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Review

Role of central serotonin and melanocortin systems in the control of energy balance

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ABSTRACT

Body weight homeostasis is critically dependent upon the convergence and integration of multiple central and peripheral signalling systems that collectively function to detect and elicit physiological and behavioural responses to nutritional state. To date, only a minority of these signals have been pharmacologically targeted for the treatment of human obesity. One signal that has been effectively manipulated to reduce body weight is the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT); however, the relevant downstream signalling pathways are incompletely understood. Recently, the melanocortin system, a nexus for multiple modulators of energy balance, has emerged as one key mediator of serotonin's effects on appetite. Here we review the serotonin and melanocortin systems with reference to their roles in energy balance and discuss the evidence that the two systems are functionally linked.

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1. The obesity epidemic

It is estimated that 400 million people are currently obese worldwide, and, largely because of the intimate link between obesity and insulin resistance, this is projected to translate into an increase in rates of type 2 diabetes and atherosclerosis, among other complications. For this reason, concerted efforts are underway to develop novel, effective pharmacotherapies capable of reducing body weight. One class of drugs that has proved effective in clinical practice are compounds targeting the serotonergic (5-hydroxytryptamine; 5-HT) system. However, despite their therapeutic efficacy, some of the most promising of these anti-obesity compounds have been associated with side effects that have led to their withdrawal from clinical use. (±)-N-Ethyl-α-methyl-m-[trifluoromethyl]phenethylamine HCl (fenfluramine), a serotonin reuptake inhibitor and facilitator of serotonin release, was the first such example, becoming widely used before being withdrawn from the market in the late 1990s due to its implication in pulmonary hypertension and cardiac valvular fibrosis. More recently, a current treatment for obesity, the serotonin and norepinephrine reuptake inhibitor 1-(4-Chlorophenyl)-N,N-dimethyl-alpha-(2-methylpropyl)cyclobutanemethanamine HCl monohydrate (sibutramine) was suspended from use in Europe (although not in the United States) due to its association with increased rates of stroke and heart attack in patients with high cardiovascular risk. Nevertheless, the clinically effective weight loss promoting properties of these compounds suggest that the serotonergic system remains a viable target for the treatment of obesity. A major priority is to establish the downstream effector pathways whereby serotonin influences energy balance and to determine whether they are functionally distinct from those serotonergic pathways influencing cardiovascular function. Indeed, different serotonin receptors have been implicated in the therapeutic and cardiovascular side effects of serotonergic obesity compounds, supporting the possibility that these effects may be dissociated.

2. The serotonin system

Serotonin is a neurotransmitter synthesized in both peripheral tissues and the brain and is involved in multiple physiological and behavioural processes. For example, peripheral serotonin has been implicated in platelet aggregation, vascular tone, hypertension and intestinal motility, while brain serotonin has been associated with temperature regulation, sleep–wake rhythms, emesis, sexual behaviour, aggression, nociception, mood and energy balance, to name a few (Berger et al., 2009; Jonnakuty and Gragnoli, 2008; Lam et al., 2008; Mohammad-Zadeh et al., 2008; Tecott, 2007; Weiger, 1997). This review will focus on serotonin's role in energy homeostasis.

2.1. Serotonin synthesis and release

Serotonin is a phylogenetically conserved monoamine found in a wide range of species from nematodes to humans (Tecott, 2007). In higher animals, serotonin is synthesized from the diet-derived essential amino acid tryptophan. The initial and rate limiting step is the conversion of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase, of which there are two isoforms. Tryptophan hydroxylase 1 predominates in the periphery whereas tryptophan hydroxylase 2 is located in the brain (Walther and Bader, 2003). The second and final step is the conversion of 5-hydroxytryptophan to serotonin by aromatic l-amino acid decarboxylase. Following synthesis, serotonin is stored in synaptic vesicles and is released into the synaptic cleft by regulated exocytosis, whereupon it binds to pre- and/or post-synaptic serotonin receptors. Reuptake of extracellular serotonin by the presynaptic neuron may occur via membrane-bound serotonin transporters.

2.2. Serotonin receptors

At least eighteen serotonin receptor genes have been identified in vertebrates (Table 1), which, together with a host of splice variants, give rise to a wide repertoire of receptor isoforms (Lam and Heisler, 2007). Serotonin receptors are broadly grouped into seven families (termed 5-HT_1 receptors through 5-HT_7 receptors) based upon sequence homology and associated second messenger systems (Filip and Bader, 2009; Hoyer et al., 2002). The majority of serotonin receptors are G-protein coupled; however 5-HT_3 receptors are ligand-gated non-selective cation channels.

2.3. Central nervous system serotonin projections

The distribution of central serotonin-synthesizing cells is restricted to the raphe nuclei of the brainstem, specifically nine areas termed B1 to B9. Axonal projections from serotonin neurons are widespread,

Table 1
Serotonin receptor subtypes and their intracellular messenger systems. Serotonin receptors are grouped into seven families based on sequence homology and second messengers employed. The mechanisms utilized by 5-HT_5 receptors have not been definitively determined. AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; PLC, phospholipase C; IP3, inositol trisphosphate; DAG, diacylglycerol; GIRK, G-protein coupled inwardly rectifying potassium channel. An upwards arrow (↑) represents activation or upregulation and a down arrow (↓) represents inhibition or downregulation.

