Obesity continues to be a burgeoning health problem worldwide. Before their removal from the market, fenfluramine and the more active enantiomer dexfenfluramine were considered to be among the most effective of weight loss agents. Much of the weight loss produced by fenfluramine was attributed to the direct activation of serotonin 5-HT_{2C} receptors in the central nervous system via the desmethyl-metabolite of fenfluramine, norfenfluramine. Norfenfluramine, however, is non-selective, activating additional serotonin receptors, such as 5-HT_{2A} and 5-HT_{1B}, which likely mediated the heart valve hypertrophy seen in many patients. Development of highly selective 5-HT_{2C} agonists may recapitulate the clinical anti-obesity properties observed with fenfluramine while avoiding the significant cardiovascular and pulmonary side effects.
Introduction

Obesity has developed into one of the most preventable causes of death in the United States and is reaching epidemic proportions in many developed nations throughout the world (1). Obesity is most often characterized by the measure of body-mass index (BMI) [i.e., weight (kg)/height (m2)]. Individuals with a BMI equal to or greater than thirty are classified as being obese, whereas those who have BMI values ranging between twenty-five and thirty are termed “overweight.” The prevalence of diabetes, hypertension, coronary artery disease, sleep apnea, cholelithiasis, and certain cancer types is highly correlated with elevations in BMI (2). Even a relatively small reduction in BMI, such as a decrease in body weight of five percent, can lead to meaningful decreases in the incidences of cardiovascular events and type II diabetes.

In the simplest terms, obesity results from an imbalance between kilocalories consumed and utilized for energy expenditure. Excess kilocalories end up being stored as fat in adipose tissue, liver, and muscle. Although controlling body weight is conceptually simple—effected by reduced caloric intake and increased energy expenditure through exercise—many people cannot achieve significant and lasting reductions in BMI. Thus, drug-based therapy may help some individuals to obtain clinically beneficial declines in body weight and a concomitant reduction in comorbidities.

Currently available pharmacotherapies include the prescription drugs orlistat, sibutramine, and phentermine, as well as a variety of over-the-counter products (3). Most of these agents have fallen short of delivering significant weight loss and have been limited by significant side effects. The lack of highly efficacious anti-obesity agents has led to a significant drug discovery effort in the pharmaceutical industry focusing on centrally and peripherally expressed G protein–coupled receptor (GPCR) targets and enzymes involved in fat and carbohydrate metabolism (4–6). Although much of this research is in its early days, many drug targets with longer histories, such as the neuropeptide Y and melanocortin receptors, have failed to reach far enough into the clinic to establish good proof of principle (7, 8). Currently, only the CB1 antagonist rimonabant seems to be likely to enter the market in the near term (9).

Fenfluramine and 5-HT2c Receptor Activation

(±) Fenfluramine (Pondimin) entered the market in the early 1970s and was followed, in the early 1990s, by the introduction of the more active enantiomer dexfenfluramine (Redux). Dexfenfluramine was the first anti-obesity drug to be approved for duration of usage in excess of three months, following a series of clinical trials making up the INDEX study (12). Redu produced only a three-percent (placebo subtracted) reduction in body weight; however, the number of patients achieving more than a ten-percent reduction in body weight was twice as great as the number doing so on placebo (11, 13) (Figure 1). In addition to reducing body weight, according to evidence from some clinical trials, treatment with fenfluramine resulted in improvements to glycemic control, lipid parameters, and blood pressure (14–16).

Because fenfluramine is a serotonin (5-HT) transporter substrate, it can function to elevate 5-HT levels, both by competing for uptake (transporter inhibition) and by promoting 5-HT efflux via the reversal of the transporter (releasing effect) (17). The anorectic action of fenfluramine was long presumed to be the activation of multiple receptors resulting from the increased synaptic levels of 5-HT. Indeed, several 5-HT receptor subtypes are implicated in the regulation of feeding (18). The elevated release mechanism was called into question, however, with the observations that neither...
the depletion of 5-HT stores by p-chloroamphetamine (20, 21) not blockade of the 5-HT transporter by fluoxetine (22) inhibited the anorectic activity of fenfluramine in rats.

The depletions produced by fenfluramine, norfenfluramine, and reserpine differentially affect serotonergic neurons, and the consequences are dependent upon the time course of the depletions. The present study indicates that the acute anorectic activity of fenfluramine in rats.

