Protein, amino acids, vagus nerve signaling, and the brain1–4

Daniel Tomé, Jessica Schwarz, Nicolas Darcel, and Gilles Fromentin

ABSTRACT
Dietary protein and amino acids, including glutamate, generate signals involved in the control of gastric and intestinal motility, pancreatic secretion, and food intake. They include postprandial meal-induced visceral and metabolic signals and associated nutrients (eg, amino acids and glucose), gut neuropeptides, and hormonal signals. Protein reduces gastric motility and stimulates pancreatic secretions. Protein and amino acids are also more potent than carbohydrate and fat in inducing short-term satiety in animals and humans. High-protein diets lead to activation of the noradrenergic-adrenergic neuronal pathway in the brainstem nucleus of the solitary tract and in melanocortin neurons of the hypothalamic arcuate nucleus. Moreover, some evidence indicates that circulating concentrations of certain amino acids could influence food intake. Leucine modulates the activity of energy and nutrient sensor pathways controlled by AMP-activated protein kinase and mammalian target of rapamycin in the hypothalamus. At the brain level, 2 afferent pathways are involved in protein and amino acid monitoring: the indirect neural (mainly vagus-mediated) and the direct humoral pathways. The neural pathways transfer preabsorptive and visceral information through the vagus nerve that innervates part of the orosensory zone (stomach, duodenum, and liver). Localized in the brainstem, the nucleus of the solitary tract is the main projection site of the vagus nerve and integrates sensory information of oropharyngeal, intestinal, and visceral origins. Ingestion of protein also activates satiety pathways in the arcuate nucleus, which is characterized by an up-regulation of the melanocortin pathway (α melanocyte-stimulating, hormone-containing neurons) and a down-regulation of the neuropeptide Y pathway. Am J Clin Nutr 2009;90(suppl):838S–43S.

INTRODUCTION
Dietary protein and amino acids, including glutamate, are believed to generate signals involved in the control of gastric and intestinal motility, pancreatic secretion, and food intake. According to current understanding, multiple and redundant signals associated with nutrients, gut neuropeptides, and hormones activate specific areas in the brain either indirectly through vagus-mediated pathways or directly after their release into the peripheral blood (Figure 1). However, the precise mechanisms involved in these signaling processes for protein and amino acids from gut to brain are not completely understood.

PROTEIN AND AMINO ACIDS GENERATE SIGNALS ACTING ON GUT PHYSIOLOGY AND FOOD INTAKE
Protein is known to reduce gastric motility and to stimulate pancreatic secretion by a mechanism mediated through cholecystokinin. This leads to an increase in the proportion of pancreatic enzymes as proteases, presumably to increase protein digestion. It has been shown, for example, that the [106–169] fragment of milk κ-casein dose dependently stimulates pancreatic secretion by a cholecystokinin-dependent mechanism (1). It is also established that protein and amino acids are more potent than carbohydrate and fat in inducing short-term satiety in animals and humans. The quantity of protein or amino acids in the meal or diet is determinant. Increasing dietary protein usually reduces energy intake in animals and humans. This effect is not due to a conditioned taste aversion.

There is a control of protein ingestion and, when given the opportunity, animals usually select a relatively constant amount of protein and percentage of protein energy in their diet. Despite the low palatability of a high-protein diet, adult rats allowed to self-select macronutrients increase their protein intake to 35–50% energy, reduce total energy intake, and do not show aversive behavior. Interestingly, it seems that the spontaneous amount of protein ingestion does not correspond to the minimal percentage of protein energy (10–12% in the adult rat) required for nitrogen balance, but is usually much higher, and varies with a multiplicity of factors, including age, physiologic state, and the type of food. This suggests that different, complex, and redundant mechanisms are involved in the control of protein ingestion (2–4).

