

SYMPOSIUM REPORT

Pharmacological targeting of the serotonergic system for the treatment of obesity

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The attenuation of food intake as induced by an increase in serotonergic (5-hydroxytryptamine, 5-HT) efficacy has been a target of antiobesity pharmacotherapies. However, the induction of tolerance and/or side-effects limited the clinical utility of the earliest serotonin-related medications. With the global prevalence of obesity rising, there has been renewed interest in the manipulation of the serotonergic system as a point of pharmacological intervention. The serotonin_{2C} receptor (5-HT_{2C}R), serotonin_{1B} (rodent)/serotonin_{1Dβ} (human) receptor (5-HT_{1B/1Dβ}R) and serotonin₆ receptor (5-HT₆R) represent the most promising serotonin receptor therapeutic targets. Canonical serotonin receptor compounds have given way to a myriad of novel receptor-selective ligands, many of which have observable anorectic effects. Here we review serotonergic compounds reducing ingestive behaviour and discuss their clinical potential for the treatment of obesity.

(Received 30 September 2008; accepted after revision 20 November 2008; first published online 24 November 2008)

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Early pharmacological manipulations identified an inverse relationship between the biogenic amine neurotransmitter serotonin and food intake. More specifically, a selective reduction in serotonin bioavailability was associated with hyperphagia and subsequent weight gain, whilst diminished food intake was induced by an increase in serotonin efficacy (Saller & Stricker, 1976; Fletcher & Paterson, 1989). Researchers further sought to clarify which of the 14 distinct serotonin receptors (5-HTRs) identified in vertebrates (clustered into 7 ‘families’, 5-HT₁–5-HT₇, based on sequence homology and effector pathways) are critically involved in serotonin’s effects on ingestive behaviour. Using pharmacological and genetic tools, the 5-HT_{1B}R, 5-HT_{2C}R and 5-HT₆R subtypes were shown to be the principal mediators through which serotonin exerts its anorectic effects in rodents, and as such, these receptors have been investigated as pharmacotherapeutic targets for the treatment of obesity. Here we review the serotonergic compounds currently lending themselves to the treatment and investigation of obesity (Table 1).

Efforts to elucidate a site of action for 5-HTR-mediated ingestive behaviour highlighted the hypothalamus as a

key component (Breisch *et al.* 1976; Fletcher & Paterson, 1989; Heisler *et al.* 2002). The physiological manifestations of appetite, and subsequent responses to caloric intake, involve the integration of multiple central and peripheral signals at this site. Perturbed feeding behaviour and consequential effects on body weight effectuated by surgical ablation of specific hypothalamic nuclei was the first unequivocal evidence as to the fundamentality of this brain region in modulating appetite. Much of the neurochemistry that underpins this function has begun to be elucidated. Niche populations of peptidergic neurones, principally within the arcuate (ARC), ventromedial (VMH) and paraventricular (PVH) nuclei and lateral hypothalamic area (LHA), form an intricate feeding circuit reactive to numerous appetitive signals, and initiate neuroendocrine and behavioural responses to food intake.

Acute administration of serotonergic compounds altered the expression of such peptidergic appetitive effectors within the hypothalamus, namely an increase in anorectic pro-opiomelanocortin (POMC) mRNA and a decrease in orexigenic neuropeptide Y (NPY) mRNA (Choi *et al.* 2006), both of which are synthesized within discrete neuronal populations of the ARC. Recently, it has been shown that manipulation of these first order hypothalamic POMC/cocaine and amphetamine regulated transcript (CART) and agouti-related protein (AgRP)/NPY neurones is a mechanism through which

This report was presented at a Physiological Society Themed Meeting on *Orchestration of metabolism in health and disease*, which took place at the University of Oxford, UK, 9–11 September 2008.

Table 1. Serotonergic compounds inducing hypophagia

Compound	Mechanism	Manufacturer (clinical status)	Selected reference
Inc. bioavail.			
5-HTP	5HT precursor	—	Fletcher & Burton (1986)
Fenfluramine	SSRI and releaser	Servier (Withdrawn in 1997)	Halford <i>et al.</i> (2007)
Fluoxetine	SSRI	Eli Lilly (Withdrawn from Phase III)	Yen <i>et al.</i> (1987)
Paroxetine	SSRI	GlaxoSmithKline	Konkle <i>et al.</i> (2003)
Sertraline	SSRI	Pfizer	Nielsen <i>et al.</i> (1992)
Fluvoxamine	SSRI	Solvay Pharmaceuticals	Nonogaki <i>et al.</i> (2007)
Sibutramine	SNRI	Knoll/Abbott (Registered therapy)	Connoley <i>et al.</i> (1995)
5-HT₁			
CP-93, 129	5-HT _{1B} R agonist	Pfizer	Macor <i>et al.</i> (1990)
CP-94, 253	5-HT _{1B} R agonist	Pfizer	Koe <i>et al.</i> (1992)
RU-24969	5-HT _{1A/1B} R agonist	Organon	Kennett <i>et al.</i> (1987)
5-HT_{2C}			
Ro 60-0175	agonist	Organon/Hoffman-La Roche	Martin <i>et al.</i> (1998)
Ro 60-0332	agonist	Organon/Hoffman-La Roche	Martin <i>et al.</i> (1998)
Org-12962	agonist	Organon (Phase II as antidepressant)	Nilsson (2006)
Org-37684	agonist	Organon	Schreiber & De Vry (2002)
VER-3323	agonist	Vernalis/Roche	Bentley <i>et al.</i> (2004)
VER-23779	agonist	Vernalis/Roche	Somerville <i>et al.</i> (2007)
VER-2692	agonist	Vernalis/Roche	Adams <i>et al.</i> (2006)
VER-5584	agonist	Vernalis/Roche	Bentley <i>et al.</i> (2004)
VER-5593	agonist	Vernalis/Roche	Bentley <i>et al.</i> (2004)
VER-8775	agonist	Vernalis/Roche	Dourish <i>et al.</i> (2004)
BVT.933	agonist	Biovitrum (Withdrawn from Phase IIb)	Svartengren <i>et al.</i> (2003a)
BVT-X	agonist	Biovitrum	Lam <i>et al.</i> (2008)
YM348	agonist	Yamanouchi Pharm.	Hayashi <i>et al.</i> (2004a)
APD-356	agonist	Arena Pharm. (Phase IIb completed)	Thomsen <i>et al.</i> (2008)
ATHX-105	agonist	Athersys	Nilsson (2006)
WAY-163909	agonist	Wyeth	Dunlop <i>et al.</i> (2005)
WAY-161503	agonist	Wyeth	Rosenzweig-Lipson <i>et al.</i> (2006)
WAY-629	agonist	Wyeth	Sabb <i>et al.</i> 2004
LY448100	agonist	Eli Lilly	Nilsson (2006)
IL-639	agonist	Bristol Myers Squibb	Nilsson (2006)
PNU-22394	agonist	Pharmacia (Phase I)	McCall <i>et al.</i> (2001)
5-HT_{1/2}			
mCPP	5-HT _{1B/2C} R agonist	Now distributed by multiple vendors	Kennett & Curzon (1988)
TFMPP	5-HT _{1B/2A/2C} R agonist	Now distributed by multiple vendors	Kennett <i>et al.</i> (1987)
MK-212	5-HT ₂ R agonist	Now distributed by multiple vendors	Halford <i>et al.</i> (1997)
DOI	5-HT ₂ R agonist	Now distributed by multiple vendors	Simansky & Vaidya (1990)
5-HT₆			
BVT.74316	antagonist	Biovitrum (Phase I completed)	Heal <i>et al.</i> (2008)
BVT.5182	antagonist	Biovitrum	Svartengren <i>et al.</i> (2003b)
E-6837	partial agonist	Esteve	Fisas <i>et al.</i> (2006)
PRX-07034	antagonist	Epix (Phase I completed)	Shacham <i>et al.</i> (2006)
SB-271046	antagonist	GlaxoSmithKline	Svartengren <i>et al.</i> (2004)
Ro 04-6790	antagonist	Organon/Hoffman-La Roche	Woolley <i>et al.</i> (2001)
SB-399885	antagonist	GlaxoSmithKline	Perez-Garcia & Meneses (2005)
SB-357134	antagonist	GlaxoSmithKline	Perez-Garcia & Meneses (2005)

