Expert Opinion

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Screening the receptorome reveals molecular targets responsible for drug-induced side effects: focus on 'fen–phen'

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The *in vitro* pharmacological profiling of drugs using a large panel of cloned receptors (e.g., G protein-coupled receptors, ligand-gated ion channels, Na^+-dependent monoamine transporters), an approach that has come to be known as 'receptorome screening', has unveiled novel molecular mechanisms responsible for the actions and/or side effects of certain drugs. For instance, receptorome screening has been employed to uncover novel molecular targets involved in the actions of antipsychotic medications and the hallucinogenic mint extract salvinorin A. This review highlights the recent application of receptorome screening to discover why the anorexigen fenfluramine causes serious cardiopulmonary side effects. Receptorome screening has implicated *N*-deethylation of fenfluramine and serotonin 5-hydroxytryptamine 2B receptors in the adverse effects of the drug; subsequent studies corroborated this finding. The results discussed highlight the utility of determining the potential activity of drugs – and, importantly, of their *in vivo* metabolites – at as many molecular targets as possible in order to reliably predict side effect profiles. Receptorome screening represents one of the most effective methods for identifying potentially serious drug-related side effects at the preclinical stage, thereby avoiding significant economic and human health consequences.

Keywords: 5-HT_{2B} receptors, 5-hydroxytryptamine, cytochrome P450, drug metabolism, fenfluramine, mitogenesis, norfenfluramine, pulmonary hypertension, serotonin, valvular heart disease

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1. Introduction

Most therapeutic drugs and drugs of abuse exert their intended actions by modulating the activity of one or more target cellular protein(s). For instance, most antidepressants have long been thought to improve mood by inhibiting the synaptic uptake of neurotransmitters such as noradrenaline and serotonin (5-hydroxytryptamine; 5-HT) via blockade of monoamine transporters (reviewed in [1]). However, subsequent studies suggest that in addition to decreasing synaptic monoamine uptake, antidepressants improve mood by modulating the activity of other molecular targets in the brain, such as post and presynaptic neurotransmitter receptors, as well as pathways involved in neurogenesis [2-6]. Similarly, the classical notion of antipsychotic action is centred on blockade of brain dopamine and serotonin neurotransmission by the drug (reviewed in [7]). However, it is now clear that antipsychotic efficacy is a complex phenomenon, involving activation and inhibition of several other neurotransmitter receptors as well as downstream effector systems (reviewed in [8]). Thus, in order to fully understand the effects of drugs in living

systems, knowledge of the entire repertoire of systems affected by the compound, and to what extent, is critical.

In order to better understand the complex *in vivo* effects of psychoactive compounds, an approach has been pioneered that has now become known as 'receptorome screening'. Receptorome screening involves measuring the affinity of a drug (i.e., the K_i) at a large panel of cloned neurotransmitter receptors, transporters and ion channels [9]. Ultimately, a receptorome screen would entail screening compounds at all of the 'receptors in the genome' (reviewed in [9]). For those molecular targets that exhibit high-to-moderate affinity for a particular drug, the agonist or antagonist actions of the drug are assessed through *in vitro* functional assays. In this way, previously unidentified drug–target interactions can be identified. For instance, a receptorome screen was performed recently of the hallucinogenic, non-nitrogenous, lipid salvinorin A in order to understand the mechanism through which it alters perception [10]. This work identified the κ-opioid receptor as the hallucinogenic target of salvinorin A [10,11]. Information obtained from a receptorome screen of antipsychotic drugs [8] was also used to implicate 5-HT_{2A} receptors as the JC virus coreceptor [12].

An additional dimension in the complexity of the *in vivo* effects of a drug is created by drug metabolism. Like the parent compound, a drug metabolite can exhibit a complex *in vivo* pharmacology that is subtly or distinctly different from that of the parent compound. For instance, the archetypal atypical antipsychotic clozapine is metabolised in the liver, yielding *N*-desmethylclozapine or norclozapine and clozapine-*N*-oxide [13]. Recently, the agonist activity of *N*-desmethylclozapine at muscarinic acetylcholine receptors has been reported, and suggested to contribute to the unique antipsychotic efficacy of clozapine treatment [14,15].

Drug metabolism, in addition to contributing to the therapeutic effect of a medication, can also generate side effect-producing species. As an illustration of this phenomenon, research on the appetite suppressant fenfluramine, which was withdrawn from the US market due to its association with serious cardiopulmonary side effects, is discussed in this review. Through receptorome screening, the likely molecular mechanism underlying fenfluramine-associated valvular heart disease and pulmonary hypertension has been identified: activation of mitogenic 5-HT_{2B} receptors by the fenfluramine metabolite *N*-desethylfenfluramine (norfenfluramine). Later studies, which are also discussed herein, corroborated the role of norfenfluramine-mediated 5-HT_{2B} receptor activation in the adverse cardiopulmonary effects of fenfluramine. Thus, the 'fenfluramine story' nicely validates the use of receptorome screening as an approach to understand and predict the *in vivo* effects of drugs. In addition, the rise and fall of fenfluramine highlights the need to apply receptorome screens not only to current and investigational drugs, but also to their *in vivo* metabolites, in order to understand and predict the potential side effects of these drugs.

