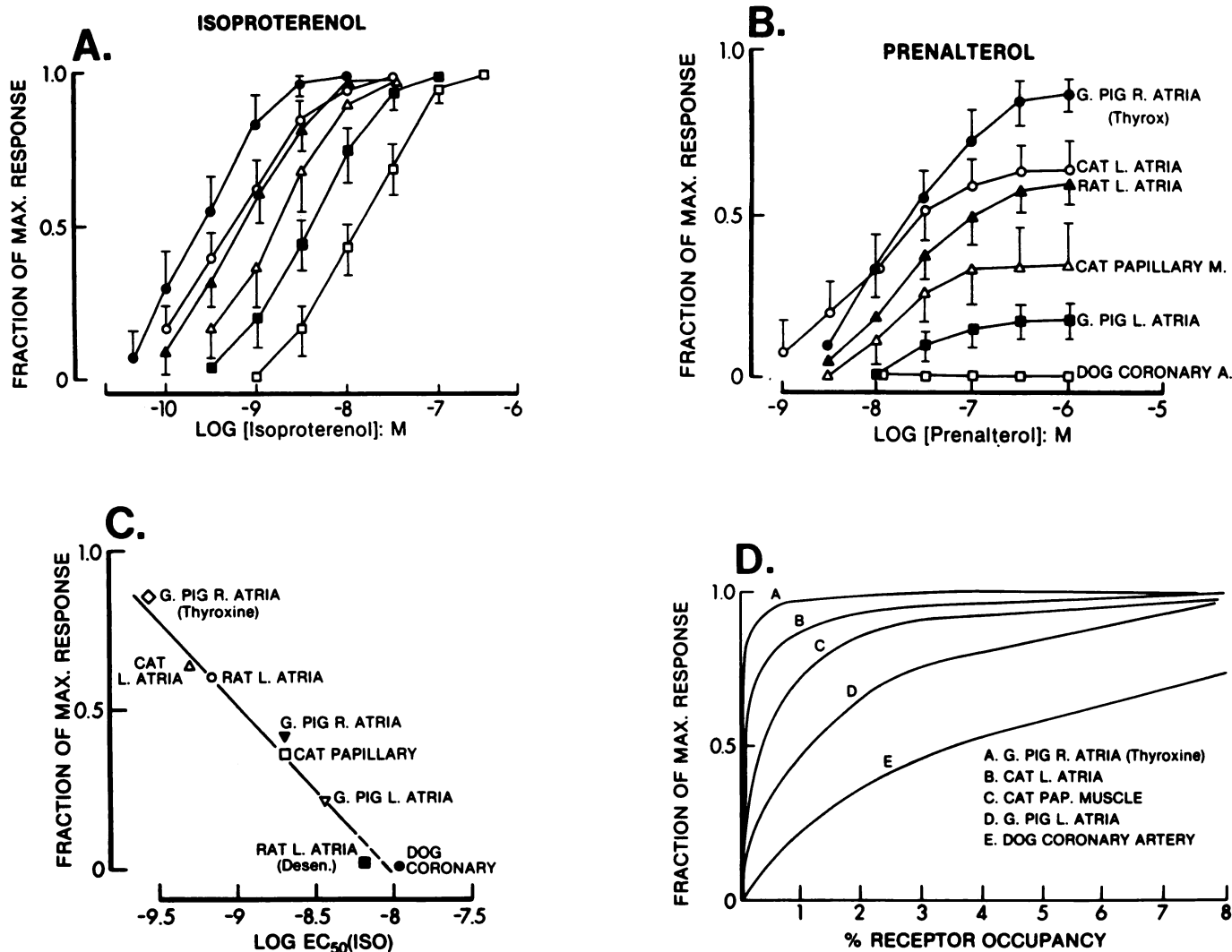
FIG. 10. Relationships between tissue responses to full and partial agonists. A. Concentration-response curves of tissues to *l*-isoproterenol. Ordinates: Fractions of maximal responses to *l*-isoproterenol. Abscissae: Logarithms of molar concentrations of *l*-isoproterenol. Responses in thyroxine-treated guinea pig right atria (●, *n* = 4), cat left atria (O, *n* = 6), rat left atria (Δ, *n* = 8), cat papillary muscle (Δ, *n* = 6), guinea pig left atria (□, *n* = 5) and dog coronary artery (□, *n* = 4). B. Responses of same tissues to prenalterol. Ordinates as for part A. Abscissae: Logarithms of molar concentrations of prenalterol. Values for *n* as for part A; bars represent S.E.M. Redrawn from Kenakin and Beek (379, 382). C. Maximal responses of tissues to prenalterol (as a fraction of the maximal response to isoproterenol) as a function of the sensitivity of the tissues to *l*-isoproterenol (expressed as the logarithm of the molar concentration of isoproterenol which produces half the maximal response). Data from parts A and B. D. Tissue responses of tissues to isoproterenol (as fractions of the maximal responses) as functions of the receptor occupancy of isoproterenol (calculated by the Langmuir isotherm assuming a homogeneous K_A of 0.2 μM).

preface to discussion of modifying stimulus-response characteristics of tissues, the need for appropriate controls should be stressed. The numerous interventions which affect responsiveness may or may not affect the nature of the drug receptors, thus experiments with competitive antagonists before and after intervention would be required. Schild analysis to indicate possible significant differences in the pK_B for a range of antagonists before and after intervention would be a useful check of receptor identity in these procedures.

A. THE MANIPULATION OF RECEPTOR NUMBER. Receptor number either can be modified chemically in vitro or with procedures which utilize the cells' own mechanisms for control of receptor number in vivo. The most widely used in vitro modification of drug receptor number

is treatment of tissues with receptor alkylating agents. As discussed in the section on affinity, such treatments preferentially depress the maximal responses to partial agonists (27, 665) allowing them to be utilized as competitive antagonists.

The cellular control of receptor number has become relevant to human disease states (19, 37, 113, 149, 162, 348), drug tolerance (146, 224, 493), drug withdrawal (142, 526, 528), and aging (404–406, 678), and a number of chronic stimuli are capable of producing alteration of receptor number in laboratory animals. For example, the implantation of osmotic mini pumps delivering 400 μg $kg^{-1}h^{-1}$ of *l*-isoproterenol was shown to decrease the number of β-adrenoceptors in rat myocardium (123, 124, 388) and correspondingly to shift the concentration-

response curve in rat atria to isoproterenol to the right and completely depress the maximal responses to the partial agonists prenalterol and pirbuterol (388) (figure 11A). In desensitized rats these drugs produced no agonist responses and therefore estimates of affinity for β_1-adrenoceptors were made by the Schild method (figure 11B). This then allowed for an estimate of the relative efficacy of these drugs by the method of Furchgott (229); see Eq. 38) in normal atria (383). Some common methods of decreasing receptor number involve chronic treatment with drugs either by repeated injection, implantation of mini osmotic pumps or pellets, or changes in the hormonal status of animals (i.e., thyroid state). Some examples of treatments for decreasing the number of receptors in various isolated tissues is given in table 6.

Increases in receptor number also can be utilized in measurements of drug receptor parameters. For example, in a poorly coupled tissue, the affinity of a weak partial agonist, which produces no agonist response, could be

estimated by the Schild method. Treatment of the animal with procedures which promote receptor proliferation would produce sensitization to full agonists and may produce a tissue which demonstrates an agonist response to the partial agonist. This concentration-response curve then could be utilized for efficacy measurements. Some interventions which have been shown to increase receptor number are given in table 7.

B. MANIPULATION OF THE EFFICIENCY OF STIMULUS-RESPONSE COUPLING. If the receptor could be though of, in electronic terms, as the preamplifier then the stimulus-response machinery of the tissues is the power amplifier which converts the pre-amp signal (receptor stimulus) into the response. There are numerous ways of adjusting the level of the power amplifier. When doing this type of experiment, care must be taken to see that the receptor profile of the tissue is not altered. Thus, control experiments with full agonists and competitive antagonists should always be done before and after the interventions which modify tissue responsiveness to test for changes in receptors by Schild analysis.

Functional (physiological) antagonism of agonist responses has often been used to depress the maximal responses of partial agonists for Schild analysis. This technique is especially useful in tissues in which a drug is needed to induce a given pharmacological tone for agonist responses to be observed. For example, guinea pig trachea requires some other intrinsic or pharmacological tone in order for relaxant responses to be studied. The degree of tone in this tissue greatly modifies the location of concentration-response curves to relaxants (105, 106, 484). Other methods of modulating agonist responses involve specific cations. For instance, Burgen and Spero (110) found that the sensitivity of guinea pig ileum was greatly dependent upon calcium and/or magnesium ion. Takeyasu and coworkers (612) found that reduction of calcium ion makes pilocarpine, normally a full agonist in guinea pig ileum, into a partial agonist. The differential utilization of calcium by blood vessels makes adjustment of calcium concentration a powerful method of adjusting the sensitivity of blood vessels to contractile agonists (329, 448). Tissue sensitivity to agonists can be decreased by a number of in vivo treatments. Table 8 lists some methods used to decrease tissue sensitivity to agonists; it should be noted that many of these treatments may decrease receptor number as a mechanism of action but since binding data corroborating this is not given in these papers, the effects will be referred to as a general decrease in sensitivity.

Potentiation of responses can occur by augmentation of some step or the inhibition of some modulatory influence in the stimulus-response chain. An example of this latter mechanism is the inhibition of phosphodiesterase to potentiate the effects of drugs which increase cellular cyclic AMP. It is well known that inhibition of this enzyme produces sinistral displacement of concentra-
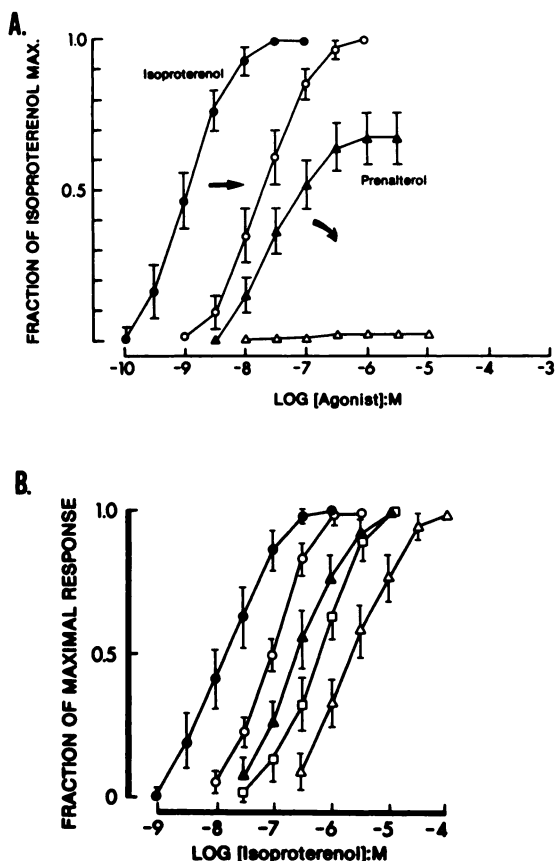
FIG. 11. The effect of chronic isoproterenol (by osmotic mini-pump delivery) on rat atrial responses to l-isoproterenol and prenalterol. A. Ordinates: Responses of paced rat left atria as fractions of the maximal responses to l-isoproterenol. Abscissae: Logarithms of molar concentrations of agonist. Responses of normal atria to l-isoproterenol ($\bullet$, n = 5) and prenalterol (Δ, n = 5) and atria from desensitized rats (400 μg kg^{-1} hr^{-1} l-isoproterenol for 4 days) (l-isoproterenol, O, n = 6; prenalterol, Δ, n = 6). From Kenakin and Ferris (388) with permission. B. Antagonism of responses of desensitized atria to l-isoproterenol by prenalterol 0 μM ($\bullet$, n = 5), 0.3 μM (O, n = 2), 1 μM (Δ, n = 3), 3 μM ($\square$, n = 2) and 10 μM (Δ, n = 3). Bars represent S.E.M. or range if n < 3. From Kenakin and Beek (383) with permission.

TABLE 6
Methods to decrease receptor number

Species	Tissue	Receptor*	Response†	Method	References
Rat	Heart	β	↓	Chronic isoproterenol	(478)
			↓	Chronic isoproterenol (miniosmotic pump)	(388)
			↓	Low dose propranolol	(33)
			—	Hypothyroid state	(36, 129, 700)
			↓	Deoxycorticosterone and salt, renal hypertensive	(705, 709)
			—	Training	(611)
			—	Alloxan-induced diabetes	(340)
		α	↓	Deoxycorticosterone and salt	(709)
		ACh	—	Hyperthyroid state	(574)
	Lung	β	↓	In vitro isoproterenol	(4)
			—	Thyroidectomy	(32)
			↓	Chronic metaproterenol	(563)
	Vas	α	—	Chronic desmethylimipramine	(696)
	Lung	ACh	—	Thyroidectomy	(32)
	Uterus	β	↓	Chronic isoproterenol	(352)
	Soleus	β	—	Chronic isoproterenol	(651)
	Tibialis	β	—	Chronic isoproterenol	(651)
	Submaxillary gland	ACh	—	Hyperthyroid state	(574)
	Submaxillary gland	β	—	Hypothyroid state	(520)
	Renal cortex	β	—	Implant pheochromacytoma	(590)
Guinea pig	Lung	β	↓	*Haemophilus influenzaa* virus	(569)
			↓	Chronic desmethylimipramine	(569)
			—	Bacterial infection	(570)
	Ileum	ACh	—	Chronic diisopropyl phosphorofluoridate	(708)
	Urinary bladder	ACh	—	Chronic diisopropyl phosphofluoridate	(708)
Frog	Erythrocyte	β	↓	In vitro isoproterenol	(452, 694)
Mouse	Small intestine	ACh	↓	Chronic diisopropyl phosphorofluoridate	(647)
			↓	Cold stress	(648)

* β, β-adrenergic; α, α-adrenergic; ACh, acetylcholine.
† ↓, Decreased response; —, response not tested.

tion-response curves to full agonists and theoretical considerations would predict increases in the maximal responses to partial agonists. This effect was used to measure the relative efficacy of drugs in guinea pig papillary muscle. In this tissue, prenalterol does not produce an agonist response and can be used as a competitive antagonist of responses to *l*-isoproterenol. The phosphodiesterase inhibitor, isobutylmethylxanthine, generates a shift to the left of the concentration-response curve to isoproterenol and produces a tissue in which prenalterol demonstrates a concentration-response curve. The estimate of affinity from the normal tissue and agonist responses from sensitized tissue allow for an estimate of relative efficacy to be made (384). The potentiation may be brought about chemically as, for example, the potentiation of agonist response in arterial tissues produced by sulfhydryl reagents (29). Chronic treatments can produce supersensitivity in tissues by receptor proliferation but also by other means (217). Thus, chronic reserpine treatment selectively potentiates responses of rabbit aorta to norepinephrine, phenylephrine, acetylcholine, and potassium but not serotonin, histamine, or angiotensin (328, 621). These effects, termed nondeviational supersensitivity by Fleming (216), can involve changes in calcium binding or flux, partial depolarization of tissues or the increase of tight junctions between muscle cells. Table 9 lists some treatments that have been found to produce supersensitivity in tissues. As with table 8, receptor number was not studied biochemically thus it is not known whether the effects are due to receptor number or to some other factor.

TABLE 7

Methods to increase receptor number

Species	Tissue	Receptor*	Response†	Method	References
Rat	Heart	β	—	Chronic guanethidine	(265)
			—	Chronic propranolol (injection)	(264)
			—	Chronic propranolol (mini-pump)	(1)
			Isoproterenol, — Norepinephrine, ↑	6-Hydroxydopamine (neonatally)	(476)
			—	Hyperthyroid state	(700)
		ACh	—	Hypothyroid state	(574)
			↑	Chronic isoproterenol	(478)
	Lung	β	—	Chronic propranolol (mini-pump)	(1)
	Salivary gland	ACh, VIP	↑, ↑	Chronic atropine	(307)
	Submaxillary gland	ACh	—	Hypothyroid state	(520)
		β	—	Hyperthyroid state	(520)
	Lymphocyte	β	—	Chronic propranolol	(1)
	Vas deferens	α	—	Chronic prazosin	(696)
Cat	Superior cervical ganglion	ACh	—	Denervation	(618)
Mouse	Small intestine	ACh	↑	Chronic hexamethonium	(649)

* β, β-adrenergic; ACh, acetylcholine; VIP, vasoactive intestinal peptide; α, α-adrenergic.

†, ↑, Increased response; — response not tested.

TABLE 8

Methods to decrease tissue sensitivity to agonists

Species	Tissue	Receptor*	Method	References
Rat	Atria	β	Immobilization stress	(710)
		ACh	Hyperthyroid state	(343)
	Aorta	β	Chronic isoproterenol (mini-pump)	(608)
		α	Chronic phenylephrine (mini-pump)	(608)
			Chronic propranolol and withdrawal	(623)
	Uterus	β	Restricted diet	(600)
	Fat cells	β	Chronic salbutamol	(224)
Guinea pig	Atria	β	In vitro isoproterenol	(381)
	Ileum	Opioid	In vitro morphine	(536)
			Subcutaneous morphine pellets	(273, 522)
	Soleus muscle	β	Terbutaline in food	(322)
			Chronic isoproterenol	(112)
	Extensor digitorum longus muscle	β	Terbutaline in food	(322)
Cat	Atria	β	In vitro isoproterenol	(362)

* β, β-adrenergic; ACh, acetylcholine; α, α-adrenergic.

D. Competitive Antagonism

1. The Schild Regression. For the most part, the definitive classification of the major drug receptor types and subtypes has been accomplished with selective competitive antagonists. In fact, antagonists are generally more selective for receptor subtypes than are agonists. There

may be chemical reasons for this phenomenon if antagonists, generally larger and more flexible molecules and often bearing the chemical structure of agonists with added lipophilic structural groups (24, 465), bind to accessory sites around the agonist binding site of the receptor. This idea, discussed as a "complimentarity principle" by Ariens and coworkers (24) dictates a sharper differentiation of receptor subtypes by antagonists rather than agonists. For larger molecules such as antagonists, variations in accessory sites, as perhaps expected with differences in the membrane constituents in various cells, may be important determinants of binding.

Ideally, the potency of a competitive antagonist depends upon its equilibrium dissociation constant (K_B) for the drug receptor, a chemical term governed only by the molecular forces that control the rate of onset and offset of the antagonist to and from the tertiary structural and cognitive components of the drug receptor protein. Therefore the K_B, like the K_I for inhibition of a competitive enzyme inhibitor for an enzyme, is a chemical term which hopefully is independent of receptor function, location, and animal species. Considering the importance of reliable estimates of K_B for antagonists it is not surprising that much pharmacological literature is concerned with efforts to make accurate estimates of K_B values.

The first independent scale for antagonist potency with theoretical relevance to equilibrium dissociation constants was devised by Schild (566) and given the name the pA scale. Within this nomenclature, the pA_2 is an empirical parameter that defines the negative loga-

TABLE 9
Methods to increase tissue sensitivity to agonists

Species	Tissue	Receptor*	Method	References
Rat	Atria	β	Chronic propranolol and withdrawal	(623)
			Acute thyroxine	(196)
		α	Hypothyroid state	(344)
		ACh	Hypothyroid state	(344)
	Aorta	β	Chronic phenylephrine (mini-pump)	(608)
	Aorta	β	Chronic propranolol and withdrawal	(623)
	Adipocytes	β	Starvation	(167)
	Lipolysis	β	Demedullation	(224)
	Uterus	α	Diet restriction	(600)
Guinea pig	Atria	β	Reserpine	(640)
			Chronic thyroxine	(382)
Rabbit	Aorta	α	^{125}I-Hypothyroidism	(547)
		5-HT	Hyperlipidemia	(711)
	Ear artery	α	Denervation	(18)
	Saphenous vein	α	Estrogen, progesterone	(545)
	Coronary artery	5-HT	Hyperlipidemia	(711)

* β, β-adrenergic; α, α-adrenergic; ACh, acetylcholine; 5-HT, 5-hydroxytryptamine.

rithm of the molar concentration of an antagonist which produces a twofold shift to the right of a concentration-response curve (564, 565).

By using Gaddum's classic formulation for simple competitive antagonism (243, 244):

$$\rho = \frac{1}{1 + \frac{K_A}{[A]}\left(1 + \frac{[B]}{K_B}\right)}. \qquad (39)$$

where fractional receptor occupancy (ρ) is a function of the molar concentrations of agonist [A] and antagonist [B] and their respective equilibrium dissociation constants (K_A and K_B), Schild derived a useful equation to calculate the K_B of a competitive antagonist (28):

$$\log(dr - 1) = n \cdot \log[B] - \log K_B \qquad (40)$$

where dr refers to the ratio of equiactive concentrations of agonist in the absence and presence of an antagonist [B]. The above equation, often referred to as the Schild equation, allows for a convenient estimation of K_B by a linear regression of a series of dose ratios [in the form $\log(dr - 1)$] obtained with a range of concentrations of antagonist (regression upon $\log[B]$). Providing the regression is linear and that the slope is unity (n = 1), the intercept is $-\log K_B$ (termed the pK_B). A Schild regression slope of unity implies a one-to-one relationship between antagonist and receptor with no substantial cooperative effects. In a comprehensive comparison of antagonist kinetics based on the standard occupation model of drug action and various cooperative models, Colquhoun (143) has shown that the Schild regressions will not differ under a variety of circumstances. However, Sine and Taylor (580A) show that the K_B calculated by

Schild analysis could differ appreciably from the antagonist binding constant in systems where the two drug molecules must bind to two cooperatively linked sites to activate a receptor (i.e., the nicotinic receptor on skeletal muscle) if the affinity of the antagonist for the two sites differs substantially. It can be seen from Eq. 40 that the zero value of the ordinate at the pK_B is obtained when dr = 2 thus the pK_B is also the empirical constant pA_2. The converse, namely that the pA_2 is also the pK_B, is very often not true, a fact that has led to periodic confusion in the classification of receptors. The Schild method has two criteria which must be met before the pA_2 can be considered to be a representation of the pK_B; the regression must be linear and have a slope of unity. In practical terms these criteria are extremely important in experimental pharmacology for two reasons. Firstly, although many drugs which are not competitive antagonists produce parallel displacement of agonist concentration-response curves in a manner identical to competitive antagonists (244), the quantitative relationship between the concentration of drug and degree of shift does not follow simple competitive kinetics. This fact often can be detected by Schild analysis where the slope of the regression will not be unity over a large concentration range. Secondly, the slope and linearity of a Schild regression can be sensitive indicators of nonequilibrium conditions in an isolated tissue. Thus, if a nonlinear Schild regression is obtained in a given isolated tissue with a known competitive antagonist, it would signify deviation from equilibrium either with respect to the concentrations of agonist and antagonist, temporal equilibration or homogeneity of the receptor population. It is worth considering each of these conditions and how they relate to Schild regressions.

A. SLOPE <1. The first experimental condition to consider is the Schild regression with a slope less than unity either over the complete concentration range or a portion of the antagonist concentrations tested. One of the most common causes of slopes less than unity are agonist uptake processes in isolated tissues. For example, in a tissue with an agonist uptake process, a fraction of the agonist added to the organ bath will not reach the receptors and a steady-state deficit of agonist between organ bath and receptor compartment concentrations of agonist will develop (i.e., see figure 4). Therefore, the control concentration-response curve to the agonist will be shifted to the right of the true curve. Then, if a concentration of antagonist produces conditions such that the concentration-response curve to the agonist is shifted far to the right and large concentrations of agonist, which saturate the uptake process, are required for responses, then uptake ceases to be a consideration for this shifted curve. In terms of the discussion for figure 4, the control curve is in region 1 and the shifted curve in regions 2 or 3 of the uptake-concentration relationship. Since uptake removes no appreciable fraction of agonist from the shifted curve, a potentiation of agonist response is effected and the antagonism is reduced by a multiple factor relating to the importance of the uptake process. This reduction of antagonism is reflected in a Schild regression (or portions thereof) with a slope less than unity. The same phenomenon can occur if the competitive antagonist for the receptors also blocks the uptake of agonist at some concentration. Where, along the $\log[B]$ axis these effects are observed is determined by the relationship between the concentrations of agonist (and antagonist) and the K_m for uptake and the equilibrium dissociation constants for the receptors. The effects on Schild regressions can be complex, from nonlinear biophasic curves to linear parallel shifts to the right. A concise model by Furchgott (232, 239) is useful for the description and prediction of these effects. Thus, the concentration of agonist in the organ bath $[A_a]$ is related to that at the receptor $[A_b]_0$ by the following equation (232):

$$[A_a] = [A_b]_0 \ (1 + [B]/K_B)$$

$$\times \left[1 + \frac{\dfrac{U_m}{k \cdot K_{AU}}}{1 + \dfrac{[A_b]_0 \ (1 + [B]/K_B)}{K_{AU}} + \dfrac{[B]}{K_B} \cdot \dfrac{K_B}{K_{BU}}} \right] \quad (41)$$

where $[B]$ refers to the molar concentration of the antagonist, K_A and K_B the equilibrium dissociation constants for the receptor of the agonist and antagonist, respectively, k the transfer rate constant of the agonist into the receptor compartment, K_{AU} the Michaelis-Menten constant of the agonist for the site of uptake, K_{BU} the equilibrium dissociation constant of the antagonist for the site of uptake, and U_m the maximal rate of uptake. This equation is based on a model which equates the rate

of entry of agonist into the receptor compartment by bulk diffusion to the rate of removal of agonist by an uptake process with Michaelis-Menten kinetics. With Eq. 41, given the K_{AU} for an uptake process and equilibrium dissociation constants for the receptors, theoretical Schild regressions may be calculated which show a variety of contours and displacements (for calculated examples see 232, 239, 380, 385, 490). The maximal rates of diffusion and uptake are not required as a prerequisite to the use of this equation since the ratio of these terms can be estimated by the maximal degree of sensitization to the agonist after complete uptake inhibition (see Eq. 10). The conversion of nonlinear into linear Schild regressions by inhibition of agonist uptake processes has been shown in a variety of isolated tissues (103, 104, 131, 232, 239, 336, 380, 385, 410, 426, 463, 490).

Virtually any mechanism that potentiates the response to the agonist (e.g., inhibition of phosphodiesterase) or produces an additional response (i.e., release of endogenous agonist) at some point in the Schild analysis can produce nonlinear Schild regressions. Chemical effects also may be relevant as in the relaxation of rabbit trachea by pH effects after addition to the organ bath of acidic solutions of histamine (381). The activation of another receptor also may produce nonlinearity and will be discussed separately.

B. SLOPE >1. Schild regressions with slopes greater than unity can be produced by inadequate periods of equilibration for the tissue with the antagonist if drug-receptor interaction and not diffusion is the rate-limiting step (369). The theoretical Schild regressions can be calculated by (369):

$$\log(dr_t - 1) = \log[B] - \log K_B$$
$$+ \log\left[\frac{1 - (\exp(-k_2([B]/K_B + 1)t))}{1 + ([B]/K_B)(\exp(-k_2([B]/K_B + 1)t))} \right] \quad (42)$$

where dr_t is the dose ratio at time t, k_2 is the rate of offset of antagonist from the receptor, and K_B is assumed to be k_2/k_1 (k_1 being the rate of onset of antagonist for receptors). Thus, over inadequate equilibration times, the fractional antagonist receptor occupancy (as compared to that at equilibrium) will be greater for higher concentrations and less for lower concentrations (near the pK_B) and a nonlinear Schild regression with portions of slope >1 is predicted. As is evident in Eq. 42, the effect is time-dependent as well as antagonist-concentration dependent thus the concentrations of antagonist over which the slope is greater than unity varies with equilibration time. The potency of antagonists has long been known to be dependent upon equilibration time (230, 247, 369, 506, 525, 564) and demonstration of time-independent pK_B estimates clearly is a prerequisite to the use of antagonists for drug and drug-receptor classification. Other experimental conditions which could produce steep slopes for Schild regressions could involve antagonist induced tissue depression if these effects are

more prominent at higher rather than lower concentrations of antagonist.