Serotonin receptor subtype	Intracellular signalling system
5-HT_{1A}	G_i-coupled ↓ AC–↓ cAMP
5-HT_{1B}	G_i-coupled ↓ AC–↓ cAMP
5-HT_{1D}	G_i-coupled ↓ AC–↓ cAMP
5-HT_{1E}	G_i-coupled ↓ AC–↓ cAMP
5-HT_{1F}	G_i-coupled ↓ AC–↓ cAMP
5-HT_{2A}	G_q-coupled ↑ PLC–↑ IP3 + DAG
5-HT_{2B}	G_q-coupled ↑ PLC–↑ IP3 + DAG
5-HT_{2C}	G_q-coupled ↑ PLC–↑ IP3 + DAG
5-HT_{3A}	Ligand gated cation channel
5-HT_{3B}	Ligand gated cation channel
5-HT_{3C}	Ligand gated cation channel
5-HT_{3D}	Ligand gated cation channel
5-HT_{3E}	Ligand gated cation channel
5-HT_4	G_s-coupled ↑ AC–↑ cAMP
5-HT_{5A}	G_i/G_o-coupled (?) ↓ AC–↓cAMP/GIRK
5-HT_{5B}	G_i/G_o-coupled (?) ↓ AC–↓cAMP/GIRK
5-HT_6	G_s-coupled ↑AC–↑ cAMP
5-HT_7	G_s-coupled ↑AC–↑ cAMP

ramifying to most regions of the central nervous system. The majority of forebrain, thalamic, hypothalamic, striatal and cortical projections as well as some cerebellar and brainstem projections originate from groups B5–B9, which are the more rostrally-positioned raphe nuclei. In contrast, groups B1–B4 which are located more caudally, project primarily to the spinal cord, medulla, pons, midbrain and cerebellum.

3. Serotonin and energy balance

Anatomical, pharmacological and genetic evidence strongly supports an intimate relationship between the serotonergic system and the control of energy balance, consistent with the effectiveness of serotonin-based drugs at inducing weight loss. However, the underlying mechanism through which serotonin influences energy balance is only partly understood.

3.1. Peripheral serotonin and energy balance

As ingested food moves through the digestive tract, several stimuli elicit the synthesis and release of serotonin from intestinal enterochromaffin cells. These stimuli include stomach distension and increased luminal pressure as well as the absorption of sugars and fatty acids (Fujimiya et al., 1997; Fukumoto et al., 2003; Li et al., 2001; Mazda et al., 2004). Peripheral exogenous serotonin administration reduces the size and duration of meals and advances the onset of satiety (Edwards and Stevens, 1991). Furthermore, oxygen consumption is increased following peripheral injections of serotonin or the serotonin releaser and reuptake inhibitor fenfluramine, suggesting an increase in metabolic rate and overall increase in energy expenditure (Le Feuvre et al., 1991; Rothwell and Stock, 1987).

Because of the poor blood brain barrier penetration of peripherally derived serotonin, it is unlikely that peripheral serotonin has significant direct central effects. However central effects could be indirectly mediated via activation of ascending vagal afferent fibres expressing 5-HT_3 receptors (Mazda et al., 2004). Supporting this possibility, intestinal serotonin released following gastric distension elicits neuronal activation in the nucleus of the solitary tract and the paraventricular nucleus of the hypothalamus, two brain structures with key roles in energy balance. However, this stimulus does not activate these structures in vagotomised animals (Mazda et al., 2004).

3.2. Brain serotonin and energy balance

The requirement for brain serotonin to maintain energy balance was demonstrated more than 30 years ago. Global depletion of central serotonin following intracerebroventricular administration of either the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Breisch et al., 1976) or the serotonergic neurotoxin 5,7-dihydroxytryptamine (Saller and Stricker, 1976) was shown to result in hyperphagia and body weight gain. Conversely, when serotonergic signalling is increased, for example through the administration of serotonin, (+)-N-Ethyl-α-methyl-*m*-[trifluoromethyl]phenethylamine hydrochloride (dexfenfluramine), sibutramine, (1S,4S)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine HCl (sertraline) or (±)-N-Methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine HCl (fluoxetine), food intake is suppressed, leading to reductions in body weight (Guy-Grand, 1995; Heisler et al., 1997; Jackson et al., 1997; Simansky and Vaidya, 1990). These reports illustrate the well established inverse relationship between food consumption and serotonin levels.

3.3. Genetic disruption of serotonergic genes and energy balance

3.3.1. Disruption of serotonin synthesis or transport

Serotonin transporter over-expressing mice are lighter and shorter than wild type littermates (Pringle et al., 2008) while serotonin transporter knockout mice develop late-onset obesity without hyperphagia (Murphy and Lesch, 2008). Mice lacking tryptophan hydroxylase exhibit growth retardation and physiological dysfunction (Alenina et al., 2009; Savelieva et al., 2008; Yadav et al., 2009) demonstrating the importance of appropriate serotonergic signalling in normal development.

