

# Evidence for Possible Involvement of 5-HT<sub>2B</sub> Receptors in the Cardiac Valvulopathy Associated With Fenfluramine and Other Serotonergic Medications

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**Background**—Serotonergic medications with various mechanisms of action are used to treat psychiatric disorders and are being investigated as treatments for drug dependence. The occurrence of fenfluramine-associated valvular heart disease (VHD) has raised concerns that other serotonergic medications might also increase the risk of developing VHD. We hypothesized that fenfluramine or its metabolite norfenfluramine and other medications known to produce VHD have preferentially high affinities for a particular serotonin receptor subtype capable of stimulating mitogenesis.

**Methods and Results**—Medications known or suspected to cause VHD (positive controls) and medications not associated with VHD (negative controls) were screened for activity at 11 cloned serotonin receptor subtypes by use of ligand-binding methods and functional assays. The positive control drugs were (±)-fenfluramine; (+)-fenfluramine; (−)-fenfluramine; its metabolites (±)-norfenfluramine, (+)-norfenfluramine, and (−)-norfenfluramine; ergotamine; and methysergide and its metabolite methylergonovine. The negative control drugs were phentermine, fluoxetine, its metabolite norfluoxetine, and trazodone and its active metabolite *m*-chlorophenylpiperazine. (±)-, (+)-, and (−)-Norfenfluramine, ergotamine, and methylergonovine all had preferentially high affinities for the cloned human serotonin 5-HT<sub>2B</sub> receptor and were partial to full agonists at the 5-HT<sub>2B</sub> receptor.

**Conclusions**—Our data imply that activation of 5-HT<sub>2B</sub> receptors is necessary to produce VHD and that serotonergic medications that do not activate 5-HT<sub>2B</sub> receptors are unlikely to produce VHD. We suggest that all clinically available medications with serotonergic activity and their active metabolites be screened for agonist activity at 5-HT<sub>2B</sub> receptors and that clinicians should consider suspending their use of medications with significant activity at 5-HT<sub>2B</sub> receptors. (*Circulation*. 2000;102:2836-2841.)

**Key Words:** valves ■ fenfluramine ■ norfenfluramine ■ receptors

The association of valvular heart disease (VHD) with the administration of phentermine and fenfluramine (phen/fen) and dexfenfluramine<sup>1</sup> led to the withdrawal of fenfluramine and dexfenfluramine from the marketplace in September 1997. Well-controlled echocardiographic prevalence studies of VHD in clinically asymptomatic patients who had taken appetite suppressants report varying case rates, ranging from no statistically significant increase compared with control subjects<sup>2,3</sup> to moderate<sup>4,5</sup> and substantially increased<sup>6</sup> case rates. Using a clinical end-point, Jick et al<sup>7</sup> reported that the use of either fenfluramine or dexfenfluramine was associated with a cumulative 5-year incidence of newly diagnosed VHD, primarily aortic regurgitation, of 35 cases per 10 000, indicating that the risk of developing clinically significant valve disease is low. The lack of any VHD cases associated with the use of phentermine alone, along with the fact that

VHD occurs in users of both phen/fen and dexfenfluramine, suggests that fenfluramine is the likely cause of the VHD.

Fenfluramine (Pondimin) is a racemic mixture of 2 enantiomers. (+)-Fenfluramine, also called dexfenfluramine, was marketed under the trade name Redux. Fenfluramine and dexfenfluramine are metabolized to (±)-norfenfluramine and (+)-norfenfluramine, respectively.<sup>8</sup> A major action of fenfluramine and its metabolites is to release neuronal serotonin (5-HT) via a carrier-mediated exchange mechanism.<sup>9</sup> In addition, fenfluramine and norfenfluramine have direct agonist actions at certain 5-HT receptors, in particular members of the 5-HT<sub>2</sub> receptor family.<sup>10</sup> Phentermine, conversely, preferentially releases dopamine.<sup>9</sup>

Serotonergic medications with various mechanisms of action are widely used to treat psychiatric disorders and are being investigated as treatments for drug dependence, gastro-

intestinal disorders, and hypertension. Fenfluramine-associated VHD has led some to propose caution "in the long-term use of other agents that act on serotonergic mechanisms, albeit by different pathways."<sup>11</sup> Uncritical acceptance of this proposal would significantly affect the treatment of psychiatric patients as well as hinder the development of new therapeutics. Thus, determining the mechanism of fenfluramine-associated VHD is likely to not only shed light on the adverse effects of this particular medication but also clarify whether this side effect might occur with other medications that act via serotonergic mechanisms.