C. SLOPE = 1, BUT SPURIOUS pK_B. If diffusion and not drug receptor interaction is the rate-limiting step in an isolated tissue, then inadequate equilibration times will not affect the slope of the Schild regression but rather will cause it to be shifted to the right of the true Schild regression. The degree of shift is inversely proportional to k_{out} (the rate of diffusion of the antagonist out of the diffusion barrier) and the equilibration time. The equation to describe these effects is (369):

$$\log(dr_t - 1) = \log[B] - \log K_B$$
$$+ \log(1 - \exp(-k_{out}t)) \quad (43)$$

where dr_t is the dose ratio at time t. Note how in Eq. 43 there is no term containing both t and $[B]$ therefore no aberration of slope should occur.

Schild regressions may be shifted to the right (with a slope of unity) by injudicious overuse of uptake inhibitors if the uptake inhibitors possess affinity for the drug receptors. For example, use of amitriptyline as an inhibitor of neuronal uptake in the rat anococcygeus muscle produces an increase in the slope of the Schild regression to phentolamine from 0.5, in the absence of uptake inhibition, to 1.0 after inhibition of uptake. However, the regression in the presence of amitriptyline, although linear with a slope not significantly different from unity, is shifted to the right of the correct one by a factor of 50 and yields a spurious pK_B (380). This is because of the significant α-adrenoceptor blocking properties of amitriptyline (380, 415). The Schild regression for a tissue possessing an agonist uptake process which is partially inhibited by an uptake inhibitor with receptor-blocking properties can be calculated by an equation similar to Furchgott's (380):

$$[A_a] = [A_b]_0\left(1 + \frac{[B]}{K_B} + \frac{[I]}{K_I} \cdot \frac{1}{\phi}\right)$$

$$\times \left[1 + \frac{\dfrac{U_m}{k \cdot K_{AU}}}{1 + \dfrac{[A_b]_0}{K_{AU}}\left(1 + \dfrac{[B]}{K_B} + \dfrac{[I]}{K_I} \cdot \dfrac{1}{\phi}\right) + \dfrac{[I]}{K_I}}\right] \quad (44)$$

where I is the uptake inhibitor, K_I the equilibrium dissociation constant of the inhibitor for the site of uptake, and ϕ the ratio of the equilibrium dissociation constants of the inhibitor for the receptor (K_{IR}) and K_I ($\phi = K_{IR}/K_I$). The other parameters are as for Eq. 41. Theoretical calculations show that the ratio of equilibrium dissociation constants of the uptake inhibitor for the receptor and site of uptake should be 20 or greater to prevent significant error in the estimation of a pK_B. Experimental results indicate that 12.5 is insufficient (380).

Finally, the corroborative nature of the Schild regression slope should be stressed in that a linear Schild regression with a slope of unity is consistent with but not proof of simple competitive antagonism of a homogeneous population of receptors. For example, the physiological antagonism of carbachol induced contractions of guinea pig trachea by l-isoproterenol produces dose ratios which yield a linear Schild regression with a slope of unity (374).

Considering the variety of ways in which a single estimate of the pA_2 can be in error with respect to the true pK_B, receptor classification on the basis of pA_2 values theoretically is unsound. Rather, a pK_B value with an adequate estimation of the slope of the Schild regression is much more preferable since the slope gives a measure of the confidence with which the intercept can be equated to the equilibrium dissociation constant of the antagonist for the receptor.

D. THE "HEMI-EQUILIBRIUM" STATE. There are kinetic conditions under which competitive antagonists shift agonist concentration-response curves to the right but also depress the maximal responses. This is most commonly encountered with persistent (low rate of offset) antagonists and low efficacy agonists. Described by Paton and Waud as a "hemi-equilibrium" state between agonist, antagonist and receptors (506A, 507), the equilibrium of the antagonist is not changed by the presence of the agonist and the agonist equilibrates with only a portion of the total receptor population. Under these conditions, the antagonist behaves as an essentially irreversible blocker and produces insurmountable antagonism. The degree of depression of the maximal response for any given dose ratio is dependent upon the intrinsic efficacy of the agonist. Figure 12 shows the depression of concentration-response curves of guinea pig ileal longitudinal smooth muscle strips to n-octyltrimethylammonium by hyoscine (527A). An estimate of the K_B of the antagonist can be made with the following equation (507):

$$\frac{1}{[A]} = \frac{1}{K_A} \cdot \frac{\rho}{(1 - \rho)} + \frac{1}{(1 - \rho)} \cdot \frac{1}{[A']} \quad (45)$$

where $[A]$ and $[A']$ refer to equiactive concentrations of agonist in the presence and absence of antagonist (B), respectively, and K_A the equilibrium dissociation constant of the agonist. Therefore, a double reciprocal regression of $1/[A]$ upon $1/[A']$ should yield a straight line with a positive intercept. The K_B then can be calculated by:

$$K_B = \frac{[B]}{(\text{slope} - 1)} \quad (46)$$

Using Eq. 45, Rang (527A) calculated the K_B for hyoscine with three alkyl-trimethylammonium compounds and found agreement with independent estimates by hyoscine antagonism of methylfurmethide.

This method is most accurate when the dissociation rate constant of the antagonist is much lower than that of agonist. As the rate constant of the antagonist in-
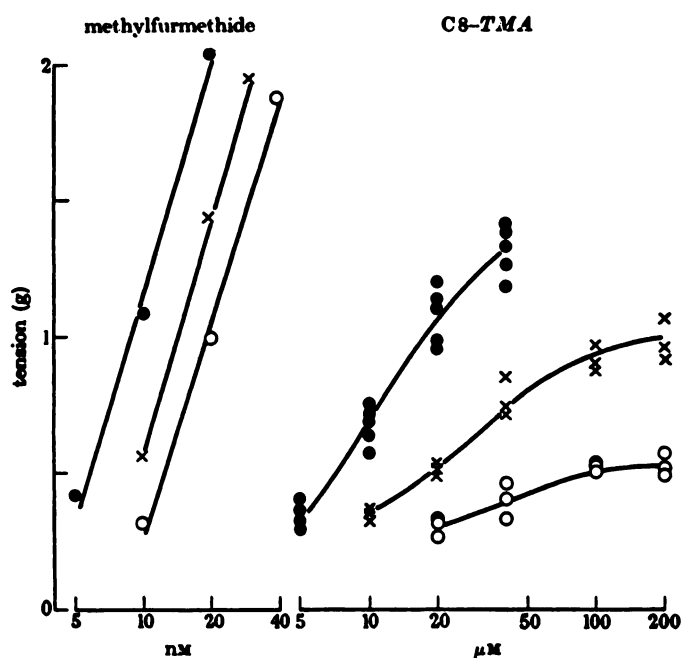
FIG. 12. Antagonism of responses of guinea pig ileal longitudinal smooth muscle to methylfurmethide and C8-tetramethylammonium. Responses in the absence (●) and presence of hyoscine 0.16 nM (×) and 0.3 nM (O). Data points for methylfurmethide are means of six responses; C3-TMA individual responses. From Rang (527A) with permission.

creases, relative to that of the agonist, simple competitive kinetics would be expected (506A).

E. RECEPTOR HETEROGENEITY AND ESTIMATIONS OF THE pK$_B$. The Schild equation predicts a linear regression with a slope of unity when a simple competitive antagonist competitively binds to a homogeneous population of receptors to chemically titrate the number of free receptors for agonist binding. The resulting pK$_B$ under these circumstances is a constant which reflects only antagonist receptor interaction and is independent of the agonist used. However, in practice, there are isolated tissues where agonist dependent Schild regressions have been obtained and under conditions which preclude consideration of agonist uptake processes (233, 234).

Figure 13A gives data from Furchgott (235) showing linear Schild regressions with slopes of unity which yield agonist dependent pK$_B$ values for propranolol in guinea pig trachea. This is a striking finding since on the surface it suggests three separate β-adrenoceptors in this tissue corresponding to the three distinct pK$_B$ values for propranolol. However, theoretical models, based on occupation theory, suggest an alternative hypothesis which explains the data in terms of a mixture of two receptor types with varying affinity for propranolol and the three agonists. The apparent pK$_B$ values would then be an amalgam of the equilibrium dissociation constants of propranolol for β$_1$- and β$_2$-adrenoceptors. The bias in terms of how much β$_1$- and how much β$_2$-adrenoceptor character this apparent pK$_B$ will have depends upon the

relative amounts of stimuli the various agonists generate from each receptor type. The apparent pK$_B$ values outwardly satisfy the requirements of true equilibrium dissociation constants for homogeneous receptors yet are artifacts of the Schild method.

This problem was first modelled by Furchgott who calculated responses from two receptors in terms of classical occupation theory assuming that the stimuli from each receptor type was additive. Response was taken to be a rectangular hyperbolic function of total stimulus (general logistic Eq. 24 where β = 1 and n = 2). In the calculations, Furchgott assumed that the agonist had equal intrinsic efficacy for the two receptor types but that both the agonist and antagonist had different affinities for the two sites. A biphasic Schild plot was calculated as shown in figure 13B. Of note here are the linear portions of the regression illustrating the potential for the observation of apparently simple kinetics (linear Schild regression with a slope of unity) in a complex system. In terms of this model, the factor which determines whether or not the Schild regression in a tissue with a heterogeneous receptor population has a slope of unity or less than unity is the concentration range of antagonist over which the analysis is carried out.

A useful model by Lemoine and Kaumann (417), which assumes a receptor reserve for both agonists, has been used recently to calculate theoretical Schild regressions in two receptor systems. The regressions were calculated by (417):

$$\log(dr - 1) = \log[B]$$
$$- \log\left\{\frac{(\sigma_Q K_{BQ} + \sigma_R K_{BR})[B] + K_{BQ}K_{BR}}{[B] + \sigma_R K_{BQ} + \sigma_Q K_{BR}}\right\} \quad (47)$$

where σ$_Q$ and σ$_R$ referred to the fractional stimuli elicited by the agonist from receptor types Q and R, respectively, and K$_{BQ}$ and K$_{BR}$ referred to the respective equilibrium dissociation constants of the antagonist for each receptor subtype. Figure 14 illustrates clearly the linear character of portions of Schild regressions calculated for tissues with heterogeneous receptor populations. The calculated regression provided an acceptable fit to data from guinea pig trachea (417).

Just as different Schild regressions can be obtained for one antagonist in a tissue bearing a heterogeneous receptor population with different agonists, so too could different Schild regressions be obtained for the same agonist-antagonist pair in different tissues if those tissues have different relative proportions of two receptor types. Assuming a population of tissues with two receptor subtypes, R$_1$ and R$_2$, the stimulus from R$_1$ produced by an agonist [A] in the presence of an antagonist [B] would be:

$$S_1 = \frac{\varepsilon_1 \cdot [R_1]}{1 + \frac{K_1}{[A]}\left(1 + \frac{[B]}{K_{B1}}\right)}. \quad (48)$$
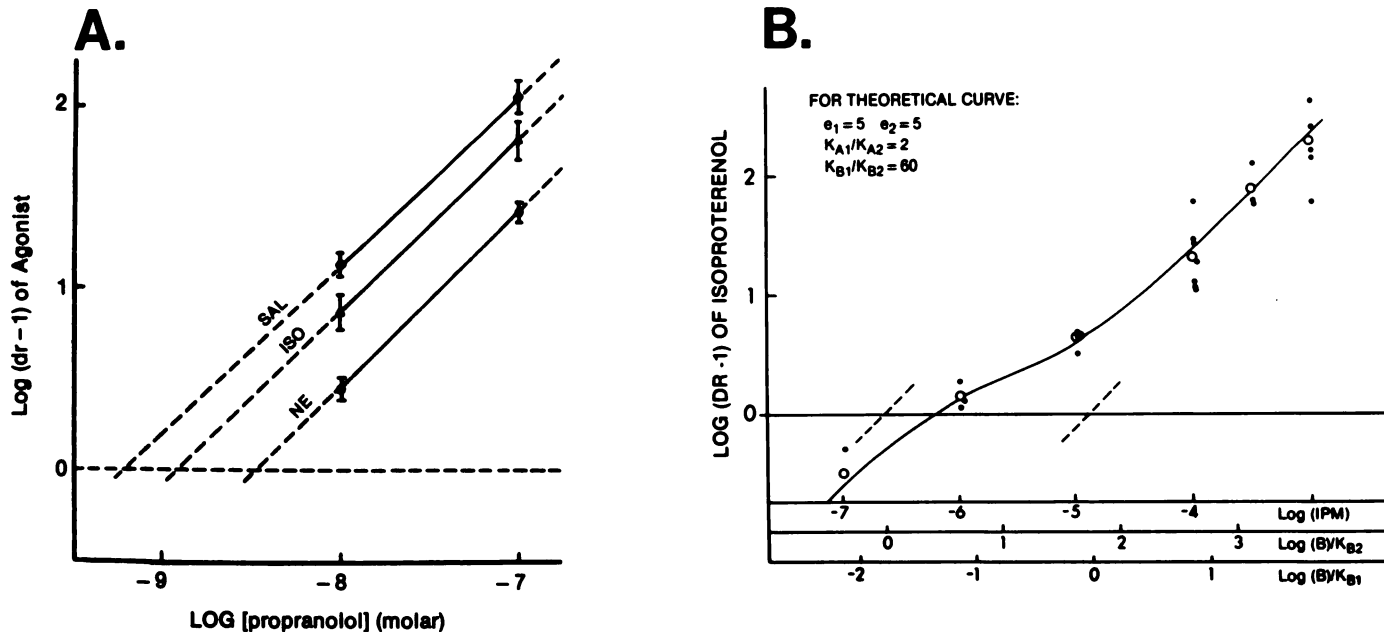
FIG. 13. Schild regressions in tissues with mixed receptor populations. A. Schild regressions in guinea pig trachea. Ordinates: Logarithms of equiactive dose-ratios-1. Abscissae: Logarithms of molar concentrations of propranolol. Antagonism of responses to salbutamol (□, SAL, $n = 5$), isoproterenol (△, ISO, $n = 2$) and norepinephrine (●, NE, $n = 4$). Bars represent S.E.M. Reprinted with permission from Furchgott (235). B. ⤺ Theoretical Schild regressions calculated for data points for isopropylmethoxamine (IPM) antagonism of responses to isoproterenol (ISO) in guinea pig trachea. Ordinates as for part A. Abscissae: Logarithms of molar concentrations of IPM; logarithms of molar concentrations of IPM as fractions of a calculated K_B for β_2-adrenoceptors and again as fractions of the K_B for β_1-adrenoceptors. Mean (O) and individual (●) data shown. Curved line calculated from model described by Furchgott (236) assuming $K_{A1}/K_{A2} = 2$ (isoproterenol has 2 times the affinity for β_2- as β_1-adrenoceptors) and $e_1 = e_2 = 5$. The two dashed lines are what would be expected if the tissue contained purely β_2-adrenoceptors (pK$_B$ = 6.63) or purely β_1-adrenoceptors (pK$_B$ = 4.85). Curved line calculated from model assuming an equal concentration of both receptor types. Reprinted with permission from Furchgott (236).

where K_1 and K_{B1} refer to the equilibrium dissociation constants of the agonist and antagonist for the receptor, respectively. Likewise for the stimulus from R_2:

$$S_2 = \frac{\varepsilon_2 \cdot [R_2]}{1 + \dfrac{K_2}{[A]}\left(1 + \dfrac{[B]}{K_{B2}}\right)}. \qquad (49)$$

Using these equations, Schild regressions for systems with various relative proportions of $[R_1]$ and $[R_2]$ were calculated. A modification of the additive stimulus assumption used by Furchgott (236) and Lemoine and Kaumann (417) was introduced into this calculation to allow for unequal coupling of receptor populations. Thus, secondary stimuli S_1' and S_2' products of saturable functions of the primary stimuli, were considered additive. This is formally identical to the subsequent stimuli hypothesis outlined by Mackay (434) where the secondary stimulus (S_Ω) is related to the primary stimulus (S_a) by:

$$S_\Omega = \frac{S_a}{aS_a + b} \qquad (50)$$

where a and b are chain constants.

In these calculations, the secondary stimulus $S_1' = f_1$ (S_1) and $S_2' = f_2$ (S_2) where f_1 and f_2 need not be identical. The function chosen for these calculations was the gen-

eral logistic with fitting constants β_1 and β_2 (Eq. 24). Note that Eq. 50 is a special case of the general logistic function ($n = a = 1$, $b = \beta$). The response was considered to be a general logistic function (fitting constant β_3) of the arithmetic sum of S_1' and S_2'. It should be noted that these mathematical expedients do not affect the outcome of predictions for the two receptor models on Schild regressions but simply build in the concepts of a nonlinear function between receptor occupancy and tissue response and unequal coupling of the two receptor populations. There is no reason a priori for two receptor populations to be coupled with equal efficiency such that there is a direct correspondence between relative numbers of the two receptor populations and the relative stimulus derived from each.

With this model, theoretical Schild regressions for an agonist with equal efficacy and affinity for R_1 and R_2 and an antagonist with 100 times the affinity for R_1 as R_2 were calculated in a range of tissues with varying relative proportions of R_1 and R_2 ($[R_1]/[R_2] = 1000$ to 0.01). For illustrative purposes, equal coupling of R_1 and R_2 for the response mechanism was assumed for this example but it should be stressed that the relationship between the observed pA$_2$ and the ratio of $[R_1]/[R_2]$ is a direct result of this assumption and therefore is not meaningful. However, there is value in this calculation since it shows the relationship between the location of the Schild regression
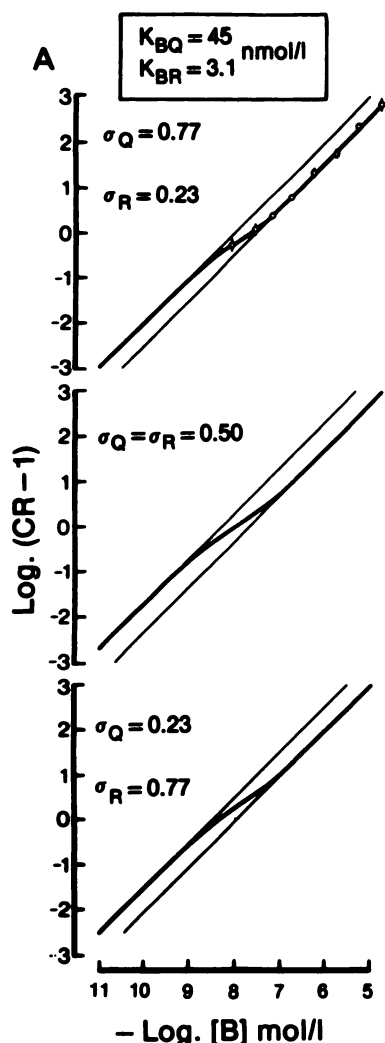
FIG. 14. Effects on Schild regressions of variable fractional stimulus from a heterogeneous receptor population. Ordinates and abscissae as for figure 13A. Data points from (+)bupranolol antagonism of guinea pig tracheal responses to (−)norepinephrine. Non-linear Schild regressions calculated from Eq. 47. Top panel: Data points fitted by Eq. 47 assuming that 77% of the norepinephrine stimulus was due to a receptor Q with low affinity for (+)bupranolol (K_{BQ} = 45 nM) and 23% to a receptor R with a high affinity for (+)bupranolol (K_{BR} = 3.1 nM). Middle panel: Schild regression for agonist which produced equal stimuli from receptors Q and R. Lower panel: Schild regression for agonist which produced 23% of stimulus from receptor Q and 77% from receptor R. Reproduced by permission from Lemoine and Kaumann (417).
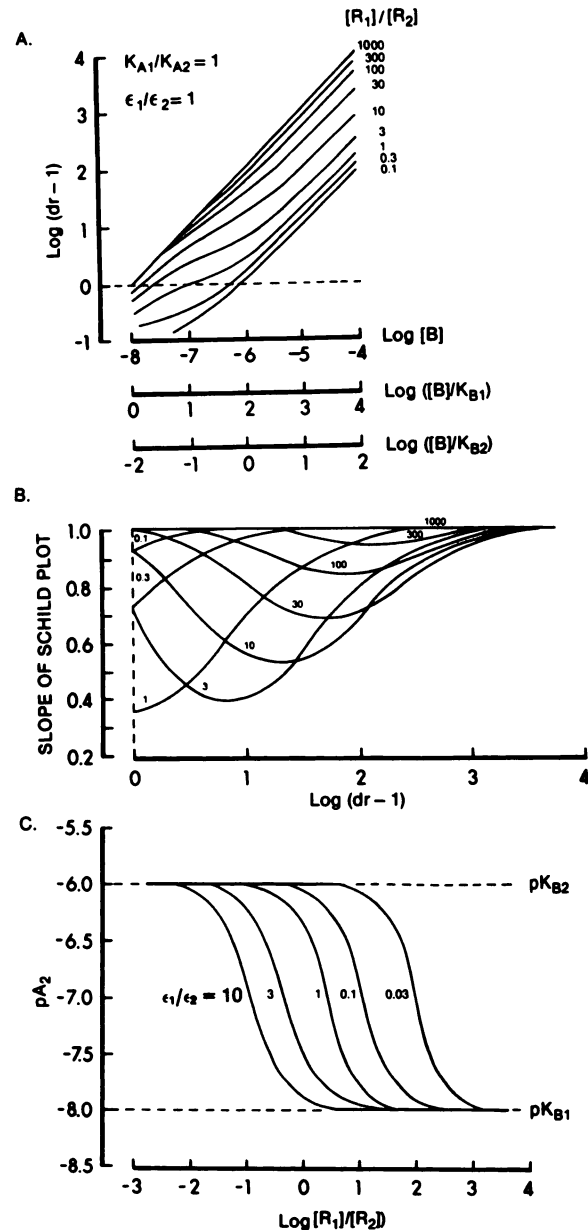
FIG. 15. Theoretical Schild regressions for heterogeneous receptor populations. A. Ordinates and abscissae as for figure 13A. Schild regression calculated for an antagonist with 100 times the affinity for receptor 1 over receptor 2 (K_{B1}/K_{B2} = 0.01) producing antagonism of responses to an agonist with equal affinity and efficacy for both receptors. Different regressions are from a range of tissues with varying fractional predominance of one receptor over the other ($[R_1]/[R_2]$ = 1000 to 0.01). It is assumed that the receptors are coupled equally in all tissues ($\beta_1 = \beta_2$). B. Slopes of the Schild regressions shown in part A. Ordinates: Slopes of the Schild regressions. Abscissae: Logarithms of the dose ratios −1. Slopes shown for varying fractional predominance of receptor populations ($[R_1]/[R_2]$ = 1000 to 0.01). C. Calculated pA$_2$ values for Schild regressions from tissues with heterogeneous receptor populations. Ordinates: Observed pA$_2$. Abscissae: Logarithm of relative proportions of receptors types 1 and 2 in a given tissue. Calculations made for agonists with varying selectivities for one of the receptor types.

and the slope as it relates to receptor heterogeneity. This relationship is not dependent upon the relative affinity and efficacy of the drugs for the receptor types or the relative efficiency of coupling of the receptor types. Figure 15A shows the effects of changing relative receptor number on the Schild regression. The designated K_B for R_1 is 10 nM and for R_2 is 1 μM (K_{B2}/K_{B1} = 100). At the extremes of nearly homogeneous populations of R_1 ($[R_1]/[R_2]$ = 1000) or R_2 ($[R_1]/[R_2]$ = 0.01), the regressions are linear with slopes of unity and yield the correct pK_B for the respective receptor types. However, at intermediate mixtures of receptor types, the regressions are

displaced with a linear portion generally at log (dr − 1) >1 and a nonlinear segment near the pA$_2$. Figure 15B shows the slopes of the regressions at various ratios of

receptor types. The calculations indicate that while slopes less than unity could be expected at dose ratios from 2 to 30, larger dose ratios yield regressions in which nonlinearity would be difficult to detect. This is in agreement with numerous published Schild regressions in tissues with heterogeneous receptor populations which have slopes of unity (235,485–487,554,555,558). In terms of satisfying the conditions for simple competitive antagonism, a linear Schild regression with a slope of unity would constitute evidence for the intercept to be considered the pK_B. However, as the foregoing analysis indicates, the intercept would reflect an artifactual pK_B value as a weighted average the pK_B for two receptor types in a tissue containing two types of receptor. Figure 15C shows the influence of relative efficacy of the agonist for two receptors on the observed pK_B of Schild regressions in tissues with varying relative amounts of the two receptor types ($K_{B2}/K_{B1} = 100$). The actual value of the pK_B equated to a given ratio of $[R_1]/[R_2]$ depends upon the coupling of the two receptor populations to the tissue response machinery. The calculations in Figure 15C assumed equal coupling.

There are an increasing number of tissues found to have heterogeneous receptor subpopulations as measured by binding studies (97,305,318,457–459,466,471,556). In some cases the receptor heterogeneity found in binding studies can be corroborated in pharmacological studies with isolated tissues, but in others the heterogeneous receptor subpopulations found by binding are not reflected in tissue responses. For example, in the rabbit uterus binding studies showed that α_1- and α_2-adrenoceptors were coexistent but responses appeared to be mediated only by the α_1-adrenoceptors (318).

Pharmacological experiments in isolated tissues indicate a heterogeneous population of β-adrenoceptors in guinea pig trachea (233–235,240,241,485), cat heart (117,118), and rat adipose tissue (298). Heterogeneous populations of postsynaptic α-adrenoceptors may be present in dog basilar artery (558), rat perfused hind quarters (657), dog saphenous vein (148,607), and rat tail artery (312).

The foregoing theoretical analyses suggest that a spectrum of apparently linear Schild regressions could be expected from a range of tissues with varying relative quantities of two discrete receptor types which subserve the same type of response. Alternatively, if accessory binding sites around the active site of the receptor are required for antagonist binding, then a continuous spectrum of binding constants might be expected with differing lipid constituents of biological membrane which could, in turn, affect the conformation of the receptor protein. This scheme of "multiple environment" as opposed to "multiple receptors" has been proposed to explain the profusion of opiate receptors (465). This hypothesis would predict a grey area of binding constants between two extremes which would reflect a range of pK_B values for a spectrum of receptor configurations. This concept differs from the discrete two-receptor idea which would predict chimerical or artifactual pK_B values within this grey area.

2. Other Methods to Calculate pK_B. The Schild method is by far the most ubiquitous in pharmacology for the measurement of equilibrium dissociation constants of competitive antagonists (374). Useful guidelines for statistical manipulations with this method have been given by MacKay (432) and Tallarida and coworkers (613).

There are other methods available for the calculation of pK_B values. A method utilizing the "L transformation" has been proposed by MacKay and Wheeler (435). A method with theoretical advantages over the Schild regression is one utilizing the "Clark plot" (603,604). Brazenor and Angus (92) have shown that estimates of the pK_B using the Clark plot and Schild regression can differ significantly. A method based on an equation by Ariens termed the "dynamic approach" has been proposed by Amidon and Buckner (10). In this method, a fixed ratio Q of agonist $[A']$ and antagonist $[B]$ are physically mixed and the mixture used to obtain a concentration-response curve in a tissue. This curve is compared to a control curve to the agonist alone $[A]$ and equiactive agonist concentrations are equated with the following relationship:

$$\frac{1}{[A]} = \frac{Q}{K_B} + \frac{1}{[A']} \tag{51}$$

Thus, the reciprocal of the intercept of a double reciprocal plot of $1/[A]$ versus $1/[A'] \times Q$ yields an estimate of the K_B.