3.3.2. 5-HT_{2C} receptor

Mice lacking functional 5-HT_{2C} receptors exhibit hyperphagia and develop late-onset obesity (Nonogaki et al., 1998; Tecott et al., 1995). Further analysis reveals that the increase in food intake in 5-HT_{2C} receptor null mice is accounted for by an elevation in meal size, not meal frequency and that the changes in body weight are due to an elevation in fat mass and a reduction in lean mass (Xu et al., 2008). Secondary to the development of obesity, 5-HT_{2C} receptor knockout mice display insulin resistance and impaired glucose tolerance (Nonogaki et al., 1998). Moreover, 5-HT_{2C} receptor deficient mice demonstrate an attenuated response to dexfenfluramine (Vickers et al., 1999), implicating this receptor subtype specifically in mediating at least some of serotonin's effects on energy homeostasis.

3.3.3. 5-HT_{1B} receptor

Mice lacking functional 5-HT_{1B} receptors display modest elevations in food intake and body weight; however they do not exhibit obesity (Bouwknecht et al., 2001). These mice also demonstrate an attenuated response to dexfenfluramine (Lucas et al., 1998) suggesting that, like 5-HT_{2C} receptors, these receptors are important in mediating serotonin's effects on energy homeostasis.

3.3.4. 5-HT_6 receptor

While some report that antisense knockdown of 5-HT_6 receptors in rats is associated with decreased food intake and attenuated body weight gain (Woolley et al., 2001), others do not (Bourson et al., 1995; Hamon et al., 1999; Yoshioka et al., 1998). In mice, genetic inactivation of the 5-HT_6 receptor is not associated with a lean phenotype on a chow diet (Bonasera et al., 2006), but is protective against dietary-induced obesity on a high fat diet (Frassetto et al., 2008). Despite similarities in the sequence homology of the 5-HT_6 receptor in the rat and mouse, differences in the brain distribution and predicted binding pocket of 5-HT_6 receptors exist (Hirst et al., 2003). The rat profile is more consistent with the human profile and thereby may represent a better model of 5-HT_6 receptor function in humans. Further characterization of the role of 5-HT_6 receptors in the serotonergic modulation of energy balance is needed.

3.3.5. Other 5-HT receptors

Disruption of other serotonin receptor subtypes does not appear to significantly perturb body weight (Garfield and Heisler, 2009; Lam and Heisler, 2007), supporting the notion that 5-HT_{2C} and 5-HT_{1B} receptors, possibly along with 5-HT_6 receptors, are the prime mediators of serotonin's anorectic action and its negative influence on energy balance.

3.4. Serotonin projections with relevance to energy balance

Serotonin produced in the raphe nuclei is transported to and released at numerous brain loci implicated in the control of energy balance. These key loci include the arcuate, paraventricular and lateral nuclei of the hypothalamus, the nucleus of the solitary tract, and the parabrachial nucleus (Botchkina and Morin, 1993; Kiss et al., 1984; Petrov et al., 1992; Steinbusch, 1981; Steinbusch and Nieuwenhuys, 1981). It is possible that serotonin affects energy balance through action at any or all of these sites. For example, injection of serotonin or dexfenfluramine directly into the paraventricular nucleus of the hypothalamus of rats decreases feeding (Shor-Posner et al., 1986), with dexfenfluramine also reported to suppress carbohydrate intake when injected into the ventromedial and dorsomedial hypothalamic nuclei (Weiss et al., 1990).

Moreover, injection of 5-HT_{1B} receptor agonists into the paraventricular nucleus of the hypothalamus (Lee et al., 1998; Macor et al., 1990) and parabrachial nucleus (Lee et al., 1998) of rats reduces food intake.

However, a nucleus with an established role in energy balance, the arcuate nucleus of the hypothalamus, appears to be a key site of serotonin's action on appetite. Approximately 90% of arcuate nucleus of the hypothalamus neurons are sensitive to serotonin *in vitro*, although the observed responses suggest both direct and indirect effects (Kang et al., 2004). More significantly, in a mouse model lacking functional 5-HT_{2C} receptors, the observed phenotype of hyperphagia, hyperactivity, obesity and attenuated responses to anorectic doses of the serotonergic drugs *m*-chlorophenylpiperazine (mCPP) and dexfenfluramine, can be normalized by re-expression of 5-HT_{2C} receptors solely on pro-opiomelanocortin neurons, a cell type found exclusively in the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract in adult animals (Xu et al., 2008). Because pro-opiomelanocortin expressing neurons represent a vital component of the melanocortin signalling pathway, these data also strongly suggest that serotonin may exert its actions on energy balance via recruitment of downstream melanocortin pathways.

3.5. Serotonergic signalling and energy expenditure

It is important to note that serotonin affects not only energy intake but also energy expenditure, evidenced, for example, by the increase in oxygen consumption induced by peripheral administration of serotonin or fenfluramine (Le Feuvre et al., 1991; Rothwell and Stock, 1987). However, the relationship between serotonin and energy expenditure is complex and incompletely understood: while serotonin and various serotonin receptor agonists influence locomotor activity and thermogenesis, the direction and magnitude of these effects depend on the agonist selected, its dose, and/or the site of administration.

