Cell Metabolism
Review

Serotonin and the Orchestration of Energy Balance

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The phylogenetically ancient signaling molecule serotonin is found in all species that possess nervous systems and orchestrates diverse behavioral and physiological processes in the service of energy balance. In some instances, the manner in which serotonin signaling influences these processes appears comparable among invertebrate and vertebrate species. Within mammalian species, central nervous system serotonergic signaling influences both behavioral and physiological determinants of energy balance. Within the gastrointestinal tract, serotonin mediates diverse sensory, motor, and secretory functions. Further examinations of serotonergic influences on peripheral organ systems are likely to uncover novel functions consistent with an apparently pervasive association between serotonergic signaling and physiological substrates of energy balance.

The need to acquire food and balance energy intake with expenditure is ubiquitous among animal phyla and provides a driving force for natural selection. The maintenance of energy balance requires the regulation of many behavioral and physiological processes, including those required to forage for food, recognize nutrient sources, consume food, digest food, and utilize/store energy. Thus, the maintenance of energy balance requires the organism to regulate both its relationship to the external environment (behavior) and the modulation of its internal environment. Throughout phylogeny, these regulatory functions are coordinated to a large extent by nervous systems. It is therefore intriguing that the monoamine signaling molecule 5-hydroxytryptamine (5-HT, serotonin), which is found in all phyla that possess nervous systems (Weiger, 1997), orchestrates behavioral and physiological determinants of energy balance in species as disparate as nematodes and humans. Serotonergic influences on some of these processes appear to be generalized throughout phylogeny. In other instances, however, serotonergic signaling appears to produce differing effects in vertebrate and invertebrate species.

Serotonergic Regulation of Invertebrate Energy Balance

Consideration of the serotonergic regulation of invertebrate energy balance reveals several themes that are generalized among diverse species. In contrast to the marked clustering of serotonergic neurons in the vertebrate brain, serotonergic neurons are generally dispersed throughout the nervous systems of invertebrates (Gillette, 2006). Their activation promotes appetitive states through the coordinated regulation of diverse physiological and behavioral processes, such as the central pattern generators that mediate foraging and ingestion, digestive enzyme release, gut expansion, and energy storage. These themes are briefly illustrated by consideration of serotonergic mechanisms regulating energy balance in several invertebrate species.

Insects

An illustrative example of the serotonergic coordination of feeding-related physiological events is provided by the South American "kissing bug" *Rhodnius prolixus*, an obligatory blood feeder (and vector for the parasite causing Chagas' disease). This insect is capable of ingesting 300 mg of blood in 15 min, increasing its body mass by a factor of 10 (Orchard, 2006). Serotonin, which is widely distributed throughout insect nervous systems, has been found to orchestrate remarkably diverse feeding-related physiological events in *Rhodnius*, both as a neurotransmitter/neuromodulator and as a neurohormone.

As *Rhodnius* feeding begins, serotonergic nerves stimulate the release of saliva, which contains substances that block hemostasis of the host's blood, permitting continuous ingestion (Orchard, 2006). Simultaneously, serotonergic nerves of the body wall trigger softening (plasticization) of the cuticle, allowing the body wall to expand as the crop (anterior midgut) fills with blood. The crop also receives serotonergic afferents, and these induce contractions and increased fluid transport into the circulatory system (hemolymph). As this occurs, serotonin is released from an abdominal nerve plexus directly into the hemolymph, elevating circulating serotonin levels up to 15-fold (Lange et al., 1989). Serotonin in the hemolymph acts as a neurohormone at the animal's Malpighian tubules, inducing a massive diuresis that is essential for the animal to reduce its mass and thereby regain its mobility.

Annelids

Rhodnius is not alone as a creature that relies heavily on serotonergic signaling for successful blood gorging. The blood-sucking annelid *Hirudo medicinalis*, the medicinal leech, provides another striking example of the serotonergic coordination of diverse processes associated with feeding. *Hirudo* is a segmented worm-like creature possessing an anterior sucker that contains a mouth with a set of tripartite jaws. Hungry leeches patrol the surface of ponds and swim toward the source of disturbances in the water (Lent et al., 1991). When they encounter warm

surfaces, they bite using their three serrated jaws, and rhythmic pharyngeal contractions direct the blood meal into the digestive organ (crop) (Lent, 1985). A single feeding episode may increase their mass 8-fold (Lent et al., 1988), followed by a prolonged period of satiation (up to 1 year). Satiated leeches remain relatively lethargic and seek cover in deep water.

