Mechanisms of Food Intake Repression in Indispensable Amino Acid Deficiency

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Abstract

Animals reject diets that lead to indispensable amino acid (IAA) depletion or deficiency. This behavior is adaptive, as continued IAA depletion is incompatible with maintenance of protein synthesis and survival. Following rejection of the diet, animals begin foraging for a better IAA source and develop conditioned aversions to cues associated with the deficient diet. These responses require a sensory system to detect the IAA depletion and alert the appropriate neural circuitry for the behavior. The chemosensor for IAA deprivation is in the highly excitable anterior piriform cortex (APC) of the brain. Recently, the well-conserved general AA control non-derepressing system of yeast was discovered to be activated by IAA deprivation via uncharged tRNA in mammalian APC. This system provides the sensory limb of the mechanism for recognition of IAA depletion that leads to activation of the APC, diet rejection, and subsequent adaptive strategies.

Contents

INTRODUCTION

Anterior piriform cortex (APC): located in the anterior ventro-lateral forebrain, the APC houses the chemosensor for IAA deficiency, also known as the area tempestas

To survive, all organisms must maintain a full complement of the amino acids (AAs) that are the precursors for protein synthesis. Nearly half of the AAs present in protein cannot be synthesized or stored in metazoans because the genes for their synthesis were lost early in evolution (41); these are the essential or dietary indispensable amino acids (IAAs). The inability of incomplete proteins (i.e., those missing or having inadequate IAA balance in the food) to support human health was appreciated as early as the 1800s (14). For generalist herbivores and omnivores, rejection of an inadequate diet and selection (of a complete food or at least one containing a complementary IAA profile) must provide a full supply of IAA in a timely fashion or general protein synthesis is halted and degradation exceeds synthesis (62). The time course for repletion via complementation varies with the limiting IAA, but it is short. Degradation of protein in brain begins within 2 h in animals prefed a basal (6%–8% crude protein equivalent) diet and then provided a diet missing a single IAA (41, 78).

When animals are given an IAA-deficient or imbalanced diet, they fail to grow because they simply won't eat the diet. That the growth failure is due to rejection of the diet, rather than toxicity, was shown in the 1930s (33, 34, 41, 49, 50, 81, 94, 95).

The behavioral responses to differing IAA proportions in the diet have been well studied (3, 33, 34, 36, 37, 41, 49, 50, 65, 92, 94, 95). Rejection of a test diet and dietary choice in laboratory animals continue to be useful nutritional tools for evaluating protein quality and IAA balance.

BEHAVIORAL RESPONSES

Rejection of IAA-Deficient Food

The behavioral strategies for dealing with limiting amounts of IAA include meal termination, altered food choice, foraging for foods that will complement or correct the deficiency, development of a learned aversion to a deficient or imbalanced food in order to avoid that food in the future, and memory for the taste, smell, or place associated with repleting food (24, 28, 29, 33, 34, 40, 65–68, 84, 102). The first three of these strategies will help the animal obtain a complete meal only if they occur as a consequence of sensing the IAA deficiency within that meal. The last two are adaptive in the longer term and are associated with learning, subsequent to the sensing of the deficiency and termination of the meal.

Many of the parts are in place to complete the puzzle of how IAA deficiencies affect food intake. Neural links from the chemosensory brain area (anterior piriform cortex, APC) to the circuits for food intake and learning are known (1, 44) (see below). The brain circuits associated with the motor behaviors that

control feeding have been reviewed (9, 13). Thoroughly documented models are available for "conditioned taste aversion" (24, 28, 29, 32–34, 40, 111) and "long-term potentiation" (28, 86), for the learned aversions and memory for place, respectively. Here we address the mechanisms that provide sensing of the IAA deficiency (36, 41, 48, 80) and activation of the neurons in the APC (36, 97–100).

Dietary Selection and Food Choice

The most parsimonious method of obtaining a balanced profile of IAA in the diet is available to carnivores, because each meal contains animal-source foods with all the IAA required for maintenance and growth. Where animal-source foods are not available, or there are cultural histories of famine or vegetarian cultural food preference, complementary nonanimal-source proteins, such as rice and beans, are used routinely by omnivores. These dietary practices in humans predate the discovery of IAA (41), and animals including birds, pigs, and rodents select appropriate complimentary proteins (41, 65). In rats, the threshold for sensing IAA, with a variety of limiting IAA, is in the range of 90–120 ppm (54, 55), showing exquisite sensitivity to IAA depletion. If rats are given even a limited choice, as with near-basal levels of lysine, the more adequate diet is chosen at lysine levels that do not decrease food intake (55). Therefore, given a choice, rats will switch diets rather than stop eating. Because they continue eating an alternative food, it is safe to assume that they are neither sick nor satiated. A constructive behavioral response is seen when animals reject the deficient food and, in the absence of another food source, begin foraging. This is seen as increased locomotor activity and digging in the food cup with increased spillage, and begins rapidly as well (65). With repletion, a rapid rate of eating continues to satiation, seen as the satiety sequence: a set of behaviors including grooming and sleep that correlate with the end of a normal meal (59).

