CALL FOR PAPERS | Physiological Regulation of Appetite

Physiological regulation of food intake

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In this issue of the Journal are published six papers that were submitted in response to the Call for Papers “Physiological Regulation of Appetite” (7, 17, 25, 27, 66, 86). In addition, four other papers in this series have been published in previous issues (13, 31, 47, 48). This Call for Papers celebrates the journal’s strong commitment to basic and translational research that contributes to understanding of the regulation of body weight in humans and other animals. Among the numerous and broad areas covered by American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, this field stands out both for the number of studies published and for their excellence. It is clear that body weight is closely regulated under most conditions (43), and this implies that energy consumption, the amount and quality that is eaten, must be matched to energy expenditure. If an imbalance develops between energy intake and output, then the consequences for the organism can be severe. A recent review has pointed out that availability of oxidizable fuel is a major regulator of fertility in female mammals (80). However, in a time of unprecedented wealth worldwide, we as a species are developing unprecedented girth. This too has profound consequences; as noted by Davy and Hall (21), there is a strong and presumably causal relationship between weight gain and development of hypertension.

Active areas of research include signaling of body status to the brain, including both energy content and of digestive status; the properties of food—its constituents and taste; central processing of these and other inputs; and interactions between ingestive and other behaviors. The unit of food intake, and to a large extent fluid intake, is the meal. Consequently an accepted definition of what exactly constitutes a meal is a matter of some importance. Current methods for defining meals in rodents have been challenged recently. Zorrilla et al. (86) have developed a meal definition that performs significantly better than previous methods.

The importance of both appetitive behavior (acquiring food) and consummatory behavior (eating what you have acquired and stored) was highlighted in several studies that employed different species of hamsters. Thus in Syrian hamsters, food deprivation induces hoarding rather than hyperphagia when food is restored. Leptin administered during the period of food deprivation attenuated the subsequent hoarding (8). Likewise in Siberian hamsters, injections into the third cerebral ventricle of agouti-related peptide (AGRP) induced a robust and prolonged increase in hoarding that was apparent at one tenth the dose required to induce feeding, suggesting that the primary effect of AGRP in this species is to trigger the search for food (22). A somewhat similar result was obtained after peripheral injection of ghrelin. Ghrelin is an orexigenic and appetite-stimulating hormone that is secreted largely by the stomach. Exogenous ghrelin, at reasonable doses, induced an early increase in feeding and a prolonged increase in appetitive behaviors (39).

Diet-induced obesity (DIO) refers to the propensity for some animals to overeat and gain weight (fat) when given a palatable diet that is rich in calories. Selective breeding has shown that the DIO phenotype is not simply the result of a primary hyperphagia (45). Outbred DIO rats demonstrate reduced anorectic sensitivity to leptin (62, 65) and to insulin (15). Studies with selectively bred DIO rats confirm the reduced leptin sensitivity (45) and suggest that this is a preexisting defect and not related to the reduction of leptin transport that occurs upon development of obesity. Other investigators tested this conclusion by adrenoviral delivery of leptin gene into the third ventricle in DIO and diet-resistant (DR) rats (81). High-fat feeding reduced the responses to leptin in DR rats [as also for insulin sensitivity (15)] but abolished both anorectic and metabolic responses to leptin in DIO rats. Consistent with these findings, insulin injection resulted in rapid increases in circulating leptin in both DIO and DR rats but reduced food intake only in DR animals (70). An attempt to develop a mouse model of leptin resistance was complicated by strain- and sex-dependent responses and by thermoregulatory imperatives in singly housed mice (6, 36).

A major clinical problem in treatment of obesity is recidivism, the weight regain after dieting or food restriction that can exceed the amount lost during the period of restriction. A number of potential contributing factors have been addressed. A single 20-min restraint stress significantly altered subsequent responses of DIO, but not DR, rats. While intake of chow or high-energy diet was not altered by the stress, weight gain was reduced in DIO rats on chow and increased in DIO rats on high-energy diet (50). Both caloric restriction and exercise reduced food intake, body weight gain, and circulating leptin in male DIO rats; however, they had divergent effects on circulating insulin and on expression of orexigenic and anorexigenic hypothalamic neuropeptides (43). Exercising rats subjected to caloric restriction also displayed reduced, or at least delayed, weight gain when ad libitum feeding was restored (42). Diet-induced hyperphagia is greater in female than male rats, and attenuation of this hyperphagia by exercise is more complete in males (24). MacLean et al. (47, 48) studied outbred Wistar DIO rats, while Evans et al. (27) studied two other outbred strains—Long-Evans and Sprague-Dawley rats—that are susceptible to DIO. All three strains show enhanced metabolic efficiency during caloric restriction. All show rapid weight...
regain when caloric restriction is ended; however, they achieve this by different routes. Wistar and Sprague-Dawley rats maintain the increased metabolic efficiency and show relatively modest hyperphagia (27, 47, 48); Long-Evans rats correct the metabolic efficiency more rapidly but show prolonged hyperphagia (27). Studies in humans also show complex, and rather slow, compensation to altered energy expenditure and input. Compensation for an energy deficit was more robust than for an energy surplus although both were predicted to take 2–4 wk (75). As in the rats, compensation included adjustments of both energy intake and energy expenditure.