Many aspects of these processes are subject to regulation by massive (50–100 μm diameter) serotonergic neurons (Retzius cells) that reside in pairs within each of the 32 ganglia comprising the animal's segmented nervous system. The serotonin content of these neurons reflects their state of satiation (Lent et al., 1991). Typically, hungry leeches have high Retzius cell serotonin levels. These levels drop during feeding (Lent, 1985) and remain low during the period of satiation. In accord with this, pharmacological depletion of serotonin reduces both approach behavior and biting directed toward food-related stimuli (Lent and Dickinson, 1984). Conversely, leeches treated with serotonin will consume larger meals, and recently sated leeches may be induced to resume biting in the presence of serotonin (Lent, 1985). Retzius neuron firing rates increase when the lip of the anterior sucker contacts a warm surface (Groome et al., 1995). These neurons innervate the jaws, pharynx, salivary glands, and body wall musculature, where they stimulate biting, pharyngeal contractions, production of saliva (containing the anticoagulant hirudin), and body wall muscle relaxation permitting expansion of the crop. Crop distension, in turn, has been shown to suppress feeding behavior, ganglionic serotonin content, and Retzius cell firing rates (Groome et al., 1995). Thus, these serotonergic neurons respond to both external and internal ingestion-related stimuli to coordinate diverse responses to food.

Gastropod Mollusks

Of course, the presence of systems employing serotonin to regulate ingestive behavior is not restricted to obligate blood feeders. Among the most phylogenetically conserved actions of serotonin in the regulation of feeding is its role as a modulator of feeding-related central pattern generators (CPGs) (Harris-Warrick and Marder, 1991). CPGs are neuronal circuits that are intrinsically capable of generating rhythmic, stereotyped motor activity. Feeding-related CPGs have been extensively studied in gastropod mollusks such as *Aplysia*, *Helisoma*, and *Lymnaea* (Elliott and Susswein, 2002). Food consumption in these species is accomplished by the repetitive protraction and retraction of the radula, a hardened tissue that conveys food into the buccal cavity. The radula is innervated by neurons of the buccal ganglia that function as CPGs mediating its rhythmic movement (Murphy, 2001). This rhythm is determined by the intrinsic physiological properties of CPG neurons, their synaptic interconnections, and their innervation by extrinsic modulators such as serotonin.

Serotonin has been found to facilitate the motor output of a wide variety of rhythm-generating circuits (Harris-Warrick and Marder, 1991), including those regulating food consumption in gastropod mollusks. The sole serotonergic innervation of the buccal ganglion CPGs arises from a pair of serotonergic cerebral giant cells. These neurons may be activated by contact of the animal with food stimuli. Activation of these neurons, as well as application of serotonin, can elicit rhythmic motor activity. Serotonergic neurotransmission has been found to produce long-term depolarizing effects on CPG neuronal membrane potentials, to regulate bursting activity, and to enhance "postinhibitory rebound" properties of CPG neurons (Straub and Benjamin, 2001). It is noteworthy that facilitating influences of serotonergic neurotransmission have also been found in mammalian brainstem CPGs that regulate mastication.

Nematodes

Recent studies of the genetically tractable nematode *Caenorhabditis elegans* further highlight the phylogenetic prevalence of serotonin utilization for the coordination of feeding-related processes. *C. elegans* can be found in the soil, foraging for bacterial food sources. When they encounter a patch of palatable food, their locomotor behavior patterns change from "roaming" (relatively rapid and straight locomotor paths) to "dwelling" (movement characterized by frequent stops and changes in direction) (Shtonda and Avery, 2006). *C. elegans* ingest bacteria through a pharyngeal pumping mechanism, and pumping rates are known to increase in the presence of food (Avery and Horvitz, 1990). In times of plenty, worms can store energy as intestinal fat. In the absence of food, roaming behavior is favored, but prolonged periods of starvation can induce a "dauer" state, in which animals become immobile and metabolically inactive.

Remarkably, each of these behavioral and physiological processes is subject to serotonergic regulation. Application of exogenous serotonin suppresses roaming activity and stimulates pharyngeal muscle activation and pumping, characteristics of feeding worms (Horvitz et al., 1982; Raizen et al., 1995). Moreover, food-induced dwelling in starved worms is markedly increased by the serotonin reuptake blocker fluoxetine (Prozac) and suppressed by serotonin receptor antagonists, indicating that endogenous serotonin regulates this behavior (Sawin et al., 2000). Additional insights have been obtained from studies of worms that lack serotonin due to a null mutation of the serotonin-synthetic enzyme tryptophan hydroxylase (*tph-1*) (Sze et al., 2000). In addition to displaying feeding behavior abnormalities consistent with prior pharmacological studies (Sze et al., 2000), *tph-1* mutants exhibit signs of perturbed metabolic regulation. This is indicated by their enhanced propensity to enter the dauer state and their increased intestinal fat accumulation. The latter phenotype was found to result from serotonergic regulation of the TGF-β and insulin-like signaling pathways known to influence fat metabolism in *C. elegans* (Kimura et al., 1997).