Perhaps by analogy with the ability of fenfluramine to increase synaptic levels of 5-HT,<sup>9</sup> investigators proposed that fenfluramine produces VHD via a serotonergic mechanism: increases in plasma 5-HT (see review<sup>12</sup>). However, as noted elsewhere, fenfluramine decreases platelet and plasma 5-HT in animals and humans, and phen/fen treatment lowers plasma 5-HT in humans.<sup>13</sup> Therefore, another explanation must be sought to clarify how fenfluramine could cause VHD.

In light of the established role of 5-HT as a mitogen,<sup>14</sup> we undertook the present study to determine whether fenfluramine [(±)-fenfluramine, (+)-fenfluramine, (−)-fenfluramine] or its metabolites [(±)-norfenfluramine, (+)-norfenfluramine, (−)-norfenfluramine] might activate mitogenic 5-HT receptors. Several other drugs were included in the study to provide both positive and negative controls. Additional "positive controls" included methysergide, its active metabolite methylethylergonovine,<sup>15</sup> and ergotamine. Methysergide and ergotamine are well known to produce primarily left-sided VHD affecting the mitral valve.<sup>16,17</sup> Negative controls included phentermine, fluoxetine, and its metabolite norfluoxetine, which have not been associated with VHD. We included the antidepressant trazodone and its active metabolite *m*-chlorophenylpiperazine (mCPP) as an additional negative control. In addition to having activity at a wide range of 5-HT receptors,<sup>18</sup> mCPP shares with fenfluramine the ability to release brain 5-HT via a carrier-mediated exchange mechanism.<sup>19</sup> Trazodone is not associated with VHD. Our working hypothesis was that the "positive control" drugs would share in common the ability to activate a particular 5-HT receptor expressed in heart valves that is mitogenic, and that the "negative control" drugs would not. We called this the commonly activated serotonin receptor, or CASR.

## Methods

### Materials

The National Institute of Mental Health's Chemical Synthesis and Drug Supply Program provided the following compounds: (±)-norfenfluramine, (+)-norfenfluramine, and (−)-norfenfluramine. (±)-Fenfluramine and (+)-fenfluramine were obtained from the NIDA Drug Supply Program (Rockville, Md). (−)-Fenfluramine, fluoxetine, and norfluoxetine were purchased from Research Biochemicals Inc. Phentermine, mCPP, methysergide, and methylethylergonovine were purchased from Sigma Chemical Co. Trazodone was supplied by the NIMH Psychoactive Drug Screening Program.

### Radioligand Binding Assays and Sources of cDNA Clones

Radioligand binding assays for 5-HT receptors were performed as previously detailed with cloned human (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>,

5-HT<sub>1E</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>) or rat (5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>) cDNAs expressed in COS-7 cells.<sup>20</sup> The h5HT<sub>1A</sub> cDNA was obtained from John Raymond (Medical University of South Carolina), the h5-HT<sub>1B</sub>, h5HT<sub>1D</sub>, h5HT<sub>6</sub>, and h5HT<sub>7</sub> cDNAs were from Mark Hamblin (University of Washington), the rat 5HT<sub>2A</sub> and rat 5HT<sub>2C</sub> cDNAs were from David Julius (University of California San Francisco), and the h5HT<sub>5A</sub> cDNA was from Rene Hen (Columbia University).

