Evidence for Possible Involvement of 5-HT\textsubscript{2B} 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 (\(\pm\))-fenfluramine; (+)-fenfluramine; (−)-fenfluramine; its metabolites (\(\pm\))-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. (\(\pm\)), (+), and (−)-Norfenfluramine, ergotamine, and methylergonovine all had preferentially high affinities for the cloned human serotonin 5-HT\textsubscript{2B} receptor and were partial to full agonists at the 5-HT\textsubscript{2B} receptor.

Conclusions—Our data imply that activation of 5-HT\textsubscript{2B} receptors is necessary to produce VHD and that serotonergic medications that do not activate 5-HT\textsubscript{2B} 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\textsubscript{2B} receptors and that clinicians should consider suspending their use of medications with significant activity at 5-HT\textsubscript{2B} receptors.

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Key Words: valves ■ fenfluramine ■ norfenfluramine ■ receptors

The association of valvular heart disease (VHD) with the administration of phentermine and fenfluramine (phen/fen) and dexfenfluramine\textsuperscript{1} 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\textsuperscript{2,3} to moderate\textsuperscript{4,5} and substantially increased\textsuperscript{6} case rates. Using a clinical end-point, Jick et al\textsuperscript{7} 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 (\(\pm\))-norfenfluramine and (+)-norfenfluramine, respectively.\textsuperscript{8} A major action of fenfluramine and its metabolites is to release neuronal serotonin (5-HT) via a carrier-mediated exchange mechanism.\textsuperscript{9} In addition, fenfluramine and norfenfluramine have direct agonist actions at certain 5-HT receptors in particular members of the 5-HT\textsubscript{3} receptor family.\textsuperscript{10} Phentermine, conversely, preferentially releases dopamine.\textsuperscript{9}

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.” 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 sympathetic levels of 5-HT, investigators proposed that fenfluramine produces VHD via a serotonergic mechanism: increases in plasma 5-HT (see review12). However, as noted elsewhere, fenfluramine decreases platelet and plasma 5-HT in animals and humans, and phen/phen treatment lowers plasma 5-HT in humans. 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, 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 methylergonovine, and ergotamine. Methysergide and ergotamine are well known to produce primarily left-sided VHD affecting the mitral valve.16,17 Negative controls included phentermine, fluoxetine, and its active 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,18 mCPP shares with fenfluramine the ability to release brain 5-HT via a carrier-mediated exchange mechanism.19 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 methylergonovine 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-HT1A, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3A, 5-HT3B, 5-HT3C, 5-HT4, 5-HT5) or rat (5-HT1D, 5-HT1E) cDNAs expressed in COS-7 cells.20 The h5HT1A cDNA was obtained from John Raymond (Medical University of South Carolina), the h5-HT2A, h5-HT3A, and h5HT3B cDNAs were from Mark Hamblin (University of Washington), the rat 5HT2A and rat 5HT3C cDNAs were from David Julius (University of California San Francisco), and the h5HT3A cDNA was from Rene Hen (Columbia University).

The h5-HT2A cDNA was obtained by amplification from human brain cDNA (Quickclone cDNA; Clonetech) with Pfu polymerase and subcloned in-frame into the pTag2A eukaryotic expression vector (Stratagene). The h5HT1C cDNA was obtained by amplification of human genomic DNA (Clonetech) with Pfu polymerase and subcloned into the pcDNA3.0 eukaryotic expression vector. The sequences of the h5HT2A and h5HT3B 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.20,21a

For initial screening, compounds were tested at concentrations of 10 μmol/L; K_i 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_i values were calculated with the LIGAND program as previously detailed.20–23

Functional Assays: Phosphoinositide Hydrolysis

Phosphoinositide hydrolysis assays were performed with stably (5-HT2A, 5-HT3C) or transiently (5-HT2B) expressed receptors plated in 24-well culture plates as previously detailed. In brief, transfected cells were loaded with [3H]inositol (15 Ci/mmol; 1 μCi/mL) overnight in isoinositol-free DMEM without serum. The next day, 3H-inositol phosphate accumulation assays were performed in a modified Krebs-bicarbonate buffer as previously detailed. 20,21