In addition to the primary behavioral responses to food described above, a recent study indicates how serotonergic neurons can participate in appetitive learning processes (Zhang et al., 2005). Following exposure to either pathogenic (aversive) or nonpathogenic bacteria, worms were provided a choice of these and unfamiliar bacterial

strains. Animals were found to migrate preferentially toward the familiar nonaversive strain and to avoid the familiar pathogenic strain. A serotonergic contribution to this learned aversive behavior was indicated by the poor performance of *tph-1* mutants in the choice assay. In an elegant series of experiments, a critical role of a serotonergic sensory neuron (termed AFD) in learned food aversion was elucidated. Exposure to pathogenic bacteria was observed to increase serotonin within AFD, and rescue of serotonergic function selectively within the AFD of serotonin-deficient strains restored learned aversive behavior. Altogether, this study demonstrates how serotonin can regulate food aversion learning, a critical behavior that is highly conserved throughout phylogeny.

Altogether, these and other invertebrate studies illustrate the widespread recruitment of serotonin systems for the regulation of key behavioral and physiological determinants of energy balance: foraging behavior, recognition of nutrient sources, food consumption and digestion, and energy storage. In comparison to the organisms discussed above, vertebrates possess expanded genomes and a proliferation of neuromodulators other than serotonin. It is therefore remarkable that this particular invertebrate signaling molecule remains called upon for widespread participation in the diverse determinants of energy balance in vertebrates such as mammals.

Central Serotonergic Regulation of Energy Balance

In contrast to invertebrates, in most vertebrate species, the global activation of central serotonin systems appears to suppress feeding. For years, the prototypical agent used in studies of serotonergic suppression of feeding has been fenfluramine, which, along with the stimulant phentermine, was a component of the "fen-phen" diet cocktail (removed from the market 10 years ago due to induction of cardiac valve disease). Fenfluramine is believed to globally enhance serotonergic neurotransmission by stimulating synaptic serotonin release and blocking its reuptake into presynaptic terminals (Rowland and Carlton, 1986). In rats with ad libitum access to food, fenfluramine reduces the sizes of individual meals, reduces feeding rates, and increases intervals between meals (Blundell and Leshem, 1975; Grinker et al., 1980). Similar effects of fenfluramine on feeding behavior have been demonstrated in a variety of mammalian species, including humans (Foltin and Moran, 1989; McGuirk et al., 1991; Rogers and Blundell, 1979). Conversely, treatments that reduce serotonergic neural activity, such as intraventricular injections of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) and lesions of the serotonergic raphe B8 cell group, produce chronic hyperphagia and weight gain (Geyer et al., 1976; Saller and Stricker, 1976).

Organization of Central Serotonin Systems

The anatomical organization of central serotonin systems is remarkably similar among vertebrate species (Parent, 1981). Serotonergic neurons are predominantly clustered into two major groups, the rostral (mesencephalic) and caudal (metencephalic) nuclei (Parent, 1981). In mammals, the rostral cluster contains a distinguishable dorsal raphe nucleus (DRN), in close proximity to a midline median raphe nucleus. Highly divergent serotonergic processes arise from these locations, providing the predominant serotonergic innervation of forebrain structures. In addition, a series of caudal raphe nuclei innervate brainstem structures and the spinal cord. Throughout the rostrocaudal extent of the brain, virtually all brain nuclei implicated in energy balance regulation receive serotonergic afferents. Thus, the anatomical organization of the central serotonin system provides few clues for the identification of those neural pathways most essential to serotonergic influences on energy balance.

Another major obstacle to unraveling neural mechanisms that underlie serotonergic influences on energy balance is the complexity of serotonergic signaling systems, highlighted by the identification of at least 14 functionally diverse subtypes of 5-HT receptors (Hoyer et al., 1994). The determination of those subtypes most critical to the anorectic effects of dexfenfluramine (dFen) and nonspecific 5-HT agonists was achieved through the complementary use of gene knockout technology and pharmacological agents with enhanced 5-HT receptor subtype specificity. Such studies have focused particular attention on the 5-HT_{2C} receptor (5-HT_{2C}R) as a key mediator of the serotonergic suppression of feeding. Additional 5-HT receptor subtypes are also likely to influence energy balance regulation; however, their contributions are less understood.