IAA DEFICIENCY

Indispensable amino acid (IAA)–deficient diets are associated with increased seizure susceptibility in animals [reviewed in (98)]. It is clear that the highly sensitive APC is activated by acute IAA deficiency. Given the ongoing oscillatory activity of the APC (26), the excitatory effects of signaling systems such as CaMKII (100) and ERK (97, 99), and the paucity of inhibitory elements (23, 98, 105), the findings reviewed here may account for the potentiation of the APC in response to IAA deficiency. Some seizure disorders seem intractable and seizure patients may have occasional unexplained breakthrough seizures. Because the APC can be activated within the duration of a single IAA-deficient meal, these findings offer the novel suggestion that people who suffer from poorly controlled seizure disorders might be advised of the potential seizure risk from IAA-imbalanced meals. This important clinical question deserves further study.

The Time Course for Sensing IAA Deficiency

In the absence of a choice, rats stop eating an IAA-deficient meal before they are satiated, but not so quickly that the rejection could be on the basis of taste. In one Skinner box study, the fastest-responding rat stopped bar pressing for threonine-devoid pellets in 28 min (37). Using computerized online meal-pattern analysis and a threonine-devoid diet, Koehnle and colleagues found that 90% of the rats eating an IAA-deficient meal stopped eating in 20 min, whereas 50% of the control rats continued eating their balanced meal (66–68). These meal terminations are not due to satiety, as evidenced by the absence of the satiety sequence (24).

In a choice situation, the deficient diet was rejected in 15 to 30 min (37). Within five days of exposure to a lysine-deficient diet, groups of rats locate the bottle containing a bitter solution of lysine HCl among 15 bottles containing various solutions. As long as the rats remain lysine deficient, they continue to ingest enough lysine HCl solution to replete their lysine stores (83, 103, 104). Very soon

Conditioned taste aversion: single-trial long-term learning, associating malaise with a flavor, i.e., smell and/or taste, also known as bait shyness or the Garcia effect

CaMKII: calcium calmodulin dependent protein kinase II. The phosphorylated form, CaMKII-P, is the active form

tRNA: transfer ribonucleic acid

after the rats are given a diet containing adequate lysine, they switch their fluid intake to a preferred solution (83). These results replicate earlier studies with histidine HCl in solution (95). Similarly, rats rapidly increase their rate of eating within 30 min when switched from an IAA-deficient or imbalanced diet to a complete diet (94). Thus, the time to sensing IAA repletion is less than 30 min. The evidence is clear that the biochemical and neurobiological mechanisms for IAA sensing must be studied in the very short term, i.e., less than 30 min.

THE CHEMOSENSOR FOR IAA DEFICIENCY

The Anterior Piriform Cortex in the Brain

Evidence that the chemosensor for IAA deficiency is not in the gastrointestinal tract and does not involve the classical chemical senses of either taste or smell has been thoroughly reviewed (33–36, 41, 50, 94, 95). Rather, the involvement of the brain in responding to IAA depletion was first suggested by the finding that, after an IAA-imbalanced meal, the limiting IAA decreased in brain tissue as rapidly as it did in the plasma (90). Also, infusions of the limiting IAA reverse the feeding response to an IAA-imbalanced diet at a much lower concentration in the carotid artery than in the jugular vein (72). Using the classical brain-lesioning techniques that were state of the art in the 1960s, Leung and Rogers destroyed a series of brain areas associated with feeding and the food selection circuitry (33, 74, 95); the chemosensor for IAA depletion was found in the very rostral brain area called the anterior prepyriform cortex, now termed the anterior piriform cortex (APC) (73). Rats with lesions in the APC fail to reject IAA-deficient diets (73). Subsequently, this finding was replicated in the rat (87) and in the bird (25). Later microinjection and histochemical studies provided confirmatory results (10, 82, 96, 99).

THE MECHANISM

The question arising from the various behavioral observations was, "The responses are rapid and reliable, but how do the animals know the diet is deficient?" Several important transcriptional effects of AA depletion are known to occur in a matter of a few hours, and have been the subject of an excellent recent review (63). However, it is highly unlikely that gene transcription activates neuronal signaling within a half hour.

General Amino Acid–Control Pathway

The biochemical pathway in the APC that serves as the sensory limb in an IAA-detection system is conserved across eukaryotic species (48) and is activated within the requisite 20-min period for identification of the IAA sensor. The initial event, after ingestion of an IAA-imbalanced or -deficient diet, is a decrease in the concentration of the limiting IAA in the APC; the limiting IAA is decreased in APC tissue by 56% at 21 min (67) (**Figure 1**).

In the earliest steps leading to the initiation of mRNA translation, AAs are acylated (charged) to transfer ribonucleic acid (tRNA) by their cognate amino acyl tRNA synthetases. Although the dispensable AAs are by definition continuously available, in IAA depletion, the cognate tRNA may be deacylated (uncharged). The abundance of deacylated tRNA in vivo is controversial because the Kms for the amino acyl synthetases are very low (101). Dennis and colleagues (19) reported no change in deacylated tRNA after AA deprivation in serum-starved, transformed HEK293 (kidney) cells. However, experimental differences such as timing (78) may affect the results. A deficient meal is rejected by 20 min after first introduction of the diet, so the initial signal must have occurred by 20 min if it is to relate to the signal for meal termination. Differences due to tissue source (e.g., kidney versus neuron) are highly likely as well;

even in neural tissue, the responsive cells are in a very restricted area of the APC, falling in a rostro-caudal segment of the ventrolateral forebrain extending less than 1 mm in layer II of the APC (99).