3.5.1. Serotonergic effects on body temperature

Central or peripheral administration of serotonin decreases body temperature in mice (Hedlund et al., 2003; Yamada et al., 1988), with agonists of 5-HT_{1A} receptors and 5-HT_7 receptors having similar effects (Hedlund et al., 2003; Heisler et al., 1998). In contrast, discrete injections of serotonin into the paraventricular or ventromedial hypothalamic nuclei are reported to activate sympathetic fibres innervating brown adipose tissue (Sakaguchi and Bray, 1989) and serotonin immunoreactive synapses have been identified within the intermediolateral nucleus of the spinal cord that may indicate connection with sympathetic preganglionic neurons (Coote, 1990; Poulat et al., 1992) (Fig. 1). Fenfluramine is also reported to activate sympathetic fibres innervating brown adipose tissue (Arase et al., 1988).

3.5.2. Serotonergic effects on locomotion

Serotonergic agonists can also influence locomotor activity, although the direction of observed effects appears to be dependent on the receptor subtype activated. The 5-$HT_{2C/1B}$ receptor agonist mCPP decreases activity (Heisler and Tecott, 2000; Kennett and Curzon, 1988), whereas the 5-$HT_{1A/1B}$ receptor agonist 5-Methoxy-3-(1,2,3,5,6-tetrahydro-4-pyridinyl)-1 H-indole (RU24969) increases it (Tricklebank et al., 1986). Further, pharmacological and genetic evidence suggests that serotonin action at the G_q-coupled 5-HT_{2C} receptor reduces locomotor activity, whereas action at the G_i-coupled 5-HT_{1B} receptor increases activity (Heisler and Tecott, 2000; Kennett and Curzon, 1988; Xu et al., 2008). Though the effects of 5-HT_6 receptor compounds on locomotor activity have not been thoroughly characterized, one study investigating this parameter reported that 5-HT_6 receptor knockdown did not influence locomotor behaviour (Woolley et al., 2001).

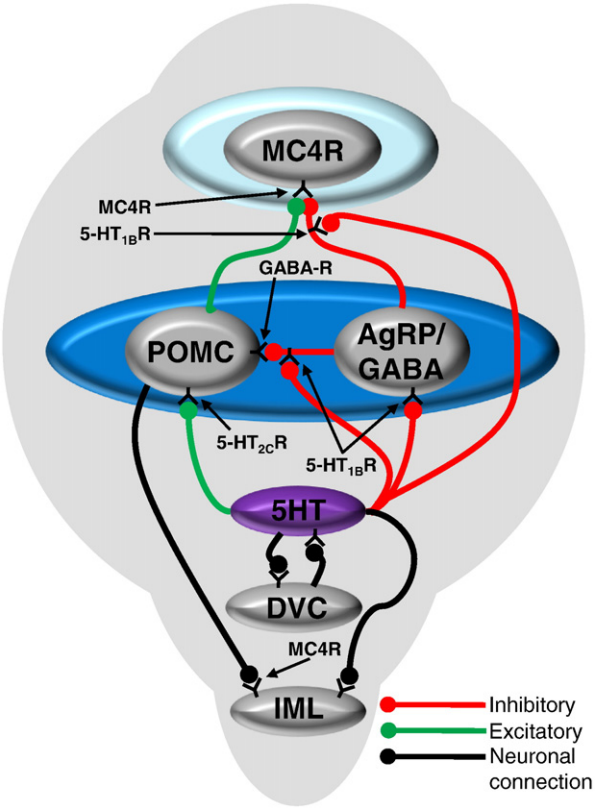

Fig. 1. Proposed neuronal connections linking the serotonin and melanocortin systems. Serotonergic (5-HT) projections from the raphe nuclei (purple) activate pro-opiomelanocortin (POMC) and inhibit agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (dark blue) via action at 5-HT_{2C} and 5-HT_{1B} receptors respectively. Activation of 5-HT_{1B} heteroreceptors by serotonin inhibits both GABAergic tone at pro-opiomelanocortin neurons and agouti-related peptide tone at melanocortin4 receptor expressing neurons (MC4R). The combined effect of serotonin action at these sites facilitates excitatory actions of pro-opiomelanocortin derived ligands at target melanocortin4 receptors (light blue). Serotonin and pro-opiomelanocortin neurons also project to sympathetic preganglionic neurons located in the intermediolateral nucleus (IML) of the spinal cord and serotonin neurons send and receive inputs to/from the dorsal vagal complex (DVC).

Taken together, these data support a role for serotonergic influence on both sides of the energy balance equilibrium. However, it is clear that further delineation of the precise mechanisms mediating these effects of serotonin, in particular on energy expenditure, is necessary. More refined pharmacological and genetic tools will facilitate the further studies required.