Serotonin 5-HT_{2C} Receptors

The Gq-coupled 5-HT_{2C}R is widely expressed throughout the neuraxis and restricted to the central nervous system (Conn et al., 1986; Julius et al., 1988; Pazos et al., 1985; Yagaloff and Hartig, 1985; Baxter et al., 1995; Wright et al., 1995). Its involvement in feeding regulation was initially suggested by the anorectic effects of the nonspecific 5-HT_{2C}R agonist *m*-chlorophenylpiperazine (mCPP) and their blockade by compounds with 5-HT_{2C}R agonist activity (Kennett and Curzon, 1988a, 1991; Kitchener and Dourish, 1994; Middlemiss and Tricklebank, 1992). A major contribution of 5-HT_{2C}Rs to serotonergic feeding suppression was further highlighted by studies employing mice bearing a null mutation of the *Htr2c* gene. These animals were found to be resistant to the anorectic effects of mCPP and fenfluramine (Tecott et al., 1995; Vickers et al., 1999).

Subsequent longitudinal studies of 5-HT_{2C}R mutant mice revealed chronically elevated food intake, commencing during the first 6 weeks of life. As young adults, this hyperphagia was not accompanied by perturbations of metabolic rate, glucose homeostasis, or leptin sensitivity (Nonogaki et al., 1998). By 5–6 months of age, body weights of mutant and wild-type littermates diverged, with mutants developing enhanced adiposity accompanied by hyperinsulinemia, reduced glucose tolerance, hyperleptinemia, and partial leptin resistance (Nonogaki et al., 1998). Moreover, the *Htr2c*$^{-}$ mutation enhanced sensitivity to high-fat feeding, leading to accelerated weight gain and the development of type 2 diabetes

mellitus. Altogether, the 5-HT_{2C}R mutant obesity syndrome appeared to be characterized by chronic hyperphagia leading to a number of physiological consequences resembling those found in common forms of human obesity.

In addition to feeding, another behavioral determinant of energy balance, physical activity, was impacted by the *Htr2c*$^{-}$ mutation (Nonogaki et al., 2003). Mutants displayed locomotor hyperactivity that persisted throughout the period during which their body weights diverged from wild-type levels. Moreover, hyperactivity of the mutants was markedly enhanced by food deprivation (Nonogaki et al., 2003). Relationships between locomotor activity and feeding behavior were further examined by behavioral monitoring. In C57BL/6J mice, approximately 80% of the animals' total daily locomotor activity occurred within 2 min of visits to the feeder. This tight temporal relationship between physical activity and feeding was maintained in mutants, even during periods of food deprivation. Thus, their enhanced locomotor responses to food deprivation were tightly associated with frequent visits to the location where they had previously found food. Altogether, these findings raised the possibility that the hyperactivity of 5-HT_{2C}R mutant mice reflects enhanced foraging (food-seeking) behavior.

Insights regarding the late onset of obesity in 5-HT_{2C}R mutant mice were provided by studies examining age-related effects of the mutation on the relationship between physical activity and metabolic rate (Nonogaki et al., 2003). Simultaneous determinations of locomotor activity levels and oxygen consumption were obtained in young adult ("preobese") and older moderately obese mutant mice (Nonogaki et al., 2003). No phenotypic differences were observed in the total oxygen consumption or resting metabolic rates of young adults. Surprisingly, in older animals, the *Htr2c* mutation reduced total energy expenditure while increasing locomotor activity levels 2-fold. Correlational analysis of locomotor activity and oxygen consumption revealed no phenotypic difference in resting metabolic rate; however, the energy costs of physical activity appeared to be substantially reduced in the mutants. These results raised the possibility that central serotonin systems can regulate the energy efficiency of physical activity in an age-dependent manner. It will be of interest to determine whether serotonergic mechanisms contribute to the age-related increases in adiposity observed in many mammalian species.

A contribution of 5-HT_{2C}Rs to human energy balance is highlighted by a recent study of Prader-Willi syndrome (PWS), which is characterized by hypogonadism, mental retardation, hyperphagia, and obesity (Kishore and Stamm, 2006). PWS results from the loss of paternally expressed genes on human chromosome 15q11-13. Among the genes lost in PWS is the small nucleolar RNA *HBII-52*. Kishore and Stamm (2006) demonstrated that HBII-52 masks an exonic silencer within the fifth exon of 5-HT_{2C}R preRNA. Its absence in PWS promotes expression of alternatively spliced versions of the 5-HT_{2C}R with reduced function. The pathophysiology of PWS has been attributed to this phenomenon, and the marked hyperphagia and obesity that are characteristic of PWS are consistent with such a mechanism.

Serotonergic Regulation of Hypothalamic Feeding Circuits

Much attention has focused on the hypothalamus as a locus of serotonergic influences on feeding. Systemic administration of dFen and fluoxetine increases extracellular hypothalamic serotonin levels, and microinjections of serotonin into the paraventricular nucleus (PVN) have been found to suppress feeding by reducing meal size and feeding rate (Hutson et al., 1988; Paez and Leibowitz, 1993; Schwartz et al., 1989; Shor-Posner et al., 1986). Similar effects were also observed with microinjection of serotonin into the ventromedial (VMN) and dorsomedial (DMN) nuclei (Leibowitz et al., 1990).

