During the past few years, understanding of the role and mechanisms of the peripheral sympathetic nervous system has been advanced by the development of techniques of sympathectomy and through the use of an array of pharmacologic agents. Nevertheless, central noradrenergic mechanisms have been more difficult to assess. The diffuse nature of the noradrenergic system (Carlsson et al., 1962) has made discrete lesioning of norepinephrine cells in brain virtually impossible. Furthermore, pharmacologic agents used to study central mechanisms have had the disadvantage of altering not only the catecholamine systems in the central nervous system but also in the periphery. A pharmacologic agent which could be introduced directly into the central nervous system and which would destroy the central noradrenergic system without altering the peripheral system would offer obvious advantages. Recent observations suggest that 6-hydroxydopamine may prove to be such an agent (Tranzer and Thoenen, 1968; Uretsky and Iversen, 1969).

The observation by Porter et al. (1963) and Laverty et al. (1965) that 6-hydroxydopamine induced a long-lasting depletion of norepinephrine in peripheral organs with sympathetic innervation led to the hypothesis that 6-hydroxydopamine destroyed norepinephrine binding sites (Porter et al., 1963). Recently, Tranzer and Thoenen (1968) have confirmed and extended these observations showing that two weeks after treatment with 6-hydroxydopamine virtually all adrenergic fibers were absent from sympathetically innervated tissues. These reports of peripheral chemical sympathectomy by 6-hydroxydopamine (Thoenen and Tranzer, 1968; Tranzer and Thoenen, 1968) led to investigation of the possibility that 6-hydroxydopamine might also produce selective destruction of brain catecholamine neurons when introduced into the central nervous system (Uretsky and Iversen, 1969).
Bloom et al., 1969; Anagnoste et al., 1969; Traylor and Breese, 1970). Ungerstedt (1968) reported that 6-hydroxydopamine produced degeneration of central catecholamine neurons when injected into a localized area of brain. Utilizing electron micrographic examination of areas rich in adrenergic neurons, Bloom and associates (1969) demonstrated that 6-hydroxydopamine administered into cerebrospinal fluid caused degeneration of central noradrenergic fibers.

The present experiments were designed to investigate the depletion of brain catecholamines after intracisternally administered 6-hydroxydopamine, to determine the effect of this procedure on the retention and metabolism of intracisternally administered H3-norepinephrine and to measure the effect of this derivative on brain tyrosine hydroxylase. Experiments were performed on animals with or without pargyline pretreatment. Our data further support the view that 6-hydroxydopamine can produce selective degeneration of central catecholamine-containing neurons.

Methods. Male Sprague-Dawley rats (140–180 g) were given from 25 to 500 µg of 6-hydroxydopamine hydrobromide intracisternally as described previously (Schanberg et al., 1966). The compound was dissolved in Elliott's “B” solution (Baxter Laboratories, Inc., Morton Grove, Ill.) containing 1 mg/ml of ascorbic acid to prevent oxidation of the 6-hydroxydopamine; total volume administered was 25 µl. In an attempt to decrease deamination of 6-hydroxydopamine (Breese et al., 1969), some animals received 50 mg/kg of pargyline i.p. 30 minutes before the 6-hydroxydopamine injection. A second dose of 6-hydroxydopamine (200 µg) was administered to a few animals seven days after the first injection; pargyline was administered with the first but not with the second dose. After the surgical procedure, each animal received 0.05 ml of benzathine penicillin G suspension i.m.