4. The melanocortin system

The melanocortin system is phylogenetically well conserved, having been identified in invertebrates, fish, and amphibians as well as in mammals (Hadley and Haskell-Luevano, 1999; Salzet et al., 1997; Takahashi and Kawauchi, 2006). In mammals, components of the system exist in both the brain and the periphery and are involved in a diverse range of physiological processes including pigmentation, sexual function, anxiety, renal function, circadian rhythms and anticipatory behaviour, inflammation and energy homeostasis (Begriche et al., 2009; Bertolini et al., 2009; Chaki and Okuyama, 2005; Cone, 2005; Humphreys, 2007; Maaser et al., 2006). Moreover, genetic evidence demonstrating that disruption of the melanocortin system induces hyperphagia and early onset obesity in humans (Farooqi and O'Rahilly, 2008) identifies the melanocortin system as a critical component of energy homeostasis, and consequently an

attractive target for weight loss pharmacotherapy. In contrast to the serotonin system, which has been targeted by several weight loss drugs, no weight loss therapies based on manipulation of melanocortin signalling are yet used for the treatment of human obesity. However, drug discovery efforts have been made in this direction. Indeed, more than fifteen patent applications for melanocortin4 receptor ligands demonstrating anti-obesity properties were filed in the US in 2008 alone (Garfield et al., 2009).

4.1. Endogenous melanocortin ligands

The physiological functions of the melanocortin network are defined via the counter-regulatory actions of both endogenously produced melanocortin receptor agonists (the pro-opiomelanocortin derived ligands) and antagonists (agouti and agouti-related peptide). Alpha-, beta- and gamma-melanocyte-stimulating hormone and adrenocorticotrophic hormone are all cleaved from the common precursor pro-opiomelanocortin and act as agonists at the five G-protein coupled melanocortin receptors (termed the melanocortin1, 2, 3, 4 and 5 receptors). Postnatally, brain pro-opiomelanocortin synthesizing neurons are located exclusively within the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract. Within the arcuate nucleus of the hypothalamus, pro-opiomelanocortin has been reported to co-localize with cocaine-and-amphetamine-regulated transcript, choline acetyl transferase and dynorphin (Elias et al., 1998; Maolood and Meister, 2008; Meister et al., 2006). In contrast, pro-opiomelanocortin cells in the nucleus of the solitary tract do not co-express any of these substances (Ellacott et al., 2006; Maolood and Meister, 2008; Meister et al., 2006).

The melanocortin receptor antagonist/inverse agonist agouti is expressed peripherally (Bultman et al., 1992), whereas agouti-related peptide demonstrates a predominantly central expression profile (Ollmann et al., 1997). Concordant with central pro-opiomelanocortin expression, agouti-related peptide is also synthesized within cells of the hypothalamic arcuate nucleus. However, these cells are completely distinct from those synthesizing pro-opiomelanocortin and are known to co-express the orexigenic peptide neuropeptide-Y (Broberger et al., 1998; Hahn et al., 1998) and the inhibitory neurotransmitter gamma-aminobutyric acid (GABA).

4.2. Melanocortin receptors

Five melanocortin receptors have been identified and all belong to the G-protein-coupled superfamily. These receptors share a common mechanism in that they each couple to G_s. The melanocortin1, 2, and 5 receptors are expressed in the periphery, whereas the melanocortin3 and 4 receptors demonstrate a predominantly central distribution (Ellacott and Cone, 2006).

4.3. Central melanocortin projections

Although melanocortin receptors and their ligands are present in the periphery and peripheral signals influence central melanocortin signalling, this review will focus on the actions of centrally located pro-opiomelanocortin-derived agonists and agouti-related peptide. Despite the restricted distribution of pro-opiomelanocortin and agouti-related peptide synthesizing neurons within the central nervous system, axonal projections of these neurons are widespread, as would be predicted based on the melanocortin receptor distributions (Kishi et al., 2003; Liu et al., 2003; Mountjoy et al., 1994; Roselli-Rehfuss et al., 1993). Multiple brain regions including the hypothalamus, thalamus, septum, amygdala and brainstem receive innervations from pro-opiomelanocortin neurons (Jacobowitz and O'Donohue, 1978). Compared to projections arising from pro-opiomelanocortin neurons, projections containing agouti-related peptide demonstrate a more restricted distribution, both in terms of the number of nuclei innervated and the density of those innervations (Haskell-Luevano et al., 1999).

5. Melanocortins and energy balance

Few pathways have been demonstrated to be as critical to energy homeostasis as the melanocortin system. The central melanocortin pathway appears to be a key conduit through which many energy balance-related neurotransmitters and peptides signal (Garfield et al., 2009). Therefore, pharmacological manipulation of this pathway for the treatment of obesity has garnered a great deal of pharmaceutical interest.

5.1. Brain melanocortin pathway and energy balance

Located as they are in the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract, it is unsurprising that neurons synthesizing melanocortin receptor ligands are influenced not only by numerous central factors but also by a broad range of peripheral hormones and nutrients. The arcuate nucleus of the hypothalamus is positioned adjacent to both the third ventricle and the median eminence and may itself be able to function as a circumventricular organ (Cone, 2005). Similarly, the nucleus of the solitary tract is located adjacent to the area postrema (another circumventricular organ) within the dorsal vagal complex, a site involved in the integration of peripheral gut signals via vagal afferent fibres.