A role for uncharged tRNA in sensing IAA deficiency by animals was shown directly by microinjecting nmol amounts of tRNA synthetase inhibitors (amino alcohols) precisely into the rat APC and observing the behavioral response (36, 41, 48). Amino-alcohols inhibit their respective amino-acyl-tRNA synthetases, increasing the concentration of deacylated tRNA (45). The effects of L-amino alcohols are stereospecific, competitive, and selective for their respective AA (36, 41, 48). Injection of nmol amounts of L-threoninol or L-leucinol, but neither D-threoninol nor a dispensable AA, into the APC decreases food intake at 20 min, the same as eating an IAA-devoid diet. These are the reciprocal of studies in which nmols of the limiting IAA, microinjected into the APC, restore feeding of an IAA-deficient or -imbalanced diet (10, 82, 96).

The next step in the yeast GCN pathway is the activation of the GCN2 kinase (52), which dimerizes and autophosphorylates when uncharged tRNA binds to a nonselective histidine tRNA site (HisRS; 88, 110). When a threonine-devoid diet is fed to naïve mice lacking the gene encoding the GCN2 kinase (*GCN2*$^{-/-}$ mice), they continue eating this IAA-depleting diet (48) while naïve intact mice reject it. Maurin and colleagues (80) confirmed this finding in their report that mice having a brain-specific deletion of *GCN2* also fail to reject an IAA-deficient diet and apparently do not develop the usual learned aversion to the IAA-depleting diet. Thus, conditioned aversion to an IAA-depleting diet may also be affected in the knockout mice. Of course, if the animals don't recognize that there is something amiss with the diet, they are unlikely to develop an aversion to it. In accord with this, lesions of the hippocampus, an area involved in learning, interfere with learning in rats fed IAA-imbalanced diets; the expected conditioned taste aversion is delayed (71). Other brain areas associated with IAA deficiency and aversion learning have been studied with similar results (34).

The activated kinase, GCN2-P, phosphorylates the alpha subunit of eukaryotic initiation factor 2 (eIF2α) (20, 109), a pivotal factor in the control of the initiation of translation in protein synthesis (5, 6, 109). The intact mouse not only rejects a threonine-devoid diet, but also responds by increasing the phosphorylation of eIF2α in APC neurons (48, 80), as does the rat (42). In contrast, the *GCN2*$^{-/-}$ mice neither reject the diet nor phosphorylate eIF2α (36, 41, 48).

GCN2 has interactions with the target of rapamycin (TOR) and its mammalian form (mammalian target of rapamycin; mTOR) in several models (5, 15, 53, 58). Not all cell types regulate mTOR through charging levels of tRNA, as some are resistant to activation by amino alcohols (93). To our knowledge, mTOR, despite its role in sensing rich sources of leucine and other nutrients in brain (18), has not been shown to have a direct role in detection of IAA deficiency (36). Still, IAA depletion, by limiting the mTOR agonists, could reduce its activation via a number of different pathways (113). To address this question, Hao and colleagues (46, 85) injected rapamycin into the APC and saw no effect on intake either of a control (basal) or IAA-deficient (threonine-devoid) diet from 20 min to 21 h after diet introduction. Therefore, the feeding response to IAA deficiency in the rat is not sensitive to rapamycin. Still, a rapamycin-insensitive mTOR complex (mTORC2) could be involved in the APC's responses to IAA deficiency, as mTORC2 is associated with the cytoskeleton and an actin-binding protein, IMPACT, inhibits activation of GCN2 (91). As noted above, mTOR is activated in the hypothalamus by injections of leucine; the feeding result is an inhibition of food intake by 4 h (18). Because mTOR is activated by many nutrients and energy sources, including glucose

General amino acid nonderepressing kinase 2 (GCN2): the eIF2α kinase activated by deficiencies of IAA, via uncharged tRNA

GCN2$^{-/-}$ mice: transgenic mice lacking the gene for GCN2

eIF2α: eukaryotic initiation factor 2 alpha. The phosphorylated form, eIF2α-P, is activated by phosphorylation on the alpha subunit at serine 51

mTOR: mammalian target of rapamycin

mTORC1/2: mammalian target of rapamycin complexes 1 and 2

GABA: gamma amino butyric acid

eIF2α-P: blocks initiation of translation at the formation of the ternary complex

Methylated amino-isobutyric acid (MeAIB): the classical substrate and indicator of System A amino acid transporter activity

Sodium-dependent neutral amino acid transporter (SNAT): formerly called ATA, System A amino acid transporter

and ATP, but in some models it is unaffected by AA deprivation (19), mTOR seems unlikely to be activated by depletion of a single IAA. However, the absence of mTOR's kinase activities such as phosphorylation of ribosomal proteins (4) in IAA deficiency could initiate signaling in other pathways. Alternatively, because other IAAs are increased, at least relatively, when one is depleted as in IAA imbalanced or single-IAA-devoid diets, the imbalance could cause activation of mTOR and the resulting decrease in food intake. This question remains open inasmuch as a role for mTOR no longer can be ruled out by rapamycin insensitivity.