2. Fenfluramine background

2.1 Fenfluramine's history as an appetite suppressant and the birth of 'fen–phen'

Racemic fenfluramine was approved by the US FDA for use as an appetite suppressant in 1973. A little more than two decades later, the more potent (+) rotamer was approved. Before its introduction into the US market, the potential weight loss efficacy of fenfluramine as an adjunct to calorie restriction and exercise had been demonstrated in many clinical trials [16-31], most of which were short-term studies (i.e., > 6 months). Globally, these studies demonstrated that fenfluramine therapy in addition to diet and exercise was more effective than diet and exercise alone, conferring an additional 0.3- to 4.6-kg weight loss/month. In addition, although the studies that examined treatments for > 6 months revealed an apparent decrease in the efficacy of fenfluramine, patients whose fenfluramine therapy was withdrawn regained more weight than patients continuing to take the drug [17,23,29]. This later observation suggests that patients did not develop a complete tolerance to fenfluramine. Thus, fenfluramine appeared in these studies to be an effective drug to help obese patients lose weight and maintain the loss.

In addition to weight loss, fenfluramine induced other salutary effects. For example, obese patients taking fenfluramine exhibited decreases in blood glucose, insulin, noradrenaline, glycosylated haemoglobin, free fatty acids, cholesterol, triglycerides, high-density lipoproteins and both systolic and diastolic blood pressure [23,25,28,30]. Because some of these effects were detectable after very short-term (hours to days) treatments, the effects were likely due to fenfluramine and not secondary to weight loss [32,33].

The weight loss effects of fenfluramine described above are similar to those induced by the drug phentermine, which had been in use since its approval by the FDA in 1959. For instance, Munro *et al.* [34] reported a 13% reduction of initial body weight in dieting patients following 36 weeks of phentermine treatment, compared with a 5.2% reduction in dieting patients taking placebo. Although the appetite-suppressing effects of phentermine and fenfluramine are similar, their adverse effect profiles are not: phentermine causes insomnia and irritability, and fenfluramine induces drowsiness, diarrhoea and dry mouth.

The impetus for the surge in fenfluramine use during the 1990s was two studies conducted by researchers at Rochester University. In the first of these, Weintraub *et al.* followed obese, dieting patients treated with fenfluramine and phentermine in combination over the course of several months [19]. The rationale for the combination was that the sedative side effects of fenfluramine would counter the stimulant side effect of phentermine. Weintraub and colleagues also showed that by combining fenfluramine and phentermine, less of each drug could be used without compromising weight loss efficacy [19]. The group followed up the short-term fenfluramine–phentermine study with a longer-term (2-year) study of the

weight loss efficacy of the combination [35,36]. At the end of the 2-year study, fenfluramine–phentermine-treated patients lost an average of 10.8 ± 0.8 kg, thus demonstrating the sustained, long-term effectiveness of the combination as an adjunct to diet and exercise. These two studies precipitated the widespread prescription of the fenfluramine–phentermine combination, which came to be known as 'fen–phen'. Many fen–phen prescriptions were issued from diet clinics that hired physicians willing to prescribe the two drugs together. In 1996, the total number of fen–phen prescriptions issued was > 18 million [37].

2.2 Fenfluramine's link to potentially serious cardiopulmonary side effects

In 1997, the fen–phen boom took a turn for the worse. Researchers from the Mayo Clinic reported 24 newly documented cases of heart valve abnormalities in patients taking fenfluramine alone or fen–phen for 1 – 28 months. Echocardiography studies revealed unusual valve morphology and regurgitation on both sides of the heart, although in all patients at least one left-sided valve was affected. Eight of the patients (a third) also exhibited pulmonary hypertension, a frequency much higher than that in the population at large; this side effect was particularly common among women [38]. Notably, phentermine, which had long been used alone as an appetite suppressant, was not known to be associated with cardiopulmonary side effects. These findings prompted the FDA to solicit physician reports of valvular heart disease and pulmonary hypertension associated with fenfluramine use. The resulting survey of 284 patients revealed that 34% of patients taking fenfluramine for a median of 14 months exhibited signs of valvular heart disease [39] – a prevalence higher than that found in the population at large [40]. In light of these data and at the urging of the FDA, American Home Products, the makers of fenfluramine, voluntarily withdrew the drug from the market. This withdrawal was accompanied by at least US$20 billion in liability – the largest so far for a single medication.