The vast array of upstream factors influencing melanocortin signalling include peripherally derived leptin, cholecystokinin, ghrelin, peptide YY and insulin, as well as centrally derived melanin-concentrating hormone, pituitary adenylate cyclase-activating peptide, neuropeptide Y, the orexins/hypocretins and serotonin (Garfield et al., 2009). In addition, glucose and fatty acids are also reported to modulate melanocortin signalling (Lopez et al., 2005; Marty et al., 2007).

5.2. Genetic disruption of melanocortin genes and energy balance

5.2.1. Pro-opiomelanocortin deficiency

Several melanocortin receptor ligands are cleaved from the common precursor pro-opiomelanocortin. Mice deficient in pro-opiomelanocortin are obese and hyperphagic, particularly when fed a high fat diet (Challis et al., 2004; Tung et al., 2007; Yaswen et al., 1999). Similarly, humans with mutations that lead to deficiency in pro-opiomelanocortin-derived products are hyperphagic and obese (Coll et al., 2004; Farooqi et al., 2006; Krude et al., 1998), though the number of identified human cases is extremely low (Farooqi and O'Rahilly, 2008).

5.2.2. Agouti mutation and agouti-related peptide deficiency

Agouti is a peripherally expressed melanocortin receptor antagonist and the single genetic exponent of the A^y mouse phenotype. As a consequence of the ectopic overexpression of agouti in all tissues (including the brain) these spontaneous heterozygous mutants exhibit hyperphagia, obesity and hyperinsulinaemia (Klebig et al., 1995; Kublaoui et al., 2006), which is consistent with antagonism of central melanocortin receptors. It is now understood that within a physiological context, these central melanocortinergic pathways are regulated by an analogous centrally expressed melanocortin antagonist; a theory supported by the subsequent cloning of agouti-related peptide, an endogenous antagonist/inverse agonist of central melanocortin3 and 4 receptors (Fong et al., 1997; Haskell-Luevano and Monck, 2001; Nijenhuis et al., 2001; Ollmann et al., 1997; Shutter et al., 1997).

Further studies involving the constitutive overexpression of agouti-related peptide confirmed its role in the central regulation of energy homeostasis (Graham et al., 1997; Ollmann et al., 1997). Interestingly however, mice with a targeted germline deletion of the

agouti-related peptide gene did not exhibit the expected hypophagic and lean phenotype (Qian et al., 2002). More recent observations in a separate genetic line now indicate that late onset reductions in body weight and adiposity may occur, although in the absence of hypophagia (Wortley et al., 2005). That these animals are also reported to have higher volumetric oxygen consumption, hyperlocomotion and increased core body temperature suggests that increased energy expenditure may account for the reductions in body weight and adiposity (Wortley et al., 2005).

Unlike the germline agouti-related peptide knockout mouse, postnatal destruction of agouti-related peptide synthesizing neurons via targeted toxins elicits a severely hypophagic or aphagic phenotype with associated reductions in body weight and adiposity that can lead to starvation and death (Bewick et al., 2005; Gropp et al., 2005; Luquet et al., 2005). Behavioural analyses indicate that the phenotype of agouti-related peptide neuron ablated mice appears to be due to a combined dysregulation of both the initiation of feeding and the size of the meal consumed (Wu et al., 2008). Of note, destruction of agouti-related peptide neurons thereby eliminates all other co-expressed factors, such as neuropeptide-Y and GABA. Likewise, this removes the inhibitory GABAergic tone arising from agouti-related peptide neurons onto local pro-opiomelanocortin neurons (Wu et al., 2008), which presumably promotes additional signalling of pro-opiomelanocortin-derived factors at target sites. Indeed, increased c-FOS mRNA expression in pro-opiomelanocortin and agouti-related peptide target sites such as the paraventricular hypothalamic nucleus and the nucleus of the solitary tract has been reported following agouti-related peptide neuronal ablation (Wu et al., 2008).

5.2.3. Melanocortin4 receptor deficiency

Melanocortin4 receptor null mice display several phenotypes indicating an inability to regulate energy homeostasis appropriately. These include hyperphagia, hypolocomotion and hyperinsulinaemia, as well as significant weight gain apparent from around 5 weeks of age compared to wild type animals (Balthasar et al., 2005; Chen et al., 2000b; Huszar et al., 1997). When fed a high fat diet, these animals do not moderate their consumption to reflect the higher caloric value, rather their energy intake increases further, exacerbating body weight gain (Butler et al., 2001; Sutton et al., 2006). Recent clinical data support these findings and demonstrate the relevance of prior murine observations to human physiology. Most notably, it has been reported that up to 6% of obese patients, particularly those with severe early onset obesity, harbour mutations in the melanocortin4 receptor gene (Farooqi et al., 2003; Larsen et al., 2005; Vaisse et al., 2000). This may represent the single greatest monogenic cause of obesity identified to date. However, not all clinical studies examining melanocortin4 receptor mutations report similar data. For example, the prevalence of melanocortin4 receptor mutations was no higher in obese individuals compared to non-obese controls in a Japanese sample (Ohshiro et al., 1999), and pathogenic mutations of the melanocortin4 receptor gene had a prevalence of less than 1% in a Swedish cohort (Jacobson et al., 2002). It is possible therefore that the relationship between melanocortin4 receptor mutation and obesity may have a component linked to ethnicity. Alternatively, the differences observed may be due to the large diversity in identified mutations (Farooqi et al., 2003).