Taken together, the evidence suggests a clear pathway for the first four steps in the mechanism for sensing IAA deficiency in the APC (**Figure 1**). Several attractive hypotheses have the potential to explain step 5, neuronal depolarization. Still, the precise mechanism for activating APC neurons in IAA deficiency has not yet been determined.

Biochemical Events Downstream of eIF2α Phosphorylation

Excitability of the APC. As a member of the olfactory cortical system (43), the APC is continually being activated by each breath in the respiratory rhythm, apart from its role in olfaction (26). Although destruction of the APC abolishes the animal's ability to reject an IAA-deficient diet (73), olfactory bulbectomy does not (70), so the IAA sensor in the APC is not dependent on the sense of smell. Another aspect of the APC, in its role as the IAA chemosensor, is that this brain area has the lowest threshold of any area in the brain for chemical stimulation by GABAergic antagonists (21, 22, 31). Indeed, its alternative name is the area tempestas (23). Such sensitivity is likely because the APC contains a paucity of inhibitory elements (23) and recurrent excitatory circuitry (43). This combination of neuronal elements sets up the probability for easy activation of the APC (61). A variety of signaling pathways affected by eIF2α and related kinases in the responses to IAA deficiency could be involved in exciting this highly sensitive brain area.

Transporter activation. The effects of IAA limitation on AA transporters have been very well reviewed (16, 57, 63, 64, 76, 89). A role for AA sensing also has been proposed for a calcium receptor (17). Although this receptor responds stereospecifically to most of the standard L-AA that are used in protein synthesis, selectivity for any particular IAA cannot be assumed. The cationic AA transporters are inducible by limitation for any IAA and not by dispensable AA, but there is a delay of 2 h for induction and a dependence on phosphorylation of eIF2α, suggesting that synthesis of new transporters is involved (51). Such delays and dependence on eIF2α-P indicate that these transporters are downstream from IAA sensing, although they help remediate the IAA deficiency in response to other signals by importing limiting IAA into the cell.

The classical system A amino acid transporter family is sodium dependent and was defined using 2 (methyl-amino) isobutyric acid (MeAIB) (11, 51, 76, 89). Recent studies have cloned and renamed the System A transporters (formerly ATA) the sodium-dependent neutral amino acid transporters SNAT1, 2, and 4. Both SNAT1 and 2 are found in brain (76). The IAA-sensitive cells in APC are the glutamatergic pyramidal cells (61) and so may use either SNAT1 or 2 as their System A transporters. Although threonine is not the preferred substrate for either SNAT1 or SNAT2, it is carried by System A (2).

MeAIB-blockable transport of labeled threonine is activated within 10 min after exposing neuron-rich cultures from the IAA-sensitive APC to a threonine-devoid medium (11, 36, 41). This rapid activation is likely due to recruitment rather than gene expression (75). Consistent with this finding, the rapid MeAIB-sensitive uptake of threonine in threonine-deficient APC neurons is

dependent on phosphorylation for activation and movement to the membrane, placing it downstream of eIF2α-P (11). Increased translation of mRNA for SNAT2 in mouse embryonic fibroblasts deprived of IAA for 1 h also depends on phosphorylation of eIF2α (30). Of interest here, the increased intracellular sodium from cotransport by system A [either SNAT1 or SNAT2 (ATA1 or ATA2)] is electrogenic (2, 114), so it can depolarize a neuron, such as the glutamatergic output cells of the APC.

MAPK/ERK1/2 signaling. If the neurons of the APC are activated by increased intracellular sodium via the SNATs (11, 30), which require activation by eIF2α-P (30), then mitogen-activated protein kinase (MAPK)/ERK1/2 (27) should be involved as well. The cells colocalizing MAPK-P and eIF2α-P appear in a narrow (<1 mm) rostrocaudal segment of the cell body layer of the pyramidal output neurons of the APC (99). These MAPK-P + eIF2α-P-positive neurons may be the primary sensory cells that recognize IAA deficiency in the APC. In addition, the ERK1/2 signaling system is associated with neuronal excitation, giving another mechanism for excitation of signaling in the APC (97, 99). An APC neuron positive for ERK1/2 antibodies may be seen in **Figure 2**.

Glutamatergic activity. Glutamate is the transmitter of the primary output cells of the APC (61). Releasable glutamate could be increased in the cells by transamination (56) of the IAAs that are in relative excess with the relative imbalance and that cannot be used for protein synthesis because translation is inhibited in the presence of eIF2α-P (4, 5, 109). Other sources of glutamatergic activity include the glutamate-glutamine cycle, which provides glutamate for neural transmission (56). Evidence supports the importance of glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors in the behavioral and biochemical responses to IAA deficiency, secondary to the initial signal in the APC (39, 47, 100). When rats are injected with an AMPA receptor antagonist 1 h prior to receiving an IAA-deficient meal, they do not reject the meal, as do saline-injected rats (47). Therefore, metabolic activation of the glutamate output cells of the APC after ingestion of an IAA-deficient diet also has potential importance for signaling IAA depletion.