The h5-HT<sub>2B</sub> cDNA was obtained by amplification from human brain cDNA (Quickclone cDNA; Clontech) with Pfu polymerase and subcloned in-frame into the pTag2A eukaryotic expression vector (Stratagene). The h5HT<sub>1E</sub> cDNA was obtained by amplification of human genomic DNA (Clontech) with Pfu polymerase and subcloned into the pcDNA3.0 eukaryotic expression vector. The sequences of the h5HT<sub>2B</sub> and h5HT<sub>1E</sub> cDNAs were verified by automatic DNA sequencing (Cleveland Genomics, Inc). Detailed protocols for transfection, using FUGENE6, as well as complete details of all the radioligand binding assays, are available.<sup>20,21a</sup>

For initial screening, compounds were tested at concentrations of 10 μmol/L; *K<sub>i</sub>* determinations using 7 concentrations of unlabeled ligand spanning 4 orders of magnitude were obtained on compounds that gave >50% inhibition at 10 μmol/L. *K<sub>i</sub>* values were calculated with the LIGAND program as previously detailed.<sup>20–23</sup>

### Functional Assays: Phosphoinositide Hydrolysis

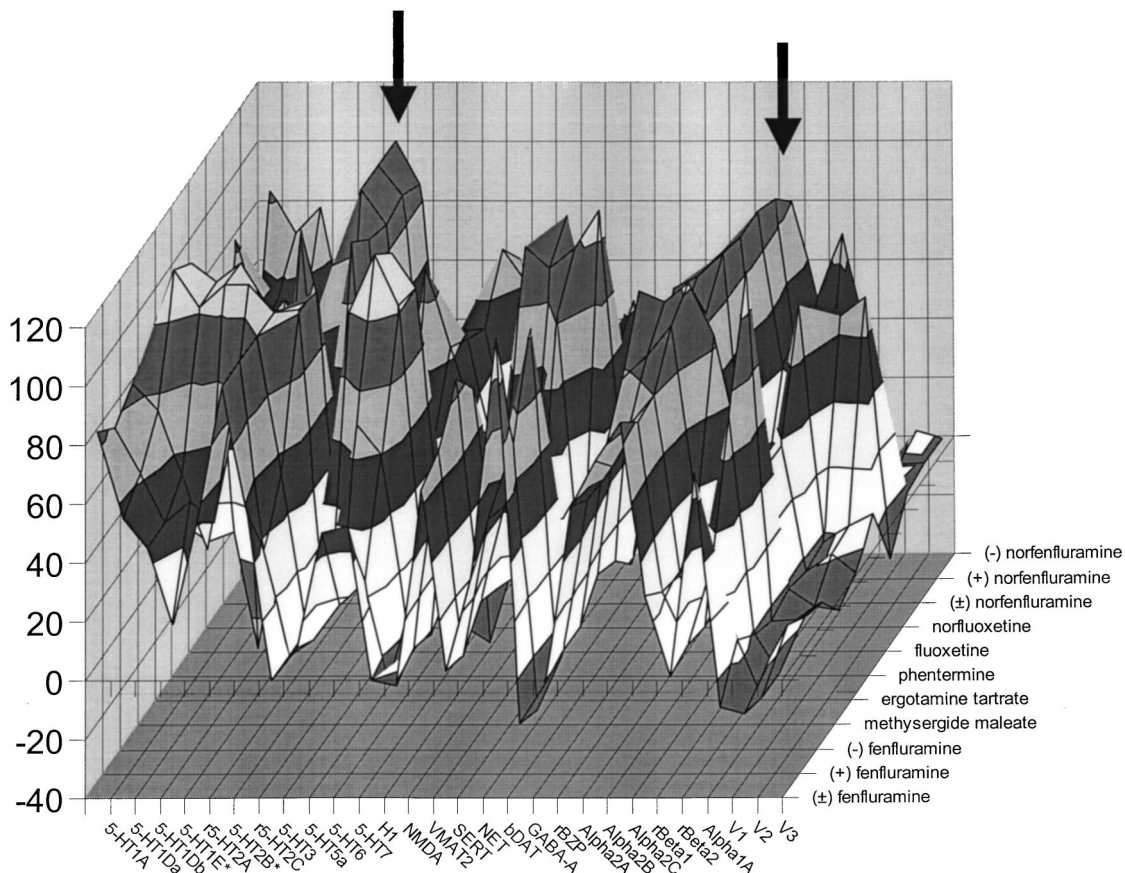
Phosphoinositide hydrolysis assays were performed with stably (5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>) or transiently (5-HT<sub>2B</sub>) expressed receptors plated in 24-well culture plates as previously detailed.<sup>21b,22</sup> In brief, transfected cells were loaded with [<sup>3</sup>H]inositol (15 Ci/mmol; 1 μCi/mL) overnight in inositol-free DMEM without serum. The next day, <sup>3</sup>H-inositol phosphate accumulation assays were performed in a modified Krebs-bicarbonate buffer as previously detailed. *K<sub>act</sub>* (nmol/L) and percent *V<sub>max</sub>* (relative to 5-HT) values were calculated as previously described.<sup>21b–23</sup>