For the measurement of brain concentrations of norepinephrine, dopamine and serotonin, animals were killed by cervical fracture and decapitated at various times after injection. Brains were removed, rinsed in cold water, homogenized in 10 ml of ice-cold 0.4 N perchloric acid solution, frozen and analyzed within 24 hours. After thawing and centrifugation of the homogenate, an aliquot of the supernatant was transferred to a chromatographic column and washed with 10 ml of 0.4 N sodium acetate solution (pH 8.6) and with 20 ml of glass-distilled water. The catecholamines were eluted from the alumina with 7 ml of 0.2 N acetic acid. Endogenous norepinephrine in the acetic acid eluate was assayed according to the method of Haggendal (1963) except that mercaptoethanol was substituted for British anti-lewisite. Dopamine was assayed according to the method of Anton and Sayre (1964). Values for endogenous amines were uncorrected for recovery which averaged 99% for norepinephrine and 96% for dopamine. Brain serotonin was isolated from an aliquot of the supernatant by the method of Bogdanski et al. (1966) and measured by the fluorescent method of Snyder et al. (1965).

Some animals were given norepinephrine-H3 (5 µc, 7.1 c/mmol) intracisternally in a volume of 25 µl. After the procedure outlined above for killing and homogenization, labeled norepinephrine and its metabolites were isolated with the method of Schanberg et al. (1967). When tyrosine-H3 (34.7 c/mmol) was injected either intracisternally (15 µc) or i.v. (40 µc), the animals were sacrificed one hour later; radioactive norepinephrine and dopamine formed from tyrosine-H3 were isolated according to the method of Sedvall et al. (1968). Less than 0.5% dopamine was noted in the norepinephrine fraction and vice versa. Radioactive amine values were uncorrected for recovery which was 80% for norepinephrine and 70% for dopamine. All radioactivity was measured by scintillation spectrometry; an internal standard of toluene-H3 was used to correct for counting efficiency.

Dissection of brain parts was performed on a moistened filter paper placed on a chilled glass plate. The term “brainstem” as used in this manuscript refers to the area of brain caudal to a cut made behind the superior colliculi after removal of the cerebellar cortices. The term “caudate” refers to that area of brain which lies adjacent to the lateral ventricle and is bounded by the radiation of the corpus callosum. Access to the caudate mass, which contained portions of the globus pallidus, was obtained through the lateral ventricle after hemisection of the whole brain. After removal of the caudate, the remainder of the brain tissue was assayed and is referred to as “rest of brain.”

Tyrosine hydroxylase was isolated from whole brain and brain parts according to the method of Musacchio et al. (1969) except that the enzyme was precipitated from a 1:1 mixture of sample and ammonium sulfate solution. Enzyme activity was determined by measuring the formation of tritiated water from 3,5-ditritiotyrosine in a modified procedure of Nagatsu et al. (1964). The incubation mixture for whole brain, rest of brain and...
brainstem consisted of tyrosine (100 mmmol), $2.9 \times 10^6$ cpm of ditritiotyrosine, 0.5 mmol of 2-amino-4-hydroxy-6,7 dimethylytetrahydropteridine in 0.1 ml of 1 M mercaptoethanol and 0.5 ml of enzyme preparation. For measurement of activity in caudate, half quantities of each reactant were used with the final volume measuring 0.5 ml. Incubation was at $37^\circ$C for 30 minutes. The reaction was terminated by the addition of 0.25 ml of 6% trichloroacetic acid. The mixture was centrifuged and the supernatant placed on a column of Dowex-50 (NH₄⁺) (3 by 0.8 cm). The effluent and one wash were combined and mixed with 10 ml of phosphor-Triton (1:1) and counted. Monoamine oxidase activity was measured according to the method of Wurtman and Axelrod (1964).

The 6-hydroxydopamine HBr was purchased from Regis Chemical Company (Chicago, Ill.) and used without further purification. Pargyline was kindly supplied by Abbott Laboratories (North Chicago, Ill.) H⁷-tyrosine (34.7 c/mmol), H⁷-norepinephrine (7.1 c/mmol) and C⁷-tryptamine (5 mc/mmol) were purchased from New England Nuclear Corporation (Boston, Mass.). All doses of the compounds used in this study are expressed as their free base.