Over the long term, the ingestion of a high-protein diet most frequently promotes satiety, facilitates weight loss, and improves body composition. A high-protein diet is usually associated with a decrease in food intake in rats and human (5–8). When rats previously adapted to a diet providing normal amounts of protein are offered a high-protein diet, they immediately reduce total food intake, and then progressively increase intake on succeeding days, although notably not to the amount before presentation of the high-protein diet (9). A decrease in food intake has also been observed in rhesus monkeys fed a high-protein diet (10). This initial decrease in the intake of the high-protein diet has been suggested to arise from the palatability, the time required for metabolic adaptation, or the satiating effect of the macronutrient.

1 From the AgroParisTech (DT, JS, and ND) and INRA (GF), UMR914 Nutrition Physiology and Ingestive Behavior, Paris, France.
3 Supported by AgroParisTech, INRA, and the EEC Marie Curie Research Program NuSisCO.
4 Address correspondence to D Tomé, AgroParisTech, 16 rue Claude Bernard, 75005 Paris, France. E-mail: tome@agroparistech.fr.
First published online July 29, 2009; doi: 10.3945/ajcn.2009.27462W.

838S
Despite previous hypotheses, it is unlikely that a high-protein diet induces a conditioned taste aversion (ie, makes the animal sick) (11).

It is currently established that under most conditions protein is more satiating than the isoenergetic ingestion of carbohydrate or fat in animals and humans (12–14) and that a high-protein diet most frequently improves weight loss and body composition (5, 15). A high-protein preload was shown to increase the delay between the preload and the test meal compared with a high-fat or a high-carbohydrate preload, respectively (13, 16). A high-protein preload was also shown to lower the ad libitum intake of a test meal in comparison with a high-carbohydrate preload and was independent of the interval between the preload and the test meal (30 or 120 min) (14). In contrast, a high-protein breakfast compared with a high-carbohydrate breakfast did not modify the intake of a test meal served 3 h later (17).

PROTEIN AND AMINO ACIDS GENERATE PRE- AND POSTABSORPTIVE SIGNALS

Proteins and amino acids are very likely to generate preabsorptive signals while still in the digestive tract. Chemo-receptors located in the small intestine detect luminal nutrients (carbohydrates, peptides, amino acids, fatty acids, triacylglycerols) and trigger the release of gut hormones from mucosal enteroendocrine cells in response. These hormones act on gut vagal receptors or are released into the blood and reach the central nervous system. Absorbed amino acids can also induce metabolic signal produced in the periphery or directly in some specific brain area.

Dietary proteins and amino acids induce the release of the anorexigenic gut hormones cholecystokinin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY); the involvement of ghrelin, however, remains uncertain (18, 19). Cholecystokinin is released in response to the presence of protein and fat in the duodenum and has a well-established peripheral role in digestion, causing gallbladder contraction and the secretion of pancreatic enzymes. It is also the first gut hormone shown to influence food intake. In the periphery, cholecystokinin directly modulates exocrine pancreatic secretion and possibly gastric motility. In addition, the proximity of vagal afferent axons and cholecystokinin-secreting cells in the gut supports the idea that cholecystokinin released from enteroendocrine cells acts on local vagal sensory fibers. By that route, cholecystokinin could act on gastric motility through a vago-vagal loop and also stimulate satiety through low-affinity vagal cholecystokinin receptors that signal the brain. Indeed, cholecystokinin is known to inhibit food intake, mediating its anorexigenic effect mostly by cholecystokinin type 1 receptors on the vagus nerve, which in turn project into the brainstem (20–22). GLP-1 is also an important inhibitor of food intake that seems to be involved in protein and amino acid signaling (18, 23, 24). The vagus nerve is important in mediating the anorectic effects of GLP-1, because vagotomy abolishes its effect on food intake.
Furthermore, PYY release is stimulated by dietary protein and amino acids in rodents, but it probably acts directly on the hypothalamic arcuate nucleus (ARC) to induce satiety (25).