Abbreviations: SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and noradrenalin reuptake inhibitor.

serotonergic compounds reduce food intake (Fig. 1). Specifically, the serotonin system concomitantly regulates the antagonistic functions of POMC/CART and AgRP/NPY neurones through neurotransmitter binding

of two spatially distinct G-protein coupled receptor subtypes: depolarizing POMC/CART neurones via action at G_q-coupled 5-HT_{2C}Rs (Heisler *et al.* 2002) and hyperpolarizing AgRP/NPY neurones through action

at G_i -coupled 5-HT_{1B} Rs (Heisler *et al.* 2006; Fig. 1). Furthermore, the anorectic effect of compounds increasing serotonergic bioavailability and 5-HT_{2C} R and 5-HT_{1B} R agonists is contingent upon the downstream activation of the melanocortin 4 receptors (MC4Rs) (Heisler *et al.* 2006; Lam *et al.* 2008). It is noteworthy that these serotonergic compounds, which are highly effective in reducing food intake, are rendered ineffective by pharmacological or genetic inactivation of this single downstream melanocortin receptor target (Heisler *et al.* 2006; Lam *et al.* 2008). These data elucidate that the melanocortin pathway is a key downstream target for serotonergic compound hypophagia. Recent research has further clarified that the key population of MC4Rs influencing appetite is expressed in the PVH and/or amygdala (Balthasar *et al.* 2005).

In addition to serotonergic modulation of neurones containing the melanocortin agonist and antagonist, serotonin may also directly influence the activity of MC4R-containing cells. For example, infusion of serotonin into the PVH of rats reduces food intake (Fletcher & Paterson, 1989). Corticotropin-releasing hormone (CRH) neurones expressed within the PVH are directly innervated by serotonergic projections and both *c-fos* induction and CRH expression are stimulated by compounds increasing serotonergic efficacy (Liposits *et al.* 1986; Bovoetto *et al.* 1996; Choi *et al.* 2006). A subpopulation of CRH neurones have been reported to express MC4Rs (Lu *et al.* 2003), and it is possible that serotonin may directly influence the activity of these CRH MC4R-expressing cells. Additional research is required to determine if this is the case. Such further research investigating the down- and upstream pathways through which serotonin influences appetite may yield additional pharmacological targets for the treatment of obesity.

Compounds increasing 5-HT bioavailability

Fenfluramine, an amphetamine derivative lacking psychostimulant properties, was synthesized in the 1970s, and followed 20 years later by the more efficacious enantiomer, dexfenfluramine. Both compounds were successfully prescribed (often in combination with phentermine) as anorectic treatments for obesity until their withdrawal from clinical use in 1997, due to corollary incidences of cardiopulmonary complications. Mechanistically, these drugs are analogous to amphetamine, causing reversal and blockade of the serotonin transporter and a consequential increase in serotonin efflux and synaptic persistence (Crespi *et al.* 1997). Furthermore, the major metabolite of fenfluramine, norfenfluramine, is a 5-HT_{2C} R agonist (Gibson *et al.* 1993). Genetic and pharmacological studies demonstrated that action at the 5-HT_{2C} Rs and 5-HT_{1B} Rs is required for fenfluramine to influence ingestive behaviour

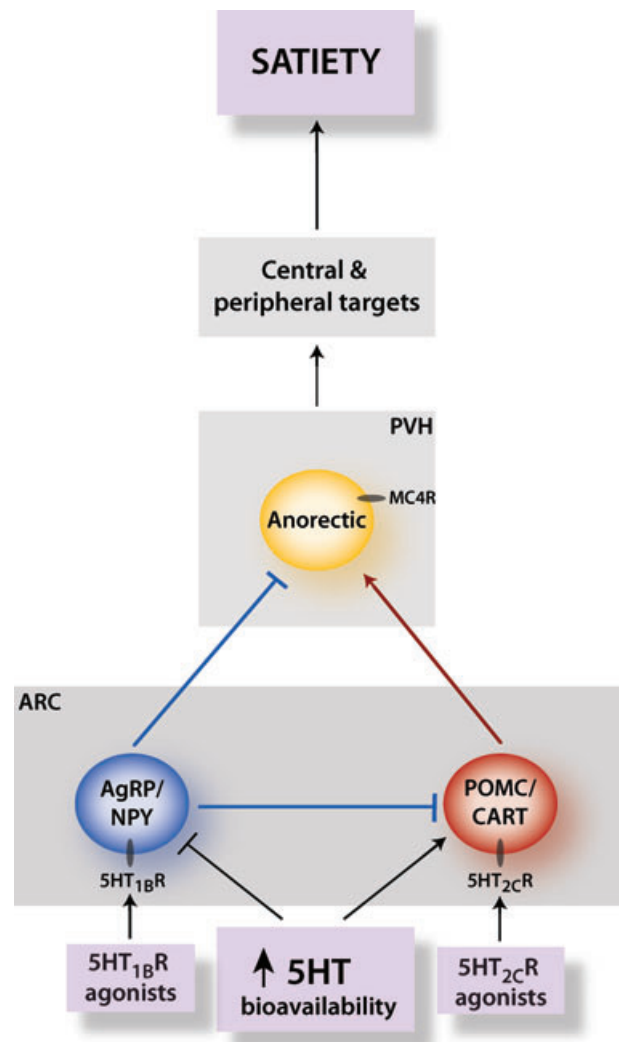


Figure 1. Proposed model of a serotonergic pathway modulating food intake

An increase in serotonin bioavailability (due to food intake or pharmacological compounds such as sibutramine and fenfluramine) or direct agonism of 5-HT_{2C} Rs and 5-HT_{1B} Rs modulates firing of pro-opiomelanocortin (POMC)/cocaine and amphetamine regulated transcript (CART) and agouti related protein (AgRP)/neuropeptide Y (NPY) neurones within the arcuate nucleus of the of the hypothalamus (ARC). Anorectic POMC neurones expressing 5-HT_{2C} R depolarize on receptor activation and release α -melanocyte-stimulating hormone (α -MSH), which in turn activates second-order melanocortin 4 receptor (MC4R) expressing neurones, principally within the paraventricular nucleus of the hypothalamus (PVH; Balthasar *et al.* 2005). Concomitant activation of 5-HT_{1B} Rs expressed on orexigenic AgRP/NPY neurones within the ARC causes membrane hyperpolarization and subsequent inhibition of neuropeptide release. Inhibitory 5-HT_{1B} R activation also attenuates inhibitory postsynaptic currents onto POMC/CART neurones further potentiating anorexigenesis. Subsequent downstream neuroendocrine signalling promotes satiety and the cessation of food intake.

(Lucas *et al.* 1998; Vickers *et al.* 1999, 2001; Simansky & Nicklous, 2002).

The effect of fenfluramine on body weight has been convincingly established by a number of experimental and clinical analyses (for a more extensive review see Halford *et al.* 2007). In obese rodents and humans, fenfluramine administration attenuated body weight gain in a manner consistent with a more rapid onset of satiety and, in the case of humans, suppressed premeal hunger (Fisler *et al.* 1993; Halford *et al.* 2007). In chronic fenfluramine trials, obese patients attained maximal weight reduction by 24 weeks, but demonstrated a considerable re-accumulation of body weight upon cessation of treatment after 3.5 years (Fernstrom & Choi, 2008). A similar effect was seen during intermittent chronic fenfluramine administration in rodents (5 days of drug followed by 5 days no drug, repeated 10 times), inasmuch as hypophagia and weight loss were only observed for the first 2 days of each treatment period (Choi *et al.* 2002). During no-drug periods animals regained body weight, due to increased food intake, before losing it again during the successive treatment period (Choi *et al.* 2002).