## Results

Figure 1 reports the results of the initial screen of compounds in the ligand-binding assays. On the basis of these results, *K<sub>i</sub>* values were determined for selected compounds. The fenfluramines and norfenfluramines had *K<sub>i</sub>* values ranging from 673 to 1950 nmol/L and lacked agonist activity at the 5-HT<sub>1A</sub> receptor (data not shown). The other positive control drugs (methysergide, ergotamine, and methylethylergonovine) had high affinities for the 5-HT<sub>1A</sub> site and were full and potent agonists (data not shown). This pattern of results, along with the fact that clinically used 5-HT<sub>1A</sub> agonists, such as buspirone, are not associated with VHD, suggests that the 5-HT<sub>1A</sub> receptor is not the CASR.

The fenfluramines and norfenfluramines had low affinity for the 5-HT<sub>1D</sub>, 5-HT<sub>1B</sub>, and 5-HT<sub>1E</sub> receptors (data not shown), and the other positive-control compounds had high affinities for these sites. Because of the low affinity of the fenfluramines for these sites, we did not conduct functional activity studies. The low affinities of the fenfluramines for the h5-HT<sub>1D/1B</sub> receptors, coupled with the observation that sumatriptan, a potent 5-HT<sub>1D/1B</sub> agonist<sup>24</sup> widely used for treating migraine headaches, is not associated with VHD, suggests that the 5-HT<sub>1D/1B</sub> receptors do not mediate fenfluramine-associated VHD.

The ergot compounds had high affinity for the 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors (Table 1). With regard to the 5-HT<sub>2A</sub> receptor, ergotamine was a full agonist, methysergide was a weak partial agonist, and methylethylergonovine was a potent, full agonist (Table 2). The fenfluramines had micro-



**Figure 1.** 3D representation of initial screen of compounds in ligand-binding assays. Data show mean percent inhibition of specific binding (along z axis) at all tested neurotransmitter receptors and channels (along x axis) by 10  $\mu\text{mol/L}$  concentration of test agents (along y axis). Left arrow shows location of 5-HT<sub>2</sub>-family receptors and fenfluramine analogues; right arrow shows location of phentermine and adrenergic receptors. Human clones were used except where noted: 5-HT<sub>1A</sub> indicates 5HT<sub>1A</sub> serotonin receptor; 5-HT<sub>1Da</sub>, 5HT<sub>1D</sub> serotonin receptor; 5-HT<sub>1Db</sub>, 5HT<sub>1B</sub> serotonin receptor; 5-HT<sub>1E</sub>, 5HT<sub>1E</sub> serotonin receptor; r5HT<sub>2A</sub>, rat 5HT<sub>2A</sub> serotonin receptor; 5-HT<sub>2B</sub>, 5HT<sub>2B</sub> serotonin receptor; r5HT<sub>2C</sub>, rat 5-HT<sub>2C</sub> serotonin receptor; 5-HT<sub>3</sub>, mouse 5HT<sub>3</sub> receptor; 5-HT<sub>5A</sub>, 5HT<sub>5A</sub> serotonin receptor; 5-HT<sub>6</sub>, 5HT<sub>6</sub> serotonin receptor; 5-HT<sub>7</sub>, 5HT<sub>7</sub> serotonin receptor; H<sub>1</sub>, histamine H<sub>1</sub> receptor; NMDA, NMDA glutamate receptor; VMAT2, vesicular monoamine transporter type II; SERT, serotonin transporter; NET, norepinephrine transporter; bDAT, bovine dopamine transporter; GABA-A, rat GABA-A receptor; rBZP, rat benzodiazepine binding site; Alpha<sub>2A</sub>,  $\alpha_{2A}$ -adrenergic receptor; Alpha<sub>2B</sub>,  $\alpha_{2B}$ -adrenergic receptor; Alpha<sub>2C</sub>,  $\alpha_{2C}$ -adrenergic receptor; rBeta<sub>1</sub>, rat  $\beta_1$ -adrenergic receptor; rBeta<sub>2</sub>, rat  $\beta_2$ -adrenergic receptor; alpha<sub>1A</sub>, rat  $\alpha_{1A}$ -adrenergic receptor; V<sub>1</sub>, vasopressin-1 receptor; V<sub>2</sub>, vasopressin-2 receptor; and V<sub>3</sub>, vasopressin-3 receptor.