Results

Figure 1 reports the results of the initial screen of compounds in the ligand-binding assays. On the basis of these results, K_i values were determined for selected compounds. The fenfluramines and norfenfluramines had K_i values ranging from 673 to 1950 nmol/L and lacked agonist activity at the 5-HT1A receptor (data not shown). The other positive control drugs (methysergide, ergotamine, and methylergonovine) had high affinities for the 5-HT1A site and were full and potent agonists (data not shown). This pattern of results, along with the fact that clinically used 5-HT1A agonists, such as buspirone, are not associated with VHD, suggests that the 5-HT1A receptor is not the CASR.

The fenfluramines and norfenfluramines had low affinity for the 5-HT1D, 5-HT1B, and 5-HT1E 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-HT1D receptors, coupled with the observation that sumatriptan, a potent 5-HT1D agonist widely used for treating migraine headaches, is not associated with VHD, suggests that the 5-HT1D receptors do not mediate fenfluramine-associated VHD.

The ergot compounds had high affinity for the 5-HT3A, 5-HT3B, and 5-HT3C receptors (Table 1). With regard to the 5-HT3A receptor, ergotamine was a full agonist, methysergide was a weak partial agonist, and methylergonovine was a potent, full agonist (Table 2). The fenfluramines had micro-
molar affinity for the 5-HT\textsubscript{2A} receptor. Although the fenflu-ramines were weak partial agonists, the norfenfluramines were somewhat more potent partial agonists. The relatively low affinity of the norfenfluramines at the 5-HT\textsubscript{2A} receptor suggests that this site is not the CASR.

The norfenfluramines were moderately potent at the 5-HT\textsubscript{2C} 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\textsubscript{2C} 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 with the same efficacy as methylergonovine. With the exception of the findings with mCPP, these findings suggest that the 5-HT\textsubscript{2B} receptor may be the CASR. Trazodone, which binds with high affinity to the 5-HT\textsubscript{2B} receptor (Table 1), is a potent 5-HT\textsubscript{2B} antagonist (data not shown).

The fenfluramines and norfenfluramines were inactive at the 5-HT\textsubscript{5} and 5-HT\textsubscript{6} receptors (data not shown), indicating that these receptors are most likely not the CASR. Although the norfenfluramines have moderate affinity at the 5-HT\textsubscript{7} receptor, ergotamine had low affinity for this site, suggesting that the 5-HT\textsubscript{7} 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$_2$ Receptors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat 5-HT$_{2A}$</th>
<th>Human 5-HT$_{2A}$</th>
<th>Rat 5-HT$_{2C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-Fenfluramine</td>
<td>5 ± 0.3</td>
<td>413 ± 57</td>
<td>318 ± 374</td>
</tr>
<tr>
<td>(+)-Fenfluramine</td>
<td>11 ± 0.9</td>
<td>509 ± 64</td>
<td>624 ± 514</td>
</tr>
<tr>
<td>(−)-Fenfluramine</td>
<td>563 ± 35</td>
<td>571 ± 134</td>
<td>3415 ± 542</td>
</tr>
<tr>
<td>(±)-Norfenfluramine</td>
<td>231 ± 163</td>
<td>52.1 ± 12.3</td>
<td>557 ± 36</td>
</tr>
<tr>
<td>(+)-Norfenfluramine</td>
<td>1516 ± 88</td>
<td>11.2 ± 4.3</td>
<td>324 ± 7.1</td>
</tr>
<tr>
<td>(−)-Norfenfluramine</td>
<td>3841 ± 361</td>
<td>47.8 ± 18.0</td>
<td>814 ± 58</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>9.0 ± 0.6</td>
<td>3.0 ± 0.2</td>
<td>12.0 ± 0.9</td>
</tr>
<tr>
<td>Methysergide</td>
<td>15.0 ± 2.4</td>
<td>9.1 ± 2.9</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Methylergonovine</td>
<td>12.6 ± 0.6</td>
<td>0.49 ± 0.09</td>
<td>12.4 ± 0.6</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>299 ± 31</td>
<td>5030 ± 1152</td>
<td>50.0 ± 5.9</td>
</tr>
<tr>
<td>Norfluoxetine</td>
<td>638 ± 63</td>
<td>5063 ± 1161</td>
<td>286 ± 35</td>
</tr>
<tr>
<td>Trazodone</td>
<td>19.8 ± 1.4</td>
<td>73.6 ± 21.17</td>
<td>402 ± 26</td>
</tr>
<tr>
<td>mCPP</td>
<td>391 ± 27</td>
<td>3.2 ± 0.6</td>
<td>59 ± 6.5</td>
</tr>
<tr>
<td>5-HT</td>
<td>614 ± 43</td>
<td>4.0 ± 1.1</td>
<td>12.2 ± 0.8</td>
</tr>
<tr>
<td>Phentermine</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