Administration of exogenous serotonin and its precursors also elicits a potent anorectic effect in humans and rodents. Injections of serotonin directly into the PVH of rats resulted in a significant decrease in food intake due to a reduction in meal size and duration (Fletcher & Paterson, 1989). However, serotonin itself fails to penetrate the blood–brain barrier and as such is of little therapeutic salience. By contrast, peripheral administration of 5-hydroxytryptophan (5-HTP), the carboxylated precursor of serotonin, successfully suppressed food intake (Fletcher & Burton, 1986). Within a clinical context, 5-HTP has demonstrated observable hypophagic effects in obese subjects and an associated reduction in body weight; moreover, the attenuation of caloric intake was correlated with an enhancement of within-meal satiety (Ceci *et al.* 1989). Despite these results, 5-HTP is not a registered antiobesity therapy. However, its amino acid precursor, tryptophan, has demonstrated anorectic effects in a clinical setting and is available as an adjunctive antidepressant medication within the UK (Halford *et al.* 2007).

Another class of drugs that augment serotonin bioavailability are the serotonin reuptake inhibitors. Via the blockade of monoamine transporters, these drugs promote the synaptic persistence of serotonin and thus sustained activation of postsynaptic receptors. Such compounds are more generally associated with the treatment of depression or anxiety; however, in light of their indiscriminate effect on serotonin concentrations, many also exhibit anorectic properties in rodents. For example, the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Prozac[®]) significantly reduced food intake

and body weight gain in the rat (Yen *et al.* 1987; Heisler *et al.* 1997; Heisler *et al.* 1999). Other SSRIs demonstrating therapeutic potential for obesity treatment include fluvoxamine (Nonogaki *et al.* 2007), paroxetine and sertraline (Halford *et al.* 2007).

Sibutramine (Reductil[®]) is a serotonin and noradrenalin reuptake inhibitor (SNRI) and a registered antiobesity treatment. Rodent studies demonstrated that sibutramine enhanced satiety (Halford *et al.* 1995) and induced significant hypophagia and weight loss (Connoley *et al.* 1995). Interestingly, studies using obese models (*ob/ob* mice and Zucker rats) revealed that chronic administration of sibutramine can elicit significant weight loss without a prolonged effect on food intake, suggesting that this compound also influences energy expenditure (Connoley *et al.* 1995; Day & Bailey, 1998; Golozoubova *et al.* 2006). Consistent with rodent work, clinical studies of sibutramine administration demonstrated that through the enhancement of satiety, chronic dosing leads to attenuated food intake and subsequent weight loss in the order of 10% (for review see Halford *et al.* 2007). Furthermore, these effects could be potentiated by a conjunctive low calorie diet, cogently demonstrating the need for life-style counselling in addition to pharmacological intervention (Apfelbaum *et al.* 1999). In long-term human studies, maximal weight loss was observed at 6 months followed by a stabilized body weight significantly lower than baseline levels or placebo controls (McNeely & Goa, 1998). During one such trial, food intake was still significantly reduced after 10 months of treatment, indicating the continued efficacy of the drug (Barkeling *et al.* 2003). At present, sibutramine represents the standard in serotonergic antiobesity therapeutics and is the yard stick by which the efficacy of newly developed compounds will be judged.

Compounds targeting serotonin receptors

5-HT_{1A}R and 5-HT_{1B}R compounds. 5-HT_{1A}R activation results in membrane hyperpolarization via G_i mediated inhibition of cAMP production (for review see Lam & Heisler, 2007). 5-HT_{1A}Rs, principally located on serotonergic efferents and cell bodies, are characterized autoreceptors which suppress action potential firing. Consistent with this function, administration of the 5-HT_{1A}R agonist 8-OH-DPAT elicited an increase in rodent feeding (Hutson *et al.* 1988). A perhaps more critical role for the 5-HT_{1A}Rs in the serotonergic regulation of anxiety and depression has been supported by murine genetic studies (Abenhaim *et al.* 1996; Heisler *et al.* 1998; Parks *et al.* 1998; Ramboz *et al.* 1998; Gross *et al.* 2002), and drug discovery efforts related to this receptor have thus far not been focused on obesity.

Of the rodent 5-HT₁Rs, it is the 5-HT_{1B}R subtype that is the most intimately associated with appetitive

control. The human homologue of the rodent 5-HT_{1B}R is the 5-HT_{1D β} R. 5-HT_{1B}R knockout mice exhibit an approximate 9–17% elevation in body weight compared to wild-type littermates and an increase in feeding that is consistent with increased body weight (Bouwknicht *et al.* 2001; see Table 2). 5-HT_{1B}R knockout mice also displayed attenuated responses to the anorectic effect of fenfluramine and the classic 5-HT_{1A/1B}R agonist RU24969 (Lucas *et al.* 1998). Complementing these genetic studies, the selective 5-HT_{1B}R pyridine agonists CP-93,129 and CP-94,253 have both demonstrated significant acute anorectic effects in rodents (Macor *et al.* 1990; Koe *et al.* 1992), effects blocked by pretreatment with 5-HT_{1B}R antagonists (Lee *et al.* 2002; Heisler *et al.* 2006). Prolonged administration of CP-94,253 decreased food intake in rats and reduced body weight gain, although drug desensitization was apparent within a week (Koe *et al.* 1992). CP-93,129, despite being significantly more selective than CP-94,253, demonstrated poor blood–brain barrier penetration and thus a lack of anorectic properties when peripherally administered (Macor *et al.* 1990; Lee & Simansky, 1997). The anorectic effect of these 5-HT_{1B}R agonists appears to involve hyperpolarization of ARC AgRP/NPY neurones and a disinhibition of POMC/CART neurones, and ultimately, activation of the downstream MC4Rs (Heisler *et al.* 2006).

5-HT_{2C}R compounds. In light of compelling pharmacological and genetic evidence, manipulation of 5-HT_{2C}R signalling has been a focus of serotonin-related obesity drug discovery efforts. The established involvement of this particular receptor subtype in the broader pharmacokinetic activities of dexfenfluramine, sibutramine and 1-3(chlorophenyl)piperazine (mCPP) elegantly highlight its therapeutic pertinence. Furthermore, genetic abrogation of 5-HT_{2C}R expression in mice engendered marked hyperphagia and middle age onset obesity (Tecott *et al.* 1995; see Table 2).

Two older compounds, Ro 60-0175 and Ro 60-0332, both described as full 5-HT_{2C}R agonists, demonstrated significant anorectic properties in a paradigm of palatable food consumption in normal rats (Martin *et al.* 1998). Continuous subcutaneous infusion of the former revealed that hypophagia only ensued for the first 10 days of a 2 week regime. Moreover, body weight accumulation was markedly reduced compared to vehicle from 2 days postimplantation and persisted throughout the duration of the study (Vickers *et al.* 2000). The authors suggested that the prolonged weight-reducing effect in the absence of sustained hypophagia was due to drug-induced hyperthermia, leading to increased energy expenditure. Such a thermogenic effect was also observed by Hayashi *et al.* (2005) during chronic oral administration of Ro 60-0175, although interestingly in this case, attenuated weight gain and hypophagia persisted concomitantly. Consistent with

these effects being transmitted via the 5-HT_{2C}R, SB242084 antagonist pretreatment negated the hypophagic and hyperthermic properties of Ro 60-0175 (Hayashi *et al.* 2005). However, Ro 60-0175 also appears to have binding affinity for the 5-HT_{2B}R, which may limit its therapeutic potential given that action at peripheral 5-HT_{2B}R is thought to underlie the cardiopulmonary complications associated with some serotonergic drugs (Martin *et al.* 1998; Fitzgerald *et al.* 2000).

The Yamanouchi Pharmaceutical Company's 5-HT_{2C}R agonist YM348 also elicited a robust dose-dependent decrease in food consumption in Zucker rats, with almost complete aphagia at some concentrations (Hayashi *et al.* 2004a). However, continuous infusion failed to sustain a hypophagic effect, with treated animals returning to control levels by day 10, although body weight gain remained significantly lower than controls (Hayashi *et al.* 2004a). This observation is potentially explained by augmented energy and calorie expenditure, as these physiological indices were not desensitized by prolonged YM348 treatment (Hayashi *et al.* 2004a,b). However, despite promising antiobesity properties, the affinity of this drug for the 5-HT_{2B}R is only 2-fold lower than that for 5-HT_{2C}R, again alluding to the potential for adverse clinical side-effects.