A number of recent papers in the journal have addressed issues of gustation—how the animal tastes what it is eating or drinking. Lipolysis of triglycerides by lingual lipase secreted into the mouth was shown to contribute to the tasting of fatty acids (72). Mice deficient in short-chain acyl-CoA dehydrogenase were shown by two-bottle preference tests to have normal acute orosensory response to a fat stimulus but altered postigestive responses (38). Interestingly, carbohydrates or fats delivered directly into the gut were shown to support a conditioned taste aversion, suggesting that nutrient tastes are detected in the intestine and recognized centrally (77). Certainly, jejunal infusion of linoleic acid specifically activated neurons in the paraventricular nucleus (PVN) of the hypothalamus (55). While systemic CCK also activated PVN neurons, the responses to the two stimuli were only weakly correlated. In humans, duodenal infusions of lauric acid (12 carbons) but not decanoic acid (10 carbons) reduced appetite, increased circulating level of glucagon like peptide-1 (GLP-1), and more strongly increased circulating CCK (28).

Sensing pathways for sweet substances are better understood than those for lipids. It was recently shown by microdialysis that orosensory stimulation by sucrose results in overflow of dopamine in the nucleus accumbens (35). The importance of this is that the nucleus accumbens is an important output site in reward circuitry that heavily affects ingestive behavior (29, 85). Interestingly, the Otsuka Long-Evans Tokushima fatty (OLETF) rat, which lacks CCK1 receptors and overeats to compensate, has increased sensitivity to the anorectic action of CCK (23). Conditioned taste aversion is an important survival mechanism when food may be spoiled, and experimental models are well developed in rats. With the increasing availability of genetically modified mice, it has become important to establish reliable taste aversion protocols in mice (64). One well known example of conditioned taste aversion is the response of rats to diets that lack threonine, an essential amino acid (67). Exogenous somatostatin increased consumption of this diet, perhaps through reduction of the size and number of taste buds in the tongue (67).

Of the factors that signal satiety, gastric distension and CCK released from the intestine in response to the presence of chyme are undoubtedly the best known and perhaps the best understood. Nevertheless a number of questions remain and CCK in particular has been the subject of many recent studies. Reidelberger and colleagues asked whether CCK was acting peripherally (e.g., by paracrine stimulation of vagal afferents) or centrally. They first showed that the effect of CCK to reduce gastric emptying required interaction with peripheral CCK1 receptors, and this likely involved a paracrine or neurocrine mechanism (59). Sham feeding was dose dependently inhibited by duodenal nutrient infusions, and the inhibition was attenuated by a CCK1 receptor antagonist that does not cross the blood-brain barrier, further implicating peripheral sites of action (58); similar results were obtained for true feeding (57). A subsequent study showed that abdominal vagotomy abolished the response to the impermanent antagonist, but not the response to another antagonist, devazapide, that does permeate the blood-brain barrier (61), suggesting a role in satiety for CCK1 receptors within the blood-brain barrier. The answer to their question was thus that both peripheral and central actions contribute to reduced feeding and that there is some degree of redundancy.

Given the multiple pathways known to contribute to satiation and satiety, interactions are to be expected. In humans, the effect of a low dose of CCK to reduce food intake was marginal. When combined with subthreshold gastric distension, however, food intake was substantially reduced (40). Interactions between CCK and the incretin GLP-1 were suggested in a study that reported that duodenal infusion of lauric acid increased circulating CCK and GLP-1 while reducing appetite (28). Infusion of CCK or of GLP-1 each inhibited feelings of hunger; however, a combined infusion caused a less than additive reduction of eating (33). In another study, both CCK and GLP-1 reduced gastrointestinal motility, although only CCK reduced hunger and eating (7). In neither study was there any indication of any synergistic action.