molar affinity for the 5-HT<sub>2A</sub> receptor. Although the fenfluramines were weak partial agonists, the norfenfluramines were somewhat more potent partial agonists. The relatively low affinity of the norfenfluramines at the 5-HT<sub>2A</sub> receptor suggests that this site is not the CASR.

The norfenfluramines were moderately potent at the 5-HT<sub>2C</sub> receptor and were full agonists. The fenfluramines were also full agonists but were significantly less potent than the norfenfluramines. Among the other positive-control test drugs, methysergide and methylergonovine had high affinity for the 5-HT<sub>2C</sub> receptor, with methysergide being a weak partial agonist and methylergonovine being a potent full agonist. Ergotamine, conversely, was a potent partial agonist. mCPP, a negative control drug, was a potent full agonist at the 5-HT<sub>2C</sub> receptor. As reported previously,<sup>25</sup> fluoxetine and its metabolite were moderate-potency antagonists. The observation that human heart valves have very low levels of this receptor<sup>10</sup> strongly suggests that the 5-HT<sub>2C</sub> receptor is not the CASR.

The norfenfluramines had high affinity (10 to 50 nmol/L) for the 5-HT<sub>2B</sub> receptor, in confirmation of recent studies.<sup>10,26</sup>

Functional studies demonstrated that the norfenfluramines were full agonists at the 5-HT<sub>2B</sub> site (Figure 2). The fenfluramines, in contrast, bound to the 5-HT<sub>2B</sub> receptor with  $K_i$  values of  $\approx 5 \mu\text{mol/L}$ . Ergotamine was a potent partial agonist, and methysergide was a very-low-efficacy partial agonist at the 5-HT<sub>2B</sub> receptor. Methylergonovine was a high-affinity partial agonist. Among the negative control drugs, mCPP was a moderate-potency partial agonist with the same efficacy as methylergonovine. With the exception of the findings with mCPP, these findings suggest that the 5-HT<sub>2B</sub> receptor may be the CASR. Trazodone, which binds with high affinity to the 5-HT<sub>2B</sub> receptor (Table 1), is a potent 5-HT<sub>2B</sub> antagonist (data not shown).

The fenfluramines and norfenfluramines were inactive at the 5-HT<sub>3</sub> and 5-HT<sub>6</sub> receptors (data not shown), indicating that these receptors are most likely not the CASR. Although the norfenfluramines have moderate affinity at the 5-HT<sub>7</sub> receptor, ergotamine had low affinity for this site, suggesting that the 5-HT<sub>7</sub> receptor is not the CASR (data not shown). Phentermine was inactive at all 5-HT receptors assayed here.