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$_{2A}$ receptor has 5 characteristics consistent with its being the CASR: (1) it is located on both mitral and aortic valves; (2) it mediates mitogenesis; (3) the norfenfluramines have high affinity and efficacy at the 5-HT$_{2A}$ receptor; (4) ergotamine and methylergonovine, the active metabolite of methysergide, are high-affinity partial agonists for the 5-HT$_{2A}$ 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$_{2C}$ receptor is ruled out as the CASR, primarily because few of these receptors are expressed in heart valves.

There are several observations that, at first glance, are difficult to reconcile with the hypothesis that the 5-HT$_{2B}$ receptor mediates the valvulopathy associated with fenfluramine, ergotamine, and methysergide. First, whereas (+)-fenfluramine produces primarily aortic regurgitation, ergotamine and methysergide produce primarily mitral regurgitation. Given that 5-HT$_{2B}$ receptors are found on both valves, 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. The best estimate of the incidence of clinically significant fenfluramine-associated VHD is 0.07% per year. In contrast, patients treated with methysergide developed clinically significant VHD, including new heart murmurs, with an incidence of 3%. Thus, although methylergonovine is a less effective agonist at the 5-HT$_{2B}$ receptor than norfenfluramine, it produces a more severe form of VHD than fenfluramine, a greater number of patients. Third, methysergide administration is associated with fibrosis of other anatomic sites in addition to heart valves. In contrast, fenfluramine-associated fibrosis appears to be localized to heart valves.

Fourth, the finding that mCPP has activity at 5-HT$_{2B}$ receptors must be reconciled with observations that traz-
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, which are in the range needed to activate 5-HT₂B receptors. However, trazodone is a potent 5-HT₂B receptor antagonist, and its plasma levels are ~5-fold higher than that of mCPP. Thus, trazodone would act to block activation of 5-HT₂B receptors by mCPP.

Thus, a possible explanation for the differing degrees of VHD prevalence seen among the 5-HT₂B agonists is the degree of 5-HT₂B antagonism produced by either parent drug or metabolites. Methysergide, as a very-low-efficacy 5-HT₂B agonist, would act to antagonize the agonist effects of methylergonovine (Table 2). However, methysergide is rapidly metabolized to methylergonovine, is more rapidly eliminated, and achieves blood levels 10-fold lower than methylergonovine. Because the agonist actions of methylergonovine 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-HT₂B receptor than (+)-norfenfluramine (75%) and achieve blood levels twice that of norfenfluramine. This indicates that the parent drugs would partially antagonize activation of 5-HT₂B 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-HT₂B 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-HT₂B receptor. Medications such as lithium and monoamine oxidase inhibitors produce sustained 2-fold increases in plasma 5-HT and are not associated with VHD. 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), 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, 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-HT₂B 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-HT₂B 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-HT₂B 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-HT₂B receptors by norfenfluramine. This mechanism does not necessitate the formulation of unlikely synergistic mechanisms between phentermine and fenfluramine 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, we suggest that all clinically available medications with serotonergic activity and their metabolites should be screened for agonist activity at 5-HT₂B receptors.

Note Added in Proof
Dr Roth’s laboratory has begun to measure the efficacies of clinically used serotonergic compounds at the h5-HT2B receptor and have not yet found any that are agonists.

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References


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