Diet, Monoamine Neurotransmitters and Appetite Control

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Introduction

Appetite and the acquisition of appropriate foods represent an important aspect of the overall process of growth and development in animals, and of body weight maintenance in adults. In adult animals, in which body weight has attained a steady state, energy intake and expenditure are roughly equal. The body thus appears capable of balancing energy input and output, indicating that a control mechanism must exist for continually achieving this goal. Such a mechanism must be fairly complex. Presumably it would include brain circuits for managing overall energy balance, brain circuits for elaborating behavior (the desire to eat; purposeful movement, and the acquisition and ingestion of food), and also circuits to control the use of energy by the body (as a simple example, turning up heat production when the ambient temperature is low). Further, it must have access to sensory information, such as the color, smell and taste of acquired food items, the fullness of the gut, and the concentrations of various nutrients in blood. Such information can be provided to brain by sensory nerves (e.g., optic nerve from the eye), or be sensed directly in brain (e.g., glucose in blood). And, it must be able to modulate the flow of nutrients into tissues, as well as their use (e.g., the oxidation of glucose, amino acids and fatty acids for energy). The level of complexity must be even greater, of course, because the body manages more than simply its energy balance. It also regulates its nitrogen economy (it must obtain sufficient protein to counterbalance obligatory nitrogen loss each day as well as meet essential amino acid requirements), and functions to meet its requirements for essential fatty acids, vitamins and minerals.
Added to this issue is a further complexity, namely that humans evolved in an environment very different from that experienced today, one in which food supplies were far from nutritionally optimal or easily accessible. Managing the nutrient economy of the body therefore required knowledge of food sources and their availability, whether consciously or not. It further required the development of behaviors that improved success in obtaining prey (e.g., cooperative hunting activity, sharing), and of remembering the location of important food items (e.g., sources of fruit, and the annual timing of their availability). On balance, the operation of all these mechanisms must be successful, or the species would not have survived.

The point of this preamble is that, although 50+ years of research on these issues has led to the identification of many of the complexities, it has not yet produced many revelations. Indeed, it appears that we have but begun to scratch the surface, and it is important to appreciate that the issues discussed below represent but the initiation of the elucidation of a very complex process. With this backdrop, then, we will focus on a very specific aspect of this set of issues: monoamine neurons in brain and their participation in appetite control. First, we will outline basic features of the monoamine neurons, and why they are viewed as important to appetite control. We will discuss pharmacologic data that link them to appetite control, and how this knowledge has led to the development of drugs that stimulate and suppress appetite in humans. Finally, we will consider how certain nutrients can influence the formation of monoamines in brain neurons, and whether this relationship might potentially serve as a signal to brain regarding success in acquiring particular foods from the environment.

**Monoamine Neurons in the Brain**

Essentially all drugs that have been (and are) used to control appetite act on a particular class of neurons in brain, those that synthesize and use a monoamine as a neurotransmitter. The logic behind this observation is as follows. The neuron is the principal functional unit of the brain. It is generally believed that neurons are analogous to electronic elements, and are organized into circuits to control particular functions (such as the feeling of hunger, or the control of blood pressure). In order for such circuits to operate, electrical signals must circulate through them. But neurons do not make direct cellular contact, and thus cannot share signals through direct cell membrane transfer. This problem is solved by a process of electrochemical transduction. That is, as electrical signals reach the end of a neuron, they cause the release of a compound, termed a 'neurotransmitter', that rapidly diffuses onto an adjacent neuron, causing it to generate a new electrical signal. This signal now travels the length of the affected neuron, and ultimately repeats the process on subsequent neurons in the circuit. The movement of electrical
signals in neuronal circuits is thus critically dependent on the normal release of neurotransmitter molecules from, and their action on, neurons.

Neuronal circuits in brain contain large numbers of neurons. By and large, each neuron makes and uses a particular neurotransmitter. Some use small molecules as transmitters, such as simple derivatives of single amino acids, while others use larger molecules, such as peptides, long strings of amino acids. The monoamines fit into the former class: they are simple derivatives of the amino acids tryptophan (TRP) or tyrosine (TYR). And the drugs known to modify appetite are believed to do so principally by altering the release of a monoamine neurotransmitter from one or more neurons contained in appetite control circuits in brain.