5.2.4. Melanocortin3 receptor deficiency

Melanocortin3 receptor null mice also display a phenotype of disrupted energy homeostasis. While melanocortin3 receptor deficient mice do not exhibit marked alterations in food intake or body weight like melanocortin4 receptor knockout mice, they do exhibit substantially increased fat mass and reduced lean mass (Butler et al., 2000; Chen et al., 2000a). Compared with melanocortin4 receptor null mice, melanocortin3 receptor nulls also display reduced insulin resistance associated with diet-induced obesity (Sutton et al., 2006). While the mechanism through which the melanocortin3 receptor influences energy homeostasis is incompletely understood, it is likely that it will be distinct from that associated with the melanocortin4 receptor given that mice lacking both melanocortin3 and 4 receptors display an additive obesity phenotype compared to mice with single receptor mutations (Chen et al., 2000a).

5.3. Melanocortin projections with relevance to energy balance

The melanocortin3 and 4 receptors have a widespread distribution pattern that includes several brain regions implicated in the control of energy homeostasis (Kishi et al., 2003; Mountjoy et al., 1994; Roselli-Rehfuss et al., 1993). However, one region that has received particular attention for the melanocortin4 receptor is the paraventricular nucleus of the hypothalamus. This structure expresses melanocortin4 receptors and receives innervations containing alpha-melanocyte stimulating hormone and agouti-related peptide (Bagnol et al., 1999; Cowley et al., 1999; Mountjoy et al., 1994). Discrete injection of melanocortin receptor agonists into the paraventricular nucleus of the hypothalamus inhibits feeding behaviour while antagonist infusion increases food intake (Giraudo et al., 1998; Kask and Schioth, 2000; Wirth et al., 2001). Complementing these pharmacological studies, an elegant study by Balthasar et al. (2005) demonstrated that re-expression of melanocortin4 receptors in SIM1 neurons (found in the paraventricular nucleus of the hypothalamus and other regions) normalizes the hyperphagic phenotype, re-establishes responsiveness to the melanocortin3/4 receptor agonist acetyl-(Nle^4,Asp^5,D-Phe^7, Lys^{10})-cyclo-α-MSH (4-10) amide (melanotan II) and reduces obesity levels to around 40% of the levels seen in the melanocortin4 receptor deficient animal (Balthasar et al., 2005). Interestingly, the authors report that energy expenditure was unaffected by SIM1 melanocortin4 receptor re-expression (Balthasar et al., 2005). This suggests that the melanocortin system may primarily influence energy expenditure via other chemically defined neurons. However, earlier studies have reported that direct injection of melanocortin ligands into the paraventricular nucleus of the hypothalamus does affect both energy intake and expenditure (Cowley et al., 1999; Giraudo et al., 1998).

5.4. Melanocortin signalling and energy expenditure

The melanocortin system is well positioned to affect energy expenditure via modulation of the autonomic nervous system. For example, melanocortin4 receptors are abundantly expressed in the dorsal motor nucleus of the vagus, where they are co-expressed with choline acetyltransferase, indicating that they are positioned to influence parasympathetic preganglionic neuron activity (Kishi et al., 2003). Sympathetic preganglionic neurons in the intermediolateral nucleus of the spinal cord also express melanocortin4 receptors and receive direct innervations from arcuate nucleus of the hypothalamus pro-opiomelanocortin neurons (Elias et al., 1998; Kishi et al., 2003) (Fig. 1). Moreover, given the distribution of melanocortin4 receptors, there are multiple candidate routes by which melanocortin signalling might affect energy homeostasis.

6. Interaction of the serotonin and melanocortin systems influencing energy state

Several lines of evidence indicate that the melanocortin signalling system is an important downstream mediator of serotonin's negative action on energy balance. Serotonergic terminals make synaptic contacts with arcuate nucleus of the hypothalamus pro-opiomelanocortin and agouti-related peptide neurons (Heisler et al., 2006; Kiss et al., 1984), indicating that the serotonin system is anatomically positioned to influence melanocortin neuron activity (Fig. 1). This may be achieved via action at G_q-coupled 5-HT_{2C} receptors, which are co-expressed with pro-opiomelanocortin neurons and G_i-coupled

5-HT_{1B} receptors, which are co-expressed with agouti-related peptide neurons (Heisler et al., 2002, 2006; Lam et al., 2008).