Intracellular calcium. In addition to sodium, ERK1/2, and glutamate, both intra- and extracellular sources of calcium are involved in the acute responses of the APC to IAA depletion. There are changes in intracellular calcium in the APC with changes in IAA but not dispensable AA (77), as well as increased phosphorylation of calcium calmodulin-dependent protein kinase II (CaMKII) in the cell body layer that houses the primary output neurons of the APC (100). CaMKII-P is a well-known indicator of increased intracellular calcium, and it is elevated within the 20 min window for the behavioral rejection of an IAA-depleting diet (**Figure 2**). CaMKII, among other functions, activates the glutamate AMPA receptor, GluR1, by phosphorylation in the postsynaptic density (106). Colocalization of CaMKII-P and the glutamate AMPA receptor subunit (GluR1) as GluR1-P in APC neurons, both already increased within 20 min after the introduction of an IAA-deficient diet, provides evidence for both calcium and glutamate in signaling IAA deficiency in the APC (100). CaMKII-P appears in far more APC cells than does eIF2α-P, and its increase in an IAA-depleted APC slice preparation depends on extracellular calcium (unpublished observation). Therefore, this phosphorylated kinase is likely to be in the postsynaptic (secondary) cells of the APC, which are activated in the positive feedback loop that characterizes the piriform cortex (43, 69) as well as in the primary chemosensory cells. The output signals are likely enhanced by such recruitment.

MAPK: mitogen-activated protein kinase; MAPK-P, the phosphorylated form

ERK1/2: extracellular signal-related kinase 1/2, a MAPK, active when phosphorylated

GABAergic signaling. Gamma amino butyric acid (GABA) is the primary source of inhibitory neurotransmission in the brain (79) and is crucially important in maintaining normal levels of neuronal activity in the APC (21, 22), providing damping of the recurrent excitatory circuitry. Agonists and antagonists of the GABAergic system injected into the APC of animals fed IAA-depleting diets have provided results correlating activity of the GABA system with food intake (105). This suggests that inhibitory transmission is associated with controlling the downstream effects of APC signaling, more directly involved in the food intake response, rather than with the chemosensory role of the APC. Recent results suggest that both the $GABA_A$ receptor and its associated potassium-chloride cotransporter are downregulated in the membranes of the APC 20 min after rats are offered a threonine-deficient meal (98). In addition, the β-3 subunit of the GABA receptor is downregulated at 2 h after an IAA-imbalanced meal (105). These proteins have short half-lives and require new translation for their continued presence in the membrane. In the presence of phosphorylated eIF2α, translation is blocked (4–6, 36, 41, 109), so the loss of rapidly turning-over membrane proteins, including GABA receptors, may be prolonged. The loss of GABAergic inhibition could be a major factor in exciting the APC (21, 22, 31, 98).

In sum, several downstream signaling pathways are candidates for an activating role in APC signaling of IAA deficiency. These include (*a*) sodium cotransport by System A transporters (11), dependent on ERK1/2 (27, 97, 99); (*b*) increased glutamate neurotransmission with either transamination or the glutamate-glutamine cycle, based on AA metabolism in the cells (56); (*c*) increased intracellular calcium from intracellular and extracellular sources (100); and (*d*) loss of GABAergic inhibition due to a rapid turnover of the protein and absence of new translation in the presence of eIF2α-P (48, 98, 109).

NEURAL CIRCUITRY AND INVOLVEMENT OF OTHER BRAIN AREAS

The APC is located in the anterior ventrolateral forebrain, and the neural circuitry associated with the feeding responses to IAA depletion has been reviewed elsewhere (34, 35, 65). Glutamatergic signaling originates in the APC, resulting from intracellular signals generated in response to IAA deficiency, such as those described above (34, 35, 39, 41). In addition to these activating systems, several neurotransmitters interact with glutamate in the APC, and inhibition of at least one receptor subtype each for serotonin, dopamine, norepinephrine, and for glutamate itself has been shown to reverse the feeding depression to IAA-deficient diets (34). This suggests that any inhibition of the glutamate output cells in the highly excitable APC will decrease signaling to food-intake-inhibitory systems and restore feeding. Taken together with the APC circuitry and oscillatory activity described above, one has a system that is poised for activation and is ideal for rapid responses to a dietary IAA deficiency.

Projections to Other Brain Areas

The APC has long been known to project to areas of the brain that are important for the control of food intake (1, 35, 44) (**Figure 3**). Yet, several laboratories have indicated that other areas of the brain, particularly the hypothalamus (12, 83, 103, 104), are involved in the IAA response. The lateral hypothalamus is involved in the hyperphagia to 10% (moderately low) protein (112). The vagus projects AA-related information to the lateral hypothalamus (60). However, roles for the ventromedial hypothalamus and lateral hypothalamus (LH) as primary sensors of IAA deficiency were ruled out by the ablation studies of the 1970s (94). Data from Monda et al. (82) and Blevins et al. (12) suggest that the lateral hypothalamus acts secondarily, receiving signals generated in the APC (axons

from the APC can be seen ending in the LH in **Figure 3**). The amygdala is important in the conditioned aversive responses, such as those for taste (33–35, 84, 95, 108). AA response elements have been studied in human hippocampal cells (63), where the much of the work on long-term potentiation is done. The most likely reconciliation of these data is that the sensing of IAA deficiency occurs in the APC and is signaled via its projections (12, 22, 35, 65, 82) to affect other brain areas, such as hypothalamic nuclei and hippocampus, or amygdala, for the feeding-motivational responses, and to motor circuits for motor acts associated with food intake, such as approach to the food cup, chewing, and swallowing (**Figure 3**).