The monoamines include the catecholamines dopamine (DA), norepinephrine (NE) and epinephrine (E), and serotonin (5-HT). DA and NE are synthesized from TYR, while 5-HT derives from TRP. In its respective neurons, each transmitter is synthesized from the appropriate amino acid and then stored in the nerve terminal in vesicles. When the neuron is depolarized, stored transmitter is released, and ultimately interacts with specific receptors on adjacent neurons to generate an electrical signal. Thereafter, the released transmitter is cleared from the intercellular space (termed the ‘synapse’), in order that it can be reset for the next signal. In monoamine neurons, the transmitter is removed by the neuron that originally released it, in a process termed ‘reuptake’. Reuptake is accomplished by very efficient uptake carriers, located on the neuronal cell membrane (Fig. 1).

Drugs that act as appetite suppressants generally increase the amount of a particular monoamine in the synapse, and thus promote electrical signal transfer. In circuits containing neurons that utilize a monoamine as a neurotransmitter, they thus tend to promote signal flow. In an appetite circuit, they would therefore tend to suppress appetite. Such agents are known to act by causing the release of a transmitter (fenfluramine releases 5-HT, phentermine releases NE, amphetamine releases DA), or by blocking its reuptake (fenfluramine blocks 5-HT reuptake; sibutramine blocks the reuptake of both 5-HT and NE into their respective neurons). These issues are discussed below in greater detail.

Monoamine neurons also share another biochemical feature of interest: the rate at which they synthesize their neurotransmitters is readily influenced by how much precursor amino acid is available to the neuron. This effect follows from the fact that the initial and rate-limiting step in each pathway is controlled by an enzyme that is only partly saturated with substrate at normal brain amino acid concentrations. This means that raising or lowering the concentration of TRP or TYR can rapidly influence the synthesis of the neurotransmitters 5-HT, or DA and NE, respectively. Much has been made of this relationship (and still is) in the appetite area, since the ingestion of proteins and carbohydrates can directly influence how much of each amino acid is available to brain neurons, and thus influence the production of these
transmitters in neurons, some of which are presumed to reside in appetite circuits in brain. As a result, some believe that the brain may sense the intake of certain macronutrients by such a mechanism, while others have suggested that this relationship allows these amino acids to serve as natural appetite suppressants. These notions will be discussed further below.

In summary, neurons in brain are organized into functional circuits. One or more such circuits control appetite, and some of the neurons in these circuits use a monoamine as a neurotransmitter. As a consequence, drugs that promote the movement of a monoamine into the synapse can influence appetite, while foods that cause more or less TYR or TRP to be available to neurons, by directly influencing the production of monoamines in appetite circuits, may also influence appetite, or at least serve as a signal to brain regarding the recent ingestion of particular macronutrients.
Monoamines and Appetite-Suppressant Drugs

Often, important clinical properties of drugs are discovered long before a mechanism of action is defined. Such was true for amphetamine (AMPH), which was known in the 1930s for its appetite-suppressing effect, well before a mechanism of action could be identified [1]. Presently, it is understood that AMPH has multiple effects on NE and DA synapses, all focused on increasing the amount of these transmitters in the synapse, and thus their stimulation of NE and DA receptors. These effects are to act as (a) a direct releasing agent of NE and DA from nerve terminals, (b) an inhibitor of the reuptake of DA and NE, and (c) a weak inhibitor of the enzyme that blocks NE and DA metabolism, monoamine oxidase [2]. As a sympathomimetic agent (since it releases, and blocks the reuptake of, NE and DA, it ‘mimics’ effects seen when the sympathetic nervous system discharges and releases NE from its peripheral nerve terminals), AMPH was noted for undesirable side effects, such as rapid increases in blood pressure. It also was found to be a behavioral stimulant, to be addictive and to produce insomnia; these undesirable effects were believed to be produced by actions in the brain. Because the appetite-suppressant effect was considered useful, a great deal of effort was expended by pharmaceutical companies in the middle of the last century to identify AMPH-like appetite suppressants that lacked the undesirable side effects [3, 4]. Several such drugs were found, including phenylpropanolamine, phentermine and fenfluramine (which are or have been popular appetite suppressants). By the time these agents emerged, it was recognized that AMPH produces appetite suppression via actions on NE and DA neurons in the brain. The newer drugs were thus examined for like pharmacologic actions. Phenylpropanolamine and phentermine were ultimately found to produce their effects via actions on NE neurons. Fenfluramine was shown to produce appetite suppression by a selective action on 5-HT neurons (this was not surprising, since other evidence had accumulating linking 5-HT neurons to appetite regulation) [5–7].