Both *in vivo* and *in vitro* pharmacological studies and genetic evidence provide support for the functional importance of 5-HT_{2C} receptors co-expressed with pro-opiomelanocortin neurons in serotonin's effects on energy balance. 5-HT_{2C} receptor agonists influence both pro-opiomelanocortin gene expression and activity. For example, one week subcutaneous infusion of the 5-HT_{2C} receptor agonist BVT.X increased arcuate nucleus of the hypothalamus pro-opiomelanocortin mRNA expression in a dose-related manner (Lam et al., 2008). Acute treatment with anorectic concentrations of dexfenfluramine or mCPP dose-dependently increased c-FOS immunoreactivity, a marker of neuronal activation, within arcuate nucleus of the hypothalamus pro-opiomelanocortin neurons (Heisler et al., 2002). This effect on pro-opiomelanocortin gene expression and activity appears to be direct since *in vitro* applications of serotonin, dexfenfluramine, and 5-HT_{2C} receptor agonists mCPP and 6-chloro-2-(1-piperazinyl)pyrazine hydrochloride (MK212) increased action potential frequency of pro-opiomelanocortin neurons (Heisler et al., 2002). The functional significance of pro-opiomelanocortin neurons on serotonin's effects on energy balance was investigated in mice lacking 5-HT_{2C} receptors. In these animals, the energy balance phenotype was normalized by re-expression of 5-HT_{2C} receptors exclusively in pro-opiomelanocortin neurons (Xu et al., 2008). However, given that pro-opiomelanocortin is also developmentally expressed (Padilla et al., 2010), the site of pro-opiomelanocortin neurons underlying these effects remains to be determined. Together, these studies illustrate that serotonergic compounds influence pro-opiomelanocortin neuronal activity and that this modulation of pro-opiomelanocortin neurons is sufficient for serotonin, via G_q-coupled 5-HT_{2C} receptors, to impact energy balance.

Agouti-related peptide neurons also receive synaptic contacts from serotonin neurons (Fig. 1) and co-express G_i-coupled 5-HT_{1B} receptors (Heisler et al., 2006). Consistent with this anatomical localization, serotonin and 5-HT_{1B} receptor agonists influence the activity of agouti-related peptide neurons. Specifically, application of serotonin or the 5-HT_{1B} receptor agonist 5-Propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo [3,2-b]pyridine hydrochloride (CP94253) hyperpolarizes agouti-related peptide cells, an effect that can be blocked with a 5-HT_{1B} receptor antagonist. Interestingly, CP94253 and another selective 5-HT_{1B} receptor agonist 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrol[3,2-b]pyridin-5-one dihydrochloride (CP93129), also reduce the number of inhibitory inputs received by arcuate nucleus of the hypothalamus pro-opiomelanocortin neurons (Heisler et al., 2006). Collectively these data suggest that endogenous activation of 5-HT_{1B} receptors may increase melanocortinergic signalling in two ways. Firstly, serotonin, via action at post-synaptic 5-HT_{1B} receptors located on agouti-related peptide neurons, can decrease the likelihood of agouti-related peptide release. Secondly, via action at 5-HT_{1B} heteroreceptors located on GABAergic terminals, serotonin can reduce the local inhibitory input onto pro-opiomelanocortin neurons arising from agouti-related peptide neurons (Fig. 1). The combined effect of these actions is presumably an increase in alpha-melanocyte stimulating hormone release together with reduced agouti-related peptide release, reducing competitive antagonism at melanocortin3 and 4 receptor expressing target cells.

The functional importance of the melanocortin pathway in serotonin's effects on energy balance was also assessed via pharmacological or genetic inactivation of melanocortin receptors. Specifically, dexfenfluramine's anorectic effects are attenuated in agouti mice and rats pretreated with the melanocortin3/4 receptor antagonist Ac-Nle-cyclo(-Asp-His-D-2-Nal-Arg-Trp-Lys)-NH_2 (SHU9119) (Heisler et al., 2002, 2006). This effect appears to be mediated via the melanocortin4 receptor since dexfenfluramine-induced hypophagia in melanocortin3 receptor deficient mice was comparable to that seen in wild type mice, whereas dexfenfluramine was ineffective in reducing appetite in melanocortin4 receptor knockout mice (Heisler et al., 2006). Further studies clarified that melanocortin4 receptors expressed on SIM1 neurons underlie these effects (Xu et al., 2010). Specifically, in melanocortin4 receptor null mice, selective re-expression of these receptors in SIM1 neurons restores the anorectic efficacy of dexfenfluramine. The consistent finding that functional melanocortin4 receptors are required for dexfenfluramine to elicit reductions in food intake implicates this pathway as a critical mediator of serotonin's anorectic action and its negative influence on energy balance.

7. Concluding remarks

Technological advances in recent years, particularly in the field of genetics, have enabled scientists to identify, and/or generate, a wide array of animal models that have vastly improved our understanding of physiology. However, the ability of developmental adaptation and compensation to mask the phenotypic consequences of germline genetic perturbation means that genetic knockdown/knockout studies should be interpreted with caution. Nevertheless, the body of anatomical, pharmacological and genetic evidence implicating serotonergic signalling in the control of energy balance and the melanocortin signalling system as a downstream component of this effect is now compelling.

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