Since 1997, several retrospective, controlled studies have attempted to assess the increased risk of valvular heart disease associated with fenfluramine use. The studies have yielded disparate results, with prevalence findings in the range of 0 – 25% [41-45]. A recent meta-analysis estimates the risk to be ~ 12% [46]; thus, although fenfluramine use is almost always found to confer a statistically significant increase in risk for developing valvular heart disease, the risk is less than that resulting from the FDA survey.

In recent years, researchers have sought to understand the mechanism(s) underlying fenfluramine-induced valvular heart disease and pulmonary hypertension. Although several approaches have been employed to address this issue (i.e., retrospective clinical analyses, molecular pharmacological approaches, genetic studies and animal models), the main focus of this review is data from *in vitro* pharmacological approaches that led to a molecular level understanding of fenfluramine-induced valvular heart disease and pulmonary hypertension. Among the earliest such reports was one from the authors of this review, in which a receptorome screen of fenfluramine and its major *in vivo* metabolite, norfenfluramine, was performed [47]. The results of the receptorome screen predicted that norfenfluramine, via activation of 5-HT_{2B} receptors, was the likely causative agent behind valvular heart disease and pulmonary hypertension. Later data, which is described in this review, supported the norfenfluramine-5-HT_{2B} receptor hypothesis, thus validating the *in vitro* receptorome screening approach. Thus, as discussed in this review, receptorome screening of drugs and their metabolites represents a feasible method both to discover the mechanisms responsible for and to predict drug-induced side effects. The fenfluramine story aptly demonstrates the need to perform receptorome screens of investigational drugs destined for human use at the preclinical stages.

3. The success of receptorome screening: implicating the fenfluramine metabolite norfenfluramine and 5-HT_{2B} receptors in fenfluramine-induced valvular heart disease and pulmonary hypertension

3.1 The initial receptorome screen

In the late 1990s, it was hypothesised that fenfluramine-induced valvular heart disease and pulmonary hypertension resulted from the interaction of the drug and/or its metabolite with a G protein-coupled receptor (GPCR), ligand-gated ion channel and/or monoamine transporter. Molecules belonging to these families were deemed likely participants in drug- and/or drug metabolite-induced valvular heart disease and pulmonary hypertension for the following reasons: i) other drugs known to cause valvular heart disease and pulmonary hypertension (e.g., ergotamine and methysergide) exhibit agonist activity at several GPCRs, ion channels and monoamine transporters, particularly those whose cognate ligand is 5-HT [47] (see also the National Institute of Mental Health Psychoactive Drug Screening Programme K_i database [101] for a comprehensive *in vitro* pharmacological profile of dihydroregotamine and methysergide); ii) the aforementioned molecular targets are known to modulate mitosis, and both valvular heart disease and pulmonary hypertension are characterised by proliferative cell lesions within the heart valves [37,48] and pulmonary artery wall [49-51]; and iii) patients with 5-HT-secreting tumours (carcinoid syndrome) often develop heart valve and pulmonary artery proliferative lesions identical to those observed in fenfluramine-induced disease [37,48].

Thus, Rothman and colleagues performed a receptorome screen of fenfluramine and its *in vivo* metabolite norfenfluramine, along with the valvular heart disease- and pulmonary hypertension-associated ergoline methysergide and its *in vivo* metabolite methylergonovine [47]. As negative controls, the serotonin transporter inhibitor fluoxetine and its *in vivo*