Metabolic signals are also suspected to be involved in protein and amino acid signaling. It has been suggested that elevated concentrations of blood or plasma amino acids are directly detected by specific areas of the hypothalamus. Intracerebroventricular administration of leucine or an increase in dietary leucine reduces food intake and body weight and improves glucose and cholesterol metabolism in rats and mice (26, 27). This effect appears to be specific to leucine, because leucine alone has the same effect on food intake as a mixture of amino acids (28). Other metabolic signals, including an increase in energy expenditure and the production of glucose through gluconeogenesis, have been also hypothesized to mediate the effects of dietary protein and amino acids on food intake. It also appears that, in humans, protein stimulates diet-induced thermogenesis to a greater extent than other macronutrients (29). This is due for a part to the utilization of $^{31}P$ bonds to incorporate each amino acid into protein. The de novo synthesis of glucose in the liver from gluconeogenic precursors, including amino acids, is stimulated by a high-protein diet in the fed state (30). This process could be involved in the satiating effect of protein through a modulation of glucose homeostasis and glucose signaling to the brain. The main gluconeogenic organ is the liver, and liver gluconeogenesis is probably the main site of glucose production from amino acids, whereas intestinal gluconeogenesis, previously hypothesized as an alternative mechanism, remains to be confirmed (30–34).

**VAGUS-MEDIATED ACTIVATION OF NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT BY PROTEIN AND AMINO ACIDS**

The vagus nerve responds to luminal and hepato-portal signals, including nutrient and non-nutrient substances. Protein, peptides and amino acids including glutamate are believed to elicit a visceral vagus-mediated activation of neurons in the nucleus of the solitary tract (NTS). Protein and amino acids infused into the duodenum activate vagal afferents in the rat, mainly through
a cholecystokinin-dependent mechanism (Figure 2) (35). First, protein and amino acids in the intestinal lumen induce the secretion of gut neuropeptides (cholecystokinin, GLP-1), which act on vagus nerve receptors. Nutrients are also sensed by hepatoportal sensors (amino acids, glucose).

Amino acid sensors in the duodeno-intestinal and hepatoportal regions have been shown to increase or decrease the activity of hepatic vagal afferent fibers, depending on the amino acid (37–40). Signals are passed along hepatic vagal afferent fibers to food intake-regulating areas of the brain. Individual amino acids appear either to excite or inhibit vagal hepatic afferents (41–43). Excitatory amino acids include L-alanine, L-arginine, L-leucine, L-lysine, L-serine, L-tryptophan, L-valine, and monosodium glutamate. Inhibitory amino acids include L-cysteine, L-glycine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, and L-threonine. Glutamate sensors are also found in the oral cavity, the intestinal wall, and the gastric wall. The infusion of monosodium glutamate into the stomach, duodenum, and portal vein increases afferent activity in the vagal gastric, celiac, and hepatic nerves, suggesting the existence of glutamate sensors in the gastric wall, intestinal wall, and hepatoportal region (44). Their activation induces reflex activation of efferent discharges in the vagal gastric and pancreatic nerve.

The involvement of vagal afferent pathways in protein sensing and signaling to the brain is supported by the finding that intraduodenal protein activates vagal afferent fibers and that high-protein feeding induces c-Fos expression in neurons within the NTS (20–49). The decrease in food intake observed after a high-protein load (compared with a normal-protein load) results from the activation of the noradrenergic neurons in the NTS which are related to cholecystokinin-induced anorexia (46). In contrast, neurons expressing GLP-1 in the NTS are not activated, which is consistent with the fact that protein-induced satiety is not associated with aversive behavior and conditioned taste aversion (11, 46).