VI. Operational Concepts in Receptor Classification

The basis of drug and drug receptor classification is the unequivocal measurement and comparison of parameters which depend only upon drug and receptor interaction. The philosophical step from such data to the postulate of a new receptor type or agonist/antagonist selectivity is, in the end, still subjective (432). The soundest approach would appear to be to eliminate as many obfuscating factors as possible that make organ selectivity appear to be due to receptor selectivity (373,376) and quantitatively compare, with appropriate statistical procedures, the drug receptor parameters. A popular guideline set forth by Furchgott (232) for distinguishing receptors with competitive antagonists is the postulate that a threefold difference in K_B values constitutes evidence for differences in receptors. Another possibility is the use of analysis of covariance of regression lines (591) to compare linear regression lines with respect to slope and elevation (385). Thus, all of the data in Schild regressions could be utilized instead of only the intercept. This procedure lends itself to any method that utilizes linear regressions.

VII. Relevance to New Drugs

Isolated tissues are widely used in industry for the finding of new therapeutic entities for the treatment of diseases in man. A valuable byproduct of this process is the discovery of selective drugs for the classification of drug receptors (445,575–577). An effective, if labor intensive, approach towards this end is the random screening of many chemical compounds in appropriate in vitro test systems. In this context, the term random refers to the a priori lack of rationale for the choice of chemical to be tested; this opens the doors to the finding of a novel drug (445). The methodological concepts which refer to the proper preservation of tissue viability and caveats to universal ascription of drug selectivity observed in a given tissue to all tissues have relevance to these screening procedures. However, some theoretical concepts under the vague heading of receptor theory may be helpful in the search for new drugs. Intuitively, it might be supposed that a screening program should be targeted to the finding of the most active and selective drug possible but there may be exceptions to both of these criteria.

Firstly, the most potent agonist may not be the most useful if the potency is related mainly to a high efficacy rather than a high affinity. As shown in figure 10B, drugs of high affinity but low efficacy are much more susceptible to the efficiency of receptor coupling than are drugs of high efficacy. Support for this idea can be found in the wide range of agonist activities of prenalterol, a β-adrenoceptor agonist of low efficacy in different tissues (figure 12B). Therefore, organ selectivity in vivo may be better achieved by choosing the agonist of lower efficacy.

In the early formulations of receptor theory, Clark and Raventos (134) distinguished "...the capacity to bind and the capacity to excite...." There are numerous studies that show the structure activity relationships for affinity and efficacy to be quite different (553–555,599). Figure 16 shows the separate structure-activity relationships of some drugs for α-adrenoceptors and highlights the independence of the properties of affinity and efficacy in drugs. For example, synephrine (4-OH, figure 16A) and 3-hydroxytolazoline (3-OH, figure 16B) show comparable activity in guinea pig aorta (555) and it would be predicted that these agonists would be equiactive full agonists in tissues with large receptor reserves (i.e., a potency ratio of 1.88 by Eq. 23). However, the agonist profiles of these two agonists would be very different (i.e., synephrine ⋗ 3-hydroxytolazoline) in tissues with little receptor reserve. This would be due to the differences in efficacy of the two drugs. Without knowledge of the relative efficacy of these drugs, the screening results on different tissues could be misleading. For example, if screened on tissues with large receptor reserves, the drugs would be assumed to be equiactive with a corresponding assumption that this profile would be true in vivo. Alternatively, if screened on a tissue with a low receptor reserve, the disparate agonist profiles might suggest artifactual receptor selective effects. Knowledge of both efficacy and affinity could be useful in the predictions of agonist effects in man.

Secondly, the concept of high selectivity may be overstressed as well. A screening program targeted to the finding of a drug with a single unique action presupposes the existence of a unique and convenient hitherto undetected receptor or mechanism that will subserve the desired activity. This is, of course, possible but there are elements of wishful thinking in this approach. Another possibility would be the conscious design of two properties within one molecule to produce a drug which, when interacting with organs possessing the two mechanisms with which the drug has activity, produces a selective effect. The selectivity would stem from the varying relative importance the two mechanisms may have in different organs. For example, metanephrine is an inhibitor of the extraneuronal uptake of catecholamines (108) and also is a β-adrenoceptor blocking agent (367,371). Since the former mechanism sensitizes some tissues to catecholamines and the latter mechanism produces dextral displacement of concentration-response curves to catecholamines, there is a potential for self cancellation. However, diffusional and uptake characteristics of different tissues make extraneuronal uptake of catecholamines (uptake$_2$) of varying importance. For example, inhibition of uptake$_2$ produces no significant sensitization of guinea pig atria (706) but a 5- to 30-fold sensitization of guinea pig trachea (367). Thus metaphrine produces organ selective β-blockade in these tissues; 1 mM metanephrine produces a dose ratio of 3 in guinea pig trachea and 25 in guinea pig atria.

Two activities in one molecule may be critical to the selectivity or overall activity of that molecule in vivo. For example, it is probable that the antihypertensive activity of labetalol relates to the combined α- and β-adrenoceptor blocking properties of the molecule. The weak partial agonist activity of dobutamine for α-adrenoceptors coupled with stronger β-adrenoceptor agonist activity may be critical to the selective inotropy observed with this drug in vivo (370).

The elucidation of these mechanisms may be important to the process of finding new drugs. It may be more beneficial for a medicinal chemist to know that a given molecule is selective in vivo because of a combination of activities as opposed to the presumption that it stimulates some hitherto unknown new receptor. Also, the design of drugs with two or more activities increases the chemical starting points for a medicinal chemist in the design of new molecules.

There are indications that combining two properties in one molecule may be useful. Baldwin and coworkers (35) term this the "symbiotic approach" and have used the idea to successfully produce a vasodilator-β-blocking drug. Unfortunately, the full utility of this agent could not be elucidated because of observed teratogenicity.

A. PHENYLETHYLAMINES

RELATIVE EFFICACY | pK_A

EFFICACY

AFFINITY

$CH-CH_2-N-CH_3$
OH H
R

1.0 — 6
0.5 — 5
— 4
0 — 3

Non Phenol | 4-OH | 3-OH | 3,4 diOH

B. IMIDAZOLINES

RELATIVE EFFICACY | pK_A

AFFINITY

EFFICACY

CH_2 N
R N
H

1.0 — 6
0.5 — 5
— 4
0 — 3

Non Phenol | 4-OH | 3-OH | 3,4 diOH

C.

RELATIVE EFFICACY | pK_A

EFFICACY

AFFINITY

1.2 — 9
1.0
0.8 — 8
0.6
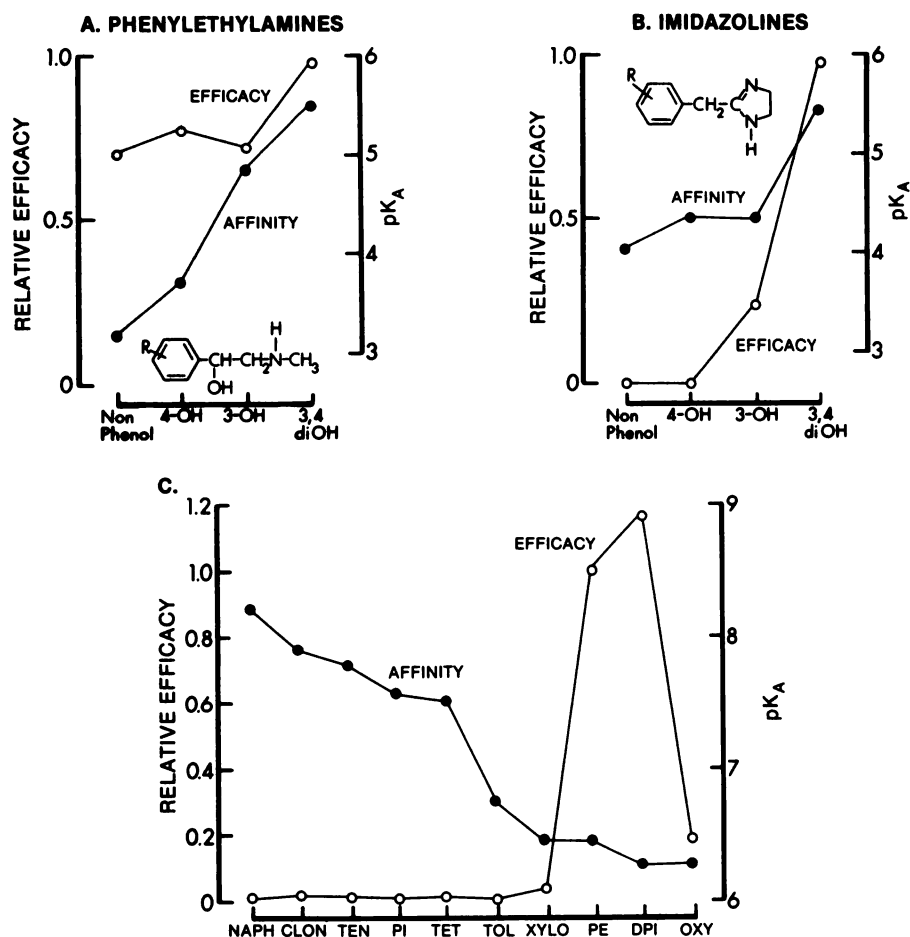0.4 — 7
0.2
0 — 6

NAPH CLON TEN PI TET TOL XYLO PE DPI OXY

FIG. 16. Structure-activity relationships for efficacy and affinity of α-adrenoceptor agonists. pK_A equals the negative logarithm of the equilibrium dissociation constant of the agonist for the receptor. Abscissae for part C: Naphazoline (NAPH), clonidine (CLON), tenaphtoxaline (TEN), 2-(phenylimino)-immidazoline (PI), tetrahydrozoline (TET), tolazoline (TOL), xylometazoline (XYLD), phenylephrine (PE), (3,4-dihydroxyphenylamino)-2-imidazoline (DPI), and oxymetazoline (OXY). Data for parts A and B (guinea pig aorta) from Ruffolo and Waddell (555) and for part C (rat aorta) from Ruffolo et al. (553), reprinted with permission.

VIII. Conclusions

This paper reviews some of the large body of knowledge concerning the process of isolating a tissue and keeping it viable and stable for a period of time sufficient for the testing of drugs. Pharmacological experience shows that very often the observed responses to drugs do not reflect drug receptor events but rather are related to the gauntlet of hazards the drugs must overcome on the way to the receptor or the complex translation of the receptor events by the tissue. Various null methods have been devised with simple kinetic models which, theoretically at least, provide parameters for drugs relating only to receptor action. If these parameters can be measured accurately and reliably they should provide the basis for the classification of drugs and receptors. Also, the collection of quantitative data describing drug affinity and efficacy should be useful for the creative design of new and better drugs for man. The superiority of isolated tissues for the quantification of agonist efficacy coupled with the economy of effort involved in the procedures and the wealth of experience available in the literature make in vitro experimentation in isolated tissues important in this process.

REFERENCES

1. AARONS, R. D. AND MOLINOFF, P. B.: Changes in the density of beta adrenergic receptors in rat lymphocytes, heart and lung after chronic treatment with propanolol. J. Pharmacol. Exp. Ther. **221:** 439–443, 1982.
2. ABERG, G., ADLER, G., AND ERICSSON, E.: The effect of age on β-adrenoceptor activity in tracheal smooth muscle. Br. J. Pharmacol. **47:** 181–182, 1973.
3. ABDULLAH, F. M., FRENCH, A. M., AND SCOTT, N. C.: Evidence of indirect action of histamine and PEA in guinea-pig and rat atria. Br. J. Pharmacol. **76:** 247p., 1982.
4. ABBRACCHIO, M. P., CATTABENI, F., COEN, E., TORRES-HERNANDES, Y., AND OMINI, C.: β-Adrenoceptor desensitization in rat lung: Functional and biochemical aspects. Eur. J. Pharmacol. **89:** 35–42, 1983.
5. AHMED, M. I., AND NAYLOR, I. L.: The effects of α_1 and α_2 agonists and antagonists on rat splenic smooth muscle strips. Br. J. Pharmacol. **78:** 44, 1983.
6. AKUBUE, P. I.: The site of action of drugs on the isolated taenia caeci from the guinea-pig. Br. J. Pharmacol. Chemother. **27:** 347–365, 1966.
7. ALLY, A. I., AND NAKATSU, K.: Adenosine inhibition of isolated rabbit ileum and antagonism by theophylline. J. Pharmacol. Exp. Ther. **199:** 208–215, 1976.
8. ALTURA, B. M., AND ALTURA, B. T.: Heterogeneity of drug receptors in different segments of rabbit thoracic aorta. Eur. J. Pharmacol. **12:** 44–52, 1970.
9. ALTURA, B. M., AND ALTURA, B. T.: Magnesium ion and contraction of vascular smooth muscles: Relationship to some vascular diseases. Fed. Proc. **40:** 2672–2679, 1981.
10. AMIDON, G. L., AND BUCKNER, C. K.: On the use of a dynamic approach to the estimation of dissociation constants for reversible competitive antagonists. J. Pharmacol. Exp. Ther. **216:** 352–356, 1981.

11. ANDERSON, G. F., AND MARKS, B. H.: Spare cholinergic receptors in the urinary bladder. J. Pharmacol. Exp. Ther. **221:** 598–603, 1982.

12. ANDERSSON, R., HOLMBERG, S., SVEDMYR, N., AND ABERG, G.: Adrenergic α- and β-receptors in coronary vessels in man. Acta Med. Scand. **191:** 241–244, 1972.

13. ANGUS, J. A., AND BLACK, J. W.: Analysis of anomalous pK$_\beta$ values for metiamide and atropine in the isolated stomach of the mouse. Br. J. Pharmacol. **67:** 59–65, 1979.

14. ANGUS, J. A., AND BLACK, J. W.: Pharmacological assay of cardiac H$_2$-receptor blockade by amitriptyline and lysergic acid diethylamide. Circ. Res. **46:** I-64–I-69, 1980.

15. ANGUS, J. A., BLACK, J. W., AND STONE, M.: Estimation of pK$_{B2}$ values for histamine H$_2$-receptor antagonists using an *in vitro* acid secretion assay. Br. J. Pharmacol. **68:** 413–423, 1980.

16. ANGUS, J. A., BRAZENOR, R. M., AND LE DUC, M. A.: Verapamil: A selective antagonist of constrictor substances in dog coronary artery: Implications for variant angina. Clin. Exp. Pharmacol. Physiol. **6:** 15–28, 1982.

17. APRIGLIANO, O., AND HERMSMEYER, K.: *In vitro* denervation of the portal vein and caudal artery of the rat. J. Pharmacol. Exp. Ther. **198:** 568–577, 1976.

18. ARAKI, H., SU, C., AND LEE, T. J.-F.: Effect of superior cervical ganglionectomy on the sensitivity of rabbit ear artery and cerebral arteries of rabbit and cat to vasoactive agents. J. Pharmacol. Exp. Ther. **220:** 49–55, 1982.

19. ARCHER, J. A., GORDEN, P., AND ROTH, J.: Defect in insulin binding to receptors in obese man. J. Clin. Invest. **55:** 166–174, 1975.

20. ARIËNS, E. J.: Affinity and intrinsic activity in the theory of competitive inhibition. Arch. Int. Pharmacodyn. Ther. **99:** 32–49, 1954.

21. ARIËNS, E. J.: Molecular Pharmacology, vol. 1, Academic Press, New York, 1964.

22. ARIËNS, E. J.: The structure-activity relationships of *beta* adrenergic drugs and *beta* adrenergic blocking drugs. Ann. N.Y. Acad. Sci. **139:** 606–631, 1967.

23. ARIËNS, E. J.: Drug levels in the target tissue and effect. Clin. Pharmacol. Ther. **16:** 155–175, 1974.

23A. ARIËNS, E. J.: Personal communication.

24. ARIËNS, E. J., BELD, A. J., DE MIRANDA, J. F. R., AND SIMONIS, A. M.: The Pharmacon-receptor-effector concept. A basis for understanding the transmission of information in biological systems. *In* The Receptors, a Comprehensive Treatise, ed. by R. D. O'Brien, pp. 33–91, Plenum Press, New York, 1979.

25. ARIËNS, E. J., AND SIMONIS, A. M.: Receptors and receptor mechanisms. *In* Beta-Adrenoceptor Blocking Agents, ed. by P. R. Saxena and R. P. Forsyth, pp. 3–27, North-Holland Publishing Company, Amsterdam, 1976.

26. ARIËNS, E. J., AND SIMONIS, A. M.: Physiological and pharmacological aspects of adrenergic receptor classification. Biochem. Pharmacol. **32:** 1539–1545, 1983.

27. ARIËNS, E. J., VAN ROSSUM, J. M., AND KOOPMAN, P. C.: Receptor reserve and threshold phenomena. Arch. Int. Pharmacodyn. Ther. **127:** 459–478, 1960.

28. ARUNLAKSHANA, O., AND SCHILD, H. O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. **14:** 48–58, 1959.

29. ASANO, M., AND HIDAKA, H.: Alterations in pharmacological receptor activities of rabbit arteries by sulfhydryl reagents. Jap. J. Pharmacol. **33:** 227–240, 1983.

30. ASH, A. S. F., AND SCHILD, H. O.: Receptors mediating some actions of histamine. Br. J. Pharmacol. Chemother. **27:** 427–439, 1966.

31. ATLAS, D., STEER, M. L., AND LEVITZKI, A.: Affinity label for β-adrenergic receptor in turkey erythrocytes. Proc. Natl. Acad. Sci. **73:** 1921–1925, 1976.

32. BAKER, S. P.: Effect of thyroid status on β-adrenoreceptors and muscarinic receptors in the rat lung. J. Auton. Pharmacol. **1:** 269–277, 1981.

33. BAKER, S. P., AND KATOVICH, M. J.: Chronic propanolol treatment decreases cardiac β-adrenoceptors in spontaneously hypertensive rats. Eur. J. Pharmacol. **78:** 479–482, 1982.

34. BAKER, S. P., AND PITHA, J.: Irreversible blockade of *beta* adrenoreceptors and their recovery in the rat heart and lung *in vivo*. J. Pharmacol. Exp. Ther. **220:** 247–251, 1982.

35. BALDWIN, J. J., LUMMA, W. C., JR., LUNDELL, G. F., PONTICELLO, G. S., RAAB, A. W., ENGELHARDT, E. L., HIRSCHMANN, R., SWEET, C. S., AND SCRIABINE, A.: Symbiotic approach to drug design: Antihypertensive β-adrenergic blocking agents. J. Med. Chem. **22:** 1284–1290, 1979.

36. BANERJEE, S. P., AND KUNG, L. S.: β-Adrenergic receptors in rat heart: Effects of thyroidectomy. Eur. J. Pharmacol. **43:** 207–208, 1977.

37. BAR, R. S., GORDEN, P., ROTH, J., KAHN, C. R., AND DE MEYTS, P.: Fluctuations in the affinity and concentration of insulin receptors on circulating monocytes of obese patients. Effects of starvation, refeeding, and dieting. J. Clin. Invest. **58:** 1123–1135, 1976.

38. BARABÉ, J., DROUIN, J.-N., REGOLI, D., AND PARK, W. K.: Receptors for bradykinin in intestinal and uterine smooth muscle. Can. J. Physiol. Pharmacol. **55:** 1270–1285, 1977.

39. BARABÉ, J., MARCEAU, F., THÉRIAULT, B., DROUIN, J.-N., AND REGOLI, D.: Cardiovascular actions of kinins in the rabbit. Can. J. Physiol. Pharmacol. **57:** 78–91, 1979.

40. BARABÉ, J., PARK, W. K., AND REGOLI, D.: Application of drug receptor theories to the analysis of the myotropic effects of bradykinin. Can. J. Physiol. Pharmacol. **53:** 345–353, 1975.

41. BARGER, G., AND DALE, H. H.: Chemical structure and sympathomimetic action of amines. J. Physiol. (Lond.) **51:** 19–59, 1910.

42. BARKER, K. A., HARPER, B., AND HUGHES, I. E.: Possible subdivisions among α-adrenoceptors in various isolated tissues. J. Pharm. Pharmacol. **29:** 129–134, 1977.

43. BARKER, L. A., AND HOUGH, L. B.: Selectivity of 4-methylhistamine at H$_1$- and H$_2$-receptors in the guinea-pig isolated ileum. Br. J. Pharmacol. **80:** 65–71, 1983.

44. BARLOW, R. B., SCOTT, K. A., AND STEPHENSON, R. P.: An attempt to study the effects of chemical structure on the affinity and efficacy of compounds related to acetycholine. Br. J. Pharmacol. **21:** 509–522, 1967.

45. BARNES, P. J., SKOOGH, B.-E., NADEL, J. A., AND ROBERTS, J. M.: Postsynaptic *alpha$_2$*-adrenoceptors predominate over *alpha$_1$*-adrenoceptors in canine tracheal smooth muscle and mediate neuronal and hormonal *alpha*-adrenergic contraction. Mol. Pharmacol. **23:** 570–575, 1983.

46. BARON, G. D., SPEDEN, R. N., AND BOHR, D. F.: Beta-adrenergic receptors in coronary and skeletal muscle arteries. Am. J. Physiol. **223:** 878–881, 1972.

47. BARSOUM, G. S., AND GADDUM, J. H.: The pharmacological estimation of adenosine and histamine in blood. J. Physiol. (Lond.) **85:** 1–14, 1935.

48. BARSTAD, J. A. B.: Cholinesterase inhibition and the effect of anticholinesterases on indirectly evoked single and tetanic muscle contractions in the phrenic nerve-diaphragm preparation from the rat. Arch. Int. Pharmacodyn. Ther. **128:** 143–168, 1960.

49. BARTLETT, V., STEWART, R. R., AND NAKATSU, K.: Evidence for two adenine derivative receptors in rat ileum which are not involved in the noradrenergic, noncholinergic response. Can. J. Physiol. Pharmacol. **57:** 1130–1137, 1979.

50. BAYER, B.-L., MENTZ, P., AND FÖRSTER, W.: Characterization of the adrenoceptors in coronary arteries of pigs. Eur. J. Pharmacol. **29:** 58–65, 1974.

51. BECKER, E., INGEBRETSEN, W. R., JR., AND MAYER, S. E.: Electrophysiological responses of cardiac muscle to isoproterenol covalently linked to glass beads. Circ. Res. **41:** 653–659, 1977.

52. BELD, A. S., AND ARIËNS, A. J.: Stereospecific binding as a tool in attempts to localize and isolated muscarinic receptors. II. Binding of (+)-benzetimide, (−)-benzetimide and atropine to a fraction from bovine tracheal smooth muscle and to bovine caudate nucleus. Eur. J. Pharmacol. **25:** 203–209, 1974.

53. BELD, A. J., VAN DEN HOVEN, S., WOUTERSE, A. C., AND ZEGERS, M. A. P.: Are muscarinic receptors in the central and peripheral nervous system different? Eur. J. Pharmacol. **30:** 360–363, 1975.

54. BELLEAU, B.: A molecular theory of drug action based on induced conformational perturbations of receptors. J. Med. Chem. **7:** 776–784, 1964.

55. BENFEY, B. G., YONG, M. S., BELLEAU, B., AND MELCHIORRE, C.: Cardiac muscarinic blocking and atropinic blocking effects of a tetramine disulfide with α-adrenoceptor blocking activity. Can. J. Physiol. Pharmacol. **57:** 41–47, 1979.

56. BENNETT, V., O'KEEFE, E., AND CUATRECASAS, P.: Mechanisms of action of cholera toxin and the mobile receptor theory of hormone receptor-adenylate cyclase interactions. Proc. Natl. Acad. Sci. **72:** 33–37, 1975.

57. BENTLEY, P., AND DICKER, S. E.: Effects of transient changes of acidity on the isolated rat's uterus, with reference to the assay of oxytocic activity. Br. J. Pharmacol. **10:** 424–428, 1955.

58. BERGMAN, R. N., AND HECHTER, O.: Neurohypophyseal hormone-responsive renal adenylate cyclase. J. Biol. Chem. **253:** 3238–3250, 1978.

59. BERKSON, J.: Estimating the bio-assay with quantal response. J. Am. Stat. Soc. **48:** 565–597, 1953.

60. BERTACCINI, G., AND CORUZZI, G.: Effect of some new histamine H$_2$-receptor antagonists on the guinea-pig papillary muscle. Naunyn-Schmeideberg's Arch. Pharmacol. **317:** 225–227, 1981.

61. BERTACCINI, G., ZAPPIA, L., BEZZI, E., AND POTENZONI, D.: Histamine receptors in the human ureter. Pharmacol. Res. Commun. **15:** 157–166, 1983.

62. BERTRAM, J. F., GOLDIE, R. G., PAPADIMITRIOU, J. M., AND PATERSON, J. W.: Correlations between pharmacological responses and structure of human lung parenchyma strips. Br. J. Pharmacol. **80:** 107–114, 1983.

63. BÉRUBÉ, A., MARCEAU, F., DROUIN, J. N., RIOUX, F., AND REGOLI, D.: The rabbit mesenteric vein: A specific bioassay for substance P. Can. J. Physiol. Pharmacol. **56:** 603–609, 1978.

64. BESSE, J. C., AND FURCHGOTT, R. F.: Dissociation constants and relative efficacies of agonists acting on alpha adrenergic receptors in rabbit aorta. J. Pharmacol. Exp. Ther. **197:** 66–78, 1976.

65. BEVAN, J. A., BEVAN, R. D., OSHER, J. V., AND SU, C.: Distribution of components of ^{3}H-noradrenaline uptake in the wall of the rabbit aorta. Eur. J. Pharmacol. **19:** 239–245, 1972.

66. BEVAN, J. A., BEVAN, R. D., PURDY, R. E., ROBINSON, C. P., SU, C., AND WATERSON, J. G.: Comparison on adrenergic mechanisms in an elastic and a muscular artery of the rabbit. Circ. Res. **30:** 541–548, 1972.

67. BEVAN, J. A., AND TÖRÖK, J.: Movement of norepinephrine through the media of rabbit aorta. Circ. Res. **27:** 325–331, 1970.

68. BEVAN, J. A., AND VERITY, M. N.: Sympathetic nerve-free vascular muscle. J. Pharmacol. Exp. Ther. **157:** 117–124, 1967.

69. BEVAN, J. A., AND WATERSON, J. G.: Biphasic constrictor response of the rabbit ear artery. Circ. Res. 28: 655–661, 1971.

70. BICKERTON, R. K.: The response of isolated strips of cat spleen to sympathomimetic drugs and their antagonists. J. Pharmacol. Exp. Ther. 142: 99–110, 1963.

71. BIETH, N., ROUOT, B., SCHWARTA, J., AND VELLY, J.: Comparison of pharmacological and binding assays for ten β-adrenoceptor blocking agents and two β-adrenoceptor agonists. Br. J. Pharmacol. 68: 563–569, 1980.

72. BIRMINGHAM, A. T., PATERSON, G., AND WOJCICKI, J.: A comparison of the sensitivities of innervated and denervated rat vasa deferentia to agonist drugs. Br. J. Pharmacol. 39: 748–754, 1970.

73. BLACK, J. W.: Receptors in the future. Postgrad. Med. J. 57: 110–112, 1981.

74. BLACK, J. W.: Receptor function and control. In Catecholamines in the Non-Ischaemic and Ischaemic Myocardium, ed. by R. Riemersma and W. Oliver, pp. 3–12, Elsevier/North-Holland Biomedical Press, Amsterdam, 1982.

75. BLACK, J. W., DUNCAN, W. A. M., DURANT, C. J., GANELLIN, C. R., AND PARSONS, E. M.: Definition and antagonism of histamine H$_2$-receptors. Nature (Lond.) 236: 385–390, 1972.

76. BLACK, J. W., JENKINSON, D. H., AND KENAKIN, T. P.: Antagonism of an indirectly acting agonist: Block by propranolol and sotalol of the action of tyramine on rat heart. Eur. J. Pharmacol. 65: 1–10, 1981.