Vernalis, in collaboration with Hoffmann-La Roche, have synthesized a number of 5-HT_{2C}R compounds with demonstrable anorectic activity. VER-3323, VER-5593 and VER-5348 are all indoline-based analogues similar in structure to Ro 60-175, although with greater 5-HT_{2C}R selectivity. Both subcutaneous and oral administration of these compounds elicited hypophagia in food deprived rats (Bentley *et al.* 2004). A subsequent study in Siberian hamsters revealed that VER-3323, whilst decreasing food intake, had no observable effect on energy expenditure in the form of thermogenesis or locomotion (Schuhler *et al.* 2005). A more recent Vernalis compound, VER-2692, exhibited a greater selectivity profile than the indoline derivatives and also resulted in hypophagia when administered acutely to food deprived rats, a response blocked by 5-HT_{2C}R antagonist pretreatment (Adams *et al.* 2006). A perhaps more promising Vernalis compound is VER-8775, a piperazine derivative with 15- and 476-fold selectivity for the 5-HT_{2C}R over 5-HT_{2A}R and 5-HT_{2B}R, respectively (Nilsson, 2006). VER-8775 significantly reduced food intake in fasted rats and mice and induced weight loss in diet-induced obese (DIO) mice during a 28-day oral regime (Dourish *et al.* 2004).

The Wyeth compound WAY-161503 has exhibited potent anorectic and weight reducing effects in both mouse and rat models of obesity (Rosenzweig-Lipson *et al.* 2006). During chronic trials in Zucker rats, drug tolerance was not observed, with both food intake and weight accumulation remaining significantly decreased throughout the 15 day study. While these results were

Table 2. Feeding and body weight phenotypes of 5-HT knockout mice

Receptor	Feeding & body weight phenotypes of mutant animals	Additional comments	References
5-HT _{1A}	Increased intake of sucrose solution in females (potentially sex-hormone related). No alterations in homecage feeding or body weight reported.	Increased anxiety related behaviours and reduced depression-related behaviours	Heisler <i>et al.</i> (1998); Parks <i>et al.</i> (1998); Ramboz <i>et al.</i> (1998); Bechtholt <i>et al.</i> (2008)
5-HT _{1B}	Mildly increased body weight and relative increase in food intake	Reduced sensitivity to d-fen and mCPP induced hypophagia. Compensatory reduction in 5HT _{2C} R function	Lucas <i>et al.</i> (1998); Bouwknecht <i>et al.</i> (2001); Clifton <i>et al.</i> (2003); Lee <i>et al.</i> (2004)
5-HT _{2A}	No alterations in homecage feeding, novelty suppressed feeding or body weight reported.	Increased anxiety related behaviours	Weisstaub <i>et al.</i> (2006)
5-HT _{2B}	Not reported	Mutants died perinatally due to incomplete heart development	Nebigil <i>et al.</i> (2000)
5-HT _{2C}	Marked hyperphagia throughout life and increased body weight gain from around 12 weeks	Increased locomotor activity and subsequent age-dependent reductions in energy cost of physical activity. Dysregulation of HPA axis and reduced anxiety-related behaviour	Tecott <i>et al.</i> (1995); Nonogaki <i>et al.</i> (2003); Heisler <i>et al.</i> (2007a,b)
5-HT ₃	No observed differences in body weight or food intake	Dysregulation of the HPA axis in response to stress	Bhatnagar <i>et al.</i> (2004)
5-HT ₄	Modestly reduced weight gain in homecage environment, despite normal food intake. Food intake increased after restraint induced stress	This model of stress induced anorexia has been associated with an increase in CART expression	Compan <i>et al.</i> (2004); Jean <i>et al.</i> (2007)
5-HT ₅	Normal body weight. No data on food intake reported	Increased exploratory behaviour that was independent of an effect on anxiety	Grailhe <i>et al.</i> (1999)
5-HT ₆	Normal body weight. No data on food intake reported	Altered responses to ethanol	Bonasera <i>et al.</i> (2006)
5-HT ₇	Normal body weight. No data on food intake reported	Failed to exhibit the expected hypothermic response to serotonin administration	Hedlund <i>et al.</i> (2003)

Abbreviations: d-fen, dexfenfluramine; HPA, Hypothalamic-Pituitary-Adrenal; CART; cocaine and amphetamine regulated transcript.

promising, functional selectivity assays demonstrated that WAY-161503 offered no apparent selectivity over 5-HT_{2B}Rs, although SB215505 (5-HT_{2B}R antagonist) pretreatment did not ablate the anorectic properties of this drug (Rosenzweig-Lipson *et al.* 2006). A more recent Wyeth compound, WAY-163909, is characterized as a full 5-HT_{2C}R agonist and partial 5-HT_{2B}R agonist, despite being 46-fold more selective for the former (Dunlop *et al.* 2005). In acute feeding paradigms, WAY-163909 reduced food intake in lean and obese rodent models. Significantly, no tolerance to this compound was observed after a 10-day regime and furthermore animals exhibited a 46% reduction in body weight as compared to controls (Dunlop *et al.* 2005).

Preclinical data on BVT.933 (generated by Biovitrum) indicated that chronic dosing of this compound was successful in attenuating both food intake and weight accumulation in DIO rats, and furthermore, that these results were correlated with a reduction in adiposity (Svartengren *et al.* 2003a). The compound was entered into clinical trials, but was withdrawn from Phase IIb. A subsequent compound developed by Biovitrum, BVT.X, has recently been characterized. Lam *et al.* (2008) demonstrated that acute administration of BVT.X was effective in reducing food intake in two models of murine obesity. However, chronic dosing via osmotic minipumps in leptin deficient obese *ob/ob* mice revealed that this anorectic effect was relatively short lived,

with treated animals returning towards control levels just 2 days through the 7 day trial. Interestingly, despite desensitization to hypophagia, body weight gain was significantly attenuated at the end of the trial. Lam *et al.* (2008) investigated the central pathways associated with BVT.X's effect on energy balance and observed an up-regulation of POMC expression in the ARC and further determined that downstream activation of MC4Rs was required for this compound to exert its anorectic effect (see Fig. 1). The stimulation of this melanocortin anorexigenic pathway is consistent with a previously asserted model of dexfenfluramine and CP-94 253 anorexia (Heisler *et al.* 2002, 2006).

One of the most recently reported 5-HT_{2C}R compounds is Arena's APD-356 (Lorcaserin®). This benzapine molecule exhibited robust functional selectivity over 5-HT_{2A}Rs and 5-HT_{2B}Rs (18- and 104-fold, respectively; Thomsen *et al.* 2008). Acute oral dosing of APD-356 significantly reduced cumulative food intake in non-deprived rats for up to 22 h, although *post hoc* analysis attributed this observation to potent hypophagia during the first 2 hours and the absence of subsequent rebound hyperphagia (Thomsen *et al.* 2008). This effect was readily ablated by 5-HT_{2C}R antagonist pretreatment, suggesting the functional selectivity of the compound for the 5-HT_{2C}R. In chronic assessments, APD-356 successfully attenuated both food intake and body weight gain in DIO rats (Thomsen *et al.* 2008). Although anorectic desensitization was observed at 13 days, body weight remained significantly lower than controls throughout the course of the study, indicating further effects on additional modalities of weight loss. During the 4 week withdrawal period, treated animals exhibited rebound hyperphagia and a subsequent return to control body weight (Thomsen *et al.* 2008). Significantly, high dosing of both Sprague–Dawley rats and cynomolgus monkeys over 3 months failed to induce any cardio-pulmonary side-effects, consistent with the compound's low selectivity for peripheral 5-HT_{2B}R (Smith *et al.* 2006). APD-356 is currently in Phase 3 clinical trials having successfully demonstrated weight reducing effects during a 12 week Phase IIb study. Drug treated obese patients exhibited a statistically significant 1.3 kg reduction in body weight. No valvopathies or pulmonary complications were observed in these patients or other clinical cohorts (Smith *et al.* 2006).

Additional 5-HT_{2C}R compounds with observed anti-obesity properties include PNU-22394, a relatively non-selective Pharmacia/Pfizer compound with anorectic effects in rodents and humans (McCall *et al.* 2001); ATHX-105 and ATH-188651, from Athersys; and IL639 from Bristol-Myers Squibb which demonstrated a 250-fold selectivity over 5-HT_{2A/2B}Rs and is orally efficacious in rat feeding paradigms (Nilsson, 2006).