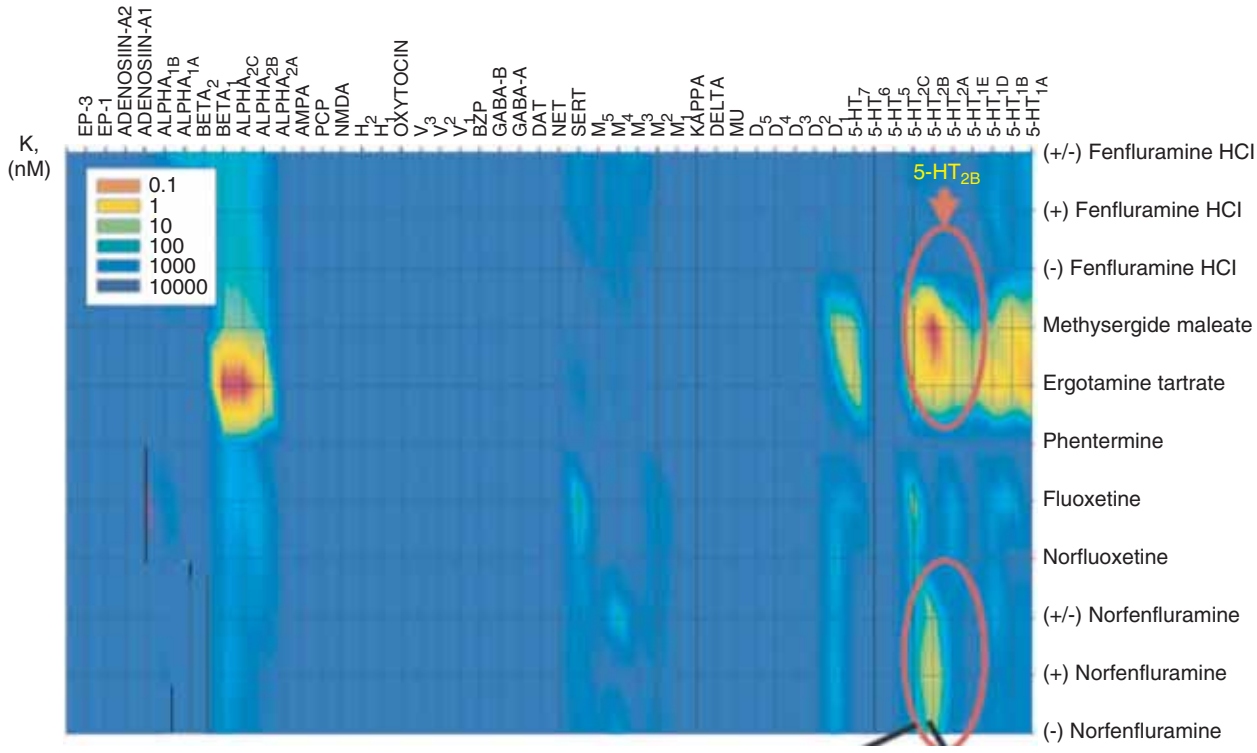

Figure 1. Results of the fenfluramine receptorome screen. The affinity constants (K_i values) for the drugs were measured as described previously [47] and colour-coded. Blue indicates the lower affinity (larger K_i value); red indicates the higher affinity (smaller K_i value). Intermediate K_i values were mapped to colours in between. Note that the fenfluramine metabolite norfenfluramine, like methylergonovine (a metabolite of the valvular heart disease- and pulmonary hypertension-associated drug methysergide) and dihydroergotamine (also associated with valvular heart disease and pulmonary hypertension) are characterised by high-affinity binding to 5-hydroxytryptamine 2B receptors; fluoxetine (not associated with the diseases) and the metabolite norfluoxetine, do not. The drugs associated with cardiopulmonary disease also share high-affinity binding to α_{2B}-adrenergic receptors; however, several therapeutic drugs target that receptor and are not associated with either valvular heart disease or pulmonary hypertension.

metabolite *N*-desmethylfluoxetine (norfluoxetine) were included, as long-term fluoxetine treatment has not been reported to cause valvular heart disease or pulmonary hypertension [47]. The results of the receptorome screen indicated that the *N*-dealkylated metabolites of fenfluramine and methysergide (i.e., norfenfluramine and methylergonovine, respectively), but neither fluoxetine nor norfluoxetine, both shared high affinity for only one molecular target that could not be excluded as mediating cardiopulmonary side effects: the 5-HT_{2B} receptor [47] (Figure 1). Further *in vitro* studies revealed that both norfenfluramine and methylergonovine were potent and efficacious 5-HT_{2B} receptor agonists [47]. At around the same time, Fitzgerald *et al.* [52] independently reported that norfenfluramine was a potent 5-HT_{2B} receptor agonist, thus corroborating the results of the fenfluramine receptorome screen *vis-à-vis* the 5-HT_{2B} receptor.

Fenfluramine itself also displayed low affinity and agonist potency at 5-HT_{2B} receptors. However, pharmacodynamic data obtained in healthy human volunteers given fenfluramine doses similar to those used clinically suggest that fenfluramine would not significantly activate 5-HT_{2B} receptors. For example, the *in vitro* potency of fenfluramine at recombinant 5-HT_{2B} receptors (i.e., the ED_{50} or the concentration of fenfluramine required to activate 5-HT_{2B} receptors to 50% of the maximal activation, E_{max}, induced by 5-HT) was determined to be 380 nM [47]. The maximum fenfluramine plasma concentration in healthy human volunteers given a therapeutic dose of fenfluramine (15 – 30 mg/kg) was ~ 90 nM 4 h after the dose, and decayed rapidly thereafter [53]. The potency of the fenfluramine metabolite norfenfluramine at 5-HT_{2B} receptors was found to be 18 nM [47]; peak plasma norfenfluramine levels in humans given a therapeutic dose of fenfluramine were ~ 40 nM (i.e., twice the ED_{50} and enough to activate the receptor to 50% of the E_{max} for 5-HT) 4 h after drug administration, and remained at a plateau for an additional 4 – 6 h [53]. Thus, based on the *in vitro* pharmacological data and the *in vivo* pharmacodynamic data referenced above, norfenfluramine is likely to significantly activate 5-HT_{2B} receptors *in vivo*, whereas fenfluramine would not be predicted to do so (Figure 2). This is precisely what was predicted based on the receptorome screen of fenfluramine.