77. BLACK, J. W., AND LEFF, P.: Operational models of pharmacological agonism. Proc. R. Soc. Lond. Biol. Sci. 220: 141–162, 1983.

78. BLACK, J., TURNER, A., AND SHAW, J.: α-Adrenoceptors in human peripheral lung. Eur. J. Pharmacol. 72: 83–86, 1981.

79. BLINKS, J. R.: Convenient apparatus for recording contractions of isolated heart muscle. J. Appl. Physiol. 20: 755–757, 1965.

80. BLINKS, J. R.: Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. J. Pharmacol. Exp. Ther. 151: 221–235, 1966.

81. BLINKS, J. R.: Evaluation of the cardiac effects of several beta adrenergic blocking agents. Ann. N.Y. Acad. Sci. 139: 673–685, 1967.

82. BLINKS, J. R., AND KOCH-WESER, J.: Physical factors in the analysis of the actions of drugs on myocardial contractility. Pharmacol. Rev. 15: 531–599, 1963.

83. BLOOM, B. M., AND GOLDMAN, I. M.: The nature of catecholamine-adenine mononucleotide interactions in adrenergic mechanisms. In Advances in Drug Research, vol. 3, ed. by N. J. Harper, and A. B. Simmonds, pp. 121–169, Academic Press, London, 1966.

84. BOEYNAEMS, J. M., AND DUMONT, J. E.: Quantitative analysis of the binding of ligands to their receptors. J. Cyclic Nucleotide Res. 1: 123–142, 1975.

85. BOEYNAEMS, J. M., AND DUMONT, J. E.: The two-step model of ligand-receptor interaction. Mol. Cell. Endocrinol. 7: 33–47, 1977.

86. BOHR, D. F.: Adrenergic receptors in coronary arteries. Ann. N.Y. Acad. Sci. 139: 799–807, 1967.

87. BOKESOY, T. A., ERCAN, Z. S., ERSOY, F., ZENGIL, H., AND TÜRKER, R. K.: Possible involvement of endogenous histamine in the mystropic effect of clonidine on isolated rabbit aorta. Agents Actions 8: 478–483, 1978.

88. BORDA, E., AGOSTINI, M. D. C., STERIN-BORDA, L., GIVENO, M. F., AND GIMENO, A. L.: Inhibitory effects of some catecholamines on contractions of uterine strips isolated from estrus and spayed rats. Influence of endogenous and exogenous prostaglandins on the action of methoxamine. Eur. J. Pharmacol. 69: 55–62, 1981.

89. BOREICHA, I., AND ROCHA E SILVA, M.: Occurrence of H$_2$-receptors for histamine in the guinea-pig intestine. Biochem. Pharmacol. 24: 1215–1219, 1975.

90. BORKOWSKI, K. R., AND PORTER, M.: Modulation of vasoconstriction by β-adrenoceptors in isolated perfused rat mesenteric arteries. Br. J. Pharmacol. 80: 508P, 1983.

91. BOYLE, F. C., AND DIGGES, K. G.: Responses to catecholamines of the rat isolated uterus throughout the natural oestrous cycle. Naunyn-Schmeideberg's Arch. Pharmacol. 321: 56–62, 1982.

92. BRAZENOR, R. M., AND ANGUS, J. A.: Ergometrine contracts isolated canine coronary arteries by a serotonergic mechanism: No role for alpha adrenoceptors. J. Pharmacol. Exp. Ther. 218: 530–536, 1981.

93. BRINK, C., DUNCAN, P. G., AND DOUGLAS, J. S.: The lung parenchymal strip: a reappraisal. J. Pharmacol. Exp. Ther. 219: 1–6, 1981.

94. BRISTOW, M., SHERROD, T. R., AND GREEN, R. D.: Analysis of beta receptor drug interactions in isolated rabbit atrium, aorta, stomach and trachea. J. Pharmacol. Exp. Ther. 171: 52–61, 1970.

95. BROADLEY, K. J.: The Langendorff heart preparation-reappraisal of its role as a research and teaching model for coronary vasoactive drugs. J. Pharmacol. Meth. 2: 143–156, 1979.

96. BROADLEY, K. J., AND WILLIAMS, R. G.: In vitro and ex vivo examination of irreversible antagonism of cardiac β-adrenoceptors. Br. J. Pharmacol. 74: 828P, 1981.

97. BRODDE, O. E., LEIFERT, F. J., AND KREHL, H.: Coexistence of β$_1$- and β$_2$-adrenoceptors in the rabbit heart: Quantitative analysis of the regional distribution by (−)-^{3}H-dihydroalprenoloe binding. J. Cardiovasc. Pharmacol. 4: 34–43, 1982.

98. BRODDE, O.-E., MEYER, F.-J., SCHEMUTZ, W., AND FREISTÜHLER, J.: Demonstration of specific vascular dopamine receptors mediating vaso-

99. BRODY, T. M., AND DIAMOND, J.: Blockade of the biochemical correlates of contraction and relaxation in uterine and intestinal smooth muscle. Ann. N.Y. Acad. Sci. 139: 772–780, 1967.

100. BROWN, C. M., AND COLLIS, M. G.: Adenosine contracts the isolated rat tail artery by releasing endogenous 5-hydroxytryptamine. Eur. J. Pharmacol. 76: 275–277, 1981.

101. BROOKES, N., AND MACKAY, D.: Diffusion of labeled substances through isolated rat diaphragm. Br. J. Pharmacol. 41: 367–378, 1971.

102. BROWN, C., BURNSTOCK, G., AND COCKS, T.: Effects of adenosine 5'-triphosphate (ATP) and β-γ-methylene ATP on the rat urinary bladder. Br. J. Pharmacol. 65: 97–102, 1979.

103. BUCKNER, C. K., BIRNBAUM, J., AND O'CONNER, M.: Factors influencing observed β-adrenergic receptor antagonism in guinea-pig trachea. Pharmacol. 26: 198–203, 1974.

104. BUCKNER, C. K., AND PATIL, P. N.: Steric aspects of adrenergic drugs. XVI. Beta-adrenergic receptors of guinea-pig atria and trachea. J. Pharmacol. Exp. Ther. 176: 634–649, 1971.

105. BUCKNER, C. K., AND SAINI, R. K.: On the use of functional antagonism to estimate dissociation constants for beta adrenergic receptor agonists in isolated guinea-pig trachea. J. Pharmacol. Exp. Ther. 194: 565–574, 1975.

106. BUCKNER, C. K., TORPHY, T., AND COSTA, D. J.: Studies on β-adrenoceptors mediating changes in mechanical events and adenosine 3',5'-monophosphate levels. Rat atria. Eur. J. Pharmacol. 47: 259–271, 1978.

107. BURGEN, A. S. V.: Conformational changes and drug action. Fed. Proc. 40: 2723–2728, 1981.

108. BURGEN, A. S. V., AND IVERSEN, L. L.: The inhibition of noradrenaline uptake by sympathetic amines in the rat isolated heart. Br. J. Pharmacol. 25: 34–49, 1965.

109. BURGEN, A. S. V., AND SPERO. L.: The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. Br. J. Pharmacol. 34: 99–115, 1968.

110. BURGEN, A. S. V., AND SPERO, L.: The effects of calcium and magnesium on the response of intestinal smooth muscle to drugs. Br. J. Pharmacol. 40: 492–500, 1970.

111. BURN, J. H., FINNEY, D. F., AND GOODWIN, L. G.: In Biological Standardization, Oxford University Press, London, 1950.

112. BUUR, T., CLAUSEN, T., HOLMBERG, E., JOHANSSON, U., AND WALDECK, B.: Desensitization by terbutaline of β-adrenoceptors in the guinea-pig soleus muscle: Biochemical alterations associated with functional changes. Br. J. Pharmacol. 76: 313–317, 1982.

113. BURSZTAJN, S., McMANAMAN, J. L., ELIAS, S. B., AND APPEL, S. H.: Myasthenic globulin enhances the loss of acetylcholine receptor clusters. Science 219: 195–197, 1983.

114. BUSBY, R. C., AND TALLARIDA, R. J.: On the analysis of straight line data in pharmacology and biochemistry. J. Theor. Biol. 93: 867–879, 1981.

115. BYNKE, G., HAKANSON, R., HÖRIG, J., AND LEANDER, S.: Bradykinin contracts the pupillary sphincter and evokes ocular inflammation through release of neuronal substance P. Eur. J. Pharmacol. 91: 469–475, 1983.

116. CAMILION DE HURTADO, M. C., ARGEL, M. I., AND CINGOLANI, H. E.; Influence of acid-base alterations on myocardinal sensitivity to catecholamines. Naunyn-Schmeideberg's Arch. Pharmacol. 317: 219–224, 1981.

117. CARLSSON, E., ABLAD, B., BRÄNDSTRÖM, A., AND CARLSSON, B.: Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. Life Sci. 11: 953–958, 1972.

118. CARLSSON, E., AND HEDBERG, A.: Are cardiac effects of noradrenaline and adrenaline mediated by different β-adrenoceptors? Acta Physiol. Scand. Suppl. 440: 47, 1976.

119. CARPENEDO, F., INFANTINO, A., FLOREANI, M., AND DODI, G.: The relaxing effect of caerulein on isolated human internal and sphincter. Eur. J. Pharmacol. 87: 271–276, 1983.

120. CARUSO, T. P., TAKEMORI, A. E., LARSON, D. L., AND PORTOGHESE, P. S.: Chlorooxymorphamine, an opioid receptor site-directed alkylating agent having narcotic agonist activity. Science 204: 316–318, 1979.

121. CATT, K. J., AND DUFAU, M. L.: Spare gonadotrophin receptors in rat testis. Nature (Lond.) New Biol. 244: 219–221, 1973.

122. CHANG, A., AND DETAR, R.: Oxygen and vascular smooth muscle contraction revisited. Am. J. Physiol. (Heart Circ. Physiol. 7) 238: H716–H728, 1980.

123. CHANG, H.-Y., KLEIN, R. M., AND KUROS, G.: Selective desensitization of cardiac beta-adrenoceptors by prolonged in vivo infusion of catecholamines in rats. J. Pharmacol. Exp. Ther. 221: 784–789, 1982.

124. CHANG, H.-Y., AND KUNOS, G.: In vivo desensitization of cardiac β-adrenoceptors. Pharmacologist 23: 183, 1983.

125. CHANGEUX, J. P., THIÉRY, J., TUNG, Y., AND KITTEL, C.: On the cooperativity of biological membranes. Proc. Natl. Acad. Sci. U.S.A. 57: 335–341, 1967.

126. CHENG, J. B., AND TOWNLEY, R. G.: Comparison of muscarinic and beta adrenergic receptors between bovine peripheral lung and tracheal smooth muscles: A striking difference in the receptor concentration. Life Sci. 30: 2079–2086, 1982.

127. CHIBA, S., FURUKAWA, Y., AND KOBAYASHI, M.: Adenosine-guanosine interactions in the isolated dog atrium. Jpn. J. Pharmacol. 30: 122–125, 1980.

128. CHIU, T. H.: Age-dependent changes in the myocardial adenylate cyclase of

normotensive and spontaneously hypertensive rat. Pharmacol. 22: 183–188, 1981.

129. CIARALDI, T., AND MARINETTI, G. V.; Thyroxine and propylthiouracil effects *in vivo* on alpha and beta adrenergic receptor in rat heart. Biochem. Biophys. Res. Commun. 74: 984–991, 1977.

130. CLANACHAN, A. S., JOHNS, A., AND PATON, D. M.: Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in the rat vas deferens. Neuroscience 2: 597–602, 1977.

131. CLANACHAN, A. S., AND MULLER, M. J.: Effect of adenosine uptake inhibition on the nature and potency of theophylline as a presynaptic adenosine receptor antagonist. Can. J. Physiol. Pharmacol. 58: 805–809, 1980.

132. CLARK, A. J.: The Mode of Action of Drugs on Cells. Edward Arnold and Co., London, 1933.

133. CLARK, A. J.: General pharmacology. *In* Heffter's Handbuch d-exp. Pharmacol. Erg.-band4, Springer, Berlin, 1937.

134. CLARK, A. J., AND RAVENTOS, J.: The antagonism of acetylcholine and of quaternary ammonium salts. Q. J. Exp. Physiol. 26: 375–391, 1937.

135. COHEN, M. L., AND BERKOWITZ, B. A.: Age-related change in vascular responsiveness to cyclic nucleotides and contractile agonists. J. Pharmacol. Exp. Ther. 191: 147–155, 1974.

136. COHEN, M. L., AND WILEY, K. S.: Specific enhancement of norepinephrine-induced contraction in rat veins after *beta* adrenergic antagonists. J. Pharmacol. Exp. Ther. 201: 406–416, 1977.

137. COHEN, M. L., AND WILEY, K. S.: Comparison of arteries with longitudinal and circular venous muscle from the rat. Am. J. Physiol. 232: H131–H139, 1977.

138. COHEN, M. L., AND WILEY, K. S.: Rat jugular vein relaxes to norepinephrine, phenylephrine and histamine. J. Pharmacol. Exp. Ther. 205: 400–409, 1978.

139. COHEN, M. L., FULLER, R. W., AND WILEY, K. S.: Evidence for 5-HT$_2$ receptors mediating contraction in vascular smooth muscle. J. Pharmacol. Exp. Ther. 218: 421–425, 1981.

140. COLEMAN, R. A.: Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. Br. J. Pharmacol. 57: 51–57, 1976.

141. COLEMAN, R. A., AND LEVY, G. P.: A non-adrenergic inhibitory nervous pathway in guinea-pig trachea. Br. J. Pharmacol. 52: 167–174, 1974.

142. COLLIER, H. O. J.: Tolerance, physical dependence and receptors. A theory of the genesis of tolerance and physical dependence through drug-induced changes in the number of receptors. *In* Advances in Drug Research, vol. 3, ed. by N. J. Harper, and A. B. Simmonds, pp. 171–188, Academic Press, London, New York, 1966.

143. COLQUHOUN, D.: The relationship between classical and cooperative models for drug action. *In* Drug Receptors, ed. by H. P. Rang, pp. 149–182, University Park Press, Baltimore, 1973.

144. COLQUHOUN, D.: How fast do drugs work? Trends Pharmacol. Sci. 2: 212–217, 1981.

145. COLQUHOUN, D., AND TATERSALL, M. L.: Rapid histamine assays: A method and some theoretical considerations. Br. J. Pharmacol. 38: 241–252, 1970.

146. COLUCCI, W. S., ALEXANDER, R. W., WILLIAMS, G. H., RUDE, R. E., HOLMAN, B. L., KONSTAM, M. A., WYNNE, J., MUDGE, G. H., AND BRAUNWALD, E.: Decreased lymphocyte beta-adrenergic-receptor density in patients with heart failure and tolerance to the beta-adrenergic agonist pirbuterol. N. Engl. J. Med. 305: 185–190, 1981.

147. CONSTANTINE, J. W., AND LEBEL, W.: Complete blockade by phenoxybenzamine of α_1- but not of α_2-vascular receptors in dogs and the effects of propanolol. Naunyn-Schmeideberg's Arch. Pharmacol. 314: 149–, 1980.

148. CONSTANTINE, J. W., LEBEL, W., AND ARCHER, R.: Functional postsynaptic α_2- but not α_1-adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. Eur. J. Pharmacol. 85: 325–329, 1982.

149. CONTI-TRONCONI, B. M., FUMAGALLI, G., SCOTTI, A., BRIGONZI, A., SHER, E., MORGUTTI, M., AND CLEMENTI, F.: Myasthenia gravis: An example of receptor disease. Recept. Neurotrans. Pep. Horm. 18: 473–488, 1980.

150. COOK, D. A.: The absence of a significant histamine receptor reserve in vascular smooth muscle. J. Pharm. Pharmacol. 22: 63–64, 1970.

151. COOK, D. A.: Blockade by phenoxybenzamine of the contractor response produced by agonists in the isolated ileum of the guinea-pig. Br. J. Pharmacol. 43: 197–209, 1971.

152. COOK, D. A., AND IWANOW, D. M.: Responses of rabbit renal artery to histamine: Receptor type and temperature dependence. Can. J. Physiol. Pharmacol. 58: 306–309, 1980.

153. CORET, I. A., AND HUGHES, M. J.: A quantitative description of chronotropic effects of histamine on rabbit heart. Arch. Int. Pharmacodyn. Ther. 208: 117–137, 1974.

154. COUPAR, I. M., AND TURNER, P.: Relative potencies of sympathetic amines in human smooth muscle. Br. J. Pharmacol. 36: 213P–214P, 1969.

155. COUTURE, R., FOURNIER, A., MAGNAN, J., ST-PIERRE, S., AND REGOLI, D.: Structure-activity studies on substance P. Can. J. Physiol. Pharmacol. 57: 1427–1436, 1979.

156. COUTURE, R., GAUDREAU, P., ST-PIERRE, S., AND REGOLI, D.: The dog common carotid artery: A sensitive bioassay for studying vasodilator effects of substance P and of kinins. Can. J. Physiol. Pharmacol. 58: 1234–1244, 1980.

157. CRANEFIELD, P. F., AND GREENSPAN, K.: The rate of oxygen uptake of quiescent cardiac muscle. J. Gen. Physiol. 44: 235–249, 1960.

158. CRANK, J.: The Mathematics of Diffusion, Oxford University Press, London, 1956.

159. CRANKSHAW, D. J.: Receptor binding studies on smooth muscle subcellular fraction. *In* Calcium and Smooth Muscle Contractility, ed. by E. E. Daniel, and A. K. Grover, Humana Press, Clifton, NJ, in press.

160. CUATRECASAS, P.: Membrane receptors. Annu. Rev. Biochem. 43: 169–214, 1974.

161. CUATRECASAS, P., HOLLENBERG, M. D., CHANG, K. J., AND BENNETT, V.: Hormone receptor complexes and their modulation of membrane function. Recent Prog. Horm. Res. 31: 37, 1975.

162. CULL-CANDY, S. G., MILEDI, R., AND TRAUTMANN, A.: Acetylcholine-induced channels and transmitter release at human endplates. Nature (Lond.) 271: 74–75, 1978.

163. CURRO, F. A., GREENBERG, S., VERBEURAN, T. J., AND VANHOUTTE, P. M.: Interaction between alpha adrenergic and serotonergic activation of canine saphenous veins. J. Pharmacol. Exp. Ther. 207: 936–949, 1978.

164. CUTHBERT, A. W., AND DUNANT, Y.: Diffusion of drugs through stationary water layers as the rate limiting process in their action at membrane receptors. Br. J. Pharmacol. 40: 508–521, 1970.

165. DAINTY, J., AND HOUSE, C. R.: "Unstirred layer" in frog skin. J. Physiol. (Lond.) 182: 66–78, 1966.

166. DANILOV, A. F., ZAVJALOVA, N. E., AND LAVRENTIERA, V. V.: On α-bungarotoxin and cobra α-toxin in estimating affinity and efficacy of full agonists. Gen. Pharmacol. 11: 107–111, 1980.

167. DAX, E. M., PARTILLA, J. S., AND GREGERNAR, R. I.: Increased sensitivity to epinephrine stimulated lipolysis during starvation: Tighter coupling of the adenylate cyclase complex. Biochem. Biophys. Res. Commun. 101: 1186–1192, 1981.

168. DE JONGH, D. K., VAN PROOSDIJ-HARTZEMA, E. G., AND JANSSEN, P.: Substituted phenylpropylamines. II. Pharmacological properties of basic butyronitrites and butyramides. Arch. Int. Pharmacodyn. Thér. 103: 100–119, 1955.

169. DE LA LANDE, I. S.: Adrenergic mechanisms in the rabbit ear artery. Blood Vessels 12: 137–160, 1975.

170. DE LA LANDE, I. S., FREWIN, D., AND WATERSON, J. G.; The influence of sympathetic innervation on vascular sensitivity to noradrenaline. Br. J. Pharmacol. 31: 82–93, 1967.

171. DE LA LANDE, I. S., HARVEY, J. A., AND HOLT, S.: Response of the rabbit coronary arteries to autonomic agents. Blood Vessels 11: 319–337, 1974.

172. DE LA LANDE, I. S., AND JELLET, L. B.: Relationship between the roles of monoamine oxidase and sympathetic nerves in the vasoconstrictor response of the rabbit ear artery to noradrenaline. J. Pharmacol. Exp. Ther. 180: 47–55, 1972.

173. DE LA LANDE, I. S., AND RAND, M. J.: A simple isolated nerve-blood vessel preparation. Aust. J. Exp. Biol. Med. Sci. 43: 639–656, 1965.

174. DE LA LANDE, I. S., AND WATERSON, J. G.: Site of action of cocaine on the perfused artery. Nature (Lond.) 214: 313–314, 1967.

175. DE LEAN, A., MUNSON, P. J., AND RODBARD, D.: Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. Am. J. Physiol. 235: E97–E102, 1978.

176. DE LEAN, A., STADEL, J. M., AND LEFKOWITZ, R. J.: A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase coupled β-adrenergic receptor. J. Biol. Chem. 255: 7108–7117, 1980.

177. DE MEY, J., AND VANHOUTTE, P. M.: Uneven distribution of postjunctional alpha$_1$- and alpha$_2$-like adrenoceptors in canine arterial and venous smooth muscle. Circ. Res. 48: 875–884, 1981.

178. DEAN, P. M.: Investigation into the mode of action of histamine on the isolated rabbit heart. Br. J. Pharmacol. 32: 65–77, 1968.

179. DEL CASTILLO, J., AND KATZ, B.: Center-action at end-plate receptors between different choline derivatives. Proc. R. Soc. Lond. Biol. Sci. 146: 369–381, 1957.

180. DETAR, R.: Mechanism of physiological hypoxia-induced depression of vascular smooth muscle contraction. Am. J. Physiol. (Heart Circ. Physiol. 7) 238: H761–H769, 1980.

181. DIGGES, K. G.: Review: Adrenoreceptors in uterus. J. Autonom. Pharmacol. 2: 53–67, 1982.

182. DIXON, W. J.: Efficient analysis of experimental observations. Annu. Rev. Pharmacol. Toxicol. 20: 441–462, 1980.

183. DOCHERTY, J. R., AND O'MALLEY, K.: Age-related changes in pre- and postsynaptic α-adrenoceptors in rat as deferens. Br. J. Pharmacol. 80: 464P, 1981.

184. DOWNIE, J. W., AND DEAN, D. M.: The contribution of cholinergic postganglionic neurotransmission to contractions of rabbit detrusor. J. Pharmacol. Exp. Ther. 203: 417–425, 1977.

185. DRAZEN, J. M., FANTA, C. H., AND LACOUTURC, P. G.: Effect of nifedipine on constriction of human tracheal strips *in vitro*. Br. J. Pharmacol. 78: 687–691, 1983.

186. DREW, G. M., AND LEVY, G. P.: Characterization of the coronary vascular β-adrenoceptor in the pig. Br. J. Pharmacol. 46: 348–350, 1972.

187. DREW, G. M., HILDITCH, A., AND LEVY, G. P.: Effect of labetalol on the uptake of [^{3}H]-(−)-noradrenaline into the isolated vas deferens of the rat. Br. J. Pharmacol. 63: 471–474, 1978.

188. DROUIN, J.-N., ST-PIERRE, S. A., AND REGOLI, D.: Receptors for bradykinin and kallidin. Can. J. Physiol. Pharmacol. **57**: 375–379, 1979.

189. EBNER, F.: The positive inotropic effect of (−)-noradrenaline and (±)-isoprenaline after chemical sympathectomy: Evidence in favour of differences at a postsynaptic site. Naunyn-Schmeideberg's Arch. Pharmacol. **316**: 8–18, 1981.

190. EBNER, F.: The inhibition by (±)-propanolol of the positive inotropic effects of (±)-isoprenaline and (−)-noradrenaline. Naunyn-Schmeideberg's Arch. Pharmacol. **316**: 96–107, 1981.

191. EBNER, F., AND WAUD, D. R.: The role of uptake of noradrenaline for its positive inotropic effect in relation to muscle geometry. Naunyn-Schmeideberg's Arch. Pharmacol. **303**: 1–6, 1978.

192. EDVINSSON, L., HARDEBO, J. E., McCULLOCH, J., AND OWMAN, C.: Effects of dopaminergic agonists and antagonists on isolated cerebral blood vessels. Acta. Physiol. Scand. **104**: 349–359, 1978.

193. EDVINSSON, L., AND OWMAN, C.: Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro. Circ. Res. **35**: 835–849, 1974.

194. EDVINSSON, L., AND OWMAN, C.: A pharmacologic comparison of histamine receptors in isolated extracranial and intracranial arteries in vitro. Neurology **25**: 271–276, 1975.

195. EHRENPREIS, S.: Possible nature of the cholinergic receptor. Ann. N.Y. Acad. Sci. **144**: 720–736, 1967.

196. EIDEN, L. E., AND RUTH, J. A.: Acute thyroid hormone increases noradrenergic responsiveness of rat atria in vitro. Eur. J. Pharmacol. **74**: 91–93, 1981.

197. EMMENS, C. W.: Dose-response relations. J. Endocrinol. **2**: 194–225, 1940.

198. EMMERSON, J., AND MACKAY, D.: A test of the null equation for functional antagonism. Br. J. Pharmacol. **73**: 135–141, 1981.

199. ENDOH, M., YAMASHITA, S., AND TAIRA, N.: Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J. Pharmacol. Exp. Ther. **221**: 775–783, 1982.

200. ERCAN, Z. S., AND TÜRKER, R. K.: Histamine receptors in the isolated rat stomach fundus and rabbit aortic strips. Pharmacologist **15**: 118–126, 1977.

201. ERICSSON, E. E.: Age-dependent variations in β-receptor activity and cyclic AMP in vascular smooth muscle. Acta. Pharmacol. Toxicol. **31**: Suppl. 1, 45, 1972.

202. ERJAVEC, F., LEMBECK, F., FLORJANC-IRMAN, T., SKOFITSCH, G., DONNERER, J., SARIA, A., AND HOLZER, P.: Release of histamine by substance P. Naunyn-Schmeideberg's Arch. Pharmacol. **317**: 67–70, 1981.

203. ESCHER, E. H. F., NGUYEN, T. M. D., ROBERT, H., ST-PIERRE, S. A., AND REGOLI, D. C.: Photoaffinity labelling of the angiotension II receptor. 1. Synthesis and biological activities of the labeling peptides. J. Med. Chem. **21**: 860–864, 1978.

204. EYRE, P.: Histamine H₂-receptors in the sheep bronchus and cat trachea: the action of burimamide. Br. J. Pharmacol. **48**: 321–323, 1973.

205. FARMER, J. B., AND LEVY, G. P.: Differentiation of β-adrenoreceptors by the use of blocking agents. J. Pharm. Pharmacol. **22**: 145–146, 1970.

206. FASSINA, G.: Effects of two beta-adrenergic blocking agents, propanolol and INPEA on lipid metabolism. Arch. Int. Pharmacodyn. Thér. **166**: 281–293, 1967.

207. FEDAN, J. S., BESSE, J. C., CARPENTER, F. G., AND TEAGUE, R. S.: Motor innervation of the smooth muscle of the rat seminal vesicle. J. Pharmacol. Exp. Ther. **201**: 285–297, 1977.

208. FEDAN, J. S., HOGABOOM, G. K., AND O'DONNELL, J. P.: Photoaffinity labeling of H₁-histamine receptors in isolated smooth muscles by 4(5)-[2-(4-azido-2-nitroanilino)ethyl]imidazole: Characterization in guinea-pig aorta and lack of pharmacological antagonism in dog trachealis. Eur. J. Pharmacol. **81**: 393–402, 1982.

209. FEDAN, J. S., HOGABOOM, G. K., WESTFALL, D. P., AND O'DONNELL, J. P.: Photoaffinity labeling of P₂-purinergic and H₁-histamine receptors in intact smooth muscle. Fed. Proc. **42**: 2846–2850, 1983.