Of considerable interest are the subset of neurons in the arcuate nucleus (ARC) that express the melanocortin precursor pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART) (Heisler et al., 2002). These neurons, which mediate feeding suppression, project to the PVN, VMN, lateral hypothalamus (LH), and spinal cord sympathetic preganglionic neurons (Cowley et al., 2001; Saper et al., 2002). ARC POMC neurons express 5-HT_{2C}Rs, and their activation depolarizes POMC neurons in slice preparations (Heisler et al., 2002). A contribution of melanocortin pathways to serotonergic feeding suppression received further support from the observation that the anorectic action of dFen was suppressed in yellow agouti mice, a strain deficient in melanocortin pathway signaling (Bultman et al., 1992; Fan et al., 1997; Heisler et al., 2002; Lu et al., 1994).

Although 5-HT_{2C}R-mediated actions are best characterized in the ARC, these receptors may also influence energy balance through direct effects at other hypothalamic sites. Aside from the ARC, 5HT_{2C}R transcripts are expressed in additional hypothalamic regions strongly implicated in energy balance, including the PVN, VMN, DMN, and LH (Hoffman and Mezey, 1989; Pasqualetti et al., 1999; Wright et al., 1995). These structures all receive serotonergic innervation from the mesencephalic dorsal and/or median raphe nuclei (Petrov et al., 1992; Steinbusch and Nieuwenhuys, 1981). It is therefore apparent that the manner in which 5-HT_{2C}Rs influence the hypothalamic regulation of energy balance is highly complex.

Adding to the challenge of elucidating the serotonergic regulation of hypothalamic function is the fact that multiple 5-HT receptor subtypes other than 5-HT_{2C}R are expressed in hypothalamic regions implicated in energy balance. These include the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{7} receptor subtypes (Bruinvels et al., 1994; Hedlund and Sutcliffe, 2004; Wright et al., 1995). Of these, the 5-HT_{1B}R subtype has been most strongly implicated in the serotonergic suppression of feeding. Although mice devoid of 5-HT_{1B}Rs do not display an obese phenotype (Bouwknecht et al., 2001; Lucas et al., 1998), pharmacological or genetic inactivation of 5-HT_{1B}Rs reduces the anorectic effects of dFen (Grignaschi and Samanin, 1992; Neill and Cooper, 1989; Vickers et al., 1996). Moreover,

5-HT_{1B}R agonists promote satiation in rodent models (Halford and Blundell, 1996; Lee and Simansky, 1997), and it has been proposed that the 5-HT_{1B}R and 5-HT_{2C}R act cooperatively in the expression of serotonergic satiation (Kennett and Curzon, 1988b; Simansky, 1996).

New insights have recently been provided into hypothalamic mechanisms through which 5-HT_{1B}Rs suppress feeding (Heisler et al., 2006). Intriguingly, 5-HT_{2C}Rs and 5-HT_{1B}Rs display a complementary distribution within the ARC: whereas 5-HT_{2C}Rs are expressed in pro-anorectic POMC/CART-expressing neurons, 5-HT_{1B}Rs are found in the orexigenic NPY/AgRP neuronal population. However, in slice preparations, agonists at this Gi-coupled receptor were observed to influence the activity of both cell types in a reciprocal manner. These agents hyperpolarized NPY/AgRP neurons and also suppressed inhibitory postsynaptic potentials in POMC/CART neurons.

Taken together, these and prior findings support a model in which postsynaptic somatodendritic 5-HT_{1B}Rs inhibit NPY/AgRP neuronal activity while presynaptic 5-HT_{1B}Rs expressed on the axon terminals of these NPY/AgRP neurons suppress their GABAergic inhibition of POMC/CART neurons. This would facilitate the release of the endogenous melanocortin 4 receptor (MC4R) agonist α-MSH from terminals of POMC/CART neurons while suppressing the release of the endogenous MC4R antagonist AgRP from terminals of AgRP/NPY neurons. The importance of MC4R as an indirect target of these serotonin-responsive pathways is further highlighted by the markedly reduced anorectic effects of dFen and 5-HT_{1B}R agonists in mice bearing null mutations of the *Mc4r* gene. When considered in light of prior findings, these results reveal an elegant design through which both 5-HT_{2C}R and 5-HT_{1B}Rs coordinately regulate endogenous excitatory and inhibitory modulators of melanocortin system function (Figure 1). It is noteworthy that these 5-HT receptor subtypes are also coexpressed at brainstem locations implicated in ingestion, raising the possibility that their complementary influences on feeding circuits may not be restricted to the hypothalamus.