The GCN2 Inhibitor IMPACT

Sattlegger, Castilho, and colleagues (91) have described an actin-binding protein, IMPACT, that is preferentially expressed in brain tissue and that inhibits the activation of the eIF2α kinase GCN2. The level of IMPACT is inversely correlated with the phosphorylation of eIF2α and is higher in several hypothalamic areas than in the APC (91). This could explain why the hypothalamus, which has classically been thought to house important feeding circuits (9, 13) and where mTOR responds to intra-third ventricular injections of leucine (18), has been so difficult to associate with the sensing of IAA deficiency (35, 38, 94). The paucity of the GCN2 inhibitor IMPACT in the APC is consistent with a role for GCN2 in the APC in sensing IAA deficiency (48, 80, 91).

After c-fos expression was seen in the dorsomedial hypothalamus when animals had eaten an IAA-imbalanced diet (107), this hypothalamic region was studied as well. Expression of c-fos in neuronal cell nuclei is an accepted tool for suggesting neuronal activation; micrographs showing nuclear c-fos may be seen in **Figures 1** and **3**. Cutting fibers running anteriorly from the dorsomedial hypothalamus increases intake of an IAA imbalanced diet for the entire first day, and the nucleus itself may be involved in the first 3 h of the responses (7, 8). It will be interesting to learn if IMPACT is expressed in the neurons of the dorsomedial hypothalamus.

CONCLUSIONS

The discovery of a role for the conserved GCN pathway in the APC sensing of IAA deficiency underscores the importance of AA homeostasis as crucial to survival in eukaryotes. It is likely that two separate systems function to sense increases (mTOR) or decreases (uncharged tRNA and GCN2) of IAA in the brain (36). The several signaling pathways activated by IAA deficiency downstream of eIF2α, combined with the excitable oscillating circuitry of the APC, finally yield potential mechanisms for activating the glutamatergic output cells of the APC, the chemosensor for IAA deficiency in the brain.

SUMMARY POINTS

1. Maintenance of indispensable amino acid (IAA) homeostasis is essential for protein synthesis and survival, requiring dietary selection in omnivores.
2. For appropriate dietary selection, sensing the depletion of an IAA is a crucial first step.
3. The brain area housing the IAA sensor is the anterior piriform cortex.
4. The mechanism of IAA sensing in the APC is the conserved general amino acid control pathway.

5. The four steps of the sensory mechanism are decreased IAA, increased deacylated tRNA, activation of GC nonderepressing kinase 2, and phosphorylation of eukaryotic initiation factor 2α, which binds to eIF2B and blocks initiation of translation.
6. The APC is highly excitable and has oscillatory activity coordinated with respiration.
7. Several signal transduction systems may be involved in potentiating the output cells of the APC.

FUTURE ISSUES

1. Which of the various possible mechanisms activate the output cells of the APC? Is there redundancy in the system, such that more than one signal transduction system is involved?
2. Where, precisely, among the various projection sites of the APC output cells, are the signals that cause inhibition of feeding received?
3. In spite of many years of work worldwide, the neural circuitry that controls feeding remains incompletely understood. Exactly how the motivation to eat is translated into actual food intake behavior needs to be explained.

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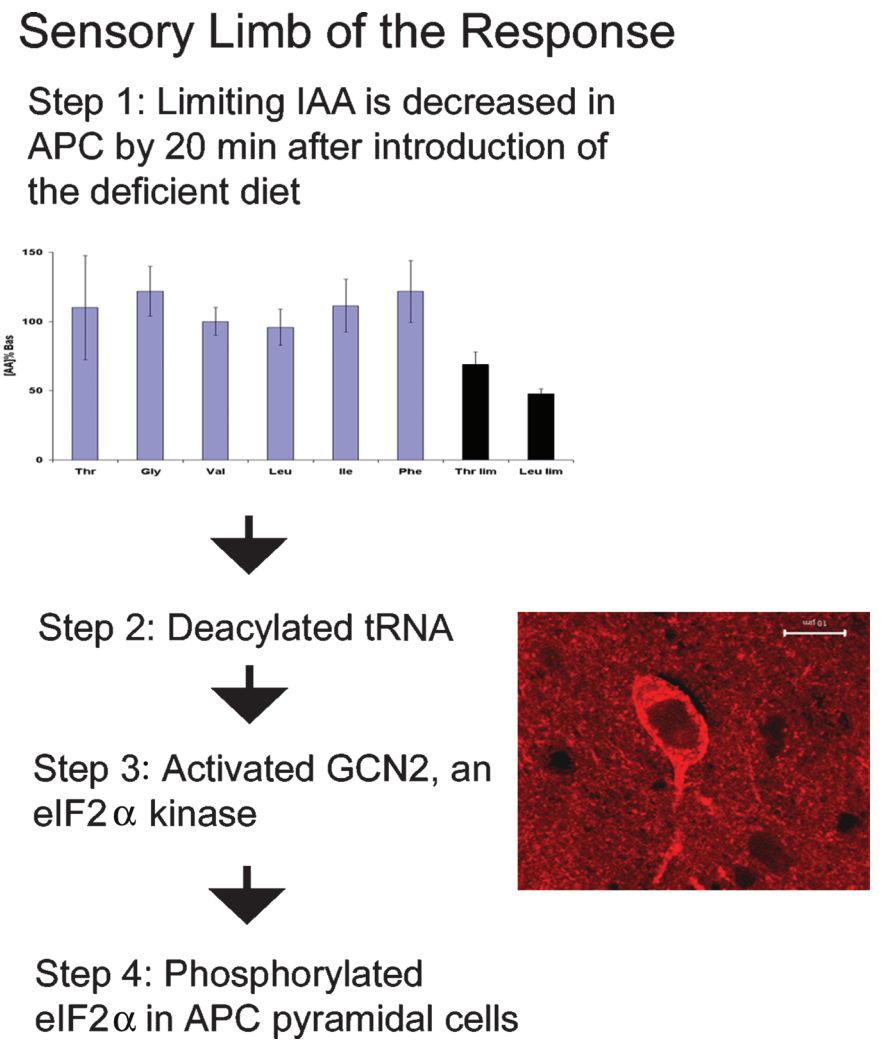