210. FINNEY, M., KARLSSON, J.-A., AND PERSSON, C. G. A.: An alternative human small airway preparation. Br. J. Pharmacol., p. 104, 1983, in press.

211. FLAIM, S. F., MINTEER, W. S., NELLIS, S. H., AND CLARK, D. P.: Chronic arteriovenous shunt: Evaluation of a model for heart failure in rat. Am. J. Physiol. **236**: H698–H704, 1979.

212. FLEISCH, J. H.: Further studies on effects of aging on β-adrenoceptor activity of rat aorta. Br. J. Pharmacol. **42**: 311–313, 1971.

213. FLEISCH, J. H., AND CALKINS, P. J.: Comparison of drug-induced responses of rabbit trachea and bronchus. J. Appl. Physiol. **41**: 62–66, 1976.

214. FLEISCH, J. H., AND HOOKER, C. S.: The relationship between age and relaxation of vascular smooth muscle in the rabbit and rat. Circ. Res. **38**: 243–249, 1976.

215. FLEISCH, J. H., MALING, H. M., AND BRODIE, B. B.: Beta-receptor activity in aorta. Circ. Res. **26**: 151–162, 1970.

216. FLEMING, W. W.: Supersensitivity in smooth muscle. Introduction and historical perspective. Fed. Proc. **34**: 1969–1970, 1975.

217. FLEMING, W. W., McPHILLIPS, J. W., AND WESTFALL, D. P.: Postjunctional supersensitivity and subsensitivity of excitable tissues to drugs. Ergeb. Physiol. **68**: 55–119, 1973.

218. FLEMING, W. W., WESTFALL, D. P., DE LA LANDE, I. S., AND JELLETT, L. B.: Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. J. Pharmacol. Exp. Ther. **181**: 339–345, 1972.

219. FLOWER, R. S.: Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. **26**: 33–67, 1974.

220. FORSTER, C., DREW, G. M., HILDITCH, A., AND WHALLEY, E. T.: Dopamine receptors in human basilar arteries. Eur. J. Pharmacol. **87**: 227–235, 1983.

221. FORSTER, C., AND WHALLEY, E. T.: Analysis of the 5-hydroxytryptamine induced contraction of the human basilar arterial strip compared with the rat aortic strip in vitro. Naunyn-Schmeideberg's Arch. Pharmacol. **319**: 12–17, 1982.

222. FOSTER, R. W.: The potentiation of the responses to noradrenaline and isoprenaline in guinea-pig isolated tracheal chain preparation by desipramine, cocaine, phentolamine, phenoxybenzamine, guanethidine, metanephrine and cooling. Br. J. Pharmacol. Chemother. **31**: 466–482, 1967.

223. FRANCO, R., COSTA, M., AND FURNESS, J. B.: Evidence for the release of endogenous substance P from intestinal nerves. Naunyn-Schmeideberg's Arch. Pharmacol. **306**: 195–201, 1979.

224. FREDHOLM, B. B., LUNELL, N.-O., PERSSON, B., AND WAGER, J.: Development of tolerance to the metabolic actions of β₂-adrenoceptor stimulating drugs. Acta Obstet. Gynecol. Scand. Suppl. **108**: 53–59, 1982.

225. FREW, R., McKENZIE, S. G., BÄR, H. P., AND HUTCHISON, K. J.: The relaxant effects of adenosine-5'-α,β-methylenediphosphonate on the longitudinal smooth muscle of the rabbit ileum. Can. J. Physiol. Pharmacol. **54**: 626–629, 1976.

226. FRUTON, J. S., STEIN, W. H., AND BERGMANN, M.: Chemical reactions of the nitrogen mustard gases. V. The reactions of the nitrogen mustard gases with protein constituents. J. Org. Chem. **11**: 559–570, 1946.

227. FURCHGOTT, R. F.: Dibenamine blockade in strips of rabbit aorta and its use in differentiating receptors. J. Pharmacol. Exp. Ther. **111**: 265–284, 1954.

228. FURCHGOTT, R. F.: The pharmacology of vascular smooth muscle. Pharmacol. Rev. **7**: 183–265, 1955.

229. FURCHGOTT, R. F.: The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. In Advances in Drug Research, vol. 3, ed. by N. J. Harper, and A. B. Simmonds, pp. 21–55, Academic Press, London, New York, 1966.

230. FURCHGOTT, R. F.: The pharmacological differentiation of adrenergic receptors. Ann. N.Y. Acad. Sci. **139**: 553–570, 1967.

231. FURCHGOTT, R. F.: Pharmacological characteristics of adrenergic receptors. Fed. Proc. Fed. Am. Soc. Exp. Biol. **29**: 1352–1361, 1970.

232. FURCHGOTT, R. F.: The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In Handbook of Experimental Pharmacology, Catecholamines, vol. 33, ed. by H. Blaschko, and E. Muscholl, pp. 283–335, Springer-Verlag, New York, 1972.

233. FURCHGOTT, R. F.: Postsynaptic adrenergic receptor mechanisms in vascular smooth muscle. In Vascular Neuroeffector Mechanisms, ed. by J. A. Bevan, pp. 131–142, S. Karger Publisher, Basel, Switzerland, 1976.

234. FURCHGOTT, R. F.: Problems encountered in the pharmacological characterization of receptors in blood vessels. In Neurogenic Control of Brain Circulation, ed. by C. Owman and L. Edvinsson, pp. 155–166, Pergamon Press, New York, 1977.

235. FURCHGOTT, R. F.: Pharmacological characterization of receptors. Its relation to radioligand-binding studies. Fed. Proc. Fed. Am. Soc. Exp. Biol. **37**: 115–120, 1978.

236. FURCHGOTT, R. F.: Adrenergic and dopaminergic peripheral receptors. Proceedings of the 4th Meeting on Adrenergic Mechanisms, Porto, Portugal, 1981.

237. FURCHGOTT, R. F., AND BHADRAKOM, S.: Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmacol. Exp. Ther. **108**: 129–143, 1953.

238. FURCHGOTT, R. F., AND BURSZTYN, P.: Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. Ann. N.Y. Acad. Sci. **139**: 882–899, 1967.

239. FURCHGOTT, R. F., JURKIEWICZ, A., AND JURKIEWICZ, N. H.: Antagonism of propranolol to isoproterenol in guinea-pig trachea: Some cautionary findings. In Frontiers in Catecholamine Research, ed. by E. Usdin, and S. H. Snyder, pp. 295–300, Pergamon Press Inc., Elmsford, New York, 1973.

240. FURCHGOTT, R. F., AND WAKADE, T. D.: Evidence for both β₁ and β₂ receptors in guinea-pig tracheal smooth muscle and variation of the β₁:β₂ ratio in different animals. Sixth International Congress of Pharmacology, 622, Helsinki, 1975, Pergamon Press, Oxford.

241. FURCHGOTT, R. F., WAKADE, T. D., SORACE, R. A., AND STOLLAK, J. S.: Occurrence of both β₁ and β₂ receptors in guinea-pig tracheal smooth muscle, and variation of the β₁:β₂ ratio in different animals. Fed. Am. Soc. Exp. Biol. **34**: 794, 1975.

242. FURCHGOTT, R. F., AND ZAWADZKI, J. V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature (Lond.) **288**: 373–376, 1980.

243. GADDUM, J. H.: The quantitative effects of antagonistic drugs. J. Physiol. (Lond.) **89**: 7P–9P, 1937.

244. GADDUM, J. H.: Biological aspects: The antagonism of drugs. Trans. Faraday Soc. **39**: 323–333, 1943.

245. GADDUM, J. H.: Log normal distributions. Nature (Lond.) **156**: 463–466, 1945.

246. GADDUM, J. H.: Bioassays and mathematics. Pharmacol. Rev. 87–134, 1953.

247. GADDUM, J. H., HAMEED, K. A., HATHWAY, D. E., AND STEPHENS, F. F.: Quantitative studies of antagonists for 5-hydroxytryptamine. Q. J. Exp. Physiol. **40**: 49–74, 1955.

248. GAIDE, M. S., FITTERMAN, W. S., WIGGINS, J. R., MYERBURG, R. J., CAMERON, J. S., AND BASSETT, A. L.: Amrinone relaxes potassium-induced contracture of failing right ventricular muscle of cats. J. Cardiovasc. Pharmacol. **5**: 335–340, 1983.

249. GALARDY, R. E., AND LA VORGNA, K. A.: Photochemical inactivation of the angiotensin receptor of rabbit aorta by Nᵃ-(2-nitro-5-azidobenzoyl)-[1-aspartic acid,5-isoleucine]angiotensin II. J. Med. Chem. **24**: 362–366, 1981.

250. GAMMELTOFT, S., AND GLIEMANN, J.: Binding and degradation of ¹²⁵I-labelled insulin by isolated rat fat cells. Biochim. Biophys. Acta **320**: 16–32, 1973.

251. GELBAND, H., AND BASSETT, A. L.: Depressed transmembrane potentials during experimentally induced venetricular failure in cats. Circ. Res. **32**: 625–634, 1973.

252. GELLAI, M., AND DETAR, R.: Evidence in support of hypoxia but against high potassium and hyperosmolarity as possible mediators of sustained vasodilation in rabbit cardiac and skeletal muscle. Circ. Res. **35**: 681–691, 1974.

253. GERO, A.: Biological stimulus and drug effect. J. Theor. Biol. **74**: 469–473, 1978.

254. GERO, A., AND TALLARIDA, R. J.: Use of the biological stimulus in determining parameters of drug action, and its relationship to the drug effect: A contribution to the theory of drug action. J. Theor. Biol. **69**: 265–274, 1977.

255. GERSTENBLITH, G., SPURGEON, J. P., FROEHLICK, J. P., WEISFELDT, M. L., AND LAKATTA, E. G.: Diminished inotropic responsiveness to ouabain in aged rat myocardium. Circ. Res. **44**: 517–523, 1979.

256. GIBSON, A.: Contractile responses of the mouse anococcygeus muscle to some α-adrenoceptor agonists. Br. J. Pharmacol. **73**: 284P–285P, 1980.

257. GILL, E. W., AND RANG, H. P.: An alkylating derivative of benzilcholine with specific and long-lasting parasympathetic activity. Mol. Pharmacol. **2**: 284–297, 1966.

258. GILLESPIE, J. S.: The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. Br. J. Pharmacol. **45**: 404–416, 1972.

259. GILLESPIE, J. S., AND McGRATH, J. C.; The response of the cat anococcygeus muscle to nerve or drug stimulation and a comparison with the rat anococcygeus. Br. J. Pharmacol. **50**: 109–117, 1974.

260. GINSBURG, R.: The isolated human epicardial coronary artery. Am. J. Cardiol. **52**: 61A–66A, 1983.

261. GINSBURG, R., BRISTOW, M. R., BILLINGHAM, M. E., STINSON, E. B., SCHROEDER, J. S., AND HARRISON, D. C.: Study of the normal a failing isolated human heart: Decreased response of failing heart to isoproterenol. Am. Heart J. **106**: 535–540, 1983.

262. GINSBURG, R., BRISTOW, M. R., STINSON, E. B., AND HARRISON, D. C.: Histamine receptors in the human heart. Life Sci. **26**: 2245–2249, 1980.

263. GINTAUTAS, J., KRAYRACK, B. J., AND RACZ, G. B.: Responsiveness of isolated cerebral arteries to various pharmacologic agents and to transmural electrical stimulation. Proc. West. Pharmacol. Soc. **23**: 63–67, 1980.

264. GLAUBIGER, G., AND LEFKOWITZ, R. J.: Elevated beta-adrenergic receptor number after chronic propanolol treatment. Biochem. Biophys. Res. Commun. **78**: 720–725, 1977.

265. GLAUBIGER, G. TSAI, B. S., LEFKOWITZ, R. J., WEISS, B., AND JOHNSON, E. M., JR.: Chronic guanethidine treatment increases cardiac β-adrenergic receptors. Nature (Lond.) **273**: 240–242, 1978.

266. GLUSA, E., AND MARKWARDT, F.: Characterization of postjunctional α-adrenoceptors in isolated human femoral veins and arteries. Naunyn-Schmeideberg's Arch. Pharmacol. **323**: 101–105, 1983.

267. GODFRAIND, T., AND MILLER, R. C.: Effects of histamine and the histamine antagonists mepyramine and cimetidine on human coronary arteries in vitro. Br. J. Pharmacol. **79**: 979–984, 1983.

268. GOKHALE, S. D., GULATI, O. D., KELKAR, L. V., AND KELKAR, V. V.; Effect of some drugs on human umbilical artery in vitro. Br. J. Pharmacol. **27**: 332–346, 1966.

269. GOLDBERG, L. I., AND TODA, N.: Dopamine induced relaxation of isolated canine renal, mesenteric and femoral arteries contracted with prostaglandin F₂ₐ. Circ. Res. **36,37**: I-97–I-102, 1975.

270. GOLDBERG, M. R., CHAPNICK, B. M., JOINER, P. D., HYMAN, A. L., AND KADOWITZ, P. J.: Influence of inhibitors of prostaglandin synthesis on venoconstrictor responses to bradykinin. J. Pharmacol. Exp. Ther. **198**: 357–365, 1976.

271. GOLDBERG, N. D.: Cyclic nucleotides and cell function. In Cell Membranes: Biochemistry, Cell Biology and Pathology, ed. by G. Weissmann and R. Claiborne, pp. 185–202, H. P. Publishing, New York, 1975.

272. GOLDIE, R. G., PATERSON, J. W., AND WALE, J. L.: Pharmacological responses of human and porcine lung parenchyma, bronchus, and pulmonary artery. Br. J. Pharmacol. **76**: 515–521, 1982.

273. GOLDSTEIN, A., AND SCHULZ, R.: Morphine tolerant-longitudinal muscle strip from guinea-pig ileum. Br. J. Pharmacol. **48**: 655–666, 1973.

274. GOODFORD, P. J., AND LEACH, E. H.: The extracellular space of the smooth muscle of the guinea-pig taenia coli. J. Physiol. (Lond.) **186**: 1–10, 1966.

275. GOSSELIN, R. E.: Drug receptor kinetics: A new model. Pharmacologist **10**: 215, 1968.

276. GOSSELIN, R. E.: Drug-receptor inactivation: A new kinetic model. In Kinetics of Drug Action, ed. by J. M. van Rossum, Springer-Verlag, Berlin, Heidelberg, New York, 1977.

277. GOTO, K., MASUDA, Y., AND KASUYA, Y.: The effect of denervation on the synchronization of contraction of the rat vas deferens. Eur. J. Pharmacol. **36**: 395–404, 1976.

278. GOVIER, W. C.: Myocardial alpha adrenergic receptors and their role in the production of a positive inotropic effect by sympathomimetic agents. J. Pharmacol. Exp. Ther. **159**: 82–90, 1968.

279. GRAHAM, J. M., AND KEATINGE, W. R.: Differences in sensitivity to vasoconstrictor drugs within the wall of the sheep carotid artery. J. Physiol. (Lond.) **221**: 477–492, 1972.

280. GREAVES, H. F.: Membrane receptor-adenylate cyclase relationships. Nature (Lond.) **265**: 681, 1977.

281. GREEN, A. L.: The kinetics of enzyme action and inhibition in intact tissues and tissue slices, with special reference to cholinesterase. J. Pharm. Pharmacol. **28**: 265–274, 1976.

282. GREEN, R. D., III, AND FLEMING, W. W.: Analysis of supersensitivity in the isolated spleen of the cat. J. Pharmacol. Exp. Ther. **162**: 254–262, 1968.

283. GRISTWOOD, R. W., LINCOLN, J. C. R., AND OWEN, D. A. A.: Effects of histamine on human isolated heart muscle: comparison with effects of noradrenaline. J. Pharm. Pharmacol. **32**: 145–149, 1980.

284. GUIMARAES, S., AZEVEDO, I., CARDOSO, W., AND OLIVEIRA, M. C.: Relation between the amount of smooth muscle of venous tissue and the degree of supersensitivity to isoprenaline caused by inhibition of catechol-O-methyl transferase. Naunyn-Schmeideberg's Arch. Pharmacol. **286**: 401–412, 1975.

285. GUIMARAES, S., AND OSSWALD, W.: Adrenergic receptors in the veins of the dog. Eur. J. Pharmacol. **5**: 133–140, 1969.

286. GUIMARAES, S., AND PAIVA, M. Q.: Two distinct adrenoceptor-biophases in the vasculature: One for α- and the other for β-agonists. Naunyn-Schmeideberg's Arch. Pharmacol. **316**: 195–199, 1981.

287. GUIMARAES, S., PAIVA, M. Q., AND MOURA, D.: Evidence for the existence of distinct biophases for α- and β-adrenoceptors in the vascular tissue. J. Cardiovasc. Pharmacol. **4**: 558–562, 1982.

288. GULATI, O. D., METHEW, B. P., PARIKH, H. M., AND KRISHNAMURTY, S. R.: Beta-adrenergic receptors of rabbit thoracic aorta in relation to age. Jap. J. Pharmacol. **23**: 259–268, 1973.

289. GUILLORY, R. J., AND JENG, S. J.: Photoaffinity labeling: Theory and practice. Fed. Proc. **42**: 2826–2830, 1983.

290. GUO, Z.-G., LEVI, R., AARONSON, L. M., AND GAY, W. A.: The isolated human pectinate muscle: A reliable preparation of human cardiac tissue. J. Pharmacol. Meth. **9**: 127–135, 1983.

291. GYANG, E. A., AND KOSTERLITZ, H. W.: Agonist and antagonist actions of morphine-like drugs on the guinea-pig isolated ileum. Br. J. Pharmacol. Chemother. **27**: 514–527, 1966.

292. HADHAZY, P., NAZY, L., JUHASZ, F., MALOMVOLGYI, B., AND MAGYAR, K.: Effects of indomethacin and prostaglandins I₂ and E₂ on the tone of human isolated mesenteric arteries. Eur. J. Pharmacol. **91**: 477–484, 1983.

293. HAGGENDAL, J., AND JOHNSSON, G.: The stability of noradrenaline in infusion solutions. Acta Pharmacol. Scand. **25**: 461–464, 1967.

294. HAGGENDAL, J., AND SVEDMYR, N.: Studies on the destruction of adrenaline in nutrient solution. Acta Pharmacol. Scand. **25**: 364–368, 1967.

295. HAHN, H. L., AND NADEL, J. A.: Methods of study of airway smooth muscle and its physiology. Pharmacol. Ther. **7**: 253–295, 1979.

296. HAND, J. M., AND BUCKNER, C. K.: Relaxant responses of the isolated guinea-pig left pulmonary artery produced by acidic histamine solutions. Eur. J. Pharmacol. **70**: 225–230, 1981.

297. HANSEN, T. R., DINEEN, D. X., AND PULLEN, G. L.: Orientation of arterial smooth muscle and strength of contraction of aortic strips from DOCA-hypertensive rats. Blood Vessels **17**: 302–311, 1980.

298. HARMS, H. H., DE VENTE, J., AND ZAAGSMA, J.: β-Adrenoceptors blocking agents a lipolysis. Br. J. Clin. Pharmacol. **13**: 181S–186S, 1982.

299. HASHIMOTO, H., NAKASHIMA, M., AND SUGINO, N.: Age-dependent differences in the positive inotropic effect of phenylephrine on rat isolated atria. Br. J. Pharmacol. **79**: 499–507, 1983.

300. HAYASHI, S., AND TODA, N.: Age-related changes in the response of rabbit isolated aortae to vasoactive agents. Br. J. Pharmacol. **64**: 229–237, 1978.

301. HAYASHI, S., AND TODA, N.: Age-related alterations in the responses of rabbit tracheal smooth musle to agents. J. Pharmacol. Exp. Ther. **214**: 675–681, 1980.

302. HAYES, R. J., AND SLOAN, T. P.: The response of human vas deferens to drugs modifying noradrenergic neurotransmission. Br. J. Pharmacol. **80**: 683P, 1983.

303. HAZRA, J.: Correlation between diisopropylfluorophosphate (DFP) inhibition of cholinesterase (CHE) of intact rat phrenic nerve-diaphragm and

effect on twitch height and injected acetylcholine (ACh). Fed. Proc. **26**: 511, 1967.

304. HEALD, S. L., JEFFS, P. W., LAVIN, T. N., NAMBI, P., LEFKOWITZ, R. L., AND CARON, M. G.: Synthesis of iodine-125 labeled (±)-15-(4-Azidobenzyl)-carazolol: A potent β-adrenergic photoaffinity probe. J. Med. Chem. **26**: 832–838, 1983.

305. HEDBERG, A., MINNEMAN, K. P., AND MOLINOFF, P. B.: Differential distribution of beta-1 and beta-2 adrenergic receptors in cat and guinea-pig heart. J. Pharmacol. Exp. Ther. **212**: 503–508, 1980.

306. HEDGES, A., AND TURNER, P.: Beta-receptors in human isolated smooth muscle. Br. J. Pharmacol. **37**: 547P–548P, 1969.

307. HEDLUND, B., ABENS, J., AND BARTFAI, T.: Vasoactive intestinal polypeptide and muscarinic receptors: Supersensitivity induced by long-term atropine treatment. Science **220**: 519–521, 1982.

308. HENSELING, M.: Kinetic constants for uptake and metabolism of ³H-(−)-noradrenaline in rabbit aorta. Naunyn-Schmeideberg's Arch. Pharmacol. **323**: 12–23, 1983.

309. HERLIHY, J. T.: Helically cut vascular strip preparation: Geometrical considerations. Am. J. Physiol. **238**: H107–H109, 1980.

310. HERMSMEYER, K., MASON, R., GRIFFEN, S. H., AND BECKER, P.: Rat cardiac muscle single cell automaticity responses to α- and β-adrenergic agonists and antagonists. Circ. Res. **51**: 532–537, 1982.

311. HESTER, R. K., WEISS, G. B., AND FRY, W. J.: Differing actions of nitroprusside and D-600 on tension and ⁴⁵Ca fluxes in canine renal arteries. J. Pharmacol. Exp. Ther. **208**: 155–160, 1979.

312. HICKS, P. E., AND LANGER, S. Z.: Antagonism by RX781094 of contractile responses to α₁- or α₂-adrenoceptor agonists in the tail artery of SHR *in vitro*. Br. J. Pharmacol. **80**: 474P, 1983.

313. HILDITCH, A., AND DREW, G. M.: Characteristics of the dopamine receptors in the rabbit isolated splenic artery. Eur. J. Pharmacol. **72**: 287–296, 1981.

314. HILL, A. V.: The mode of action of nicotine and curari, determined by the form of the contraction curve and the method of temperature coefficients. J. Physiol. (Lond.) **39**: 361–373, 1909.

315. HILL, A. V.: Diffusion of oxygen and lactic acid through tissues. Proc. R. Soc. Lond. B Biol. Sci. **104**: 39–96, 1928.

316. HILL, R., KEENAM, A. K., NELLIGAN, M., AND O'MALLEY, E.: Facilitatory presynaptic β-adrenoceptors in human atria. Br. J. Pharmacol. **80**: 620P, 1983.

317. HOBBIGER, F., AND LANCASTER, R.: The determination of acetylcholinesterase activity of brain slices and its significance in studies of extracellular acetylcholinesterase. J. Neurochem. **18**: 1741–1749, 1971.

318. HOFFMAN, B. B., LAVIN, T. N., LEFKOWITZ, R. J., AND RUFFOLO, R. R., JR.: Alpha adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. J. Pharmacol. Exp. Ther. **219**: 290–295, 1981.

319. HOLCK, M. I., JONES, C. H. M., AND HAEUSLER, G.: Differential interactions of cloridine and methoxamine with the postsynaptic α-adrenoceptor of rabbit main pulmonary artery. J. Cardiovasc. Pharmacol. **5**: 240–248, 1983.

320. HOLCK, M. I., MARKS, B. H., AND WILBERDING, C. A.: Characterization of alpha-adrenergic receptors in guinea-pig vas deferens by (³H) dihydroergocryptine binding. Mol. Pharmacol. **16**: 77–90, 1979.

321. HOLL, J. E., AND MOKLER, C. M.: Loss of H₂ histamine receptor activity in rabbit aorta after maturity. J. Cardiovasc. Pharmacol. **4**: 136–141, 1982.

322. HOLMBERG, E., JEPPSSON, A.-B., AND WALDECK, B.: Selective development of tolerance to β-adrenoceptor agonists in skeletal muscle as compared with airway smooth muscle from the guinea-pig. Clin. Exp. Pharmacol. Physiol. **8**: 49–56, 1981.

323. HOLMBERG, E., AND WALDECK, B.: Analysis of the β-receptor mediated effect on fast-contracting skeletal muscle *in vitro*. Naunyn-Schmeideberg's Arch. Pharmacol. **301**: 109–113, 1977.

324. HOLMSTEDT, B., AND LILJESTRAND, G.: Readings in Pharmacology, Pergamon Press, New York, 1963.

325. HOMBURGER, V., LUCAS, M., ROSENBAUM, E., VASSENT, G., AND BOCKAERT, J.: Presence of both beta₁- and beta₂-adrenergic receptors in a single cell type. Mol. Pharmacol. **20**: 463–469, 1981.

326. HOOKER, C. S., CALKINS, P. J., AND FLEISCH, J. H.: On the measurement of vascular and respiratory smooth musle response *in vitro*. Blood Vessels **14**: 1–11, 1977.

327. HU, E. H., AND VENTER, J. C.: Adenosine cyclic 3′,5′-monophosphate concentrations during the positive inotropic response of cat cardiac muscle to polymeric immobilized isoproterenol. Mol. Pharmacol. **14**: 237–245, 1978.

328. HUDGINS, P. M., AND FLEMING, W. W.: A relative nonspecific supersensitivity in aortic strips resulting from pretreatment with reserpine. J. Pharmacol. Exp. Ther. **153**: 70–80, 1966.

329. HUDGINS, P. M., AND WEISS, G. B.: Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. J. Pharmacol. Exp. Ther. **159**: 91–97, 1968.

330. HUGHES, I. E., AND SMITH, J. A.: The stability of noradrenaline in physiological saline solutions. J. Pharm. Pharmacol. **30**: 124–126, 1978.

331. HUGHES, J., KOSTERLITZ, H. W., AND LESLIE, F. M.: Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of

332. HUGHES, M. J.: Direct and indirect actions of impromidine (a new H₂-receptor agonist) on atrial tissue. Life Sci. **29**: 817–824, 1981.

333. HUGHES, M. J., AND CORET, I. A.: The effect of pit on rabbit atrial response to histamine. Can. J. Physiol. Pharmacol. **54**: 118–127, 1976.

334. HUGHES, M. J., AND O'BRIEN, L. J.: Liberation of endogenous compounds by tolazoline. Agents Actions **7**: 225–230, 1977.

335. HUIDOBRO-TORO, J. P., AND WAY, E. L.: Comparative study on the effect of morphine and the opioid-like peptides in the vas deferens of rodents: Species and strain differences, evidence for multiple opiate receptors. Life Sci. **28**: 1331–1336, 1981.

336. HUMPHREY, P. P. A.: The effects of uptake on α-adrenoceptor antagonist potency in dog saphenous vein. Br. J. Pharmacol. **63**: 665–669, 1978.

337. HURWITZ, L., HUBBARD, W., AND LITTLE, S.: The relationship between the drug-receptor interaction and calcium transport in smooth muscle. J. Pharmacol. Exp. Ther. **183**: 117–126, 1972.

338. HURWITZ, L., AND WEISSINGER, J.: Effects of variations in extracellular acetylcholine and calcium ion concentration on the operational level of calcium channels in intestinal smooth muscle. J. Pharmacol. Exp. Ther. **214**: 581–588, 1980.

339. IGNARRO, L. J., AND TITUS, E.: The presence of antagonistically acting *alpha* and *beta* adrenergic receptors in the mouse spleen. J. Pharmacol. Exp. Ther. **160**: 72–80, 1968.