The 5-HT2C receptor, a class A GPCR, is one of fourteen 5-HT receptors and signal transduction coupling, the receptor has been grouped with the 5-HT2A and 5-HT2B receptors into a common subfamily (24). The gene that encodes the 5-HT2C receptor is found on the X-chromosome (Xq24) and possesses four exons within the open reading frame and two in the 5-3′-untranslated region (26, 27). The open reading frame encodes a protein that is 460 amino acids in length. Alternative splicing may result from the use of an alternative 5′-donor site to yield a truncated receptor that can be expressed in a truncated action but does not bind ligand or signal through G-proteins (28). Polymorphic chain reaction strategies have localized the mRNA for the splice variant in several brain regions but its function is unknown.

Homodimerization of the full-length 5-HT2C receptor has been reported in transfected cell lines, but its role is not yet known whether the full-length receptor can dimerize with the truncated alternative splice variant of the receptor, whether mRNA editing may affect dimer formation, or if heterodimerization occurs with other GPCRs.

The 5-HT2C receptor is the only known GPCR to undergo mRNA editing (26). Editing occurs at five positions that encode amino acids within the second intracellular loop of the receptor and occurs at an allelic rate of 0.13 (28). Studies to date have not shown any influence of this polymorphism on receptor pharmacology or on obesity (30–32). A polymorphism in the promoter region (−759C/T) of the 5-HT2C receptor-encoding gene has recently received a great deal of attention. A number of studies have suggested that the polymorphism is associated with weight gain in human populations, and in rats (34, 35).

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5-HT2c Receptor Distribution

In all species examined, 5-HT2c receptor-encoding mRNA is found almost exclusively in the central nervous system, with the highest region of expression being the choroids plexus, where the physiological role of the receptor remains unclear. 5-HT2c mRNA is found in several brain regions of the rat, as determined by in situ hybridization, that are involved in feeding behavior including the nucleus of the solitary tract, dorsal medial hypothalamic nucleus, paraventricular hypothalamic nucleus, and amygdala (51). All of these regions receive significant innervation from serotoninergic neurons arising from the raphe nuclei. Receptor autoradiography studies in the rat, utilizing [3H]-mesulergine, show a similar distribution pattern to that of the mRNA labeling studies (52). In human brain slices, in situ hybridization reveals significant mRNA expression in both the hypothalamic paraventricular and ventromedial nuclei, and labeling in the amygdala is also observed (53).

Selective 5-HT2c agonists produce significant elevations in c-Fos within the arcuate and paraventricular nuclei, central amygdala, nucleus of the solitary tract, and ventral segmental area of the rat brain (54). These same regions are also activated by fenfluramine and norfenfluramine (55). Unlike fenfluramine, however, direct-acting 5-HT2c agonists do not cause elevations in c-Fos in areas such as the cortex and striatum. Interestingly, those nuclei exhibiting 5-HT2c receptor-mediated elevations in c-Fos are also activated by compounds from other neurotransmitter systems involved in feeding, such as melanocortin-4 receptor agonists and glucagon-like peptide-1 (56, 57).

In an elegant study by Heisler and colleagues, fenfluramine was found to produce c-Fos activation of pro-opiomelanocortin (POMC) neurons within the arcuate nucleus. These POMC neurons also express 5-HT2c receptor mRNA, suggesting that the 5-HT2c receptor mediates fenfluramine's action in this brain nucleus (58) (Figure 2). In addition, the authors determined that either pharmacologic or genetic inhibition of melanocortin-4 receptors prevent the efficacy of fenfluramine, suggesting that 5-HT2c activation may regulate melanocortin signaling. Identification of 5-HT2c receptors in these cells is also significant in that it places the receptor on close proximity to those cells involved in glucose, insulin, and leptin signaling (59, 60).

The 5-HT2c Receptor Knockout Mouse

While evidence for the involvement of the 5-HT2c receptor in feeding was emerging on the pharmacological level, the development of a transgenic mouse line lacking the receptor significantly heightened interest in agent drug discovery. 5-HT2c knock-out mice were first described by Tezott and colleagues in 1995. These mice develop late-onset obesity, weighing thirteen percent more than their littermate controls, as early as fifteen weeks of age, and thirty percent more after forty-two weeks (61, 62). The elevation in weight is primarily due to an increase in white adipose tissue (61). The animals are hyperphagic, and exhibit metabolic hormone changes including the development of hyperleptinemia and hyperinsulinemia (63) (Figure 3), and they are completely insensitive to the anorectic action of mCPP (a non-selective 5-HT2c agonist). Loss of 5-HT2c receptor expression appears to affect meal patterning, with an increase in both meal duration and frequency (62). Subsequent evaluation of these mice has shown the compound dexfenfluramine to be less effective at reducing feeding and to fail to modulate satiety sequences (63). In comparison, 5-HT1B knockout mice exhibit only a slight increase in body weight relative to littermate controls (64). These animals are insensitive to the anorectic action of fenfluramine, but recent evidence indicates this may be due to compensatory down-regulation of 5-HT2c receptor function (65).
The Need for Selectivity in Anorectic Drug Development

A 1997 paper from Connolly et al. identified a group of patients displaying heart valve malformations that were attributed to the use of fenfluramine and dexfenfluramine (66). Subsequently, a significant number of papers appeared confirming the association of heart valve hypertrophy and the use of the fenfluramines [for review see (67)].