BLOOD-MEDIATED ACTIVATION OF NEURONS IN THE ARC BY PROTEIN AND AMINO ACIDS

However, subdiaphragmatic vagotomy (47) and hepatic portal vein vagal deafferentation (Figure 3) do not block the reduction in food intake in rats induced by the ingestion of a high-protein diet. Hence, nonvagal signals are also involved in the behavioral responses to dietary amino acids and protein. Information from peripheral tissues and organs is centralized in the hypothalamus; the hypothalamus is a key brain region in food intake regulation and energy homeostasis (48, 49). Protein ingestion activates ARC satiety pathways, which include an up-regulation of melanocortin [α-melanocyte-stimulating hormone (α-MSH) containing] neurons and a down-regulation of neuropeptide Y (NPY) neurons. When a high-protein meal is ingested, eg, the number of ARC neurons expressing both c-Fos (a marker of neuronal activation) and α-MSH increases (46). At the same time, the number of neurons expressing c-Fos that lack α-MSH declines (46). Some of the latter ARC neurons could be NPY neurons.

The signals that modulate these pathways remain unclear. Amino acid-sensitive neurons have been detected in the lateral hypothalamus. A critical role for PYY neurons in protein-mediated satiety and body-weight regulation has also been proposed (25). This effect is mediated through the activity of the AMP-activated protein kinase (AMPK) and the mammalian target of rapamycin (mTOR) (50). High-protein diets influence AMPK and mTOR in neurons in the ARC and the hypothalamic paraventricular nucleus. AMPK and mTOR may have overlapping and reciprocal functions (26, 51). The activation of mTOR and the suppression of AMPK phosphorylation seem to modulate hypothalamic neuropeptides, with a decrease in orexigenic neuropeptides such as NPY and agouti-related peptide, and an increase in the expression of the anorexigenic peptide α-MSH (28, 50).

CONCLUSIONS

Complex pathways are involved in protein and amino acid signaling to the brain. The signals to the brain associated with the ingestion of amino acids and other energy-providing nutrients originate from the visceral and metabolic processes and involve both indirect (mainly vagus mediated) and direct (plasma concentrations of nutrients and hormones) pathways. Gut hormones (cholecystokinin, GLP-1, and PYY) presently are major signaling candidates, acting either indirectly by activation of the vagus pathway (cholecystokinin, GLP-1) or directly at the level of the hypothalamus (PYY). Amino acids are also probably directly involved in signaling the vagus pathway in the hepatoportal area and the ARC in the hypothalamus. Other signals related to amino acid metabolism include gluconeogenesis and a possible increase in energy expenditure. (Other articles in this supplement to the Journal include references 52–80.)

The authors’ responsibilities were as follows—DT: wrote the original draft of the manuscript, and participated in the protocols reported in Figures 2 and 3; ND and JS: contributed to the preparation and editing of the manuscript, and performed the experiments summarized in Figure 2; and GF: contributed to the preparation and editing of the manuscript, and participated in the protocols reported in Figures 2 and 3. The presenting author’s (DT) travel expenses associated with participation in the symposium and an honorarium were paid by the conference sponsor, the International Glutamate Technical Committee, a nongovernmental organization funded by industrial producers and users of glutamate in food. None of the authors had a conflict of interest.

REFERENCES


60. San Gabriel A, Maekawa T, Uneyama H, Torii K. Metabotropic glutamate receptor type 1 in taste tissue. Am J Clin Nutr 2009;90(suppl):743S–6S.
68. Donaldson LF, Bennett L, Baic S, Melichar JK. Taste and weight: is there a link? Am J Clin Nutr 2009;90(suppl):800S–3S.
70. Blachier F, Boutry C, Bos C, Tomé D. Metabolism and functions of γ-glutamate in the epithelial cells of the small and large intestines. Am J Clin Nutr 2009;90(suppl):814S–21S.
77. Stanley CA. Regulation of glutamate metabolism and insulin secretion by glutamate dehydrogenase in hypoglycemic children. Am J Clin Nutr 2009;90(suppl):862S–6S.
78. Hawkins RA. The blood-brain barrier and glutamate. Am J Clin Nutr 2009;90(suppl):867S–74S.
80. Fernstrom JD. Symposium summary. Am J Clin Nutr 2009;90(suppl):881S–5S.