340. INGEBRETSEN, C. G., HAWELU-JOHNSON, C., AND INGEBRETSEN, W. R., JR.: Alloxan-induced diabetes reduces β-adrenergic receptor number without affecting adenylate cyclase in rat ventricular membranes. J. Cardiovasc. Pharmacol. **5**: 454–461, 1983.

341. INSEL, P. A., AND KENNEDY, M. S.: Colchicine potentiates β-adrenoreceptor-stimulated cyclic AMP in lymphoma cells by an action distal to the receptor. Nature (Lond.) **273**: 471–472, 1978.

342. INSEL, P. A., AND STOOLMAN, L. M.: Radioligand binding to *beta* adrenergic receptors of intact cultured S49 cells. Mol. Pharmacol. **14**: 549–561, 1978.

343. ISHAC, E. J. N., AND PENNEFATHER, J. N.: The effects of altered thyroid state upon responses mediated by atrial muscarinic receptors in the rat. Br. J. Pharmacol. **79**: 451–459, 1983.

344. ISHAC, E. J. N., PENNEFATHER, J. N., AND HANDBERG, G. M.: Effect of changes in thyroid state on atrial α- and β-adrenoceptors, adenylate cyclase activity, and catecholamine levels in the rat. J. Cardiovasc. Pharmacol. **5**: 396–405, 1983.

345. IWAYAMA, Y., AND TAKAYANAGI, I.: Photoaffinity labelling of dopamine receptors in molluscar smooth muscle. J. Pharm. Pharmacol. **34**: 729–730, 1982.

346. JACOB, L. S., AND TALLARIDA, R. J.: Further studies on the action of ultraviolat light on vascular smooth muscle: Effect of partial irreversible receptor blockade. Arch. Int. Pharmacodyn. Thér. **225**: 166–176, 1977.

347. JACOBS, S., AND CUATRECASAS, P.: The mobile receptor hypothesis and 'cooperativity' of hormone binding application to insulin. Biochim. Biophys. Acta. **433**: 482–495, 1976.

348. JACOBS, S., AND CUATRECASAS, P.: Current concepts. Cell receptors in disease. N. Engl. J. Med. **297**: 1383–1386, 1977.

349. JANSSENS, W., AND VERHAEGHE, R.: Modulation of the concentration of noradrenaline at the neuro-effector junction in human saphenous vein. Br. J. Pharmacol. **79**: 577–585, 1983.

350. JENKINSON, D. H.: Partial agonists in receptor classification. *In* Proceedings of the VI International Symposium on Medicinal Chemistry, ed. by M. A. Simpkins, pp. 373–383, Cotswold Press, Oxford, 1979.

351. JOHANSSON, B.: The β-adrenoceptors in the smooth muscle of pig coronary arteries. Eur. J. Pharmacol. **24**: 218–224, 1973.

352. JOHANSSON, S. R. M., AND ANDERSSON, R. G. G.: Mechanisms of β-adrenergic desensitization in rat myometrium. Acta. Pharmacol. Toxicol. **49**: 241–247, 1981.

353. JORDAN, C. C., AND OWEN, D. G.: Dithiothreitol potentiates the depolarizing action of substance P in the frog spinal cord *in vitro*. Br. J. Pharmacol. **69**: 285P, 1979.

354. KAHN, C. R.: Membrane receptors for hormones and neurotransmitters. J. Cell. Biol. **70**: 261–286, 1976.

355. KALSNER, S.: Differential activation of the inner and outer muscle cell layers of the rabbit ear artery. Eur. J. Pharmacol. **20**: 122–124, 1972.

356. KALSNER, S.: Sensitization of effector responses by modification of agonist disposition mechanisms. Can. J. Physiol. Pharmacol. **54**: 177–187, 1976.

357. KARLIN, A.: On the application of a "plausible model" of allosteric proteins to the receptor for acetylcholine. J. Theor. Biol. **16**: 306–320, 1967.

358. KATZ, B., AND THESLEFF, S.: A study of the 'desensitization' produced by acetylcholine at the motor end-plate. J. Physiol. (Lond.) **138**: 63–80, 1957.

359. KAUMANN, A. J.: On spare β-adrenoceptors for inotropic effects of catecholamines in kitten ventricle. Naunyn-Schmeideberg's Arch. Pharmacol. **305**: 97–102, 1978.

360. KAUMANN, A. J.: Cultured heart cells as a model for β-adrenoceptors in a heart pacemaker. Chronotropic spare β-adrenoceptors and spare adenylyl cyclase for (−)-isoprenaline but not for (−)-dichloroisoprenaline in rat cardiocytes. Naunyn-Schmeideberg's Arch. Pharmacol. **320**: 119–129, 1982.

361. KAUMANN, A. J.: Yohimbine and rauwolscine inhibit 5-hydroxytryptamine-

induced contraction of large coronary arteries of calf through blockade of 5 HT$_2$ receptors. Naunyn-Schmeideberg's Arch. Pharmacol. **323:** 149–154, 1983.

362. KAUMANN, A. J., AND BIRNBAUMER, L.: Desensitization of kitten atria to chronotropic, inotropic and adenylyl cyclase stimulating effects of (−)-isoprenaline. Naunyn-Schmeideberg's Arch. Pharmacol. **293:** 199–202, 1976.

363. KAUMANN, A. J., AND BLINKS, J. R.: Stimulant and depressant effects of β-adrenoceptor blocking agents on isolated heart muscle. Naunyn-Schmeideberg's Arch. Pharmacol. **311:** 205–218, 1980.

364. KAUMANN, A. J., AND HENNEKES, R.: The affinity of atropine for muscarinic receptors in human sphincter pupillae. Naunyn-Schmeideberg's Arch. Pharmacol. **306:** 209–211, 1979.

365. KAUMANN, A. J., LEMOINE, H., MORRIS, T. H., AND SCHWEDERSKI, U.: An initial characterization of human heart β-adrenoceptors and their mediation of the positive inotropic effects of catecholamines. Naunyn-Schmeideberg's Arch. Pharmacol. **319:** 216–221, 1982.

366. KAUMANN, A. J., AND MARANO, M.: On equilibrium dissociation constants for complexes of drug-receptor subtypes. Selective and non-selective interactions of partial agonists with two plausible β-adrenoceptor subtypes mediating positive chronotropic effects of (−)-isoprenaline in kitten atria. Naunyn-Schmeideberg's Arch. Pharmacol. **318:** 192–201, 1982.

367. KENAKIN, T. P.: Errors in the measurement of agonist potency-ratios produced by uptake processes: A general model applied to β-adrenoceptors agonists. Br. J. Pharmacol. **71:** 407–417, 1980.

368. KENAKIN, T. P.: On the importance of agonist concentration-gradients within isolated tissues. Increased maximal responses of rat vasa deferentia to (−)-noradrenaline after blockade of neuronal uptake. J. Pharm. Pharmacol. **32:** 833–838, 1980.

369. KENAKIN, T. P.: Effects of equilibration time on the attainment of equilibrium between antagonists and drug receptors. Eur. J. Pharmacol. **66:** 295–306, 1980.

370. KENAKIN, T. P.: An *in vitro* quantitative analysis of the *alpha* adrenoceptor partial agonist activity of dobutamine and its relevance to inotropic selectivity. J. Pharmacol. Exp. Ther. **216:** 210–219, 1981.

371. KENAKIN, T. P.: A pharmacological method to estimate the pK$_I$ of competitive inhibitors of agonist uptake processes in isolated tissues. Naunyn-Schmeideberg's Arch. Pharmacol. **316:** 89–95, 1981.

372. KENAKIN, T. P.: The potentiation of cardiac responses to adenosine by benzodiazepines. J. Pharmacol. Exp. Ther. **222:** 752–758, 1982.

373. KENAKIN, T. P.: Organ selectivity of drugs. Alternatives to receptor selectivity. Trends Pharmacol. Sci. **3:** 153–156, 1982.

374. KENAKIN, T. P.: The Schild regression in the process of receptor classification. Can. J. Physiol. Pharmacol. **60:** 249–265, 1982.

375. KENAKIN, T. P.: Theoretical and practical problems with the assessment of intrinsic efficacy of agonists: Efficacy of reputed beta-1 selective adrenoceptor agonists for beta-2 adrenoceptors. J. Pharmacol. Exp. Ther. **223:** 416–423, 1982.

376. KENAKIN, T. P.: Receptor classification by selective agonists: Coping with circularity and circumstantial evidence. Trends Pharmacol. Sci. **4:** 291–295, 1983.

377. KENAKIN, T. P.: The relative contribution of affinity and efficacy to agonist activity: Organ selectivity of noradrenaline and oxymetazoline with reference to the classification of drug receptors. Br. J. Pharmacol. **81:** 131–143, 1984.

378. KENAKIN, T. P., AND ANGUS, J. A.: The histamine-like effects of tolazoline and clonidine: Evidence against direct activity at histamine receptors. J. Pharmacol. Exp. Ther. **219:** 474–480, 1981.

379. KENAKIN, T. P., AND BEEK, D.: Is prenalterol (H133/80) really a selective beta-1 adrenoceptor agonist? Tissue selectivity resulting from differences in stimulus-response relationships. J. Pharmacol. Exp. Ther. **213:** 406–412, 1980.

380. KENAKIN, T. P., AND BEEK, D.: The measurement of antagonists potency and the importance of selective inhibition of agonist uptake processes. J. Pharmacol. Exp. Ther. **219:** 112–120, 1981.

381. KENAKIN, T. P., AND BEEK, D.: A quantitative analysis of histamine H$_2$-receptor-mediated relaxation of rabbit trachea. J. Pharmacol. Exp. Ther. **220:** 353–357, 1982.

382. KENAKIN, T. P., AND BEEK, D.: *In vitro* studies on the cardiac activity of prenalterol with reference to use in congestive heart failure. J. Pharmacol. Exp. Ther. **220:** 77–85, 1982.

383. KENAKIN, T. P., AND BEEK, D.: Relative efficacy of prenalterol and pirbuterol for β$_1$-adrenoceptors: Measurement of agonist affinity by alteration of receptor number. J. Pharmacol. Exp. Ther., **229:** 340–345, 1984.

384. KENAKIN, T. P., AND BEEK, D.: The measurement of the relative efficacy of partial agonists in isolated tissues by selective potentiation of tissue responses: The relative efficacy of prenalterol and pirbuterol in cardiac tissue. J. Auton. Pharmacol., in press, 1984.

385. KENAKIN, T. P., AND BLACK, J. W.: The pharmacological classification of practolol and chloropractolol. Mol. Pharmacol. **14:** 607–623, 1978.

386. KENAKIN, T. P., AND COOK, D. A.: Blockade of histamine-induced contractions of guinea pig ileum by β-haloalkylamines. Can. J. Physiol. Pharmacol. **54:** 386–392, 1976.

387. KENAKIN, T. P., AND COOK, D. A.: N,N-Diethyl-2-(1-pyridyl)ethylamine, a

partial agonist for the histamine receptor in guinea pig ileum. Can. J. Physiol. Pharmacol. **58:** 1307–1310, 1980.

388. KENAKIN, T. P., AND FERRIS, R. M.: Effects of *in vivo* β-adrenoceptor down-regulation on cardiac responses to prenalterol and pirbuterol. J. Cardiovasc. Pharmacol. **5:** 90–97, 1983.

389. KENAKIN, T. P., AND LEIGHTON, H. J.: Pharmacological estimation of potencies of agonists and antagonists in the classification of adenosine receptors. *In* Methods in Pharmacology. Methods Used in Adenosine Research, ed. by D. M. Paton, Plenum Publ. Co., New York, 1984, in press.

390. KHANNA, O. P., BARBIERI, E. J., AND McMICHAEL, R. F.: The effects of adrenergic agonists and antagonists on vesicourethral smooth muscle of rabbits. J. Pharmacol. Exp. Ther. **216:** 95–100, 1981.

391. KIEFER, H., LINDSTROM, J., LENNOX, E. S., AND SINGER, S. J.: Photoaffinity labeling of specific acetylcholine-binding sites on membranes. Proc. Natl. Acad. Sci. **67:** 1688–1694, 1970.

392. KO, K. C., AND PARADISE, R. R.: The effects of substrates on contractility of isolated rat atria depressed by hydrochloric acid. Proc. Soc. Exp. Biol. Med. **136:** 178–182, 1971.

393. KOCH-WESER, J.: Influence of osmolarity of perfusate on contractility of mammalian myocardium. Am. J. Physiol. **204:** 957–962, 1963.

394. KOCH-WESER, J., AND BLINKS, J. R.: The influence of the interval between beats on myocardial contractility. Pharmacol. Rev. **15:** 601–652, 1963.

395. KOGER, Y., DOWNES, H., AND TAYLOR, S. M.: Direct and indirect actions of dopamine on tracheal smooth muscle. Naunyn-Schmeideberg's Arch. Pharmacol. **315:** 15–20, 1980.

396. KONO, T., AND BARHAM, F. W.: The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin. J. Biol. Chem. **246:** 6210–6216, 1971.

397. KOSTERLITZ, H. W., AND WATT, A. J.: Kinetic parameters of narcotic agonists and antagonists with particular reference to N-allylnoroxymorphone (naloxone). Br. J. Pharmacol. Chemother. **33:** 266–276, 1968.

398. KREBS, H. A.: Body size and tissue respiration. Biochim. Biophys. Acta **4:** 249–269, 1950.

399. KREBS, H. A., AND HENSELEIT, K.: Untersuchungen über die harnstoffbildung im tierkörper. Z. Physiol. Chem. **210:** 33–66, 1932.

400. KRNJEVIC, K., AND MITCHELL, J. F.: Diffusion of acetylcholine in agar gels and in the isolated rat diaphragm. J. Physiol. (Lond.) **153:** 562–572, 1960.

401. KUSHMERICK, M. J., AND PODOLSKY, R. J.: Ionic mobility in muscle cells. Science **166:** 1297–1298, 1969.

402. LAHER, I., AND McNEILL, J. H.: The effects of 2-(2-pyridyl)ethylamine (PEA) in the isolated guinea-pig heart. Proc. West. Pharmacol. Soc. **23:** 41–43, 1980.

403. LAI, R.-T., WATANABE, Y., AND YOSHIDA, H.: The influence of aging on α-adrenoceptors in rat heart and vas deferens. Jap. J. Pharmacol. **33:** 241–245, 1983.

404. LAKATTA, E. G.: Age-related alterations in the cardiovascular response to adrenergic mediated stress. Fed. Proc. **39:** 3173–3177, 1980.

405. LAKATTA, E. G., GERSTENBLITH, G., ANGELL, C. S., SHOCK, N. W., AND WEISFELDT, M. L.: Diminished inotropic response of aged myocardium to catecholamines. Circ. Res. **36:** 262–269, 1975.

406. LAKATTA, E. G., AND YIN, F. C. P.: Myocardial aging: Functional alterations and related cellular mechanisms. Am. J. Physiol. **242:** H927–H941, 1982.

407. LAMAR, J.-C., AND EDVINSSON, L.: 5-Hydroxytryptamine receptors. Contractile activity and mode of inhibition by methysergide in mammalian intracranial and extracranial vessels. Arch. Int. Pharmacodyn. Thér. **243:** 245–254, 1980.

408. LA MORTE, W. W., HINGSTON, S. J., AND WISE, W. E.: pH-dependent activity of H$_1$- and H$_2$-histamine receptors in guinea-pig gall bladder. J. Pharmacol. Exp. Ther. **217:** 638–644, 1981.

409. LANGENDORFF, O.: Untersuchurgen am überletenden Säugethierherzen. Pflügers Arch. Ges. Physiol. **61:** 291–332, 1895.

410. LANGER, S. Z., AND TRENDELENBURG, U.: The effect of a saturable uptake mechanisms on the slopes of dose-response curves for sympathomimetic amines and on the shifts of dose-response curves produced by a competitive antagonist. J. Pharmacol. Exp. Ther. **167:** 117–142, 1969.

411. LANGLEY, J. N.: On the physiology of the salivary secretion. J. Physiol. (Lond.) **1:** 339–369, 1878.

412. LAVIN, T. N., HEALD, S. L., JEFFS, P. W., SCHORR, R. G. L., LEFKOWITZ, R. J., AND CARON, M. G.: Photoaffinity labeling of the β-adrenergic receptor. J. Biol. Chem. **256:** 11944–11950, 1981.

413. LECLERC, G., ROUOT, B., VELLY, J., AND SCHWARTZ, J.: β-Adrenergic receptor subtypes. Trends Pharmacol. Sci. **2:** 18–20, 1981.

414. Lefebore, R. A., Blancquaert, J. P., Willems, J. L., and Bogaert, M. G.: *In vitro* study of the inhibitory effects of dopamine on the rat gastric fundus. Naunyn-Schmeideberg's Arch. Pharmacol. **322:** 228–236, 1983.

415. LEIGHTON, H. J.: Quantitative assessment of the pre- and postsynaptic *alpha* adrenoceptor antagonist potency of amitriptyline. J. Pharmacol. Exp. Ther. **220:** 299–304, 1982.

416. LEIGHTON, H. J., AND SU, Y. F.: Determination of presynaptic receptor dissociation constants using functional antagonism. J. Pharmacol. Exp. Ther., 1984, in preparation.

417. LEMOINE, H., AND KAUMANN, A. J.: A model for the interaction of competitive antagonists with two receptor-subtypes characterized by a Schild-

Plot with apparent slope unity. Naunyn-Schmeideberg's Arch. Pharmacol. **322**: 111–120, 1983.

418. LESTER, H. A.: The response to acetylcholine. Sci. Am. **236**: 106–116, 1977.

419. LEVI, R., HORDOF, A., EDIE, R., AND ROSEN, M. L.: Histamine effects on human atria (abstr.). Circ. **57,58**: suppl. II, II-105, 1978.

420. LEVIN, J. A., AND FURCHGOTT, R. F.: Interactions between potentiating agents of adrenergic amines in rabbit aortic strips. J. Pharmacol. Exp. Ther. **172**: 320–331, 1970.

421. LEVIN, R. M., AND WEIN, A. J.: Distribution and function of adrenergic receptors in the urinary bladder of the rabbit. Mol. Pharmacol. **16**: 441–448, 1979.

422. LI, C. K., AND MITCHELSON, F.: The selective antimuscarinic action of stercuronium. Br. J. Pharmacol. **70**: 313–321, 1980.

423. LIGHT, K. E., AND HUGHES, M. J.: Depressive action of clonidine on guinea-pig and rabbit atrial pairs. Res. Commun. Pathol. Pharmacol. **23**: 433–451, 1979.

424. LULICH, K. M., MITCHELL, H. W., AND SPARROW, M. P.: The cat lung strip as an *in vitro* preparation of peripheral airways: A comparison of β-adrenoceptor agonists, antacoids and araphylactic challenge on the lung strip and trachea. Br. J. Pharmacol. **58**: 71–79, 1976.

425. LULICH, K. M., AND PATERSON, J. W.: An *in vitro* study of various drugs on central and peripheral airways of the rat: A comparison with human airways. Br. J. Pharmacol. **68**: 633–636, 1980.

426. LUMLEY, P., AND BROADLEY, K. J.: Differential blockade of guinea-pig atrial rate and force response to (−)-noradrenaline by practolol—an uptake phenomenon. Eur. J. Pharmacol. **34**: 207–217, 1975.

427. MACDONALD, A., AND MCGRATH, J. C.: The distribution of adrenoceptors and other drug receptors between the two ends of the rat vas deferens as revealed by selective agonists and antagonists. Br. J. Pharmacol. **71**: 445–458, 1980.

428. MACKAY, D.: A flux-carrier hypothesis of drug action. Nature (Lond.) **197**: 1171–1173, 1963.

429. MACKAY, D.: A general analysis of the receptor-drug interaction. Br. J. Pharmacol. **26**: 9–16, 1966.

430. MACKAY, D.: A new method for the analysis of drug-receptor interactions. *In* Advances in Drug Research, ed. N. J. Harper and A. B. Simmonds, vol. 3, pp. 1–19, Academic Press, London, New York, 1966.

431. MACKAY, D.: A critical survey of receptor theories of drug action. *In* Kinetics of Drug Action, ed. by J. M. van Rossum, pp. 255–322, Springer-Verlag, Berlin, Heidelberg, New York, 1977.

432. MACKAY, D.: How should values of pA₂ and affinity constants for pharmacological competitive antagonists be estimated? J. Pharm. Pharmacol. **30**: 312–313, 1978.

433. MACKAY, D.: An analysis of functional antagonism and synergism. Br. J Pharmacol. **73**: 127–134, 1981.

434. MACKAY, D.: Dose-response curves and mechanisms of drug action. Trends Pharmacol. Sci. **3**: 496–499, 1982.

435. MACKAY, D., AND WHEELER, J.: A useful transformation for comparing dose-response curves. J. Pharm. Pharmacol. **26**: 569–581, 1974.

436. MAENGWYN-DAVIES, G. D.: The dual mode of action of histamine in the cat isolated tracheal chain. J. Pharm. Pharmacol. **20**: 572–573, 1968.

437. MAGNAN, J., AND REGOLI, D.: Characterization of receptors for angiotensin in the rat vas deferens. Can. J. Physiol. Pharmacol. **57**: 417–423, 1979.

438. MAGUIRE, M. E., ROSS, E. M., AND GILMAN, A. G.: β-Adrenergic receptor: Ligand binding properties and the interaction with adenylyl cyclase. Adv. Cyclic Nucleotide Res. **8**: 1–83, 1977.

439. MAGUIRE, M. E., VAN ARSDALE, P. M., AND GILMAN, A.: An agonist specific effect of guanine nucleotides on binding to the beta-adrenergic receptor. Mol. Pharmacol. **12**: 335–339, 1976.

440. MARANO, M., AND KAUMANN, A. J.: On the statistics of drug-receptor constants for partial agonists. J. Pharmacol. Exp. Ther. **198**: 518–525, 1976.

441. MARSHALL, I.: An inhibitory histamine H₂-receptor in the mouse vas deferens. Br. J. Pharmacol. **62**: 447P, 1978.

442. MARSHALL, P. B.: Some chemical and physical properties associated with histamine antagonism. Br. J. Pharmacol. **10**: 270–278, 1955.

443. MASTRANGELO, D., AND MATHISON, R.: Everted portal vein: A sensitive model for studies of vasoactive compounds. J. Cardiovasc. Pharmacol. **5**: 98–101, 1983.

444. MATTSSON, H., HEDBERG, A., AND CARLSSON, E.: Intrinsic sympathomimetic activity of the partial agonist prenalterol in relation to *beta* adrenoceptor interaction in various tissues *in vitro*. J. Pharmacol. Exp. Ther. **224**: 654–661, 1983.

445. MAXWELL, R. A.: The State of the Art of the Science of Drug Discovery—an Opinion. Drug. Dev. Res., 1984, in press.

446. MAXWELL, R. A., ECKHARDT, S. B., AND WASTILA, W. B.: Concerning the distribution of endogenous norepinephrine in the adventitial and media-intimal layers of the rabbit aorta and the capacity of these layers to bind tritiated norepinephrine. J. Pharmacol. Exp. Ther. **161**: 34–39, 1968.

447. MAXWELL, R. A., FERRIS, R. M., AND BURCSU, J. E.: Structural requirements for inhibition of noradrenaline uptake by phenethylamine derivatives, desipramine, cocaine, and other compounds. *In* The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines, ed. by D. M. Paton, pp. 95–153, Raven Press, New York, 1976.

448. MCCALDEN, T. A., AND BEVAN, J. A.: Sources of activator calcium in rabbit basilar artery. Am. J. Physiol. (Heart Circ. Physiol. 10) **241**: H129–H133, 1981.

449. MCELROY, W. T., JR., GERDES, A. J., AND BROWN, E. B., JR.: Effects of CO₂, bicarbonate and pH on the performance of isolated perfused guinea-pig hearts. Am. J. Physiol. **195**: 412–416, 1958.

450. MENDELSON, C., DUFAU, M., AND CATT, K.: Gonadotrophin binding and stimulation of cyclic adenosine 3':5'-monophosphate and testosterone production in isolated leydig cells. J. Biol. Chem. **250**: 8818–8823, 1975.

451. MICHELSON, M. J.; AND SHELKOVNIKOV, S. A.: Isotonic and isometric responses of different tonic muscles to agonists and antagonists. Br. J. Pharmacol. **56**: 457–467, 1976.

452. MICKEY, J. V., TATE, R., MULLIKIN, D., AND LEFKOWITZ, R. J.: Regulation of adenylate cyclase-coupled beta adrenergic receptor binding sites by beta adrenergic catecholamines *in vitro*. Mol. Pharmacol. **12**: 409–419, 1976.

453. MIKKELSEN, E., ANDERSSON, K.-E., AND PEDERSEN, O. L.: Effects of digoxin on isolated human peripheral arteries and veins. Acta. Pharmacol. Toxicol. **45**: 249–256, 1979.

454. MIKKELSEN, E. O., SAKR, A. M. R., AND JESPERSEN, L. T.: Effects of nifedipine on contractile responses to potassium, histamine, and 5-hydroxytryptamine in isolated human pulmonary vessels. J. Cardiovasc. Pharmacol. **5**: 317–320, 1983.

455. MILLER, J. W.: Adrenergic receptors in the myometrium. Ann. N.Y. Acad. Sci. **139**: 788–798, 1967.

456. MINNEMAN, K. P., FOX, A. W., AND ABEL, P. W.: Occupancy of alpha₁-adrenergic receptors and contraction of rat vas deferens. Mol. Pharmacol. **23**: 359–368, 1983.

457. MINNEMAN, K. P., HEDBERG, A., AND MOLINOFF, P. B.: Comparison of *beta* adrenergic receptor subtypes in mammalian tissues. J. Pharmacol. Exp. Ther. **211**: 502–508, 1979.

458. MINNEMAN, K. P., HEGSTRAND, L. R., AND MOLINOFF, P. B.: The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung *in vitro*. Mol. Pharmacol. **16**: 21–33, 1979.

459. MINNEMAN, K. P., HEGSTRAND, L. R., AND MOLINOFF, P. B.: Simultaneous determination of beta-1 and beta-2 adrenergic receptors in tissues containing both receptors subtypes. Mol. Pharmacol. **16**: 34–46, 1979.

460. MITTAG, T. W., EHRENPREIS, S., DETWILER, P., AND BOYLE, R.: Some properties of cholinesterases in intact rat diaphragm *in vitro*. Arch. Int. Pharmacodyn. Thér. **191**: 261–269, 1971.

461. MITTAG, T. W., EHRENPREIS, S., AND HEHIR, R. M.: Functional acetylcholinesterase of rat diaphragm muscle. Biochem. Pharmacol. **20**: 2263–2273, 1971.

462. MITTAG, T. W., EHRENPREIS, S., AND PATRICK, P.: Some properties of cholinesterase in intact guinea-pig ileum *in vitro*.. Arch. Int. Pharmacodyn. Thér. **191**: 270–278, 1971.

463. MOORE, G. E., AND O'DONNELL, S. R.: A potent β-adrenoceptor blocking drug: 4-(2-hydroxy-3-isopropylamino propoxy) indole. J. Pharm. Pharmacol. **22**: 180–188, 1970.

464. MORISHITA, H.: Distribution and characterization of the adrenoceptors in dog coronary arteries. Arch. Int. Pharmacodyn. Thér. **239**: 195–207, 1979.

465. MORLEY, J. S.: A common opiate receptor? Trends Pharmacol. Sci. **4**: 370–371, 1983.

466. MORRIS, T. H., AND KAUMANN, A. J.: The β₁-adrenoceptor is preferentially coupled to the adenylyl cyclase: Implications for heart function. Naunyn-Schmeideberg's Arch. Pharmacol., in press.

467. MOULDS, R. F. W., AND STEVENS, M. J.: Facilitatory prejunctional β-adrenoceptors in human arteries and veins. Gen. Pharmacol. **14**: 81–83, 1983.