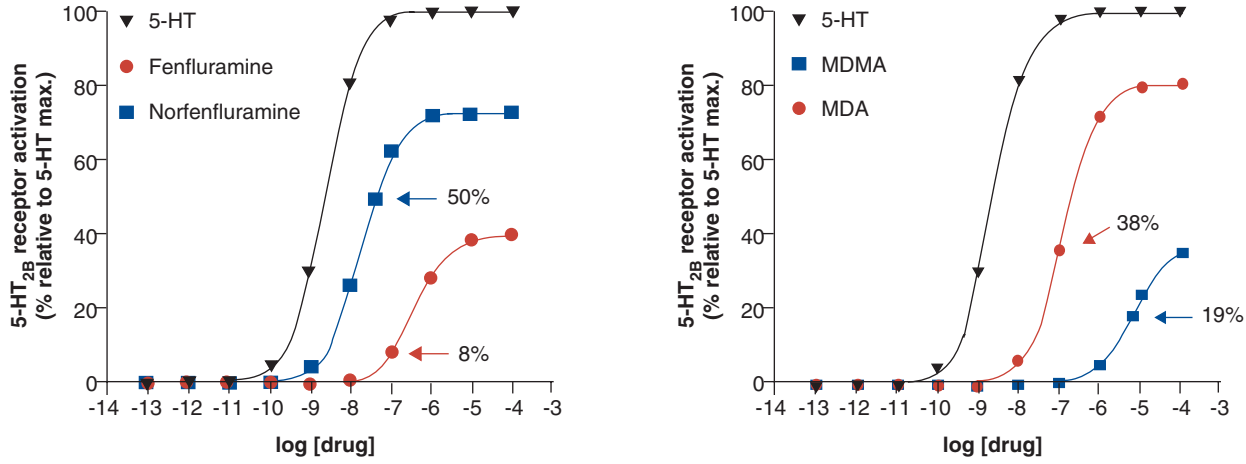

Figure 2. Theoretical dose–response curves for 5-HT-, fenfluramine- and norfenfluramine-induced (left) and 5-HT-, MDMA- and MDA-induced (right) 5-HT$_{2B}$ receptor activation. Curves were generated based on the following equation: $y = E_{max}/(1+10^{logEC50-x})$ where y is 5-HT$_{2B}$ receptor activation expressed as a per cent of the maximal activation observed with 5-HT, E_{max} is the maximal 5-HT$_{2B}$ receptor activation and EC_{50} is the concentration of drug required to reach half-maximal receptor activation for that drug (values from [47,63]), and x is the logarithm of the drug concentration. The peak plasma concentrations of fenfluramine and norfenfluramine reported in humans following administration of a therapeutic dose, along with their calculated (from the above equation) 5-HT$_{2B}$ receptor activation (relative to the 5-HT E_{max}), are indicated by the arrows. Note that, as predicted by the fenfluramine receptorome screen, the fenfluramine metabolite norfenfluramine is predicted to elicit significant 5-HT$_{2B}$ receptor activation, whereas the parent drug fenfluramine is not. For MDMA and MDA plasma levels following a recreational dose, both compounds are likely to activate 5-HT$_{2B}$ receptors *in vivo*.

5-HT: 5-Hydroxytryptamine; MDA: 3,4-Methylenedioxyamphetamine; MDMA: 3,4-Methylenedioxymethamphetamine.

In summary, the receptorome screen of fenfluramine revealed that the metabolite norfenfluramine and drugs associated with valvular heart disease and pulmonary hypertension share high affinity for 5-HT$_{2B}$ receptors. Further characterisation revealed that the metabolites of cardiopulmonary disease-linked drugs were potent, efficacious 5-HT$_{2B}$ receptor agonists; that is, they activated the receptor at low concentrations and to a large extent relative to the maximum receptor activation observed with 5-HT. These findings led to the idea that fenfluramine *N*-deethylation gives rise to the causative agent of valvular heart disease and pulmonary hypertension, norfenfluramine, which elicits its cardiopulmonary effects via activation of mitogenic 5-HT$_{2B}$ receptors. Results from molecular pharmacological, cell biological, genetic and human pharmacodynamic studies were all consistent with the fenfluramine receptorome screen-based model; however, direct proof of the model (i.e., that norfenfluramine directly induces heart valve cell proliferation through a 5-HT$_{2B}$ receptor-dependent mechanism) was lacking.