**TABLE 1.  $K_i$  Values of Test Drugs at 5-HT<sub>2</sub> Receptors**

Drug	Rat 5-HT <sub>2A</sub>	Human 5-HT <sub>2B</sub>	Rat 5-HT <sub>2C</sub>
(±)-Fenfluramine	5 216±2.5	4134±753	3183±374
(+)-Fenfluramine	11 107±1354	5099±690	6245±514
(-)-Fenfluramine	5463±352	5713±1344	3415±542
(±)-Norfenfluramine	2316±163	52.1±12.3	557±36
(+)-Norfenfluramine	1516±88	11.2±4.3	324±7.1
(-)-Norfenfluramine	3841±361	47.8±18.0	814±58
Ergotamine	9.0±0.6	3.0±0.2	12.0±0.9
Methysergide	15.0±2.4	9.1±2.9	1.8±0.1
Methylergonovine	12.6±0.6	0.49±0.09	12.4±0.6
Fluoxetine	299±31	5030±1152	50.0±5.9
Norfluoxetine	638±63	5063±1161	286±35
Trazodone	19.8±1.4	73.6±21.17	402±26
mCPP	391±27	3.2±0.6	59±6.5
5-HT	614±43	4.0±1.1	12.2±0.8
Phentermine	>10 000	>10 000	>10 000

Values are mean±SEM (n=3). Units are nanomolar.

**Discussion**

The study reported here examined the interaction of 9 medications that are associated with VHD (positive controls) and 5 medications that are not associated with VHD (negative controls) at 11 cloned 5-HT receptors. We sought to identify a mitogenic 5-HT receptor that would be activated by the positive but not the negative controls. We hypothesized that this CASR mediates fenfluramine-associated VHD. Among the receptors assayed, the 5-HT<sub>2B</sub> receptor has 5 characteristics consistent with its being the CASR: (1) it is located on both mitral and aortic valves<sup>10</sup>; (2) it mediates mitogenesis<sup>27</sup>; (3) the norfenfluramines have high affinity and efficacy at the 5-HT<sub>2B</sub> receptor; (4) ergotamine and methylergonovine, the active metabolite of methysergide, are high-affinity partial agonists for the 5-HT<sub>2B</sub> receptor; and (5) with the exception of mCPP (see below), the negative control drugs (fluoxetine, norfluoxetine, phentermine) have very low affinity for this site and lack agonist effects at this receptor. The 5-HT<sub>2C</sub> receptor is ruled out as the CASR, primarily because few of these receptors are expressed in heart valves.<sup>10</sup>

There are several observations that, at first glance, are difficult to reconcile with the hypothesis that the 5-HT<sub>2B</sub> receptor mediates the valvulopathy associated with administration of fenfluramine, ergotamine, and methysergide. First, whereas (+)-fenfluramine produces primarily aortic regurgitation,<sup>4,7</sup> ergotamine and methysergide produce primarily mitral regurgitation.<sup>16</sup> Given that 5-HT<sub>2B</sub> receptors are found on both valves,<sup>10</sup> the mechanism underlying the anatomic specificity of the valvulopathy associated with these 2 classes of drugs is enigmatic. Second, methysergide appears to produce a more severe form of VHD than fenfluramine. Patients with fenfluramine-associated VHD are clinically asymptomatic and typically do not have audible heart murmurs.<sup>2</sup> The best estimate of the incidence of clinically significant fenfluramine-associated VHD is 0.07% per year.<sup>7</sup> In contrast, patients treated with methysergide developed