**RESULTS.** Effect of 6-hydroxydopamine on brain norepinephrine, dopamine and 6-hydroxytryptamine. After the intracisternal injection of
different from that in 6-hydroxydopamine-treated animals which did not receive pargyline. Thus, pargyline pretreatment does not appear to offer any advantage in the 6-hydroxydopamine-induced depletion of brain norepinephrine.

With or without pargyline, heart norepinephrine was not affected by intracisternally administered 6-hydroxydopamine.

In contrast to the pargyline effect on norepinephrine depletion by 6-hydroxydopamine, a quite different depleting pattern was noted on dopamine (fig. 3). Without pargyline, 6-hydroxydopamine caused a dose-dependent reduction of dopamine from 25 to 100 μg. The lower doses of 6-hydroxydopamine did not cause a significant depletion of dopamine in either the pargyline or nontreated animals. However, 6-hydroxydopamine administered at the 100 μg and 200 μg doses to pargyline-treated animals had marked effects on brain dopamine, depleting the amine 66 and 76%, respectively. Animals which did not receive pargyline before intracisternally administered 6-hydroxydopamine had their brain dopamine reduced approximately 40%.

Metabolism of intracisternally administered H^-norepinephrine in 6-hydroxydopamine-treated rats. Two hours after the intracisternal administration of H^-norepinephrine, labeled norepinephrine in brain tissue was found to be reduced by 76% (fig. 4). At the same time, H^-normetanephrine was increased 264% and deaminated catechols were reduced by 83%. The O-methylated deaminated products of labeled norepinephrine were only slightly elevated in 6-hydroxydopamine-treated animals.

Formation of labeled norepinephrine and dopamine from H^-tyrosine. After the administration of radioactive tyrosine, labeled dopamine and norepinephrine are formed (Sedvall et al., 1968). Whether the label tyrosine was administered i.v. or intracisternally, animals treated with 6-hydroxydopamine were found to have reduced amounts of labeled dopamine as well as norepinephrine when compared to controls (table 1). In an attempt to determine if 6-hydroxydopamine might be interfering with the storage of amines but not synthesis, animals given H^-tyrosine i.v. were pretreated with pargyline in an attempt to spare catecholamines which might be synthesized. Nevertheless, results by the two routes of administration were
### TABLE 1

**Effect of 6-hydroxydopamine on the synthesis of H^+ catecholamines from H^+ tyrosine**

<table>
<thead>
<tr>
<th>Tyrosine Administration</th>
<th>Treatment</th>
<th>H^+ Tyrosine</th>
<th>H^+ Norepinephrine</th>
<th>H^+ Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Saline</td>
<td>243,000 ± 13,000</td>
<td>710 ± 70</td>
<td>665 ± 40</td>
</tr>
<tr>
<td></td>
<td>6-Hydroxydopamine</td>
<td>266,000 ± 16,000</td>
<td>130 ± 25*</td>
<td>140 ± 25*</td>
</tr>
<tr>
<td>Intracisternal</td>
<td>Saline</td>
<td>168,000 ± 9,500</td>
<td>940 ± 100</td>
<td>555 ± 51</td>
</tr>
<tr>
<td></td>
<td>6-Hydroxydopamine</td>
<td>174,000 ± 17,000</td>
<td>218 ± 34*</td>
<td>192 ± 13*</td>
</tr>
</tbody>
</table>

* Results expressed as counts per minute per brain and are the mean ± S.E.M. of 6 to 12 determinations. Rats received 40 μg of H^+ tyrosine i.v. or 15 μg intracisternally 21 days after the intracisternal injection of 6-hydroxydopamine (200 μg, 2 doses). Animals were sacrificed 1 hour after the labeled tyrosine.

b Animals received pargyline (50 mg/kg) 30 minutes before the administration of H^+ tyrosine.

* P < .001.

comparable. After i.v. injection of H^+ tyrosine, tritium-labeled norepinephrine formed was reduced 82% while H^+ dopamine formed was reduced 75%. The amount of radioactive tyrosine found in brain was not significantly different from its corresponding control.