3.2 Validating the results of the fenfluramine receptorome screen: evidence linking norfenfluramine-mediated activation of 5-HT$_{2B}$ receptors to fenfluramine-induced cardiopulmonary side effects

3.2.1 Valvular heart disease

Several cell biological and genetic studies of 5-HT$_{2B}$ receptors corroborated and fortified the results of the receptorome screen, which suggested a link between norfenfluramine-mediated activation of 5-HT$_{2B}$ receptors and fenfluramine-associated valvular heart disease. First, and most importantly, 5-HT$_{2B}$ receptor expression has been documented in human and porcine heart valves [52] and in the mouse heart [54-56], thus suggesting a role for 5-HT$_{2B}$ receptors in normal cardiac function. Second, activation of 5-HT$_{2B}$ receptors has been shown to regulate heart cell proliferation [57-59]. Indeed, mice lacking 5-HT$_{2B}$ receptors often die *in utero* due to severely underdeveloped hearts. Third, activation of 5-HT$_{2B}$ receptors has been shown to induce mitosis in many cellular contexts [52,55,57,58,60-64]. Thus, these reports provided strong evidence supporting the predictions of the fenfluramine receptorome screen; that is, that fenfluramine, on metabolism to norfenfluramine, activates heart valve cell 5-HT$_{2B}$ receptors, thus inducing proliferation of heart valve interstitial cells, plaque formation and valve dysfunction.

To directly test the model proposed above, the mitotic effect of norfenfluramine in primary cultures of human heart valve interstitial cells, the cells comprising fenfluramine-induced valvular lesions, was measured [63]. In this study, it was demonstrated that norfenfluramine (10 µM final concentration) induced heart valve cell proliferation by a level two-fold higher than vehicle, as measured by incorporation of [^{3}H]thymidine into replicating DNA [63]. To assess whether the effects of norfenfluramine were mediated by 5-HT$_{2B}$ receptors as the model suggested, parallel measures of

norfenfluramine-induced proliferation were performed in the presence of a 5-$HT_{2B/2C}$ receptor-selective antagonist (SB-206,553 final concentration: 10 µM). Cotreatment of heart valve cells with norfenfluramine and SB-206,553 did not result in significant proliferation compared with treatment with SB-206,553 alone [63]. These data marked the first demonstration that a valvulopathogenic drug induces (in a 5-HT_{2B} receptor-dependent manner, as 5-HT_{2C} receptors are not expressed in the heart) human heart valve cell proliferation *in vitro*: an activity reminiscent of its adverse effects *in vivo*. Furthermore, and importantly, the results of studies of norfenfluramine-treated human heart valve cells corroborated what the fenfluramine receptorome screen suggested – that the fenfluramine metabolite norfenfluramine, via 5-HT_{2B} receptor activation, is the likely causative agent of valvular heart disease.

Shortly before the publication of this work with human heart valve cells, the anti-Parkinsonian pergolide was reported to cause valvular heart disease [65-67]. Subsequently, cabergoline was also identified as a 5-HT_{2B} agonist [68] that induces valvular heart disease [69,70]. Based on the findings from the receptorome screen of the valvular heart disease and pulmonary hypertension-associated drugs, it was reasoned that pergolide, like ergotamine, methysergide and the methysergide metabolite methylergonovine, exhibited high affinity and agonist activity at 5-HT_{2B} receptors [63]. Thus, all known valvular heart disease-inducing drugs have been shown to be 5-HT_{2B} receptor agonists, further validating the results of the fenfluramine receptorome screen.

The identification of fenfluramine as a valvular heart disease- and pulmonary hypertension-inducing drug was chemically interesting, as it is the only such drug that is not a member of the ergoline structural class (amphetamine and its derivatives, such as fenfluramine, are phenylisopropylamines). At around the same time as the receptorome screen of fenfluramine was performed, a similar screen of 3,4-methylenedioxymethamphetamine (MDMA – the recreational drug 'Ecstasy') was undertaken in order discover novel molecular targets responsible for its unique entactogenic effects. In so doing and without any prior knowledge, it was discovered that MDMA exhibited moderate affinity (K_i: 500 nM) at 5-HT_{2B} receptors [63]. Further characterisation revealed the drug to be a 5-HT_{2B} receptor agonist [63]. It, like fenfluramine, also undergoes *in vivo N*-dealkylation to generate 3,4-methylenedioxyamphetamine (MDA), with peak plasma concentrations of the parent compound and metabolite being 783 and 50 nM, respectively, following ingestion of a recreational dose [71]. Compared with the experimentally determined EC_{50} and E_{max} values for MDMA (6000 nM and 38%, respectively) and MDA (100 nM and 81%, respectively), plasma levels of both species following ingestion of a recreational dose would be predicted to activate 5-HT_{2B} receptors. As such, the receptorome screen of MDMA predicts that it should elicit fenfluramine-like proliferative responses from heart valve cells *in vitro*. Indeed, both MDMA and MDA (at 10-µM final concentrations) elicited fenfluramine-like proliferative responses that were blocked by cotreatment with the 5-$HT_{2B/2C}$ receptor-selective antagonist SB-206,553. Thus, as receptorome screening had predicted for fenfluramine, *in vivo N*-dealkylation of MDMA generates a more potent, more efficacious 5-HT_{2B} receptor agonist (MDA) that induces heart valve cell proliferation *in vitro* and, thus, is likely to increase the risk for developing valvular heart disease in humans who use MDMA more or less continuously.