Subsequent work has revealed these drugs to have a major (though not exclusive) focus of their appetite-suppressing effects in specific areas of the hypothalamus, a region of brain known for its role in the control of ‘vegetative’ functions such as energy homeostasis [8–11]. In addition, other studies have identified particular NE and 5-HT receptor subtypes that appear to be important in mediating the appetite suppressant effects of these transmitters [7, 12, 13]. All of these approaches have been useful in advancing our understanding of appetite regulation and how NE and 5-HT neurons might participate in this process. However, one of the most interesting recent findings relating to this issue has been the emergence of a new appetite suppressant, sibutramine, and a number of studies focused on its mechanism of action. Sibutramine is a distant structural variant of AMPH that does not...
release transmitter, but only blocks transmitter reuptake. It might thus be predicted to be pharmacologically less potent than other weight-reducing agents, since reuptake blocking agents are generally thought to be less active than releasing agents (like phentermine and fenfluramine). However, this drug has the interesting feature of blocking the reuptakes of both 5-HT and NE (it is labeled as a combined 5-HT-NE reuptake inhibitor, or SNRI). Perhaps for this reason it has been found to be as potent in reducing body weight in humans as a releasing agent [14]. Of interest with regard to the pharmacologic features of sibutramine that make it an effective appetite suppressant is a recent report that both NE and 5-HT reuptake blocking actions are probably required for appetite suppression and weight reduction. This was effectively demonstrated in rats by showing that while the administration of a 5-HT reuptake blocker (fluoxetine) or an NE reuptake blocker (nisoxetine) alone failed to reduce food intake, the two drugs given together (which would produce the same NE + 5-HT reuptake blocking profile as sibutramine) produced as potent a reduction in food intake as sibutramine itself [15]. While this study focused on the appetite-suppressing efficacy of SNRIs (it also showed that other SNRIs suppress food intake), its findings underscored a clinical impression in the use of 5-HT reuptake blockers to suppress appetite: agents such as Prozac (fluoxetine) are not particularly efficacious when used alone in the chronic treatment of obesity [16–18]. They further suggest that a fruitful approach to identifying new appetite suppressants of this type might involve searching for agents that combine 5-HT and NE receptor stimulating actions.

An additional, relevant feature in the study of appetite suppressants has been the report that 5-HT agents like fenfluramine and fluoxetine reduce the intake of a particular macronutrient, carbohydrate [19]. This action has been disputed [20], though recent data in rats offer support for this notion [21]. Since there is no known nutritional requirement for carbohydrates (though there is for protein, energy and essential fatty acids), new data notwithstanding, it remains unclear why some 5-HT neurons in brain should devote themselves to modulating carbohydrate intake and appetite [20]. This unresolved issue serves to remind us how little we really understand about the brain’s management of the nutritional and metabolic economy of the body.

Indeed, more generally, our knowledge of appetite control circuitry, and of how the brain regulates body metabolism is so rudimentary that we cannot begin to answer other very simple questions about the drugs we currently use to suppress appetite. For example, we know that when NE is applied to different portions of the hypothalamus, it can either suppress or stimulate food intake [11, 22]. But, we also know that when drugs are given orally or systemically that promote NE transmission, such as phentermine, they suppress appetite and food intake. Since the drug should reach all NE neurons in brain, why should the suppressive action of NE dominate? As another example, we know in rats that appetite suppressants like sibutramine and fenfluramine enhance energy expenditure, presumably via an action in brain to stimulate
sympathetic nervous system activity (which increases metabolism) [23, 24]. But we know virtually nothing about the importance of this phenomenon in humans, and of the circuitry in brain that might mediate this effect. As a third example, we know that potent appetite suppressants work extremely well to reduce body weight in some individuals, but extremely poorly in others [25]. To the neuropharmacologist, appetite suppressants are potent agents; it is thus a mystery why some individuals show no responsiveness to these drugs. Finally, appetite suppressants generally appear to reduce body weight by about 10%, but no more. When these agents are given chronically, a plateau in body weight is achieved within 6 months of initiating treatment. No one knows why body weight stops falling despite continued treatment. These and other questions, all focused around this group of appetite-suppressant drugs, will no doubt form the basis for future work, the results of which will hopefully improve our knowledge of how appetite, body weight and energy balance are regulated by brain, and offer new insights into the development of new drugs for controlling appetite and body weight.