**TABLE 2. Functional Activity of Test Drugs at 5-HT<sub>2</sub> Receptors**

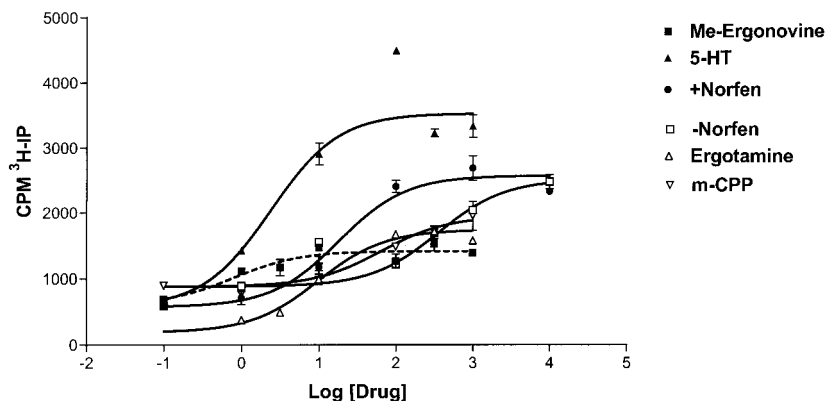
Drug	Human 5-HT <sub>2A</sub>	Human 5-HT <sub>2B</sub>	Human 5-HT <sub>2C</sub>
(±)-Fenfluramine			
$K_{act}$	4 131±14 400	ND	ND
$V_{max}$	15±2.35		
(+)-Fenfluramine			
$K_{act}$	>10 000	379±70	362±64
$V_{max}$	ND	38±8.2	80±5.9
(-)-Fenfluramine			
$K_{act}$	5 279±587	1248±252	360±91
$V_{max}$	43±4.2	47±2.9	84±7.4
(±)-Norfenfluramine	ND	ND	ND
(+)-Norfenfluramine			
$K_{act}$	630±141	18.4±5.3	13±2.4
$V_{max}$	88±5.3	73±3.5	100±6.5
(-)-Norfenfluramine			
$K_{act}$	1 565±190	357±105	18±5.3
$V_{max}$	93±5.3	71±8.8	80±10
Ergotamine			
$K_{act}$	16±2.3	9.8±1.8	5±1.76
$V_{max}$	75±1.8	56±1.8	75±8.8
Methysergide			
$K_{act}$	3.5±1.00	150±25	2.9±0.9
$V_{max}$	24±1.76	18±2.4	33±2.0
Methylergonovine			
$K_{act}$	1.3±0.2	0.8±0.3	2.5±0.7
$V_{max}$	70±4.1	40±1.8	103±4.1
Fluoxetine	ND	ND	Antagonist $K_i=616±101$
Norfluoxetine	ND	ND	Antagonist $K_i=43±10$
Trazodone	Antagonist	Antagonist	Antagonist
mCPP			
$K_{act}$	65±10	64±15	0.64±0.17
$V_{max}$	55±6.5	43±8.2	79±8.8
5-HT			
$K_{act}$	66±15	2.4±0.9	0.6±0.1
$V_{max}$	100	100	100
Phentermine			
$K_{act}$	ND	ND	1394±264
$V_{max}$			66±5.9

Values are mean±SEM (n=3).  $K_{act}$  values are nmol/L±SEM;  $V_{max}$  values are % of 5-HT±SEM. ND indicates not done.

clinically significant VHD, including new heart murmurs,<sup>16</sup> with an incidence of 3%.<sup>16</sup> Thus, although methylergonovine is a less effective agonist at the 5-HT<sub>2B</sub> receptor than norfenfluramine, it produces a more severe form of VHD in a greater number of patients. Third, methysergide administration is associated with fibrosis of other anatomic sites in addition to heart valves.<sup>28</sup> In contrast, fenfluramine-associated fibrosis appears to be localized to heart valves.

Fourth, the finding that mCPP has activity at 5-HT<sub>2B</sub> receptors must be reconciled with observations that traz-





**Figure 2.** Dose-response curves for selected agents in 5-HT<sub>2B</sub> functional assay: (+)-norfenfluramine, (–)-norfenfluramine, ergotamine, methylethylergonovine, and mCPP. Data represent mean ± SD of [<sup>3</sup>H]IP accumulation in counts per minute from HEK-293 cells transiently transfected with h5HT<sub>2B</sub> receptor from a typical experiment. Curves were generated with GraphPad Prism and represent theoretical fits of data using parameter estimates obtained from fits.

odone, from which it is metabolically derived, is not associated with VHD. Therapeutic doses of trazodone generate plasma levels of mCPP from 150 to 550 nmol/L,<sup>29</sup> which are in the range needed to activate 5-HT<sub>2B</sub> receptors. However, trazodone is a potent 5-HT<sub>2B</sub> receptor antagonist, and its plasma levels are ≈5-fold higher than that of mCPP.<sup>29</sup> Thus, trazodone would act to block activation of 5-HT<sub>2B</sub> receptors by mCPP.