5-HT_{1/2}R compounds. Compounds that demonstrate a degree of 5-HT₁R and 5-HT₂R cross-talk have often proved highly effective in modulating feeding behaviour, most likely due to their concerted action at anorexigenic and orexigenic populations of hypothalamic neurones. For example, mCPP exhibits a preferential affinity for 5-HT_{1B}R and 5-HT_{2C}R subtypes and induced robust hypophagia during both acute and chronic regimes (Kennett & Curzon, 1988; Vickers *et al.* 2003). Significant attenuation of body weight gain was also observed during a 28 day study, with no apparent drug tolerance (Vickers *et al.* 2003). Furthermore, the use of pair-fed animals in this study strongly implicated the hypophagic effect of the compound as underlying the observed reduction in weight accumulation. In human subjects, mCPP was capable of reducing caloric intake at a test meal by 30% when administered to healthy women, an effect associated with a decrease in premeal hunger ratings (Halford *et al.* 2007). A similar decline in premeal hunger and body weight was observed in obese men receiving mCPP over a 2 week period, although assessment of food consumption was not reported (Halford *et al.* 2007). 1-[3-(Trifluoromethyl) phenyl]piperazine (TFMPP), a structurally related analogue of mCPP, also elicited observable hypophagic and weight reducing effects, although drug desensitization was observed during chronic administration (Kennett *et al.* 1987; Rouru *et al.* 1993). Hypophagia induced by mCPP and TFMPP is consistent with enhancement of satiety, although at higher doses both drugs have shown to induce hypoactivity (Kennett & Curzon, 1988).

5-HT₆R compounds. 5-HT₆R distribution within the CNS includes hypothalamic regions of immediate salience to a role in appetitive control, including the ARC, PVH and VMH (Heal *et al.* 2008). Concordant with this expression profile, manipulation of 5-HT₆R signalling has been demonstrated to have potent effects on both food consumption and body weight (for review see Heal *et al.* 2008). It is of note that it is the antagonism of this receptor that is generally associated with its anti-obesity function (Heal *et al.* 2008). One of the earliest 5-HT₆R antagonists reported to induce hypophagia was Ro 04-6790 (Organon/Hoffman-La Roche), which when administered to rats on three consecutive days, resulted in a suppression of body weight gain that persisted for at least 10 days postwithdrawal (Woolley *et al.* 2001). Interestingly, the same study also demonstrated that food consumption and weight gain were decreased following antisense knockdown of 5-HT₆R, a phenotype absent in traditional 5-HT₆R knockout mice (Woolley *et al.* 2001; Bonasera *et al.* 2006).

Biovitrum's BVT.5182 5-HT₆R antagonist has also demonstrated significant anorectic properties during

both chronic and acute studies in rat and mouse models of obesity. In DIO mice, repeated subcutaneous doses elicited a 9% and 11% decrease in food consumption and body weight, respectively (Svartengren *et al.* 2003*b*). Subsequent studies in obese *ob/ob* mice and non-obese rats demonstrated that the observed hypophagia was attributable to enhanced satiety (Svartengren *et al.* 2004). This later study also reported that acute doses of the GlaxoSmithKline 5-HT₆R antagonist SB-271046 dose-dependently suppressed food consumption. However, Phase I trials of SB-271046 were apparently halted due to poor blood-brain barrier permeability. Little has been reported about Biovitrum's second 5-HT₆R compound, BVT.74316, though it did complete a Phase Ia study as an antiobesity therapy (press release of Biovitrum, 19 December 2007). PRX-07034 from Epix Pharmaceuticals has also recently completed Phase I trials (press release of Epix-Pharmaceuticals, 29 October 2007). In preclinical studies, chronic administration successfully reduced food intake and body weight in DIO rats; moreover, it would appear that no drug tolerance was observed over the course of the 5 week study (Shacham *et al.* 2006).

E-6837 (Esteve) represents a curious pharmacological paradox in that it is classified as a 5-HT₆R partial agonist, but like 5-HT₆R full antagonists, it reduces body weight (for discussion on this issue, see Heal *et al.* 2008). Specifically, chronic oral administration of E-6837 significantly attenuated body weight by day 3 and continued to do so until reaching a plateau at day 20. At the culmination of the trial, E-6837 treated animals were approximately 6% lighter than a sibutramine control group. Furthermore, whilst the sibutramine group regained control weight by day 44, E-6837 treated animals were still significantly lighter at day 71 (Fisas *et al.* 2006). Interestingly, E-6837-induced hypophagia lasted for only the first 3 weeks after which significant rebound hyperphagia, commencing at week 1 of the withdrawal analysis, was observed (Fisas *et al.* 2006). Although no thermogenic effects were reported, further elucidation of E-6837 regulated energy expenditure and metabolism is warranted.

Conclusion

Pharmacological and genetic studies have consistently demonstrated the fundamentality of the serotonergic system in the regulation of appetite and ingestive behaviour. Moreover, they have highlighted the involvement of specific serotonin receptor subtypes in mediating these effects. Historically, pharmacological compounds that augment endogenous serotonergic signalling have proved effective in suppressing food consumption and attenuating body weight gain, physiological effects of immediate clinical salience to the

ever increasing incidence of obesity. Furthermore, with many antiobesity compounds falling short of optimal therapeutic efficacy (i.e. due to minimal weight loss, adverse side-effects or long-term resistance), there is a substantial unmet clinical need for novel pharmacological therapies. Currently, the 5-HT_{2C}R and 5-HT₆R have garnered the most attention in serotonin drug discovery efforts. Both 5-HT_{2C}R agonists and 5-HT₆R compounds have entered into clinical trials. Critically, the success of any 5-HT_{2C}R compound is contingent upon its functional selectivity, in particular over the peripherally expressed 5-HT_{2B}Rs. In this regard, 5-HT₆R compounds hold an advantage, inasmuch as they demonstrate little affinity for other serotonin receptor subtypes. Although the neuro-molecular/pharmacological aspects of 5-HT₆R function remain to be fully elucidated, a number of specific compounds have demonstrated significant anti-obesity potential in preclinical studies. Given the apparent independence of 5-HT₆R mediated appetite from that of 5-HT_{2C}R, the possibility of combinational therapies that exploit both pathways bears considering.

Energy homeostasis is a critical balance between the intake and utilization of energy, such that a decrease in the former or an increase in the latter engenders a negative energy balance and an associated loss of weight in the form of fat. Although the weight-reducing effects of most serotonergic compounds are principally mediated via their anorectic properties, evidence from a number of pharmacological studies has indicated that augmented energy expenditure may also play a role (Connoley *et al.* 1995; Vickers *et al.* 2000; Hayashi *et al.* 2004*b*; Golozoubova *et al.* 2006; Lam *et al.* 2008). The melanocortin pathway, which is downstream of the homeostatic serotonergic system, has been demonstrated to promote energy expenditure. The necessity for intact MC4R signalling in the anorectic actions of a number of serotonergic compounds (Heisler *et al.* 2002, 2006; Lam *et al.* 2008) may also be of pertinence to the promotion of serotonin mediated energy expenditure. Murine genetic studies have reported the involvement of the MC4R in modulation of a number of expending processes, including locomotion, diet-induced thermogenesis and lipolysis (reviewed in Ellacott & Cone, 2006). Furthermore, the role of these mechanisms in regulating energy homeostasis can be spatially dissociated from the anorectic properties of MC4R (Balthasar *et al.* 2005). Whether the MC4Rs are a downstream component of serotonergic-mediated energy expenditure remains to be fully clarified.

In conclusion, it is apparent that the serotonergic system offers therapeutic potential in the unremitting battle against obesity. Continued pharmaceutical investment in this cause, together with more spatially refined genetic models, will afford researchers more specific tools with which to unravel the neurology of this increasingly pervasive condition.