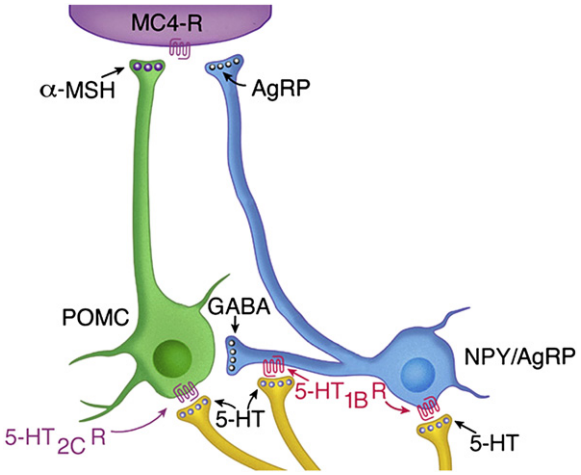

Figure 1. Serotonergic Regulation of the Arcuate Nucleus Neurons
Reproduced from Heisler et al. (2006).

Serotonergic Regulation of Brainstem Feeding Circuits

Although the hypothalamus appears to be a principal site of serotonin action, many additional brain regions are also likely to mediate serotonergic influences on energy balance. Several lines of evidence indicate that serotonin-responsive regions of the caudal brainstem warrant particular consideration (Grill and Kaplan, 2002). For example, infusions of mCPP and dFen into the fourth ventricle have been found to effectively suppress feeding, indicating that the hypothalamus may not be essential for serotonin's anorectic actions (Grill et al., 1997; Grill and Kaplan, 2002; Kaplan et al., 1998). The ability of mCPP and dFen to suppress feeding behavior in decerebrate rats lends further support for this possibility. A key role for caudal brainstem sites is also indicated by the ability of fourth ventricular administration of a partially selective 5-HT_{2C}R antagonist to suppress the anorectic actions of systemic mCPP administration (Kaplan et al., 1998).

Within the caudal brainstem, the dorsal vagal complex (DVC) and the parabrachial nuclei (PBN) have been most implicated in the serotonergic regulation of feeding (Tache et al., 1995). The DVC contains the dorsal motor nucleus of the vagus (DMV) and the nucleus tractus solitarius (NTS). The DMV contains efferent motoneurons of the vagus nerve, and the NTS receives vagal afferents originating from the gastrointestinal tract. The DVC receives serotonergic afferents from raphe nuclei of the caudal brainstem such as the raphe pallidus (RPa) and the raphe obscurus (ROb) (Thor and Helke, 1987). Stimulation of these raphe nuclei activates gastric vagal efferents, leading to gastric acid release and enhanced gastric motility (White et al., 1991). Both 5-HT_{2A}Rs and 5-HT_{2C}Rs have been implicated in these effects (Tache et al., 1995).

The PBN is considered a site where diverse modalities of sensory information, including visceral and gustatory, are integrated. Through widespread projections to regions that include the hypothalamus, amygdala, and brainstem, it participates in the regulation of feeding, drinking, respiration, and cardiovascular function (Saper and Loewy, 1980). The PBN receives serotonergic innervation from the dorsal raphe nucleus and area postrema (Petrov et al., 1992; Simansky and Nicklous, 2002) and expresses both 5-HT_{2C}Rs and 5-HT_{1B}Rs (Bruinvels et al., 1993; Wright et al., 1995). An important contribution of the PBN to dFen-induced anorexia is indicated by the ability of PBN lesions to suppress this effect (Li et al., 1994). This may occur through the activation of 5-HT_{1B}Rs, as indicated by the ability of local PBN 5-HT_{1B}R agonist administration to suppress feeding and the ability of local 5-HT_{1B}R antagonist administration to suppress dFen-induced anorexia (Simansky and Nicklous, 2002). Altogether, these findings indicate that an exclusively "hypothalamocentric" focus would fail to account for important mechanisms through which central serotonin regulates feeding behavior.

This point is further highlighted by a different brainstem function that is essential to the initial stage of digestion, mastication. Electrophysiological recordings from awake cats revealed that approximately 25% of DRN serotonergic neurons fire during repetitive oral-buccal movements, such as chewing, biting, and licking (Jacobs et al., 2002). Frequently, increases in the firing rates of these neurons occur just prior to movement initiation. Furthermore, recordings from the caudal brainstem RPa and ROb revealed that the majority of serotonergic neurons at these locations display elevated firing rates during bouts of feeding (Jacobs et al., 2002). It is believed that mastication is regulated by a brainstem CPG that drives trigeminal motoneurons (Lund et al., 1998). These motoneurons receive serotonergic projections (from the DRN, RPa, and ROb), and 5-HT_2 receptor activation has been observed to facilitate rhythmic bursts of trigeminal motoneuron activity (Kolta et al., 1993; Mori et al., 2002). It is intriguing that the serotonergic regulation of feeding-related motor pattern generators is found not only in mammals but throughout phylogeny, as illustrated by studies of CPGs in reptiles, crustaceans, and mollusks (Harris-Warrick and Marder, 1991).