Figure 1

The mechanisms underlying the sensory limb of the responses to dietary indispensable amino acid (IAA) deficiency are listed as steps 1–4. Bars in the top figure indicate the amino acid (AA) concentrations (common three-letter abbreviations on the *X* axis) of nonlimiting AAs (*blue*) or limiting IAA (*black*) in anterior piriform cortex (APC) tissue taken 20 min after introduction of a threonine- or leucine-devoid diet are given as a percent of control. *Black bars* show the decrease in the limiting IAA at 20 min after introduction of the appropriate IAA-deficient diet. Steps 2–4 describe the next three biochemical events, similar in yeast and in the rodent APC, after IAA depletion. (*Right*) Micrograph of a pyramidal cell from rat APC layer II showing increased fluorescence for eIF2alpha in the cytoplasm. The tissue was taken 20 min after introduction of a threonine-devoid meal (42).

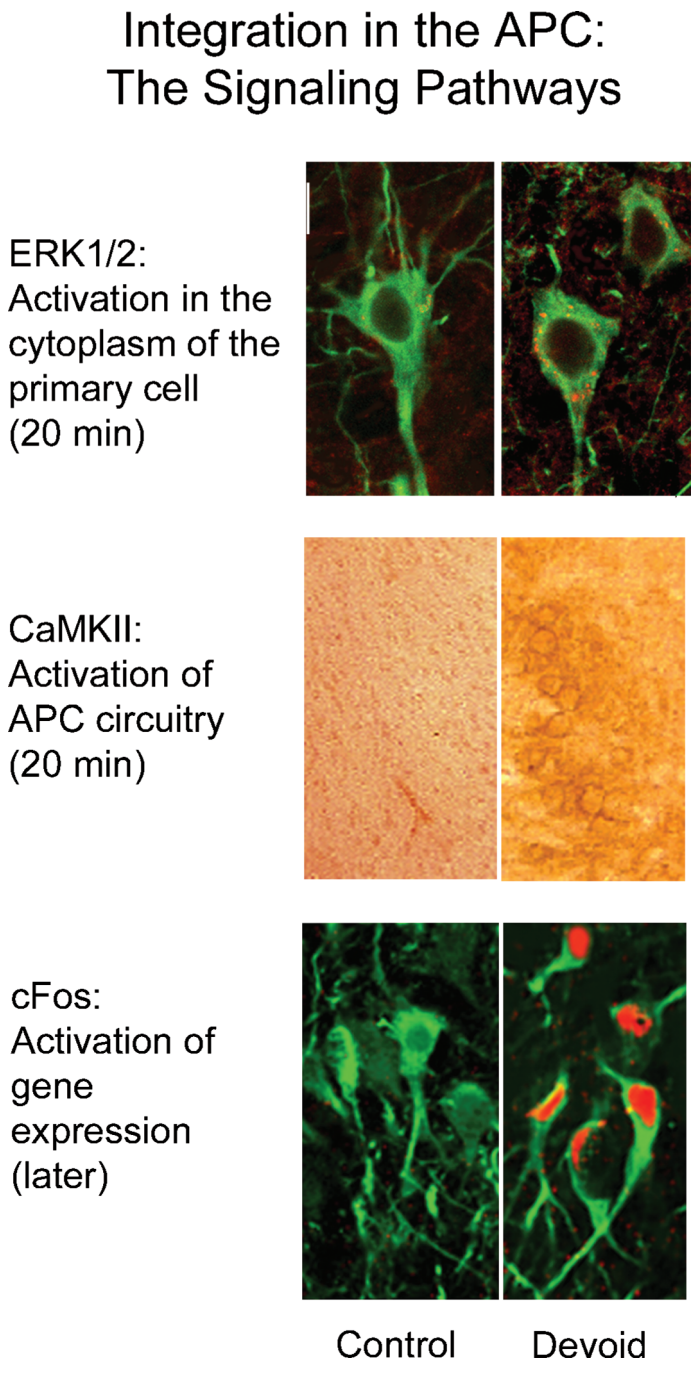

Figure 2

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Figure 2

Integration in the anterior piriform cortex (APC). Examples of micrographs show signal transduction in the pyramidal cells of the APC. (*Top*) Phosphorylated extracellular signal-related kinase 1/2 (ERK1/2) in the cytoplasm. Green is a neuronal marker; red is a fluorescence-tagged antibody to