References

- Abenhaim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G & Begaud B (1996). Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med* **335**, 609–616.
- Adams DR, Bentley JM, Benwell KR, Bickerdike MJ, Bodkin CD, Cliffe IA, Dourish CT, George AR, Kennett GA, Knight AR, Malcolm CS, Mansell HL, Misra A, Quirk K, Roffey JR & Vickers SP (2006). Pyrrolo (iso) quinoline derivatives as 5-HT_{2C} receptor agonists. *Bioorg Med Chem Lett* **16**, 677–680.
- Apfelbaum M, Vague P, Ziegler O, Hanotin C, Thomas F & Leutenegger E (1999). Long-term maintenance of weight loss after a very-low-calorie diet: a randomized blinded trial of the efficacy and tolerability of sibutramine. *Am J Med* **106**, 179–184.
- Balthasar N, Dalgaard LT, Lee CE, Yu J, Funahashi H, Williams T, Ferreira M, Tang V, McGovern RA, Kenny CD, Christiansen LM, Edelstein E, Choi B, Boss O, Aschkenasi C, Zhang CY, Mountjoy K, Kishi T, Elmquist JK & Lowell BB (2005). Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* **123**, 493–505.
- Barkeling B, Elfhag K, Rooth P & Rossner S (2003). Short-term effects of sibutramine (Reductil) on appetite and eating behaviour and the long-term therapeutic outcome. *Int J Obes Relat Metab Disord* **27**, 693–700.
- Bechtholt AJ, Smith K, Gaughan S & Lucki I (2008). Sucrose intake and fasting glucose levels in 5-HT_{1A} and 5-HT_{1B} receptor mutant mice. *Physiol Behav* **93**, 659–665.
- Bentley JM, Adams DR, Bebbington D, Benwell KR, Bickerdike MJ, Davidson JE, Dawson CE, Dourish CT, Duncton MA, Gaur S, George AR, Giles PR, Hamlyn RJ, Kennett GA, Knight AR, Malcolm CS, Mansell HL, Misra A, Monck NJ, Pratt RM, Quirk K, Roffey JR, Vickers SP & Cliffe IA (2004). Indoline derivatives as 5-HT_{2C} receptor agonists. *Bioorg Med Chem Lett* **14**, 2367–2370.
- Bhatnagar S, Sun LM, Raber J, Maren S, Julius D & Dallman MF (2004). Changes in anxiety-related behaviors and hypothalamic-pituitary-adrenal activity in mice lacking the 5-HT-3A receptor. *Physiol Behav* **81**, 545–555.
- Bonasera SJ, Chu HM, Brennan TJ & Tecott LH (2006). A null mutation of the serotonin 6 receptor alters acute responses to ethanol. *Neuropsychopharmacology* **31**, 1801–1813.
- Bouwknicht JA, van der Gugten J, Hijzen TH, Maes RA, Hen R & Olivier B (2001). Male and female 5-HT_{1B} receptor knockout mice have higher body weights than wildtypes. *Physiol Behav* **74**, 507–516.
- Bovetto S, Rouillard C & Richard D (1996). Role of CRH in the effects of 5-HT-receptor agonists on food intake and metabolic rate. *Am J Physiol Regul Integr Comp Physiol* **271**, R1231–R1238.
- Breisch ST, Zemlan FP & Hoebel BG (1976). Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. *Science* **192**, 382–385.
- Ceci F, Cangiano C, Cairella M, Cascino A, Del Ben M, Muscaritoli M, Sibilila L & Rossi Fanelli F (1989). The effects of oral 5-hydroxytryptophan administration on feeding behavior in obese adult female subjects. *J Neural Transm* **76**, 109–117.
- Choi S, Blake V, Cole S & Fernstrom JD (2006). Effects of chronic fenfluramine administration on hypothalamic neuropeptide mRNA expression. *Brain Res* **1087**, 83–86.
- Choi S, Jonak EM, Simpson L, Patil V & Fernstrom JD (2002). Intermittent, chronic fenfluramine administration to rats repeatedly suppresses food intake despite substantial brain serotonin reductions. *Brain Res* **928**, 30–39.
- Clifton PG, Lee MD, Somerville EM, Kennett GA & Dourish CT (2003). 5-HT_{1B} receptor knockout mice show a compensatory reduction in 5-HT_{2C} receptor function. *Eur J Neurosci* **17**, 185–190.
- Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J, Dumuis A, Brunner D, Bockaert J & Hen R (2004). Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT₄ receptor knock-out mice. *J Neurosci* **24**, 412–419.
- Connoley IP, Heal DJ & Stock MJ (1995). A study in rats of the effects of sibutramine on food-intake and thermogenesis. *Br J Pharmacol* **114**, P388–P388.
- Crespi D, Mennini T & Gobbi M (1997). Carrier-dependent and Ca²⁺-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylenedioxymethamphetamine, p-chloroamphetamine and (+)-fenfluramine. *Br J Pharmacol* **121**, 1735–1743.
- Day C & Bailey CJ (1998). Effect of the antiobesity agent sibutramine in obese-diabetic ob/ob mice. *Int J Obes Relat Metab Disord* **22**, 619–623.
- Dourish CT, Adams DR, Bentley J, Benwell K, Bickerdike MJ, Harrison K, Kennett GA, Knight AR, Lightowler SL, Malcolm C, Misra A, Vickers SP, Cliffe IA, Coassolo P, Frei B, Mizrahi J, Stalder H & Stephan-Guldne M (2004). Discovery and development of selective 5-HT_{2C} receptor agonists for obesity. *Fundam Clin Pharmacol* **18**, SAT S1.4, 127–134.
- Dunlop J, Sabb AL, Mazandarani H, Zhang J, Kalgaonker S, Shukhina E, Sukoff S, Vogel RL, Stack G, Schechter L, Harrison BL & Rosenzweig-Lipson S (2005). WAY-163909 [(7bR, 10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole], a novel 5-hydroxytryptamine 2C receptor-selective agonist with anorectic activity. *J Pharmacol Exp Ther* **313**, 862–869.
- Ellacott KL & Cone RD (2006). The role of the central melanocortin system in the regulation of food intake and energy homeostasis: lessons from mouse models. *Philos Trans R Soc Lond B Biol Sci* **361**, 1265–1274.
- Fernstrom JD & Choi S (2008). The development of tolerance to drugs that suppress food intake. *Pharmacol Ther* **117**, 105–122.
- Fisas A, Codony X, Romero G, Dordal A, Giraldo J, Merce R, Holenz J, Vrang N, Sorensen RV, Heal D, Buschmann H & Pauwels PJ (2006). Chronic 5-HT₆ receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. *Br J Pharmacol* **148**, 973–983.