The relative incidence of heart valve malformations has been estimated to be anywhere between two and twenty-five percent, with longer duration of use seeming to correlate with higher incidence.

In a seminal paper by Fitzgerald and colleagues at DuPont Pharmaceuticals, it was determined that mRNAs for 5-HT 2B and 5-HT2A receptors are expressed in human heart valves and that norfenfluramine is a potent full agonist for the 5-HT 2B receptor and is less potent at the 5-HT2A receptor (68). The authors hypothesized that activation of 5-HT2B receptors by d-norfenfluramine (Ki = 27 nM, EC50 = 24 nM) is responsible for the valve hypertrophy, and their work has been confirmed and extended by Roth et al. (69).

This group has found that interstitial cells from human heart valves express mitogenic signals following stimulation with norfenfluramine that can be blocked by a 5-HT2B antagonist (70). Other groups have also found the 5-HT2B receptor to be expressed in heart valves from human and other species, although there is some variability in which subtypes are expressed (71, 72). It should be noted that many groups have found 5-HT2A mRNA in valves from various species, and it is not possible to completely rule out this receptor as a potential contributor to the heart valve hypertrophy (71, 73).

Another significant side effect of the fenfluramines and other serotonin transporter substrates, such as Aminorex (pulled from the European market in the 1970s), is primary pulmonary hypertension (74). This often fatal condition is thought to arise from the co-stimulation of multiple 5-HT receptors on the pulmonary smooth muscle, leading to proliferation (75), and/or from the interaction with a K+ channel subtype after uptake through the 5-HT transporter (76).

Whatever the exact mechanism, the need for selectivity against the serotonin receptors and transporter is highlighted.

Recent Advances in the Identification of 5-HT2C Agonists

The association of heart valve hypertrophy with 5-HT2B (and potentially 5-HT2A) agonism placed a great level of emphasis on finding potent 5-HT2C agonists that are as selective as possible. The 5-HT2B receptor is 51% homologous to the 5-HT2C receptor and is 71% identical in the transmembrane domains, whereas the 5-HT2A receptor is 49% and 80% homologous overall and within the transmembrane domains, respectively (77, 78). The fact that all three receptors utilize 5-HT as endogenous agonist suggests that the specific amino acid contact sites required for agonism will prove to be conserved. The development of highly selective 5-HT2C agonists is thus quite challenging. The compounds highlighted in this section are meant to give a flavor of the relative diversity of chemotypes being pursued and the progress in finding compounds that are truly selective (Figure 4).

There are undoubtedly more selective compounds in existence than the ones highlighted here but they have yet to be disclosed.

For many years, mCPP has been used as the prototypical 5-HT2C receptor agonist. The compound reduces feeding both acutely and chronically and produces chronic reductions in body weight (79, 80). Antagonist studies have largely demonstrated that the anorectic action of mCPP is mediated by the 5-HT2C receptor, but the compound also possesses (to varying extents) 5-HT2A, 5-HT1A, 5-HT2B agonism (81). Thus, a number of other behavioral observations associated with the compound (both good and bad) may indeed be due to activation of multiple 5-HT receptors, especially at higher doses (82).
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**Figure 4.** Diverse chemotypes under investigation as selective 5-HT receptor ligands. The development of highly selective 5-HT<sub>2C</sub> agonists continues to be challenging, although selectivity of certain compounds indicates that specific receptor subtypes may be targeted in anti-obesity drug development.
The 5-HT₂C receptor represents a compelling target for the treatment of obesity. The localization of the receptor is consistent with a role in directly modulating pathways of food intake and body weight control. More significantly, such a role has been confirmed by the successes obtained with selective agonists thus far in preclinical models. The complex regulation of 5-HT₂C receptor structure by mHAT editing, the ability to signal through multiple pathways, and the high homology with the 5-HT₂A and 5-HT₂B receptors make the identification of efficacious and selective agonists most challenging. If indeed 5-HT₂C agonists can match the efficacy of fenfluramine in man, without the cardiovascular risks, they should become powerful pharmacological tools for combating the obesity epidemic. 

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