Thus, a possible explanation for the differing degrees of VHD prevalence seen among the 5-HT<sub>2B</sub> agonists is the degree of 5-HT<sub>2B</sub> antagonism produced by either parent drug or metabolites. Methysergide, as a very-low-efficacy 5-HT<sub>2B</sub> agonist, would act to antagonize the agonist effects of methylethylergonovine (Table 2). However, methysergide is rapidly metabolized to methylethylergonovine, is more rapidly eliminated, and achieves blood levels 10-fold lower than methylethylergonovine.<sup>15</sup> Because the agonist actions of methylethylergonovine are most likely not significantly blocked by its parent drug, methysergide administration would probably cause a higher prevalence of VHD. In the case of fenfluramine, (+)-fenfluramine and (–)-fenfluramine have lower efficacy (≈40%) at the 5-HT<sub>2B</sub> receptor than (+)-norfenfluramine (75%) and achieve blood levels twice that of norfenfluramine.<sup>8</sup> This indicates that the parent drugs would partially antagonize activation of 5-HT<sub>2B</sub> receptors by (+)-norfenfluramine. This may explain why the fenfluramines appear to produce a less severe form of VHD than methysergide (see above).

Taken at face value, the 5-HT<sub>2B</sub> hypothesis predicts that elevations of plasma 5-HT should produce valvulopathy in both the aortic and mitral valves. Indeed, 5-HT is the most potent and efficacious agonist at the 5-HT<sub>2B</sub> receptor. Medications such as lithium and monoamine oxidase inhibitors produce sustained 2-fold increases in plasma 5-HT and are not associated with VHD.<sup>30,31</sup> This suggests that modest elevations of plasma 5-HT are unlikely to produce this adverse effect. Patients with carcinoid syndrome develop extremely high levels of plasma 5-HT (>500 nmol/L<sup>32</sup>), and fibrotic valve lesions occur exclusively on the right side of the heart. Although some attribute the lack of left-sided VHD in carcinoid syndrome to the almost complete removal of plasma 5-HT by the lung before the blood empties into the left atrium,<sup>12</sup> this hypothesis fails to take into account the fact that the blood samples taken for analysis of 5-HT are

withdrawn from the antecubital vein in the arm, the blood of which is derived most directly from the left side of the heart. Although the right side of the heart is undoubtedly bathed in higher concentrations of 5-HT than the left side, the left side is clearly exposed to 5-HT concentrations well in excess of that necessary to completely activate the 5-HT<sub>2B</sub> receptor. Thus, it is not clear why carcinoid syndrome produces fibrotic lesions on the valves of the right side of the heart, whereas fenfluramine, methysergide and ergotamine affect primarily the valves of the left side.

Viewed collectively, these considerations suggest that activation of 5-HT<sub>2B</sub> receptors may be necessary to produce VHD. Clearly, other factors also determine the susceptibility of an individual to develop the lesion, its anatomic location, and its severity. Despite our lack of knowledge of what these factors might be, these data suggest that serotonergic medications, which do not activate 5-HT<sub>2B</sub> receptors, are unlikely to produce VHD. These findings further suggest that the simplest pathogenic mechanism to explain anorexigen-associated VHD is a direct activation of 5-HT<sub>2B</sub> receptors by norfenfluramine. This mechanism does not necessitate the formulation of unlikely synergistic mechanisms between phentermine and fenfluramine<sup>33</sup> or a role for plasma 5-HT to explain the occurrence of VHD. Finally, on the basis of these results and those recently reported by Fitzgerald et al,<sup>10</sup> we suggest that all clinically available medications with serotonergic activity and their metabolites should be screened for agonist activity at 5-HT<sub>2B</sub> receptors.

## Note Added in Proof

Dr Roth's laboratory has begun to measure the efficacies of clinically used serotonergic compounds at the h5-HT<sub>2B</sub> receptor and have not yet found any that are agonists.

## Acknowledgments

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## **Evidence for Possible Involvement of 5-HT<sub>2B</sub> Receptors in the Cardiac Valvulopathy Associated With Fenfluramine and Other Serotonergic Medications**

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