468. MÜLLER-SCHWEINITZER, E.: Tissue specific susceptibility of alpha-adrenoceptor mediated vasoconstriction to nifedipine. Naunyn-Schmeideberg's Arch. Pharmacol. **324**: 64–69, 1983.

469. MURAMATSU, I., FUJIWARA, M., AND SHIBATA, S.: Reactivity of isolated canine cerebral arteries to adenine nucleotides and adenosine. Pharmacologist **21**: 198–205, 1980.

470. NAGASAKA, M., BOUCKAERT, J., DE SCHAEPDRYVER, A. F., AND HEYMANS, C.: Adrenergic constriction in isolated guinea-pig lung revealed by nethalide. Arch. Int. Pharmacol. Thér. **149**: 237–242, 1964.

471. NAHORSKI, S. R.: α- and β-adrenoceptor coupling to adenylate cyclase. Biochem. Soc. Trans. 600th Meeting, Oxford University Press, Oxford, **10**: 498–500, 1982.

472. NEDERGAARD, O. A., WESTERMANN, E.: Action of various sympathomimetic amines on isolated stripped vas deferens of the guinea-pig. Br. J. Pharmacol. **34**: 475–483, 1968.

473. NICHOLSON, C. D., AND BROADLEY, K. J.: Irreversible β-adrenoceptor blockade of atrial rate and tension responses. Eur. J Pharmacol. **52**: 259–269, 1978.

474. NICKERSON, M.: Receptor occupancy and tissue response. Nature (Lond.) **178**: 697–698, 1956.

475. NICKERSON, M., AND GOODMAN, L. S.: Pharmacological properties of a new adrenergic blocking agent: N,N-dibenzyl-β-chloro-ethylamine (dibenamine). J. Pharmacol. Exp. Ther. **89**: 167–185, 1947.

476. NOMURA, Y., HIROKO, K., AND SEGAWA, T.: Hypersensitivity of cardiac β-adrenergic receptors after neonatal treatment of rats with 6-hydroxydopa. Eur. J. Pharmacol. **66**: 225–232, 1980.

477. NOMURA, Y., KAJIYAMA, H., AND SEGAWA, T.: Decrease in muscarinic cholinergic response of the rat heart following treatment with 6-hydroxydopa. Eur. J. Pharmacol. **60**: 323–327, 1979.

478. NOMURA, Y., KAJIYAMA, H., AND SEGAWA, T.: Alteration in sensitivity to isoproterenol and acetylcholine in the rat heart after repeated administration of isoproterenol. J. Pharmacol. Exp. Ther. **220**: 411–416, 1982.

479. NORESSON, E., THORÉN, P., AND HALLBÄCK-NORDLANDER, M.: Performance of the hypertrophied left ventricle in spontaneously hypertensive rats. Effects of adrenergic stimulation. Acta. Physiol. Scand. **114**: 497–504, 1982.

480. NORTON, J. M., AND DETAR, R.: Potassium and isolated coronary vascular smooth muscle. Am. J. Physiol. **222**: 474–479, 1972.

481. O'DONNELL, J. P., HOGABOOM, G. K., AND FEDAN, J. S.: Specific, irreversible antagonism of histamine receptors in the isolated guinea-pig vas deferens by 4(5)-[2-(4-azido-2-nitroanilino)ethyl]imidazole, a photoaffinity analog of histamine. Eur. J. Pharmacol. **73**: 261–271, 1981.

482. O'DONNELL, J. P., HOGABOOM, G. K., AND FEDAN, J. S.: Comparison of photoaffinity labeling of P_2-purinergic receptors of isolated guinea-pig vas deferens by arylazido aminopropionyl ATP and by arylazido aminobutyryl ATP. Eur. J. Pharmacol. **86**: 435–440, 1983.

483. O'DONNELL, S. R., AND WANSTALL, J. C.: The contribution of extraneuronal uptake to the trachea-blood vessel selectivity of β-adrenoceptor stimulants in vitro in guinea-pigs. Br. J. Pharmacol. **57**: 369–373, 1976.

484. O'DONNELL, S. R., AND WANSTALL, J. C.: The use of functional antagonism to determine whether β-adrenoceptor agonists must have a lower efficacy than isoprenaline to be trachea-atria selective in vitro in guinea-pigs. Br. J. Pharmacol. **60**: 255–262, 1977.

485. O'DONNELL, S. R., AND WANSTALL, J. C.: The importance of choice of agonist in studies designed to predict $\beta_2:\beta_1$ adrenoceptor selectivity of antagonists from pA_2 values on guinea-pig trachea and atria. Naunyn-Schmeideberg's Arch. Pharmacol. **308**: 183–190, 1979.

486. O'DONNELL, S. R., AND WANSTALL, J. C.: Pharmacological approaches to the characterization of β-adrenoreceptor populations in tissues. J. Auton. Pharmacol. **1**: 305–312, 1981.

487. O'DONNELL, S. R., AND WANSTALL, J. C.: Relaxation of cat trachea by β-adrenoceptor agonists can be mediated by both β_1- and β_2-adrenoceptors and potentiated by inhibitors or extraneuronal uptake. Br. J. Pharmacol. **78**: 417–424, 1983.

488. OFFERMEIER, J., AND ARIËNS, E. J.: Serotonin. I. Receptors involved in its action. Arch. Int. Pharmacodyn. Thér. **164**: 192–245, 1966.

489. OGLETREE, M. L., SMITH, J. B., AND LEFER, A. M.: Actions of prostaglandins on isolated perfused cat coronary arteries. Am. J. Physiol. **235**: H400–H406, 1978.

490. OHASHI, M., IWASAWA, Y., AND KIYOMOTO, A.: Studies on the mode of antagonism between adrenergic β-mimetics and β-blocking agents. II. Analysis by the uptake saturation model. Jpn. J. Pharmacol. **26**: 79–90, 1976.

491. OHHASHI, T., AND AZUMA, T.: Paradoxical relaxation of arterial strips induced by vaso-constrictive agents. Blood Vessels **17**: 16–26, 1980.

492. OUDART, N., SERCOMBE, R., AUBINEAU, P., BOULU, R. G., AND SEYLAZ, J.: Relaxation by dopaminergic agonists in cerebral and peripheral arteries (in vitro). Arch. Int. Pharmacodyn. Thér. **252**: 196–209, 1981.

493. OVERSTREET, D. H., AND YAMAMURA, H. I.: Minireview: Receptor alterations and drug tolerance. Life Sci. **25**: 1865–1878, 1979.

494. PAGE, E.: Cat heart muscle in vitro. J. Gen. Physiol. **46**: 201–213, 1962.

495. PAGE, E., AND BERNSTEIN, R. S.: Cat heart muscle in vitro. V. Diffusion through a sheet of right ventricle. J. Gen. Physiol. **47**: 1129–1140, 1964.

496. PALM, D., LANGENECKERT, W., AND HOLTZ, P.: Bedeutung der N- and α-methylierung für die affinität von brenzcatechinaminen zu den adrenergischin receptoren. Naunyn-Schmiedeberg's Arch. Pharmacol. **258**: 128–149, 1967.

497. PARKER, R. B.: Measurement of drug-receptor dissociation constants of muscarinic agonists on intestinal smooth muscle. J. Pharmacol. Exp. Ther. **180**: 62–70, 1972.

498. PARKER, R. B., AND WAUD, D. R.: Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. J. Pharmacol. Exp. Ther. **177**: 1–12, 1971.

499. PASCUAL, R., AND BEVAN, J. A.: Characteristics of contractile responses of the rabbit aortic strip to norepinephrine entering through the adventitia or through the intima. Proc. West. Pharmacol. Soc. **21**: 95–98, 1978.

500. PASCUAL, R., AND BEVAN, J. A.: Variation in contractile response characteristics of rabbit aorta strips with surface of drug entry. J. Pharmacol. Exp. Ther. **210**: 51–55, 1979.

501. PASCUAL, R., AND BEVAN, J. A.: Asymmetry of consequences of drug disposition mechanisms in the wall of the rabbit aorta. Circ. Res. **46**: 22–28, 1980.

502. PATIL, P. N.: Adrenergic receptors of the bovine iris sphincter. J. Pharmacol. Exp. Ther. **166**: 299–307, 1969.

503. PATIL, P. N., FUDGE, K., AND JACOBOWITZ, D.: Steric aspects of adrenergic drugs. XVIII. α-Adrenergic receptors of mammalian aorta. Eur. J. Pharmacol. **19**: 79–87, 1972.

504. PATIL, P. N., MILLER, D. D., AND TRENDELENBURG, U.: Molecular geometry and adrenergic drug activity. Pharmacol. Rev. **26**: 323–392, 1975.

505. PATIL, P. N., PATEL, D. G., AND KRELL, R. D.: Steric aspects of adrenergic drugs. XV. Use of isomeric activity ratio as a criterion to differentiate adrenergic receptors. J. Pharmacol. Exp. Ther. **176**: 622–633, 1971.

506. PATON, W. D. M.: A theory of drug action based on the rate of drug-receptor combination. Proc R. Soc. Lond. B Biol. Sci. **154**: 21–69, 1961.

506A. PATON, W. D. M., AND RANG, H. P.: The kinetic approach to drug antagonism. In Advances in Drug Research. ed. by N. J. Harper, and A. B. Simmonds, vol. 3, pp. 57–80, Academic Press, London, New York, 1966.

507. PATON, W. D. M., AND WAUD, D. R.: The margin of safety of neuromuscular transmission. J. Physiol. (Lond.) **191**: 59–90, 1967.

508. PATON, W. D. M., AND ROTHSCHILD, A. M.: The effect of varying calcium concentration on the kinetic constants of hyoscine and mepyramine antagonism. Br. J. Pharmacol. **24**: 432–436, 1965.

509. PATON, W. D. M., AND ZAR, A.: A denervated preparation of the longitudinal muscle of the guinea-pig ileum. J. Physiol (Lond.) **179**: 85P–86P, 1965.

510. PEGRAM, B. L., BEVAN, R. D., AND BEVAN, J. A.: Facial vein of the rabbit. Neurogenic vasodilation mediated by β-adrenergic receptors. Circ. Res. **39**: 854–860, 1976.

511. PENDLETON, R. G., TORCHIANA, M. L., HANSON, C. A., AND CLINE-SCHMIDT, B. V.: Studies defining 3-N-[3-[3-(1-piperidinomethyl)phenoxy)propyl]amino-4-amino-1,2,5-thiadiazole-1-oxide (L-643,441) as a competitive, irreversible histamine H_2-receptor antagonist in guinea-pig atria. Fed. Proc. **41**: 1555, 1982.

512. PENNEFATHER, J. N.: A comparison of the responses of the isolated vas deferens of the rat to field stimulation and to noradrenaline added cumulatively and non-cumulatively. Eur. J. Pharmacol. **23**: 245–250, 1973.

513. PENNEFATHER, J. N.: The effects of cocaine and diphenhydramine upon the reactivity of rat vas deferens to supramaximal doses of noradrenaline and of other agonists: The mode of action of cocaine. Eur. J. Pharmacol. **35**: 333–339, 1976.

514. PERRY, W. L. M.: Reports on biological standards. VI. The design of toxicity tests. Med. Res. Coun. (G.B.) Spec. Rep. Ser. **270**: 1–50, 1950.

515. PERSSON, H., AND JOHNSON, B.: Adrenergic receptors in the guinea-pig trachea and lung. Acta Pharmacol. Toxicol. **28**: 49–56, 1970.

516. PERT, C. B., AND SYNDER, S. H.: Opiate receptor binding of agonists and antagonists affected differentially by sodium. Mol. Pharmacol. **10**: 868–879, 1974.

517. PFEIFFER, C. C.: Optical isomerism and pharmacological action, a generalization. Science **124**: 29–31, 1956.

518. PITHA, J., MILECKI, J., CZAJKOWSKA, T., AND KUSIAK, J. W.: β-Adrenergic antagonists with multiple pharmacophores: Persistent blockade of receptors. J. Med. Chem. **26**: 7–11, 1983.

519. POCHET, R., AND DELESPESSE, G.: β-Adrenoreceptors display different efficiency on lymphocyte subpopulations. Biochem. Pharmacol. **32**: 1651–1655, 1983.

520. POINTON, S. E., AND BANERJEE, S. P.: β-Adrenergic and muscarinic cholinergic receptors in rat submaxillary glands. Effects of thyroidectomy. Biochim. Biophys. Acta **583**: 129–132, 1979.

521. POLANIN, A., AND MCNEILL, J. H.: Characterization of the histamine receptors in rabbit left atria. Can. J. Physiol. Pharmacol. **59**: 19–24, 1980.

522. PORRECA, F., AND BURKS, T. F.: Affinity of normorphine for its pharmacologic receptor in the naive and morphine-tolerant guinea-pig isolated ileum. J. Pharmacol. Exp. Ther. **225**: 688–693, 1983.

523. PORTER, W. T.: A new method for the study of the isolated mammalian heart. Am. J. Physiol. **1**: 511–518, 1898.

524. PORTOGHESE, P. S., LARSON, D. L., JIANG, J. B., CARUSO, T. P., AND TAKEMORI, A. E.: Synthesis and pharmacological characterization of an alkylating analogue (chlornaltexamine) of naltrexone with ultralong-lasting narcotic antagonist properties. J. Med. Chem. **22**: 168–173, 1979.

525. POTTER, L. T.: Uptake of propanolol by isolated guinea-pig atria. J. Pharmacol. Exp. Ther. **155**: 91–100, 1967.

526. PRICHARD, B. N. C., TOMLINSON, B., WALDEN, R. J., AND BHATTACHARJEE, P.: The β-adrenergic blockade withdrawal phenomenon. J. Cardiovasc. Pharmacol. **5**: S56–S62, 1983.

527. RANG, H. P.: Stimulant actions of volatile anaesthetics on smooth muscle. Br. J. Pharmacol. **22**: 356–365, 1964.

527A. RANG, H. P.: The kinetics of action of acetylcholine antagonists in smooth muscle. Proc. R. Soc. Lond. B Biol. Sci. **164**: 488–510, 1966.

528. RANGNO, R. E., LANGLOIS, S., AND STEWART, J.: Cardiac hyper- and hyporesponsiveness after pindolol withdrawal. Clin. Pharmacol. Ther. **31**: 564–571, 1981.

529. RANKIN, A., AND BROADLEY, K. J.: Comparison of the apparent irreversible β-adrenoceptor antagonist R_o 03-7894 with propranolol in cardiac ventricular muscle by pharmacological and radioligand binding techniques. Biochem. Pharmacol. **31**: 1325–1332, 1982.

530. REGOLI, D., BARABÉ, S., AND PARK, W. K.: Receptors for bradykinin in rabbit aortae. Can. J. Physiol. Pharmacol. **55**: 855–867, 1977.

531. REGOLI, D., AND PARK, W. W.: The pressor and myotropic effects on the antagonist properties of several analogues of angiotensin II. Can. J. Physiol. Pharmacol. **50**: 99–112, 1972.

532. REGOLI, D., PARK, W. K., AND RIOUX, F.: Pharmacology of angiotensin. Pharmacol. Rev. **26**: 69–123, 1974.

533. REGOLI, D., AND VANE, J. R.: A sensitive method for the assay of angiotensin. Br. J. Pharmacol. **23**: 351–359, 1964.

534. REIFFENSTEIN, R. J., AND TRIGGLE, C. R.: Cocaine-induced supersensitivity in the human umbilical artery. Can. J. Physiol. Pharmacol. **52**: 687–698, 1974.

535. REUTER, H., AND PORZIG, H.: Beta-adrenergic receptors and responses in the heart. Postgrad. Med. J. **57**: 62–70, 1981.

536. REZVANI, A., HUIDOBRO-TORO, J. P., HU, J., AND WAY, E. L.: A rapid and simple method for the quantitative determination of tolerance development to opiates in the guinea-pig ileum *in vivo*. J. Pharmacol. Exp. Ther. **225**: 251–255, 1983.

537. RICE, K. C., JACOBSON, A. E., BURKE, T. R., JR., BAJWA, B. S., STREATY, R. A., AND KLEE, W. A.: Irreversible ligands with high selectivity toward α or μ opiate receptors. Science **220**: 314–316, 1983.

538. RICO, J. M. G. T.: The influence of calcium on the activity of full and partial muscarinic agonists. Eur. J. Pharmacol. **13**: 218–229, 1971.

539. RIMELE, T. J., ROOKE, T. W., AARHUS, L. L., AND VANHOUTTE, P. M.: Alpha-1 adrenoceptor and calcium in isolated canine coronary arteries. J. Pharmacol. Exp. Ther. **226**: 668–672, 1983.

540. RINGDAHL, B., AND JENDEN, D. J.: Affinity, efficacy, and stereoselectivity of oxotremorine analogues for muscarinic receptors in the isolated guinea-pig ileum. Mol. Pharmacol. **23**: 17–25, 1983.

541. ROACH, P. J.: Functional significance of enzyme cascade systems. Tr. Biochem. Sci. **2**: 87–90, 1977.

542. ROBERTS, J. M., INSEL, P. A., AND GOLDFIEN, A.: Regulation of myometrial adrenoceptors and adrenergic response by sex steroids. Mol. Pharmacol. **20**: 52–58, 1981.

543. ROCHE E SILVA, M.: Influence of pH on the interaction of histamine with its receptors in the guinea pig ileum. Arch. Int. Pharmacodyn. Thér. **128**: 355–374, 1960.

544. ROCHE E SILVA, M.: Action of histamine on the smooth muscle. *In* Handbook of Experimental Pharmacology, vol. 18, pp. 225–237, Springer Publishing Co., Inc., New York, 1966.

545. RORIE, D. K., AND MULDOON, S. M.: Increased reactivity of isolated rabbit saphenous vein after treatment with estrogen and progesterone. Blood Vessels **16**: 252–258, 1979.

546. RORIE, D. K., RUSCH, N. J., SHEPHERD, J. T., VANHOUTTE, P. M., AND TYCE, G. M.: Prejunctional inhibition of norephinephrine release caused by acetylcholine in the human saphenous vein. Circ. Res. **49**: 337–341, 1981.

547. ROSENQVIST, U., AND BOREÚS, L. O.: Enhancement of the alpha-adrenergic response in aorta from hypothyroid rabbits. Life Sci. **11**: 595–604, 1972.

548. RUBIO, E., MORALES-OLIVAS, F. J., MORCILLO, E., AND ESPLUGUES, J.: Indirect sympathomimetic effect of some H₂-histamine receptor antagonists in the isolated uterus of the rat. J. Pharm. Pharmacol. **34**: 740–741, 1982.

549. RUDINGER, J., PLISKA, V., AND KREJCI, I.: Oxytocin analogues in the analysis of some phases of hormone action. Recent Prog. Horm. Res. **28**: 131–172, 1972.

550. RUDOLPH, S. A., GREENGARD, P., AND MALAWISTA, S. E.: Effects of colchicine on cyclic AMP levels in human leukocytes. Proc. Natl. Acad. Sci. U.S.A. **74**: 3404–3408, 1977.

551. RUFFOLO, R. R., JR.: Review: Important concepts of receptor theory. J. Auton. Pharmacol. **2**: 277–295, 1982.

552. RUFFOLO, R. R., JR.: The use of isolated, physiologically responding tissues to investigate neurotransmitter receptors. *In* Neuroreceptors in Health and Disease, ed. by J. Marwaha, and W. Anderson, S. Karger AG, Basel, 1983.

553. RUFFOLO, R. R., ROSING, E. L., AND WADDELL, J. E.: Receptor interactions of imidazolines. I. Affinity and efficacy for *alpha* adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. **209**: 429–436, 1979.

554. RUFFOLO, R. R., JR., WADDELL, J. E., AND YADEN E. L.: Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aorta. J. Pharmacol. Exp. Ther. **221**: 309–314, 1982.

555. RUFFOLO, R. R., JR., AND WADDELL, J. E.: Aromatic and benzylic hydroxyl substitution of imidazolines and phenthylamines: Differences in activity at alpha-1 and alpha-2 adrenergic receptors. J. Pharmacol. Exp. Ther. **224**: 559–566, 1983.

556. RUGG, E. L., BARNETT, D. B., AND NAHORSKI, S. R.: Coexistence of beta₁ and beta₂ adrenoceptors in mammalian lung: Evidence from direct binding sites. Mol. Pharmacol. **14**: 996–1005, 1978.

557. RUOHO, A. E., HALL, C. C., AND RASHIDBAIGI, A.: Use of photolabels to probe the Na,K-ATPase and the β-adrenergic receptor. Fed. Proc. **42**: 2837–2841, 1983.

558. SAKAKIBARA, Y., FUJIWARA, M., AND MURAMATOU, I.: Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. Naunyn-Schmeideberg's Arch. Pharmacol. **319**: 1–7, 1982.

559. SÁNCHEZ-BLÁZQUEZ, P., GARZÓN, J., AND LEE, N. M.: Functional opiate receptor in mouse vas deferens: Evidence for a complex interaction. J. Pharmacol. Exp. Ther. **226**: 706–711, 1983.

560. SASTRY, B. V. R., AND CHENG, H. C.: Dissociation constants of D- and L-lactoylcholines and related compounds at cholinergic receptors. J. Pharmacol. Exp. Ther. **180**: 326–339, 1972.

561. SATOH, H., AND HASHIMOTO, K.: Effect of pH on the sino-atrial node cells and atrial muscle of dog. Arch. Int. Pharmacodyn. Thér. **261**: 67–78, 1983.

562. SAYRE, L. M., TAKEMORI, A. E., AND PORTOGHESE, P. S.: Alkylation of opioid receptor subtypes by α-chlornaltrexamine produces concurrent irreversible agonistic and irreversible antagonistic activities. J. Med. Chem. **26**: 503–506, 1983.

563. SCARPACE, P. J., AND ABRASS, I. B.: Desensitization of adenylate cyclase and down regulation of *beta* adrenergic receptors after *in vivo* administration of *beta* agonist. J. Pharmacol. Exp. Ther. **223**: 327–331, 1982.

564. SCHILD, H. O.: pA, a new scale for the measurement of drug antagonism. Br. J. Pharmacol. **2**: 189–206, 1947.

565. SCHILD, H. O.: The use of drug antagonists for the identification and classification of drugs. Br. J. Pharmacol. **2**: 251–258, 1947.

566. SCHILD, H. O.: pAx, and competitive drug antagonism. Br. J. Pharmacol. **4**: 277–280, 1949.

567. SCHILD, H. O.: Drug antagonism and pAx. Pharmacol. Rev. **9**: 242–246, 1957.

568. SCHILD, H. O., HAWKINS, D. F., MONGAR, J. L., AND HERXHEIMER, H.: Reactions of isolated human asthmatic lung and bronchial tissue to a specific antigen. Histamine release and muscular contraction. Lancet **261**: 376–382, 1951.

569. SCHREURS, A. J. M., AND NIJKAMP, F. P.: *Haemophilus influenzae* induced loss of lung β-adrenoceptor binding sites and modulation by changes in peripheral catecholaminergic input. Eur. J. Pharmacol. **77**: 95–102, 1982.

570. SCHREURS, A. J. M., VERHOEF, J., AND NIJKAMP, F. P.: Bacterial cell wall components decrease the number of guinea-pig lung β-adrenoceptors. Eur. J. Pharmacol. **87**: 127–132, 1983.

571. SCHÜMANN, H. J., WAGNER, J., KNORR, A., REIDEMEISTER, J. C., SADONY, V., AND SCHRAMM, G.: Demonstration in human atrial preparations of α-adrenoceptors mediating positive inotropic effects. Naunyn-Schmeideberg's Arch. Pharmacol. **302**: 333–336, 1978.

572. SEELIG, S., AND SAYERS, G.: Isolated adrenal cortex cells: ACTH agonists, partial agonists, antagonists; cyclic AMP and corticosterone products. Arch. Biochem. Biophys. **154**: 230–239, 1973.

573. SETLOW, R. B., AND POLLARD, E. C.: Molecular Biophysics, Addison-Wesley, Inc., MA, London, 1962.

574. SHARMA, V. K., AND BANERJEE, S. P.: Muscarinic cholinergic receptors in rat heart: Effects of thyroidectomy. J. Biol. Chem. **252**: 7444–7446, 1977.

575. SHELLEY, J. H.: Creativity in drug research. I. Trends Pharmacol. Sci. **4**: 283–284, 1983.

576. SHELLEY, J. H.: Creativity in drug research. II. Trends Pharmacol. Sci. **4**: 323–325, 1983.

577. SHELLEY, J. H.: Creativity in drug research. III. Trends Pharmacol. Sci. **4**: 361–362, 1983.

578. SHEYS, E. M., AND GREEN, R. D.: A quantitative study of alpha adrenergic receptors in the spleen and aorta of the rabbit. J. Pharmacol. Exp. Ther. **180**: 317–325, 1972.

579. SHIBATA, S., CHENG, J. B., AND MURAKAMI, W.: Reactivity of isolated human cerebral arteries to biogenic amines. Blood Vessels **14**: 356–365, 1975.

580. SIEGEL, P. K., AND MCNEILL, J. H.: Antagonism with dibenamine, D-600, and Ro 3-7894 to estimate dissociation constants and receptor reserves for cardiac adrenoceptors in isolated rabbit papillary muscles. Can. J. Physiol. Pharmacol. **60**: 1131–1137, 1982.

580a. SINE, S. M., AND TAYLOR, P.: Relationship between reversible antagonist occupancy and the functional capacity of the acetylcholine receptor. J. Biol. Chem. **256**: 6692–6699, 1981.

581. SINENSKY, M., MINNEMAN, K. P., AND MOLINOFF, P. B.: Increased membrane acyl chain ordering activates adenylate cyclae. J. Biol. Chem. **254**: 9135–9141, 1979.

582. SINGER, S. J., RUOHO, AA., KIEFER, H., LINDSTROM, J., AND LENNOX, E. S.: The use of affinity labels in the identification of receptors. *In* Drug Receptors, ed. by H. P. Rang, pp. 183–191, University Park Press, London, 1973.

583. SJÖBERG, B., AND WAHLSTRÖM, B. A.: The effect of ATP and related compounds on spontaneous mechanical activity in the rat portal vein. Acta. Physiol. Scand. **94**: 46–53, 1975.

584. SKAUG, N., AND DETAR, R.: Indirect evidence for the importance of steady-state [K]ₒ in determining the extent of the effects of hyperpolarizing electrogenesis on vascular smooth muscle reactivity. Blood Vessels **17**: 117–122, 1980.

585. SKAUG, N., AND DETAR, R.: Contractility of vascular smooth muscle: Maximum ability to contract in response to a stimulus. Am. J. Physiol. **240**: H971–H979, 1981.

586. SKOLNICK, P., SCHWERI, M. M., WILLIAMS, E. F., MONCADA, V. Y., AND PAUL, S. M.: An *in vitro* binding assay which differentiates benzodiazepine 'agonists' and 'antagonists.' Eur. J. Pharmacol. **78**: 133–136, 1982.

587. SKOMEDAL, T., OSNES, J. -B., AND OYE, I.: Differences between α-adrenergic and β-adrenergic inotropic effects in rat heart papillary muscles. Acta. Pharmacol. Toxicol. **50**: 1–12, 1982.

588. SMITH, C. B.: Relaxation of the nictitating membrane of the spinal cat by sympathomimetic amines. J. Pharmacol. Exp. Ther. **142**: 163–170, 1963.

589. SMITHSON, K. W., MILLAR, D. B., JACOBS, L. R., AND GRAY, G. M.: Intestinal diffusion barrier: Unstirred water layer or membrane surface mucous coat? Science **214**: 1241–1244, 1981.

590. SNAVELY, M. D., MOTULSKY, J. H., MOUSTAFA, E., MAHAN, L. C., AND INSEL, P. A.: β-Adrenergic receptor subtypes in the rat renal cortex:

Selective regulation of β_1-adrenergic receptors by pheochromocytoma. Eur. J. Pharmacol. **82**: 37–45, 1982.