- Fisler JS, Underberger SJ, York DA & Bray GA (1993). *d*-Fenfluramine in a rat model of dietary fat-induced obesity. *Pharmacol Biochem Behav* **45**, 487–493.
- Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, Sun JH, Link JR, Abbaszade I, Hollis JM, Largent BL, Hartig PR, Hollis GF, Meunier PC, Robichaud AJ & Robertson DW (2000). Possible role of valvular serotonin 5-HT_{2B} receptors in the cardiopathy associated with fenfluramine. *Mol Pharmacol* **57**, 75–81.
- Fletcher PJ & Burton MJ (1986). Dissociation of the anorectic actions of 5-HTP and fenfluramine. *Psychopharmacology (Berl)* **89**, 216–220.
- Fletcher PJ & Paterson IA (1989). A comparison of the effects of tryptamine and 5-hydroxytryptamine on feeding following injection into the paraventricular nucleus of the hypothalamus. *Pharmacol Biochem Behav* **32**, 907–911.
- Gibson EL, Kennedy AJ & Curzon G (1993). *d*-Fenfluramine- and *d*-norfenfluramine-induced hypophagia: differential mechanisms and involvement of postsynaptic 5-HT receptors. *Eur J Pharmacol* **242**, 83–90.
- Golozoubova V, Strauss F & Malmlof K (2006). Locomotion is the major determinant of sibutramine-induced increase in energy expenditure. *Pharmacol Biochem Behav* **83**, 517–527.
- Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Geyer MA & Hen R (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT_{5A} receptor. *Neuron* **22**, 581–591.
- Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S & Hen R (2002). Serotonin_{1A} receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**, 396–400.
- Halford JC, Harrold JA, Boyland EJ, Lawton CL & Blundell JE (2007). Serotonergic drugs: effects on appetite expression and use for the treatment of obesity. *Drugs* **67**, 27–55.
- Halford JCG, Heal DJ & Blundell JE (1995). Effects in the rat of sibutramine on food-intake and the behavioral satiety sequence. *Br J Pharmacol* **114**, P387.
- Halford JC, Lawton CL & Blundell JE (1997). The 5-HT₂ receptor agonist MK-212 reduces food intake and increases resting but prevents the behavioural satiety sequence. *Pharmacol Biochem Behav* **56**, 41–46.
- Hayashi A, Sonoda R, Kimura Y, Takasu T, Suzuki M, Sasamata M & Miyata K (2004a). Antiobesity effect of YM348, a novel 5-HT_{2C} receptor agonist, in Zucker rats. *Brain Res* **1011**, 221–227.
- Hayashi A, Suzuki M, Sasamata M & Miyata K (2004b). Thermogenic effect of YM348, a novel 5-HT_{2C}-receptor agonist, in rats. *J Pharm Pharmacol* **56**, 1551–1556.
- Hayashi A, Suzuki M, Sasamata M & Miyata K (2005). Agonist diversity in 5-HT_{2C} receptor-mediated weight control in rats. *Psychopharmacology (Berl)* **178**, 241–249.
- Heal DJ, Smith SL, Fisas A, Codony X & Buschmann H (2008). Selective 5-HT₆ receptor ligands: progress in the development of a novel pharmacological approach to the treatment of obesity and related metabolic disorders. *Pharmacol Ther* **117**, 207–231.
- Hedlund PB, Danielson PE, Thomas EA, Slanina K, Carson MJ & Sutcliffe JG (2003). No hypothermic response to serotonin in 5-HT₇ receptor knockout mice. *Proc Natl Acad Sci U S A* **100**, 1375–1380.
- Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH & Tecott LH (1998). Elevated anxiety and antidepressant-like responses in serotonin 5-HT_{1A} receptor mutant mice. *Proc Natl Acad Sci U S A* **95**, 15049–15054.
- Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD & Elmquist JK (2002). Activation of central melanocortin pathways by fenfluramine. *Science* **297**, 609–611.
- Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones Z, Liu HY, Zigman JM, Balthasar N, Kishi T, Lee CE, Aschkenasi CJ, Zhang CY, Yu J, Boss O, Mountjoy KG, Clifton PG, Lowell BB, Friedman JM, Horvath T, Butler AA, Elmquist JK & Cowley MA (2006). Serotonin reciprocally regulates melanocortin neurons to modulate food intake. *Neuron* **51**, 239–249.
- Heisler LK, Kanarek RB & Gerstein A (1997). Fluoxetine decreases fat and protein intakes but not carbohydrate intake in male rats. *Pharmacol Biochem Behav* **58**, 767–773.
- Heisler LK, Kanarek RB & Homoleski B (1999). Reduction of fat and protein intakes but not carbohydrate intake following acute and chronic fluoxetine in female rats. *Pharmacol Biochem Behav* **63**, 377–385.
- Heisler LK, Pronchuk N, Nonogaki K, Zhou L, Raber J, Tung L, Yeo GS, O'Rahilly S, Colmers WF, Elmquist JK & Tecott LH (2007a). Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. *J Neurosci* **27**, 6956–6964.
- Heisler LK, Zhou L, Bajwa P, Hsu J & Tecott LH (2007b). Serotonin 5-HT_{2C} receptors regulate anxiety-like behavior. *Genes Brain Behav* **6**, 491–496.
- Hutson PH, Dourish CT & Curzon G (1988). Evidence That the Hyperphagic response to 8-OH-DPAT is mediated by 5-HT_{1A} receptors. *Eur J Pharmacol* **150**, 361–366.
- Jean A, Conductier G, Manrique C, Bouras C, Berta P, Hen R, Charnay Y, Bockaert J & Compan V (2007). Anorexia induced by activation of serotonin 5-HT₄ receptors is mediated by increases in CART in the nucleus accumbens. *Proc Natl Acad Sci U S A* **104**, 16335–16340.
- Kennett GA & Curzon G (1988). Evidence that hypophagia induced by mCPP and TFMPP requires 5-HT_{1C} and 5-HT_{1B} receptors; hypophagia induced by RU 24969 only requires 5-HT_{1B} receptors. *Psychopharmacology (Berl)* **96**, 93–100.
- Kennett GA, Dourish CT & Curzon G (1987). 5-HT_{1B} agonists induce anorexia at a postsynaptic site. *Eur J Pharmacol* **141**, 429–435.
- Koe BK, Nielsen JA, Macor JE & Heym J (1992). Biochemical and behavioral studies of the 5-HT_{1B} receptor agonist, CP-94,253. *Drug Dev Res* **26**, 241–250.
- Konkle AT, Sreter KB, Baker SL & Bielajew C (2003). Chronic paroxetine infusion influences macronutrient selection in male Sprague-Dawley rats. *Pharmacol Biochem Behav* **74**, 883–890.
- Lam DD & Heisler LK (2007). Serotonin and energy balance: molecular mechanisms and implications for type 2 diabetes. *Expert Rev Mol Med* **9**, 1–24.
- Lam DD, Przydzial MJ, Ridley SH, Yeo GS, Rochford JJ, O'Rahilly S & Heisler LK (2008). Serotonin 5-HT_{2C} receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. *Endocrinology* **149**, 1323–1328.