Peripheral Serotonin

An appreciation that at least 95% of total body serotonin content is found within the gut (Gershon, 2004) provides an additional perspective on the prominence of serotonergic signaling in the regulation of mammalian energy balance. Serotonergic cells (both neuronal and nonneuronal) are found throughout the entire rostrocaudal extent of the gastrointestinal tract, where they mediate diverse motor and sensory functions through both synaptic and paracrine signaling mechanisms. In this regard, serotonin functions within the mammalian gut in a manner not entirely dissimilar from its actions within invertebrate digestive systems.

Taste Buds

The ubiquitous nature of serotonergic contributions to feeding and energy balance is highlighted by its actions at the most proximal regions of the alimentary tract. In a diverse array of vertebrate species, serotonin has been found in taste buds, cloistered structures densely packed with taste receptor cells (TRCs) (Kim and Roper, 1995; Uchida, 1985). In mammals, serotonin is found in posterior taste buds within a subpopulation of TRCs that synapse onto afferent nerve fibers (Yee et al., 2001). In a recent study, the capacity of taste buds to release serotonin was tested using a novel biodetector system consisting of chinese hamster ovary cells stably expressing 5-HT_{2C}Rs (Huang et al., 2005a, 2005b). When preloaded with a calcium-sensitive dye and incubated with isolated mouse taste buds, these ''biosensors'' could detect serotonin release. Using this approach, exposure of taste buds to bitter, sour, and sweet tastants was found to stimulate serotonin release.

In addition to its putative role as a neurotransmitter at primary afferent nerve fibers, serotonin may also have a paracrine signaling role within taste buds. This possibility is suggested by the observation that some TRCs are themselves responsive to serotonin (Delay et al., 1997; Imendra et al., 2002; Kaya et al., 2004). It has been suggested that serotonin's actions as a neurotransmitter and as a paracrine signal may be mediated by distinct 5-HT receptors (Kaya et al., 2004). In response to tastants, paracrine release of serotonin may inactivate neighboring TRCs that express the 5-HT_{1A} receptor subtype (Kaya et al., 2004). In contrast, synaptic serotonin released by TRCs has been proposed to activate afferent nerve fibers through 5-HT_3 receptors. However, this has recently been called into question by the observation of normal taste behavior in 5-HT_{3A}R knockout mice (Finger et al., 2005). Despite recent progress, the functional significance of serotonin signaling in primary taste sensation and the mechanisms through which this may occur remain to be elucidated.

Intestinal Serotonin

In the 1930s, serotonin was identified in the intestine, and thus termed ''enteramine.'' This was subsequently found to be the same substance extracted from blood samples in the 1940s and termed serotonin. In the 1950s, serotonin was identified as a principal monoamine neurotransmitter of the gastrointestinal tract. Multiple lines of evidence support its role as a neurotransmitter within the enteric nervous system. The myenteric and submucosal plexi contain tryptophan hydroxylase and display a capacity to synthesize serotonin from L-tryptophan (Gershon and Dreyfus, 1980). Moreover, serotonin is released upon electrical stimulation of the enteric nervous system. The functional significance of enteric nervous system serotonin is indicated by the impairment of intestinal motility by the serotonergic neurotoxin 5,7-DHT.

Serotonin-synthesizing enteric cells are not restricted to neurons; in fact, 85% of total body serotonin is found within mucosal enterochromaffin (EC) cells located within enteric epithelium throughout the gastrointestinal tract (Figure 2; Gershon, 2004). EC cells appear to mediate sensory transduction, releasing serotonin into the bowel wall in response to mechanical pressure and nutrients (Gershon, 2003). The released serotonin initiates the luminal secretion of sodium chloride and fluid. These effects are mediated through excitation of intrinsic submucosal primary afferent neurons through activation of a putative 5-HT_{1P} receptor subtype that remains to be molecularly cloned (Gershon, 2003). These neurons in turn project to the myenteric plexus and activate peristaltic reflexes, a process that is facilitated by the activation of 5-HT_4 receptors. In addition, released serotonin activates extrinsic sensory neurons via 5-HT_3 receptors. These neurons, with cell bodies in vagal and dorsal root ganglia, convey visceral sensory information (e.g., nausea, pain, and bloating) to the central nervous system. This is in accord with the clinical use of 5-HT_3R antagonist compounds as antiemetic agents. Additionally, 5-HT_3 receptor-mediated activation of vagal extrinsic sensory neurons has also been implicated in pancreatic exocrine secretion (Li et al., 2000).