Neurons in several brain regions use GLP-1 as a neurotransmitter. When tested in fasted rats, microinjection of GLP-1 into PVN, hypothalamic ventromedial and dorsomedial nuclei, and lateral hypothalamus, but not into medial nucleus of the amygdala, reduced food intake (68). In the hindbrain, GLP-1-containing neurons are also found in the nucleus of the solitary tract. Activation of these neurons has been implicated in aversive pathways. Recently it was shown in rats that they are also activated by gastric distension within the physiological range, consistent with the view that they are involved in appetite control (79). Also consistent with this idea is the finding that anorexia induced by systemic administration of LPS is attenuated by delivery of the GLP-1 antagonist exendin (9–39) into hindbrain, but not forebrain (32). Interactions involving CCK and other peptides occur at both peripheral and central sites. Kobelt et al. (41) demonstrated a one-sided interaction between peripherally injected ghrelin and CCK; CCK blocked both the stimulation by ghrelin of food intake and activation of neurons in the arcuate nucleus while ghrelin did not alter CCK-dependent activation of neurons in the PVN and the nucleus of the solitary tract. Enterostatin selectively reduces fat intake in rats and acts at both peripheral and central sites. Both peripheral and central actions were shown to require activation of peripheral and central CCK1 receptors, respectively (34). Sensitivity to the anorectic action of CCK is significantly enhanced in mice deficient in the receptor tyrosine kinase c-Kit (12) and in mice having a knock-in of neurotrophin-4 (13). These two animals have complementary alterations of mechanosensors in the wall of the gastrointestinal tract. Another genetically modified mouse, this one lacking all dopamine β-hydroxylase, was used to show that the absence of norepinephrine does not alter the satiating effects of CCK (9).

Serotonin also participates in CCK actions as shown by the effects of ondansetron, an antagonist of serotonin type 3 receptors. Ondansetron attenuates the inhibition by CCK of gastric emptying and of true feeding, but not of sham feeding,
thus suggesting that these serotonin receptors contribute indirectly to satiation (37). A more direct, afferent role is suggested by the demonstration that ondansetron inhibits the anorectic response of CCK to duodenal infusion of carbohydrate by a postabsorptive, but preabsorptive mechanism (66). There are also sex-dependent differences in how serotonin affects food intake. The anorectic response to the serotonin releasing agent fenfluramine (intraperitoneal) is apparent at lower doses in female than in male rats. It is also of longer duration in females, and its magnitude is modulated by the estrous cycle, presumably by gonadal hormone status (25). Covasa and colleagues have addressed the contribution of \( N \)-methyl-D-aspartate (NMDA) receptors to the regulation of food intake. Whether administered intraperitoneally or into the fourth ventricle, an NMDA receptor antagonist increases food intake (18–20). This effect requires a peripheral cholinergic mechanism (18), is not due to interaction with CCK (19), and occurs through a mechanism independent of gastric emptying (20).

Amylin, which is co-secreted with insulin and reduces food intake, is another peripheral peptide that acts on brain orexigenic circuits. Intravenous infusion of an amylin antagonist substantially and dose dependently increased food intake in rats (60). Amylin is known to activate neurons in the area postrema (increased c-Fos expression) and subsequent pathways have also been explored. Lateral hypothalamic neurons appear to be a major target because either refeeding or exogenous amylin is able to reverse the elevation of c-Fos expression induced by food deprivation (63). Consistent with this result is the finding that orexin expression in lateral hypothalamus varies with circulating triglyceride levels (84). Another brain-gut peptide that may contribute to regulation of appetite is peptide YY (PYY). Benoit and Tschöp (3) have clearly and concisely discussed some of the divergent data and conclusions concerning the importance of PYY in regulation of energy balance. Careful dose-response studies performed in rats and using intravenous infusions of two naturally occurring variants of PYY [PYY-(1–36) and PYY-(3–36)] show that PYY-(3–36) is an order of magnitude more potent at reducing gastric emptying (11). Bolus injections in rhesus monkeys also reduced gastric emptying and food intake. However, in this case the responses were not sustained across successive days (51). Clearly the physiological roles of PYY are not yet understood.

Opioid pathways contribute significantly to appetite regulation. The nonselective opioid antagonist LY-255582 was given orally to DIO rats for 14 days. There was sustained reduction of weight gain associated with reductions of food intake and respiratory quotient, indicating stimulation of lipid use (73). Dual-injection studies revealed reciprocal, opioid-mediated communication between nucleus accumbens shell region and the ventral tegmental area, beginning the process of understanding a complex, distributed network (46). The parabrachial nucleus is a site of integration in food intake pathways, and opioid pathways therein have been implicated in feeding behavior. A recent study showed that activation of \( \mu \)- but not \( \kappa \)- or \( \delta \)-opioid signaling caused a robust hyperphagia (82). Neuropeptide FF (NPFF) and related peptides are regarded as endogenous modulators of opioid activity, and neurons that synthesize NPFF innervate the parabrachial nucleus. Injection into the parabrachial nucleus of small amounts of NPFF blocked stimulation of food intake by a \( \mu \)-opioid agonist at that site. In contrast injection of larger doses of NPFF stimulated food intake, although mechanisms remain unclear (52). The endocannabinoid pathway also strongly influences motivation to eat. Interestingly, a selective antagonist of the central cannabinoid receptor reduced weight in DIO mice, not only by reducing food intake, but also by an action to cause sustained increase in metabolic rate (56).