**Effect of 6-hydroxydopamine on brain tyrosine hydroxylase and monoamine oxidase activity.** In animals treated with 6-hydroxydopamine in combination with pargyline, whole brain tyrosine hydroxylase activity in vitro was found to be decreased by approximately 50% (table 2). This was in close agreement with the depletion of whole brain catecholamines (fig. 1; table 2). A comparable loss of tyrosine hydroxylase activity and norepinephrine depletion was also observed in brainstem. Tyrosine hydroxylase activity in the caudate, normally an active region (McGeer et al., 1967), was markedly reduced in 6-hydroxydopamine-treated rats. Dopamine concentration in the caudate of animals similarly treated was reduced by some 81% (table 2). Tyrosine hydroxylase activity in brain portions remaining after removal of the caudate was approximately 20% of control with catecholamine content being altered proportionally.

### TABLE 2

**Effect of 6-hydroxydopamine (6-OH-DA) on brain catecholamines and tyrosine hydroxylase activity**

<table>
<thead>
<tr>
<th>Brain Part</th>
<th>Norepinephrine</th>
<th>Dopamine</th>
<th>Tyrosine Hydroxylase</th>
<th>Monoamine Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>6-OH-DA (μmol/μg/hr)</td>
<td>Control</td>
<td>6-OH-DA (μmol/μg/hr)</td>
</tr>
<tr>
<td>Whole Brain</td>
<td>330 ± 0.5 (25)</td>
<td>74 ± 4* (25)</td>
<td>204 ± 13 (25)</td>
<td>55 ± 4* (25)</td>
</tr>
<tr>
<td>Brainstem</td>
<td>450 ± 18 (3)</td>
<td>91 ± 6* (3)</td>
<td>880 ± 203 (9)</td>
<td>1092 ± 33* (9)</td>
</tr>
<tr>
<td>Caudate</td>
<td>174 ± 20 (4)</td>
<td>46 ± 15* (4)</td>
<td>5880 ± 203 (9)</td>
<td>1092 ± 33* (9)</td>
</tr>
<tr>
<td>Rest of brain</td>
<td>319 ± 18 (5)</td>
<td>79 ± 10* (5)</td>
<td>206 ± 33 (5)</td>
<td>140 ± 12* (5)</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± S.E.M.; numbers in parentheses indicate the number of animals per group. Brain dissections described in Marron.

b Animals in which whole brain and brainstem were assayed were given 2 doses of 6-hydroxydopamine (200 μg) as described in Marron. Animals in which the caudate and rest of brain were assayed received 200 μg of 6-hydroxydopamine with pargyline. All animals were sacrificed 14 days after the initial treatment.

* P < .001.
Thoenen, 1968; Malmfors and Sachs, 1968) that 6-hydroxydopamine caused a chemical sympathectomy peripherally prompted several laboratories to investigate whether this compound would display the same neurotoxicity toward central catecholamine neurons (Uretsky and Iversen, 1969; Groppetti et al., 1969; Traylor and Breese, 1970). Uretsky and Iversen (1969) have found brain norepinephrine to be lowered for as long as 32 days, thus lending strong support to the view that noradrenergic degeneration could be induced by 6-hydroxydopamine. The present study shows that depletion of brain catecholamines persists at 78 days. With the use of electron microscopy, Bloom and co-workers (1969) have confirmed the presence of degenerating norepinephrine neurons within the central nervous system after administering 6-hydroxydopamine intracisternally. Several reports have indicated that sympathectic denervation decreases tyrosine hydroxylase activity in peripheral organs with adrenergic innervation (Sedvall et al., 1967; Mueller et al., 1969). Therefore, the marked reduction of tyrosine hydroxylase activity in brain (table 2) and the decreased norepinephrine synthesis from tyrosine (table 1) after 6-hydroxydopamine treatment not only add support to the view that noradrenergic neuronal degeneration occurs but also suggests that destruction is quite widespread.