In summary, the preceding paragraphs present a nice example of how receptorome screening led to the discovery that the fenfluramine metabolite norfenfluramine is the likely causative agent of valvular heart disease. Norfenfluramine and the other valvular heart disease-associated drugs (but not fluoxetine or norfluoxetine) shared high affinity for only one molecular target likely to be involved in heart valve cell proliferation (i.e., the 5-HT_{2B} receptor). Thus, an additional success of the fenfluramine receptorome screen was its revelation of the likely mechanism underlying fenfluramine-induced valvular heart disease: metabolism of fenfluramine to norfenfluramine generates plasma concentrations of the metabolite that activate mitogenic 5-HT_{2B} receptors on heart valve cells, thus leading to excessive proliferation, fibrotic lesions and valve dysfunction. In addition, the results of the fenfluramine receptorome screen were applied to the recently reported valvular heart disease-inducing drug pergolide. Consistent with the results of the fenfluramine receptorome screen, pergolide displayed 5-HT_{2B} receptor agonist activity. In addition, a receptorome screen was performed of a drug structurally similar to fenfluramine (MDMA), and it was found that it and (to a greater extent) its *N*-demethylated metabolite (MDA), like norfenfluramine, are 5-HT_{2B} receptor agonists. Although it is unknown whether MDMA abuse is associated with valvular heart disease in humans, MDMA and MDA, like norfenfluramine, elicit proliferative responses from heart valve cells *in vitro*, an activity reminiscent of that which, *in vivo*, gives rise to fibrotic lesions that compromise valve function. Future studies revealing increased risk for valvular heart disease among chronic MDMA users would represent the best validation of the receptorome screening approach, that is, the prediction that a drug and/or its metabolite causes a side effect before such an adverse consequence has been documented.

3.2.2 *Pulmonary hypertension*

The most conclusive evidence linking the fenfluramine metabolite norfenfluramine to pulmonary hypertension comes from mouse studies. Maroteaux *et al.* exposed wild-type and 5-HT_{2B} receptor knockout mice to chronic hypoxia, which induces pulmonary artery smooth muscle cell proliferation and, thus, pulmonary hypertension [72]. The authors found that only wild-type mice, and not 5-HT_{2B} receptor knockout mice, developed pulmonary hypertension following chronic hypoxia, thus implicating the receptor in the disease [72]. Further demonstrating the role of 5-HT_{2B} receptors in pulmonary hypertension, administration of the 5-HT_{2B}

receptor-selective antagonist RS-127,445 blocked hypoxia-induced pulmonary hypertension in wild-type mice [72]. When fenfluramine was chronically administered to wild-type mice under hypoxic conditions, the authors found that the ensuing pulmonary hypertension was exacerbated *vis-à-vis* chronic hypoxic control mice [72]. Through plasma fenfluramine and norfenfluramine measurements, the authors were able to ascertain that norfenfluramine was the species responsible for the worsening of hypoxia-induced pulmonary hypertension [72]. Thus, 1 year later, *in vivo* data corroborated the prediction of the fenfluramine receptorome screen that the fenfluramine metabolite norfenfluramine was the likely culprit responsible for fenfluramine-induced pulmonary hypertension. In addition, these findings predict that, assuming similar molecular and cellular bases for pulmonary hypertension in mice and men, 5-HT_{2B} antagonists might effectively treat some forms of pulmonary hypertension.

In addition to inducing pulmonary artery remodelling, the fenfluramine metabolite norfenfluramine causes vasoconstriction, which could also contribute to pulmonary hypertension. For instance, Hong *et al.* [73] showed that norfenfluramine causes more robust contraction of isolated pulmonary artery rings than does fenfluramine. This suggests that the metabolite increases pulmonary arterial pressure directly by inducing contraction, as well indirectly via induction of smooth muscle cell proliferation and increased pulmonary artery resistance [72]. Other findings suggest that norfenfluramine, but not fenfluramine, acts as a global pressor agent in rodents [74], an activity that would contribute to increases in pulmonary artery pressure. Again, data obtained after the original fenfluramine receptorome screen corroborate its implication of the metabolite norfenfluramine in fenfluramine-associated cardiopulmonary side effects.

The mechanism through which drugs and/or their metabolites cause pulmonary hypertension is likely to involve molecular targets in addition to the 5-HT_{2B} receptor. This is because the use of ergots as antimigraines (ergotamine and methysergide) and as an anti-Parkinsonian (pergolide) does not appear to be associated with increased risk for developing pulmonary hypertension, although it is not clear whether this has been adequately addressed. Because these ergots are 5-HT_{2B} receptor agonists, pulmonary hypertension-inducing drugs are likely to modulate other pathogenic pathways.