- Lee MD, Kennett GA, Dourish CT & Clifton PG (2002). 5-HT_{1B} receptors modulate components of satiety in the rat: behavioural and pharmacological analyses of the selective serotonin_{1B} agonist CP-94,253. *Psychopharmacology (Berl)* **164**, 49–60.
- Lee MD & Simansky KJ (1997). CP-94,253: a selective serotonin_{1B} (5-HT_{1B}) agonist that promotes satiety. *Psychopharmacology (Berl)* **131**, 264–270.
- Lee MD, Somerville EM, Kennett GA, Dourish CT & Clifton PG (2004). Reduced hypophagic effects of d-fenfluramine and the 5-HT_{2C} receptor agonist mCPP in 5-HT_{1B} receptor knockout mice. *Psychopharmacology (Berl)* **176**, 39–49.
- Liposits Z, Phelix C & Paull WK (1986). Adrenergic innervation of corticotropin releasing factor (CRF)-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. A combined light and electron microscopic immunocytochemical study. *Histochemistry* **84**, 201–205.
- Lu XY, Barsh GS, Akil H & Watson SJ (2003). Interaction between α -melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. *J Neurosci* **23**, 7863–7872.
- Lucas JJ, Yamamoto A, Scarce-Levie K, Saudou F & Hen R (1998). Absence of fenfluramine-induced anorexia and reduced c-Fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor knock-out mice. *J Neurosci* **18**, 5537–5544.
- Macor JE, Burkhart CA, Heym JH, Ives JL, Lebel LA, Newman ME, Nielsen JA, Ryan K, Schulz DW, Torgersen LK *et al.* (1990). 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT_{1B}) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J Med Chem* **33**, 2087–2093.
- Martin JR, Bos M, Jenck F, Moreau J, Mutel V, Sleight AJ, Wichmann J, Andrews JS, Berendsen HH, Broekkamp CL, Ruigt GS, Kohler C & Delft AM (1998). 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J Pharmacol Exp Ther* **286**, 913–924.
- McCall RB, Franklin SR, Hyslop DK, Knauer CS, Chio CL, Haber CL & Fitzgerald LW (2001). PNU-22394, a 5-HT_{2C} receptor agonist, reduced feeding in rodents and produces weight loss in humans. *Soc Neurosci Abstr* **27**, 309.302.
- McNeely W & Goa KL (1998). Sibutramine. A review of its contribution to the management of obesity. *Drugs* **56**, 1093–1124.
- Nebigil CG, Choi DS, Dierich A, Hickel P, Le Meur M, Messaddeq N, Launay JM & Maroteaux L (2000). Serotonin 2B receptor is required for heart development. *Proc Natl Acad Sci U S A* **97**, 9508–9513.
- Nielsen JA, Chapin DS, Johnson JL Jr & Torgersen LK (1992). Sertraline, a serotonin-uptake inhibitor, reduces food intake and body weight in lean rats and genetically obese mice. *Am J Clin Nutr* **55**, 185S–189S.
- Nilsson BM (2006). 5-Hydroxytryptamine 2C (5-HT_{2C}) receptor agonists as potential antiobesity agents. *J Med Chem* **49**, 4023–4034.
- Nonogaki K, Abdallah L, Goulding EH, Bonasera SJ & Tecott LH (2003). Hyperactivity and reduced energy cost of physical activity in serotonin 5-HT_{2C} receptor mutant mice. *Diabetes* **52**, 315–320.
- Nonogaki K, Nozue K, Takahashi Y, Yamashita N, Hiraoka S, Kumano H, Kuboki T & Oka Y (2007). Fluvoxamine, a selective serotonin reuptake inhibitor, and 5-HT_{2C} receptor inactivation induce appetite-suppressing effects in mice via 5-HT_{1B} receptors. *Int J Neuropsychopharmacol* **10**, 675–681.
- Parks CL, Robinson PS, Sibille E, Shenk T & Toth M (1998). Increased anxiety of mice lacking the serotonin_{1A} receptor. *Proc Natl Acad Sci U S A* **95**, 10734–10739.
- Perez-Garcia G & Meneses A (2005). Oral administration of the 5-HT₆ receptor antagonists SB-357134 and SB-399885 improves memory formation in an autoshaping learning task. *Pharmacol Biochem Behav* **81**, 673–682.
- Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D & Hen R (1998). Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* **95**, 14476–14481.
- Rosenzweig-Lipson S, Zhang J, Mazandarani H, Harrison BL, Sabb A, Sabalski J, Stack G, Welmaker G, Barrett JE & Dunlop J (2006). Antiobesity-like effects of the 5-HT_{2C} receptor agonist WAY-161503. *Brain Res* **1073–1074**, 240–251.
- Rouru J, Pesonen U, Isaksson K, Huupponen R & Koulu M (1993). Effect of chronic treatment with TFMPP, a 5-HT₁ receptor agonist, on food intake, weight gain, plasma insulin and neuropeptide mRNA expression in obese Zucker rats. *Eur J Pharmacol* **234**, 191–198.
- Sabb AL, Vogel RL, Welmaker GS, Sabalski JE, Coupet J, Dunlop J, Rosenzweig-Lipson S & Harrison B (2004). Cycloalkyl[b][1,4]benzodiazepinoindoles are agonists at the human 5-HT_{2C} receptor. *Bioorg Med Chem Lett* **14**, 2603–2607.
- Saller CF & Stricker EM (1976). Hyperphagia and increased growth in rats after intraventricular injection of 5,7-dihydroxytryptamine. *Science* **192**, 385–387.
- Schreiber R & De Vry J (2002). Role of 5-HT_{2C} receptors in the hypophagic effect of m-CPP, ORG 37684 and CP-94,253 in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* **26**, 441–449.
- Schuhler S, Clark A, Joseph W, Patel A, Lehnen K, Stratford E, Horan TL, Fone KC & Ebling FJ (2005). Involvement of 5-HT receptors in the regulation of food intake in Siberian hamsters. *J Neuroendocrinol* **17**, 276–285.
- Shacham S, Heal DJ, Cheetham SC, Jackson HC, Melendez R, Rutkowski JV, Orbach P & Gannon KS (2006). PRX-07034, a potent and selective 5-HT₆ receptor antagonist, reduces food intake and body weight in dietary-induced obese (DIO) rats. *2006 Abstract Viewer/Itinerary Planner*, Program No. 62.10. Society for Neuroscience, Washington, DC.
- Simansky KJ & Nicklous DM (2002). Parabrachial infusion of D-fenfluramine reduces food intake. Blockade by the 5-HT_{1B} antagonist SB-216641. *Pharmacol Biochem Behav* **71**, 681–690.

- Simansky KJ & Vaidya AH (1990). Behavioral mechanisms for the anorectic action of the serotonin (5-HT) uptake inhibitor sertraline in rats: comparison with directly acting 5-HT agonists. *Brain Res Bull* **25**, 953–960.
- Smith BM, Thomsen WJ & Grottick AJ (2006). The potential use of selective 5-HT_{2C} agonists in treating obesity. *Expert Opin Invest Drugs* **15**, 257–266.
- Somerville EM, Horwood JM, Lee MD, Kennett GA & Clifton PG (2007). 5-HT_{2C} receptor activation inhibits appetitive and consummatory components of feeding and increases brain c-fos immunoreactivity in mice. *Eur J Neurosci* **25**, 3115–3124.
- Svartengren J, Axelsson-Lendin P, Edling N, Fhølenhag K, Isacson R, Hillegaard V & Grönberg A (2004). The selective serotonin 5-HT₆ receptor antagonist BVT-5182 decreases food intake and body weight in both rats and mice. *2004 Abstract Viewer/Itinerary Planner*, Program No. 75.8. Society for Neuroscience, Washington, DC.
- Svartengren J, Fhølenhag K, Modiri A, Axelsson-Lendin P, Edling N, Klingström G, Larsson C & Sakariassen KS (2003a). Preclinical in vivo pharmacology of BVT-933, a selective 5-HT_{2c} receptor agonist. *2003 Abstract Viewer/Itinerary Planner*, Program No. 509.9. Society for Neuroscience, Washington, DC.
- Svartengren J, Ohman B, Edling N, Svensson M, Fhølenhag K, Axelsson-Lendin P, Klingström G & Larsson C (2003b). The Serotonin 5-HT₆ receptor antagonist BVT.5182 reduces body weight of high fat diet-induced mice. *Int J Obesity* **27**, T1: P1–P094.
- Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF & Julius D (1995). Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. *Nature* **374**, 542–546.
- Thomsen WJ, Grottick AJ, Menzaghi F, Reyes-Saldana H, Espitia S, Yuskin D, Whelan K, Martin M, Morgan M, Chen W, Al-Shamma H, Smith B, Chalmers D & Behan D (2008). Lorcaserin, a novel selective human 5-hydroxytryptamine_{2C} agonist: in vitro and in vivo pharmacological characterization. *J Pharmacol Exp Ther* **325**, 577–587.
- Vickers SP, Benwell KR, Porter RH, Bickerdike MJ, Kennett GA & Dourish CT (2000). Comparative effects of continuous infusion of mCPP, Ro 60-0175 and d-fenfluramine on food intake, water intake, body weight and locomotor activity in rats. *Br J Pharmacol* **130**, 1305–1314.
- Vickers SP, Clifton PG, Dourish CT & Tecott LH (1999). Reduced satiating effect of d-fenfluramine in serotonin 5-HT_{2C} receptor mutant mice. *Psychopharmacology (Berl)* **143**, 309–314.
- Vickers SP, Dourish CT & Kennett GA (2001). Evidence that hypophagia induced by d-fenfluramine and d-norfenfluramine in the rat is mediated by 5-HT_{2C} receptors. *Neuropharmacology* **41**, 200–209.
- Vickers SP, Easton N, Webster LJ, Wyatt A, Bickerdike MJ, Dourish CT & Kennett GA (2003). Oral administration of the 5-HT_{2C} receptor agonist, mCPP, reduces body weight gain in rats over 28 days as a result of maintained hypophagia. *Psychopharmacology (Berl)* **167**, 274–280.
- Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, Sibille E, Underwood M, Itohara S, Dauer WT, Ansorge MS, Morelli E, Mann JJ, Toth M, Aghajanian G, Sealfon SC, Hen R & Gingrich JA (2006). Cortical 5-HT_{2A} receptor signaling modulates anxiety-like behaviors in mice. *Science* **313**, 536–540.
- Woolley ML, Bentley JC, Sleight AJ, Marsden CA & Fone KC (2001). A role for 5-HT₆ receptors in retention of spatial learning in the Morris water maze. *Neuropharmacology* **41**, 210–219.
- Yen TT, Wong DT & Bemis KG (1987). Reduction of food-consumption and body-weight of normal and obese mice by chronic treatment with fluoxetine – a serotonin reuptake inhibitor. *Drug Dev Res* **10**, 37–45.

Acknowledgements

This work was supported by the NIDDK DK065171 and the Wellcome Trust (L.K.H).