Bloom and associates (1969) recently reported that norepinephrine neurons were more sensitive to depletion by 6-hydroxydopamine than dopaminergic nerve cells. The results of this present study are in full agreement with these earlier results (fig. 3). This observation might have been expected because of the report that dopaminergic neurons are resistant to depletion by intracisternally administered amphetamine derivatives which lower brain norepinephrine levels significantly (Breese et al., 1970). Preliminary evidence indicates that a multiple injection sequence of lower doses of 6-hydroxydopamine may deplete norepinephrine to an even greater extent than a single injection with minimal influence on brain dopamine levels (unpublished data).

The study of the effects of 6-hydroxydopamine peripherally indicated that selective degeneration of adrenergic nerves occurred only in the distal part of the neuron, but not in the perikaryon (Tranzer and Thoenen, 1968). In brain, Bloom and associates (1969) found depletion of norepinephrine by 6-hydroxydopamine to be greatest in areas containing axons and nerve terminals and least in those areas which contained biogenic amine cell bodies. Whereas electron microscopic evaluation of fiber endings would strongly suggest that 6-hydroxydopamine eliminates norepinephrine-containing terminals, the morphologic evidence concerning the fate of the cell bodies is not yet available (Bloom et al., 1969). In the present study, all brain areas seem to be influenced uniformly by the neuronal toxicity of 6-hydroxydopamine. Whether this increased effect may be due to adrenergic cell body destruction or to an increased effectiveness on fiber terminals was not established. Whatever may be the case, it is clear that the effects are quite prolonged (fig. 2), suggesting that axonal regeneration does not occur within the central nervous system as previously reported for the periphery (Thoenen and Tranzer, 1968).

After the monoamine oxidase inhibitor paraglyine, 6-hydroxydopamine (200 µg) was found to decrease not only brain norepinephrine but also dopamine (fig. 1). Early observations in our laboratory had indicated that brain dopamine was only moderately altered by 6-hydroxydopamine. With this in mind, it was reasoned that taking advantage of the fact that amines pass slowly from the central nervous system after monoamine oxidase inhibition (Schanberg, et al., 1968; Breese et al., 1969) this minor effect of 6-hydroxydopamine could be amplified. As shown in figure 1, this procedure has permitted consistent depletion of brain dopamine as well as norepinephrine (Breese et al., 1970). Furthermore, the results of the present study and the recent work of Uretsky and Iversen (1970) emphasize that dopaminergic neurons are not only depleted but are probably affected by the neurotoxicity of 6-hydroxydopamine. This is exemplified by the prolonged depletion of dopamine (fig. 2), by lack of in vivo synthesis of labeled dopamine from H2-tyrosine (table 1) and by the reduction of tyrosine hydroxylase activity in the basal ganglia (table 2) well known for its high dopamine content (Carlsson, 1959). In further support of this view, F. E. Bloom (personal communication) has recently found degenerating fibers in the caudate of paraglyine-treated animals which have received 6-hydroxydopamine intracisternally.
After the administration of H-\(^{3}\)-norepinephrine or norepinephrine and dopamine neurons should aid significantly in the effort to define the central role of these amines. However, the usefulness of 6-hydroxydopamine as a pharmacologic agent for evaluating the central role of catecholamines has yet to be established. In view of the central function attributed to these amines, it has been somewhat surprising to find that the general appearance of rats “centrally sympathectomized” with 6-hydroxydopamine has thus far been grossly indistinguishable from that of untreated controls except for a slight decrease in body weight and a lack of selfgrooming. Certainly these animals do not display a classical “reserpine syndrome” (Bein, 1953). Experiments are presently underway to evaluate further the role of 6-hydroxydopamine as a specific tool for the study of central adrenergic mechanisms.

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REFERENCES