Normal gastrointestinal function requires serotonin reuptake mechanisms that limit submucosal serotonin

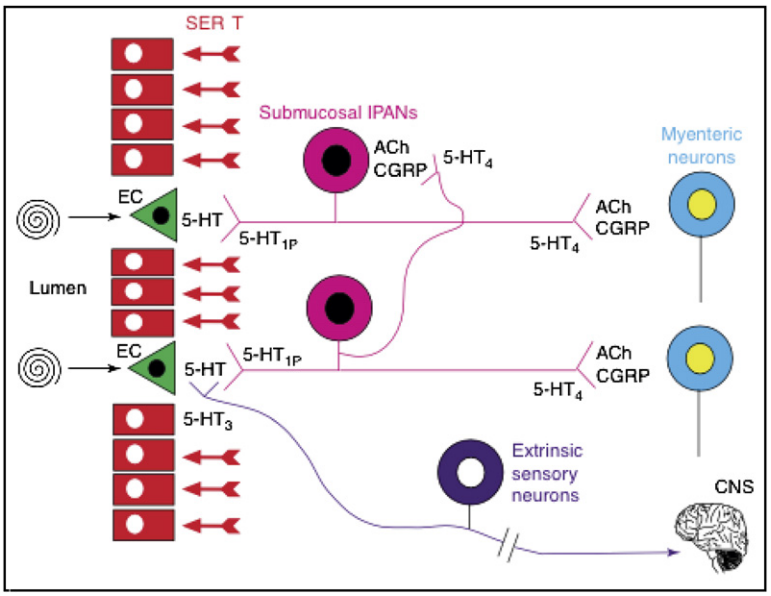

Figure 2. Serotonergic Contributions to Enteric Nervous System Function
Reproduced from Gershon (2003).

content. The bulk of this reuptake occurs within luminal enterocytes that express the serotonin transporter. Notably, serotonin transporter expression is markedly reduced by mucosal inflammation and is also reduced in irritable bowel syndrome. The resulting elevations of serotonin levels have been proposed to produce discomfort through enhanced stimulation of 5-HT_3 receptors and diarrhea through enhanced stimulation of 5-HT_{1P} and 5-HT_4 receptors (Gershon, 2004).

Conclusion

A fundamental feature of animal life is the need to acquire energy through the consumption of material derived from other organisms. This is accomplished through coordinated movements in the service of food acquisition and its transit to an internal chamber for digestion. It is perhaps fitting that one of the most phylogenetically ancient signaling molecules would be intimately involved in the regulation of processes so fundamental to animal life. Serotonergic regulation of behavioral responses to energy status, the sensing of food stimuli, the release of digestive enzymes, CPGs that mediate ingestion, gut motility, and interoceptive feedback occur widely among both invertebrate and vertebrate species. Throughout phylogeny, both synaptic and paracrine serotonergic signaling mechanisms mediate these processes. Although serotonergic influences on each of the physiological determinants of energy balance are not entirely consistent across vertebrate and invertebrate species, some of these influences do appear to generalize. This highlights the potential utility of model invertebrate organisms to provide insights relevant to serotonergic mechanisms of energy balance in mammals.

Serotonergic signaling mechanisms seem to be ubiquitous, not only throughout phylogeny but also among the diverse behavioral and physiological processes mediating energy balance within mammalian species. We are currently far from a full understanding of the functional scope of these influences. Within the brainstem, serotonergic afferents modulate structures receiving meal-associated interoceptive and systemic signals. Hypothalamic structures implicated in the homeostatic regulation of energy balance all receive serotonergic innervation and express 5-HT_{2C}Rs, among others. The contributions of other serotonin receptor subtypes that are expressed in feeding-related structures (e.g., 5-HT_4, 5-HT_6, and 5-HT_7 receptors) to feeding regulation remain to be fully explored. With regard to nonhomeostatic regulatory influences on feeding, such as palatability, motivation, appetitive learning, and craving, serotonergic pathways innervating limbic and cortical structures also warrant attention.

As described above, serotonergic signaling also mediates multiple physiological processes within the enteric nervous system. These include regulation of sensory responses to luminal stimuli, mucosal secretion, peristaltic reflexes, pancreatic exocrine function, and enteroceptive signaling to the central nervous system. It is notable that serotonin receptors are expressed in a number of additional organ systems implicated in energy balance, although detailed surveys are needed. A recent intriguing example is a study demonstrating a critical role for hepatic 5-HT_{2B} receptors in the process of liver regeneration (Lesurtel et al., 2006). In light of the ubiquitous influences of serotonin signaling on the physiology of energy balance, it would be worthwhile to assess potential serotonergic influences on processes such as pancreatic endocrine function, liver substrate utilization, and skeletal muscle insulin sensitivity. Such studies have the potential to reveal novel roles for serotonin and novel therapeutic opportunities for metabolic diseases.

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