ERK1/2. (*Middle*) Phosphorylated calcium calmodulin kinase II (CaMKII-P) in layer II of the APC. CaMKII-P is seen in many more neurons (*right panel*) than either ERK (**Figure 2**, *top*) or eIF2α-P (**Figure 1**, *bottom right*). (*Bottom*) The immediate early gene, c-Fos (red in nuclei), used here to show that 45 min after eating a threonine-devoid diet, gene expression can be seen. *Left panels*, control; *right panels*, devoid treatment.

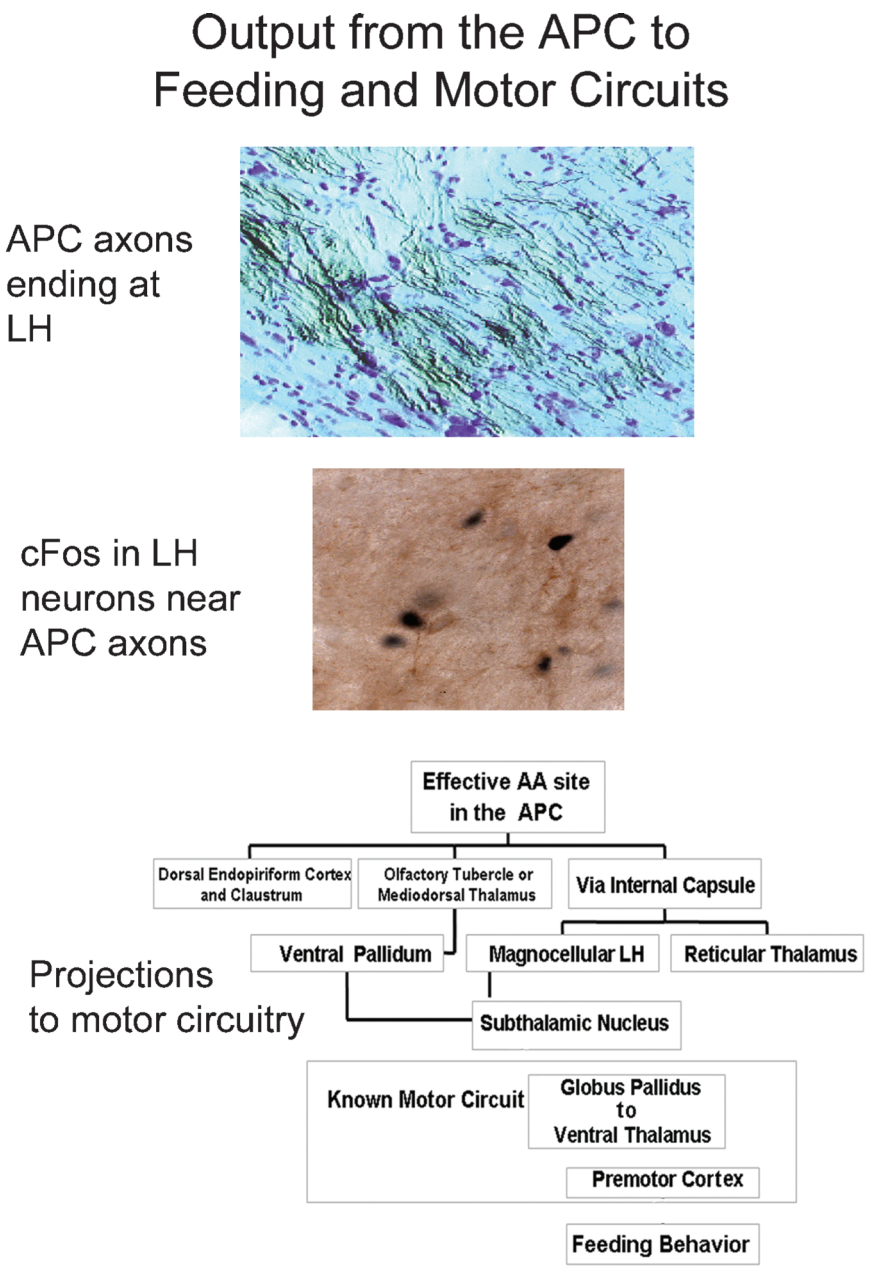

Figure 3

Representative data for output signaling from the anterior piriform cortex (APC). (*Top*) Dark lines extending from top left are biocytin-stained axons from APC neurons at their termination in the lateral hypothalamus. (*Middle*) Brown staining from upper right corner is of APC axons extending into the lateral hypothalamus and ending around or near c-Fos positive cells (*black*). (*Bottom*) Selected motor pathways traced from the APC after injections of the tract-tracing agent biocytin. Shown here are brain areas in known motor pathways where APC axon terminals were seen. AA, amino acid; LH, lateral hypothalamus.

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Errata

An online log of corrections to *Annual Review of Nutrition* chapters (if any, 1997 to the present) may be found at http://nutr.annualreviews.org/errata.shtml