Very recently, the serotonin transporter was implicated in drug-induced pulmonary hypertension. Guignabert and colleagues reported that serotonin transporter inhibition, but not antagonism of either 5-$HT_{1B/1D}$, 5-HT_{2A} or 5-HT_{2B} receptors, prevented monocrotaline-induced pulmonary hypertension in rats [75]. Monocrotaline itself is metabolised in the liver to monocrotaline pyrrole: the agent that causes pulmonary hypertension [76]. In this respect, monocrotaline is similar to fenfluramine; both seem to be prodrugs that give rise to pathological metabolites. Although it is not clear whether monocrotaline pyrrole and norfenfluramine cause pulmonary hypertension via the same pathway, the report from Guignabert and colleagues clearly implicates the serotonin transporter in monocrotaline-induced pulmonary hypertension.

The serotonin transporter also seems to play a role in primary (i.e., not secondary to drug treatment) pulmonary hypertension; for instance, a serotonin transporter gene promoter polymorphism associated with increased transcriptional activity [77] is found more frequently in pulmonary hypertension patients than in control individuals [78]. Along the same lines, serotonin transporter null mice are, like their 5-HT_{2B} receptor counterparts, resistant to hypoxia-induced pulmonary hypertension [72,79]. Thus, there is sufficient data to conclude that the serotonin transporter plays a role in various forms of pulmonary hypertension.

4. Expert opinion and conclusion

In closing, this review demonstrates how receptorome screening can be used, as it had been for fenfluramine, to identify drugs and metabolites that may induce serious side effects First, receptorome screening was applied to fenfluramine and its metabolite norfenfluramine, as well as to other drugs associated with fenfluramine-like cardiopulmonary side effects, in order to identify common molecular targets and, thus, the underlying molecular mechanisms responsible for the diseases. Once receptorome screening identified metabolism of fenfluramine to norfenfluramine and the 5-HT_{2B} receptor as likely mediators of fenfluramine-induced valvular heart disease and pulmonary hypertension [47], the prediction was corroborated using primary cultures of human heart valve cells [63]. Others demonstrated that fenfluramine metabolism to norfenfluramine is associated with pulmonary hypertension in a mouse model of the disease [72]. Thus, receptorome screening first predicted, and later studies demonstrated, that *N*-deethylation of fenfluramine to norfenfluramine generates a species that, through 5-HT_{2B} receptor activation, is responsible for the cardiopulmonary side effects of the parent compound.

Receptorome screening also led to the prediction that MDMA might be associated with fenfluramine-like cardiopulmonary side effects. For instance, through receptorome screening, it was discovered that MDMA and its *N*-demethylated metabolite MDA display 5-HT_{2B} agonist properties. It was also demonstrated that both compounds induce human heart valve cell proliferation *in vitro* [63]. Thus, receptorome screening of MDMA may have revealed an additional health consequence of the drug that is due mainly to its *N*-demethylated metabolite MDA. Risk analyses of chronic MDMA users are needed to address this issue.

The studies presented herein highlight the utility and validity of receptorome screening to identify drugs and metabolites that might be associated with side effects in humans. Based on these studies, it is our expert opinion that receptorome profiles should be obtained for as many current and investigational pharmaceuticals and drugs of abuse as possible, as well as for their metabolites. To do so for drugs already known to cause undesirable side effects may reveal the underlying

mechanisms responsible, as it did for fenfluramine-induced cardiopulmonary side effects, and help identify other such drugs. Indeed, this procedure has been used to identify histamine H_1 and 5-HT_{2C} receptors as the likely molecular targets responsible for antipsychotic-drug-induced weight gain [80]. Receptorome screens have also been employed to discover the molecular targets responsible for the actions of ephedra-related amines [81] and homocysteine-metabolites [82]. As for drugs not yet associated with any or with serious side effects, having receptorome profiles at the ready, should adverse consequences surface, would facilitate the discovery of the process(es) responsible and the identification of other compounds likely to elicit similar side effects.

As it stands, several molecular targets for serious drug-induced side effects have been identified. In addition to discovering the role of the 5-HT_{2B} receptor in drug-induced cardiopulmonary side effects, Kroeze and colleagues have identified affinity for histamine H_1 receptors as positively correlating with the large weight gain induced by atypical antipsychotic medications [80]. Others have identified activation of atrial 5-HT_4 receptors and blockade of the human ether-a-go-go-related gene inwardly rectifying potassium channel as molecular bases for drug-induced arrhythmias [83,84]. Thus, one receptorome screen of a drug and its metabolites would allow the identification of potentially serious side effects *in vivo*. In the case of currently prescribed drugs, such knowledge would allow physicians to closely monitor their patients for signs of such side effects and discontinue therapy if necessary. For compounds currently under development, receptorome screening at the preclinical stages could identify lead candidates and metabolites thereof that might, on chronic use, cause great economic loss and human suffering.

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