591. SNEDECOR, G. W., AND COCHRAN, W. G.: Statistical methods. Iowa State University Press, Ames, Iowa, 1967.

592. SOMLYO, A. V., WOO, C. -Y., AND SOMLYO, A. P.: Responses of nerve-free vessels to vasoactive amines and polypeptides. Am. J. Physiol. **208**: 748–753, 1965.

593. SONNENBLICK, E. H.: Force-velocity relations in mammalian heart muscle. Am. J. Physiol. **202**: 931–939, 1962.

594. SONNENBLICK, E. H.: Determinants of active state in heart muscle: Force, velocity, instantaneous muscle length, time. Fed. Proc. **24**: 1396–1409, 1965.

595. SPERELAKIS, N., HOSHIKO, T., KELLER, R. F., JR., AND BERNE, R. M.: Intracellular and external recording from frog ventricular fibers during hypertonic perfusion. Am. J. Physiol. **198**: 135–140, 1960.

595A. STARKE, K., REIMANN, W., ZUMSTEIN, A., AND HERTTING, G.: Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. **305**: 27–36, 1978.

596. STARR, M. S., AND WEST, G. B.: The effect of bradykinin and antiinflammatory agents on isolated arteries. J. Pharm. Pharmacol. **18**: 838–840, 1966.

597. STEPHENS, N. L., KROEGER, E., AND MEHTA, J. A.: Force-velocity characteristics of respiratory airway smooth muscle. J. Appl. Physiol. **26**: 685–692, 1969.

598. STEPHENS, N. L., MEYERS, J. L., AND CHERNIACK, R. M.: Oxygen carbon dioxide H^+ ion, and bronchial length-tension relationships. J. Appl. Physiol. **25**: 376–383, 1968.

599. STEPHENSON, R. P.: A modification of receptor theory. Br. J. Pharmacol. **11**: 379–393, 1956.

600. STERIN, A. B. F., GOLDRAIJ, A., GIMENO, M. A. F., AND GIMENO, A. L.: In vitro contractile responses of the uterus from 'restricted diet' rats to adrenoceptor agonists. Influence of cyclo-oxygenase inhibitors. Eur. J. Pharmacol. **90**: 411–417, 1983.

601. STEVENS, M. J., AND MOULDS, R. F. W.: Are the pre- and postsynaptic α-adrenoceptors in human vascular smooth muscle atypical? J. Cardiovasc. Pharmacol. **4**: S129–S133, 1982.

602. STEVENS, M. J., LIPE, S., AND MOULDS, R. F. W.: The effect of age on the responses of human isolated arteries and veins to noradrenaline. Br. J. Clin Pharmacol. **14**: 750–752, 1982.

603. STONE, M.: The Clark plot: A semi-historical case study. J. Pharm. Pharmacol. **32**: 81–86, 1980.

604. STONE, M., AND ANGUS, J. A.: Developments of computer-based estimation of pH₂ values and associated analysis. J. Pharmacol. Exp. Ther. **207**: 705–718, 1978.

605. STRICKLAND, S., AND LOEB, J. N.: Obligatory separation of hormone binding and biological response curves in systems dependent upon secondary mediators of hormone action. Proc. Natl. Acad. Sci. U.S.A. **78**: 1366–1370, 1981.

606. STRITTMATTER, W. J., DAVIS, J. N., AND LEFKOWITZ, R. J.: Alpha-adrenergic receptors in rat parotid cells. I. Correlation of [³H]dihydroergocryptive binding and catecholamine-stimulated potassium efflux. J. Biol. Chem. **252**: 5472–5477, 1977.

607. SULLIVAN, A. T., AND DREW, G. M.: Pharmacological characterization of pre- and postsynaptic α-adrenoceptors in dog saphenous vein. Naunyn-Schmiedeberg's Arch. Pharmacol. **314**: 249–258, 1980.

608. SUN, C. -L. J., AND HORNIG, J. P.: Alteration of sensitivity of adrenergic vascular responses after prolonged exposure to agonists via osomotic minipump (41584). Proc. Soc. Exp. Biol. Med. **173**: 440–444, 1983.

609. SWAMY, V. C., AND TRIGGLE, D. J.: 2-Halogenoethylamines and the role of Ca^{2+} in adrenergic α-receptor activation in the rat vas deferens. Eur. J. Pharmacol. **19**: 67–78, 1972.

610. SWILLENS, S., AND DUMONT, J. E.: A unifying model of current concepts and data on adenylate cyclase activation by β-adrenergic agonists. Life Sci. **27**: 1013–1028, 1980.

611. SYLVESTRE-GERVAIS, L., NADEAU, A., NGUYEN, M. H., TANCRÈDE, G., AND ROUSSEAU-MIGNERON, S.: Effects of physical training on β-adrenergic receptors in rat myocardial tissue. Cardiovas. Res. **16**: 530–534, 1982.

612. TAKEYASU, K., UCHIDA, S., WADA, A., MARUNO, M., LAI, R. T., HATA, F., AND YOSHIDA, H.: Experimental evidence and dynamic aspects of spare receptors. Life Sci. **25**: 1761–1772, 1979.

613. TALLARIDA, R. J., COWAN, A., AND ADLER, M. W.: Minireview: pA₂ and receptor differentiation: A statistical analysis of competitive antagonism. Life Sci. **25**: 637–654, 1979.

614. TALLARIDA, R. J., LASKIN, O. L., AND JACOB, L. S.: Perturbation of drug receptor equilibrium in the presence of competitive blocking agents. J. Theor. Biol. **61**: 211–219, 1976.

615. TALLARIDA, R. J., AND MURRAY, R. B.: Manual of Pharmacologic Calculations with Computer Programs, Springer-Verlag, New York, Heidelberg, Berlin, 1979.

616. TALLARIDA, R. J., SEVY, R. W., AND HARAKAL, C.: Relaxation methods for the determination of drug receptor affinities. Bull. Math. Biophys. **32**: 65–69, 1970.

617. TALLARIDA, R. J., SEVY, R. W., HARAKAL, C., AND LOUGHNANE, M. H.: Characteristics of photorelaxation in vascular smooth muscle: Evidence supporting the hypothesis of drug-receptor equilibrium disturbance. IEEE Trans. Biomed. Eng. **BME-22**: 493–501, 1975.

618. TANIGUCHI, T., KURAHASHI, K., AND FUJIWARA, M.: Alterations in muscarinic cholinergic receptors after preganglionic denervation of the superior cervical ganglion in cats. J. Pharmacol. Exp. Ther. **224**: 674–678, 1983.

619. TAPP, W. N., LEVIN, B. E., AND NATELSON, B. H.: Stress-induced heart failure. Psychosom. Med. **45**: 171–176, 1983.

620. TAYLOR, D. B., STEINBORN, J., TZU-CHIAU, LU: Ion exchange processes at the neuromuscular junction of voluntary muscle. J. Pharmacol. Exp. Ther. **175**: 213–227, 1970.

621. TAYLOR, J., AND GREEN, R. D.: Analysis of reserpine-induced supersensitivity in aortic strips of rabbits. J. Pharmacol. Exp. Ther. **177**: 127–135, 1971.

622. TAYO, F. M., AND ACHOLEM, M. O.: Pharmacological characterization of some receptors in the rat calcum. Arch. Int. Pharmacodyn. Thér. **254**: 20–27, 1981.

623. TENNER, T. E., JR.: Propranolol withdrawal supersensitivity in rat cardiovascular tissue, in vitro. Eur. J. Pharmacol. **92**: 91–97, 1983.

624. THOENEN, H., AND TRANZOR, J. P.: Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. Naunyn-Schmeideberg's Arch. Pharmakol. Exp. Pathol. **261**: 271–288, 1968.

625. THOMPSON, E. B., AND WEST, T. C.: Antagonism by propanolol of the cardiac chronotropic response to norepinephrine or to transmural electrical stimulation. J. Pharmacol. Exp. Ther. **161**: 232–237, 1968.

626. THRON, D. C.: Graphical and weighted regression analyses for the determination of agonist dissociation constants. J. Pharmacol. Exp. Ther. **175**: 541–553, 1970.

627. THRON, C. D.: On the analysis of pharmacological experiments in terms of an allosteric receptor model. Mol. Pharmacol. **9**: 1–9, 1973.

628. TIMMERMANS, P. B. M. W. M., DE JONGE, A., VAN MEEL, J. C. A., MATHY, M. -J., AND VAN ZWIETEN, P. A.: Influence of nifedipine on functional responses in vivo initiated at α_2-adrenoceptors. J. Cardiovasc. Pharmacol. **5**: 1–11, 1983.

629. TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: α_2 Adrenoceptors: Classification, localization, mechanisms, and targets for drugs. J. Med. Chem. **25**: 1389–1401, 1982.

630. TODA, N.: The action of vasodilating drugs on isolated basilar, coronary and mesenteric arteries of the dog. J. Pharmacol. Exp. Ther. **191**: 139–146, 1974.

631. TODA, N.: Influence of dopamine and noradrenaline on isolated cerebral arteries of the dog. Br. J. Pharmacol. **58**: 121–126, 1976.

632. TODA, N.: Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. J. Pharmacol. Exp. Ther. **226**: 861–868, 1983.

633. TODA, N., AND GOLDBERG, L. I.: Effects of dopamine on isolated canine coronary arteries. Cardiovasc. Res. **9**: 384–389, 1975.

634. TODA, N., HOJO, M., SAKAE, K., AND USUI, H.: Comparison of the relaxing effects of dopamine with that of adenosine, isoproterenol and acetylcholine in isolated canine coronary arteries. Blood Vessels **12**: 290–301, 1975.

635. TODD, M. E., LAYE, C. G., AND OSBORNE, D. N.: The dimensional characteristics of smooth muscle in rat blood vessels. Circ. Res. **53**: 319–331, 1983.

636. TOKUDOME, T., AND TAIRA, N.: Characterization of β-adrenoceptors in the dog saphenous vein. Jpn. J. Pharmacol. **31**: 731–736, 1981.

637. TOLKOVSKY, A. M., AND LEVITZKI, A.: Mode of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes. Biochemistry **17**: 3795–3810, 1978.

638. TOMIOKA, K., AND YAMADA, T.: Effects of histamine H₂-receptor agonists and antagonists on isolated guinea-pig airway muscles. Arch. Int. Pharmacodyn. Thér. **255**: 16–26, 1982.

639. TORCHIANA, M. L., PENDLETON, R. G., COOK, P. G., HANSON, C. A., AND CLINESCHMIDT, B. V.: Apparent irreversible H₂-receptor blocking and prolonged gastric antisecretory activities of 3-N{3-3[3-(1-piperidinomethyl)phenoxy]propyl}amino-4-amino-1,2,5-thiadiazole-1-oxide (L-643,441). J. Pharmacol. Exp. Ther. **224**: 514–519, 1983.

640. TORPHY, T. J., WESTFALL, D. P., AND FLEMING, W. W.: Effect of reserpine pretreatment on mechanical responsiveness and [¹²⁵I]iodohydroxybenzylpindolol binding sites in the guinea-pig right atrium. J. Pharmacol. Exp. Ther. **223**: 332–341, 1982.

641. TRENDELENBURG, U.: The effect of cocaine on the pacemaker of isolated guinea-pig atria. J. Pharmacol. Exp. Ther. **161**: 222–231, 1968.

642. TRENDELENBURG, U.: Classification of sympathomimetic amines. In Handbook of Experimental Pharmacology, Catecholamines, ed. by H. Blaschko, and E. Muscholl, vol. 33, pp. 336–362, Springer-Verlag, New York, 1972.

643. TRENDELENBURG, U.: Supersensitivity in peripheral organs. In Receptors for Neurotransmitters and Peptide Hormones, ed. by G. Pepeu, M. J. Kuhar, and S. J. Enna, vol. 21, pp. 99–105, Raven Press, New York, 1972.

644. TRINER, L., NAHAS, G. G., VULLIEMOZ, Y., OVERWEG, N. I. A., VEROSKY, M., HABIF, D. V., AND NGAI, S. H.: Cyclic AMP and smooth muscle function. Ann. N.Y. Acad. Sci. **185**: 458–476, 1971.

645. TYRODE, M. V.: Mode of action of some purgative salts. Arch. Int. Pharmacodyn. Thér. **20**: 205–210, 1910.

646. UCHIDA, K., KAMIKAWA, Y., AND SHIMO, Y.: Time-dependent augmentation of the contractile responses to adrenaline and noradrenaline of the guinea-

pig esophageal muscularis mucosae *in vitro*. Naunyn-Schmeideberg's Arch. Pharmacol. **323:** 114–120, 1983.

647. UCHIDA, S., TAKEYASU, K., MATSUDA, T., AND YOSHIDA, H.: Changes in muscarinic acetylcholine receptors of mice by chronic administrations of diisopropylfluorophosphate and papaverine. Life Sci. **24:** 1805–1812, 1979.

648. UCHIDA, S., TAKEYASU, K., NOGUCHI, Y., YOSHIDA, H., HATA, T., AND KITA, T.: Decrease in muscarinic acetylcholine receptors in the small intestine of mice subjected to repeated cold stress. Life Sci. **22:** 2197–2204, 1978.

649. UEKI, S., OKAMOTO, E., KUWATA, K., TOYOSAKA, A., NAGAI, K., UCHIDA, S., AND YOSHIDA, H.: Increases in muscarinic acetylcholine receptors in mouse intestine by hexamethonium treatment. Life Sci. **32:** 2431–2437, 1983.

650. VACCARI, A., AND MAURA, G.: Effects of drugs on the extracellular spaces of smooth muscle *in vitro*. Pharmacol. Res. Commun. **10:** 675–690, 1978.

651. VALLIERES, J., COTE, C., AND BUKOWIECKI, L.: Regulation of β-adrenergic receptors in rat skeletal muscles by catecholamines *in vivo*. Gen. Pharmacol. **10:** 63–67, 1979.

652. VAN DEN BRINK, F. G.: The model of functional interaction. I. Development and first check a new model of functional synergism and antagonism. Eur. J. Pharmacol. **22:** 270–278, 1973.

653. VAN DEN BRINK, F. G.: The model of functional interaction. II. Experimental verification of a new model: The antagonism of β-adrenoceptor stimulants and other agents. Eur. J. Pharmacol. **22:** 279–286, 1973.

654. VAN DEN BRINK, F. G.: General theory of drug-receptor interactions. Drug-receptor interaction models. Calculation of drug parameters. *In* Kinetics of Drug Action, ed. by J. M. Van Rossum, pp. 169–254, Springer-Verlag, Berlin, Heidelberg, New York, 1977.

655. VAN GINNEKEN, C. A. M.: Kinetics of drug-receptor interaction. *In* Kinetics of Drug Action, ed. by J. M. van Rossum, pp. 357–411, Springer-Verlag, Berlin, Heidelberg, New York, 1977.

656. VAN MEEL, J. C. A., DE JONGE, A., KALKMAN, H. O., WILFFERT, B., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Organic and inorganic calcium antagonists reduce vasoconstriction *in vivo* mediated by postsynaptic α₂-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. **316:** 288–293, 1981.

657. VAN MEEL, J. C. A., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: α₁- and α₂-adrenoceptor stimulation in the isolated perfused hindquarters of the rat: An *in vitro* model. J. Cardiovasc. Pharmacol. **5:** 580–585, 1983.

658. VAN MEEL, J. C. A., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Hypotensive activity of calcium entry blockers in rats. Relationship with depression of α₂-adrenoceptor-mediated vasopressor responses. Eur. J. Pharmacol. **92:** 27–34, 1983.

659. VAN MEEL, J. C. A., TOWART, R., KAZDA, S., TIMMERMANS, P. B. M. W. M., AND VAN ZWIETEN, P. A.: Correlation between the inhibitory activities of calcium entry blockers on vascular smooth muscle constriction *in vitro* after K⁺-depolarization and *in vivo* after α₂-adrenoceptor stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol. **322:** 34–37, 1983.

660. VAN NUETEN, J. M.: Comparison of isotonic and isometric measurements in isolated arterial tissues. *In* Vascular Neuroeffector Mechanisms, ed. by J. A. Bevan, T. Godfraind, R. A. Maxwell, and P. M. Vanhoutte, pp. 37–39, Raven Press, New York, 1980.

661. VAN ROSSUM, J. M.: Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Thér. **143:** 299–330, 1963.

662. VAN ROSSUM, J. M.: Different types of sympathomimetic α-receptors. J. Pharm. Pharmacol. **17:** 202–216, 1965.

663. VAN ROSSUM, J. M.: Limitations of molecular pharmacology. Some implications of the basic assumptions underlying calculations on drug-receptor interactions and the significance of biological drug parameters. *In* Advances in Drug Research, ed. by N. J. Harper, and A. B. Simmonds, vol. 3, pp. 189–234, Academic Press, London, New York, 1966.

664. VAN ROSSUM, J. M., AND ARIËNS, E. J.: Pharmacodynamics of parasympathetic drugs. Structure-action relationships in homologous series of quaternary ammonium salts. Arch. Int. Pharmacodyn. Thér. **118:** 418–446, 1959.

665. VAN ROSSUM, J. M., AND ARIËNS, E. J.: Receptor-reserve and threshold-phenomena. II. Theories on drug-action and a quantitative approach to spare receptors and threshold values. Arch. Int. Pharmacodyn. Thér. **136:** 385–413, 1962.

666. VANE, J. R.: A sensitive method for the assay of 5-hydroxytryptamine. Br. J. Pharmacol. **12:** 344–349, 1957.

667. VANHOUTTE, P. M.: Heterogeneity of postjunctional vascular α-adrenoceptors and handling of calcium. J. Cardiovasc. Pharmacol. **4:** S91–S96, 1982.

668. VANHOUTTE, P. M., AND RIMELE, T. J.: Calcium and α-adrenoceptors in activation of vascular smooth muscle. J. Cardiovasc. Pharmacol. **4:** S280–S286, 1982.

669. VANHOUTTE, P. M., AND RIMELE, T. J.: Effects of calcium entry blockers on isolated blood vessels. Prog. Pharmacol. **5:** 23–36, 1983.

670. VARMA, D. R., AND NICKERSON, M.: Beta-adrenoreceptors of the cat nictitating membrane. J. Auton. Pharmacol. **1:** 291–297, 1981.

671. VAUGHN WILLIAMS, E. M.: The individual effects of CO₂, bicarbonate and pH on the electrical and mechanical activity of isolated rabbit auricles. J. Physiol. (Lond.) **129:** 90–110, 1955.

672. VENTER, J. C.: Cardiac sites of catecholamine action: Diffusion models for soluble and immobilized catecholamine action on isolated cat papillary muscles. Mol. Pharmacol. **14:** 562–574, 1978.

673. VENTER, J. C.: High efficiency coupling between beta-adrenergic receptors and cardiac contractility: Direct evidence for "spare" beta-adrenergic receptors. Mol. Pharmacol. **16:** 429–440, 1979.

674. VENTER, J. C., ROSS, J., JR., AND KAPLAN, N. O.: Lack of detectable change in cyclic AMP during the cardiac inotropic response to isoproterenol immobilized on glass beads. Proc. Natl. Acad. Sci. **72:** 824–828, 1975.

675. VERE, D. W.: Methods to linearize the lower end of a dose-response curve. Br. J. Pharmacol. **43:** 480–481, 1971.

676. VERITY, M. A.: Morphologic studies of vascular neuroeffector apparatus. Symposium on physiological and pharmacological vascular neuroeffector systems, Interlaken, 1969, pp. 2–12, Basel, Karger, 1971.

677. VERMA, S. C., AND MCNEILL, J. H.: Investigations into the cardiac effects of tolazoline in guinea-pig atria and ventricular strips. Agents Actions **7:** 191–197, 1977.

678. VESTAL, R. E., WOOD, A. J. J., AND SHAND, D. G.: Reduced β-adrenoceptor sensitivity in the elderly. Clin. Pharmacol. Ther. **26:** 181–186, 1979.

679. WALDECK, B.: An *in vitro* method for the study of β-receptor mediated effects on slow contracting skeletal muscle. J. Pharm. Pharmacol. **28:** 434–436, 1976.

680. WALDER, D. N.: The muscularis mucosae of the human stomach. J. Physiol. (Lond.) **120:** 365–372, 1953.

681. WALKER, E. B., MCNALL, S. J., AND MANSOUR, T. E.: Photoreactivity of lysergic acid diethylamide and its possible utility as a photo affinity labeling reagent. Biochem. Pharmacol. **32:** 1251–1257, 1983.

682. WATSON, S. P.: Rapid degradation of [³H]-substance P in guinea-pig ileum and rat vas deferens *in vitro*. Br. J. Pharmacol. **79:** 543–552, 1983.

683. WATSON, S. P., SANDBERG, B. E. B., HANLEY, M. R., AND IVERSEN, L. L.: Tissue selectivity of substance P alkyl esters: Suggesting multiple receptors. Eur. J. Pharmacol. **87:** 77–84, 1983.

684. WAUD, D. R.: Pharmacological receptors. Pharmacol. Rev. **20:** 49–88, 1968.

685. WAUD, D. R.: A quantitative model for the effect of a saturable uptake on the slope of the dose-response curve. J. Pharmacol. Exp. Ther. **167:** 140–141, 1969.

686. WAUD, D. R.: On the measurement of the affinity of partial agonists for receptors. J. Pharmacol. Exp. Ther. **170:** 117–122, 1969.

687. WAUD, D. R.: Analysis of dose-response relationships. *In* Advances in General and Cellular Pharmacology, ed. by L. T. Narahashi, and C. P. Bianchi, pp. 145–178, Plenum Press, New York, 1976.

688. WAUD, D. R.: Analysis of dose-response curves. Trends Pharmacol. Sci. **2:** 52–55, 1981.

689. WAUD, D. R., AND PARKER, R. B.: Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. II. Competitive antagonists. J. Pharmacol. Exp. Ther. **177:** 13–24, 1971.

690. WAUD, D. R., SON, S. L., AND WAUD, B. E.: Kinetic and empirical analysis of dose-response curves illustrated with a cardiac example. Life Sci. **22:** 1275–1286, 1978.

691. WEILAND, G. A., MINNEMAN, K. P., AND MOLINOFF, P. B.: Fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. Nature (Lond.) **281:** 114–117, 1979.

692. WEISBRODT, N. W., AND HUG, C. C., JR., AND BASS, P.: Separation of the effects of *alpha* and *beta* adrenergic receptor stimulation on taenia coli. J. Pharmacol. Exp. Ther. **170:** 272–280, 1969.

693. WEISS, G. B.: Homogeneity of extracellular space measurement in smooth muscle. Am. J. Physiol. **210:** 771–776, 1966.

694. WESSELS, M. R., MULLIKIN, D., AND LEFKOWITZ, R. J.: Differences between agonist and antagonist binding following β-adrenergic receptor desensitization. J. Biol. Chem. **253:** 3371–3373, 1978.

695. WESTFALL, D. P., AND FLEMING, W. W.: Potentiation of the rat vas deferens to norepinephrine (NE) and acetylcholine (ACh) by cocaine. Fed. Proc. **30:** 656, 1971.

696. WETZEL, H. W., BRILEY, M. S., AND LANGER, S. Z.: ³H-WB4101 binding in the rat vas deferens: Effects of chronic treatments with desipramine and prazosin. Naunyn-Schmeideberg's Arch. Pharmacol. **317:** 187–192, 1981.

697. WHALLEY, E. T., AND WAHL, M.: Analysis of bradykinin receptor mediating relaxation of cat cerebral arteries *in vivo* and *in vitro*. Naunyn-Schmeideberg's Arch. Pharmacol. **323:** 66–71, 1983.

698. WIEGEL, F. W., AND DELISI, C.: Evaluation of reaction rate enhancement by reduction in dimensionality. Am. J. Physiol. **243:** R475–R479, 1982.

699. WILLIAMS, L. T., AND LEFKOWITZ, R. J.: Receptor Binding Studies in Adrenergic Pharmacology, Raven Press, New York, 1978.

700. WILLIAMS, L. T., LEFKOWITZ, R. J., WATANABE, A. M., HATHAWAY, D. R., AND BESCH, H. R., JR.: Thyroid hormone regulation of β-adrenergic receptor number. J. Biol. **252:** 2787–2789, 1977.

701. WILLIAMS, R. G., AND BROADLEY, K. J.: Determination of agonist affinity for cardiac β-adrenoceptors during reserpine-induced supersensitivity. Eur. J. Pharmacol. **87:** 95–105, 1983.

702. WILSON, F. A., AND DIETSCHY, J. M.: The intestinal unstirred layer: Its surface area and effect on active transport kinetics. Biochim. Biophys. Acta **363:** 112–126, 1974.

703. WINQUIST, R. J., AND BEVAN, J. A.: Relative location of α- and β-adreno-ceptors to sites of release of sympathetic transmitter in the rabbit facial vein. Circ. Res. **49:** 486–492, 1981.

704. WONG, S. K., AND BUCKNER, C. K.: Studies on β-adrenoceptors mediating changes in mechanical events and adenosine 3′,5′-monophosphate levels. Guinea-pig trachea. Eur. J. Pharmacol. **47:** 273–280, 1978.

705. WOODCOCK, E. A., FUNDER, S. W., AND JOHNSTON, C. I.: Decreased cardiac β-adrenergic receptors in deoxycorticosterone-salt and renal hypertensive rats. Circ. Res. **45:** 560–565, 1979.

706. WÖPPEL, W., AND TRENDELENBURG, U.: Temperature-dependent supersen-sitivity of isolated atria to catecholamines. Eur. J. Pharmacol. **23:** 302–305, 1973.

707. WYSE, D. G.: On the "normalization" of active developed force of isolated helical strips of muscular and elastic arteries for variation in wall thick-ness. J. Pharmacol. Meth. **4:** 313–326, 1980.

708. YAMADA, S., ISOGAI, M., OKUDAIRA, H., AND HAYASHI, E.: Correlation between cholinesterase inhibition and reduction in muscarinic receptors and choline uptake by repeated diisopropylfluorophosphate administra-tion: Antagonism by physostigmine and atropine. J. Pharmacol. Exp. Ther. **226:** 519–525, 1983.

709. YAMADA, S., YAMAMURA, H. I., AND ROESKE, W. R.: Alterations in central and peripheral adrenergic receptors in deoxycorticosterone/salt hyperten-sive rats. Life Sci. **27:** 2405–2416, 1980.

710. YAMAGUCHI, I., TORDA, T., HIRATA, F., AND KOPIN, I. J.: Adrenoceptor desensitization after immobilization stress or repeated injection of isopro-terenol. Am. J. Physiol. **240:** H691–H696, 1981.

711. YOKOYAMA, M., AKITA, H., MIZUTANI, T., FUKUZAKI, H., AND WATANABE, Y.: Hyperreactivity of coronary arterial smooth muscles in response to ergonovine from rabbits with hereditary hyperlipidemia. Circ. Res. **53:** 63–71, 1983.

712. YOSHIDA, H., TAKEYASU, K., AND UCHIDA, S.: Muscarinic acetylcholine receptor and smooth muscle contraction. Trends Pharmacol. Sci. **1:** 34–36, 1979.

713. ZAAGSMA, J., OUDHOF, R., VAN DEN HEIJDEN, P. J. C. M., AND PLANTJÉ, J. F.: Subheterogeneity of β-adrenoceptors in the pulmonary and the cardiac system of the guinea pig. Fourth International Catecholamine Symposium, pp. 435–437, Pergamon Press, New York, 1979.

714. ZABOROWSKY, B. R., McMAHAN, W. C., GRIFFIN, W. A., NORRIS, F. H., AND RUFFOLO, R. R., JR.: Computerized graphic methods for determining dissociation constants of agonists, partial agonists, and competitive an-tagonists in isolated smooth muscle preparations. J. Pharmacol. Meth. **4:** 165–178, 1980.