Central oxytocin contributes to a wide variety of behaviors, including inhibition of food intake. Microinjections at several hypothalamic sites of peptide histidine isoleucine reduced food intake and activated oxytocin neurons in the PVN (53). Blevins et al. (5) defined a descending pathway in which oxytocin neurons in parvocellular PVN that project to the nucleus of the solitary tract are activated by leptin. The result is increased sensitivity to peripheral satiety signals of neurons in the nucleus of the solitary tract. However, Mantella et al. (49) showed by use of an oxytocin knockout mouse that anorectic effects of systemic CCK and fenfluramine are intact in the absence of oxytocin. There is also current discussion concerning the exact role of oxytocin in regulating thirst and salt appetite (30, 74).

Neuropeptide Y (NPY) is of course a primary orexigenic signal within the hypothalamus. When neonatal rats received multiple intracerebroventricular injections of NPY, the females but not the males showed reduced weight gain out to 4 mo of age that was associated with markedly reduced NPY content in the arcuate nucleus and the PVN and with reduced food intake (78). The cause of the sex specificity of imprinting was unclear. Further evidence of the plasticity of hypothalamic NPY comes from studies of acutely food-deprived vs. chronically food-restricted rats. In this situation NPY expression was differently regulated in arcuate and dorsomedial nuclei and in the latter was not under control of leptin signaling (4). Photoperiod modulates food intake in many mammals. In sheep, increased food intake during long days was associated with increased NPY expression in the arcuate nucleus, but not with expression of other neuropeptides (14). Consistent with this, in Siberian hamsters the anorectic response to an agonist of melanocortin-4 receptors did not differ between long and short days (69). Ageing and senescence are times of important changes in regulation of food intake. Coppola et al. tested the prediction that reduced expression of NPY receptor subtypes underlay the anorexia of ageing. They found that receptor expression was preserved and suggested broader hypothalamic dysfunction as an explanation for the anorexia that accompanies terminal senescence, which is typically a period of \( \sim \)10 days (16). These studies were extended and showed that orexigenic responses to PVN injection of the GABA-A agonist muscimol also failed in senescent rats (17). Others have also reported reduced orexigenic response to NPY in old (although not senescent) rats (83). These authors, however, also showed enhanced orexigenic responses to AGRP in very old rats.

Prolactin releasing peptide is expressed in the hypothalamic dorsomedial nucleus and in the nucleus of the solitary tract. Single injections into the lateral ventricle reduce food intake and increase metabolic rate, although tachyphylaxis occurs in response to repeated injections over 3 days (26). Both the anorectic and metabolic responses require activation of corticotropin-releasing hormone receptors, but not melanocortin-4 receptors (42). Neurons that express orexins also have very restricted distribution in the lateral hypothalamus. It is clear that considerable integration occurs at the level of these neu-
rons. As noted above, orexin expression is fat sensitive (84). There is important activating input from the limbic system (85), and there is also clock and metabolic input (76) into the orexigenic signal conveyed by orexins. The peptides encoded from cocaine- and amphetamine-regulated transcript (CART) are most often thought of as hypothalamic, although CART is also expressed in brain stem areas involved in regulation of food intake. Infusion of CART peptide into the fourth ventricle reduced both gastric emptying and sucrose consumption (71). However, these two actions were only loosely coupled as the effect on gastric emptying, but not that on sucrose consumption, was blocked by corticotropin-releasing hormone.

A moment’s reflection suffices to show that there is considerable circadian periodicity in food intake. The master circadian clock resides in the suprachiasmatic nucleus and is primarily entrained by photoperiod through restricted feeding schedules can do so as well (10). Even if the suprachiasmatic clock is completely dysfunctional, scheduled feeding can generate a food-entrained oscillator (54). Such a food-entrained oscillation is reflected in neuron activation patterns (c-Fos) in a variety of hypothalamic and hindbrain sites (1, 2). In the hypothalamus these patterns persisted through 3 days of fasting and were interpreted to be central to food-entrained oscillation (1). In contrast those in the hindbrain did not persist through fasting and were interpreted to arise from peripheral inputs (2). Suffice it to say that the physiological regulation of appetite is a particularly rich area of research and that work published in the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology is contributing largely to answering many of the important questions that have been posed and that continue to be thrown up.

REFERENCES

31. Fox EA and Byerly MS. A mechanism underlying mature-onset obesity: evidence from the hyperphagic phenotype of brain-derived neurotrophic factors.