In sum, research spawned by the discovery of drugs that suppress appetite in humans has led to the findings that NE, DA and 5-HT neurons participate in circuits that govern appetite, and that in general, when given orally or systemically, drugs that enhance NE, DA and/or 5-HT neurotransmission suppress appetite.

**Dietary Effects on Brain Monoamines**

Pharmacologic data were the first to suggest that catecholamine (NE and DA are catecholamines) and 5-HT neurons in brain are involved in appetite regulation. A more recent line of investigation, which approaches this link from a nutritional and physiological perspective, adds important support to this connection. This line of evidence begins with the recognition that these neurotransmitters are synthesized from amino acid precursors, and that the synthesis rates are influenced by the local availability of amino acid. Amino acid availability can be directly affected by the diet, thus linking diet to the production of neurotransmitters in brain thought to be important in appetite regulation.

*Serotonin*: 5-HT is synthesized from TRP, an essential amino acid. The enzyme that governs the rate of 5-HT synthesis (Fig. 2), tryptophan hydroxylase, is only partly saturated with substrate at normal brain TRP concentrations. For this reason, raising or lowering brain TRP concentrations was found to alter the saturation of this enzyme with substrate, and thereby the rate of 5-HT synthesis [26]. This recognition led to studies of factors that might modify brain TRP concentrations, diet being an obvious candidate. Subsequent studies revealed that the ingestion of a carbohydrate load by fasting rats would rapidly raise brain TRP (and stimulate 5-HT synthesis),
Fig. 2. Biosynthesis of serotonin and norepinephrine in neurons. **A** Norepinephrine (NE) synthesis from the non-essential amino acid tyrosine (TYR). TYR is hydroxylated to dihydroxyphenylalanine (DOPA) in a reaction catalyzed by tyrosine hydroxylase; DOPA is then decarboxylated to dopamine (DA) in a reaction mediated by aromatic L-amino acid decarboxylase. DA is converted to NE by the enzyme dopamine-β-hydroxylase. In NE neurons, the entire pathway is present. In neurons that make and use DA as their neurotransmitter, the last enzyme is absent. In NE neurons, the principal NE metabolite is methoxy-hydroxyphenylethylene glycol-sulfate (MOPEG-SO₄). Once released into the synapse, NE interacts with receptors, and is then rapidly reabsorbed into the releasing nerve terminals, where it is either reused or metabolized. TYR is taken up into brain by a competitive transport carrier, located at the blood-brain barrier, which it shares with several other large neutral amino acids (LNAA: phenylalanine, tryptophan, leucine, isoleucine, valine). The asterisk indicates the rate-limiting step in NE synthesis, TYR hydroxylation. **B** Serotonin (5-HT) synthesis from the essential amino acid tryptophan (TRP). Tryptophan is hydroxylated to 5-hydroxytryptophan (5-HTP) in a reaction catalyzed by tryptophan hydroxylase; 5-HTP is then converted to 5-HT by aromatic L-amino acid decarboxylase. When released into the synapse, 5-HT interacts with receptors, and is then rapidly reabsorbed into the presynaptic terminals, where it is either restored and reused, or metabolized to 5-hydroxyindoleacetic acid (5-HIAA).
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while the ingestion of a protein-containing meal, particularly when it followed a few hours after a carbohydrate meal, would drive down brain TRP and 5-HT synthesis [27] (Fig. 3). The negative effect of the protein meal on brain TRP was paradoxical, since the ingestion of protein introduces substantial amounts of exogenous TRP into the circulation. But this curious finding was ultimately explained by an examination of the mechanism by which TRP gains access to brain. TRP is a large neutral amino acid (LNAA); other LNAA include TYR, phenylalanine (PHE), and the branched-chain amino acids leucine, isoleucine and valine. TRP is taken up into brain across the blood-brain barrier (BBB) via a competitive transport carrier it shares with the other LNAA. When carbohydrates alone are consumed, TRP concentrations in blood do not fall (humans) or rise (rats), while the concentrations of the other LNAA (particularly the branched-chain amino acids) fall, an effect of insulin secretion and action. As a result, TRP gains a competitive advantage for transport into brain, and brain TRP concentrations rise. When proteins are consumed in food, plasma TRP increases, but now so do the concentrations of the other LNAA, particularly the branched-chain amino acids, since they are not metabolized in liver. As a consequence, the plasma concentrations of TRP’s transport competitors rise more in blood than does TRP; TRP entry into brain is thus inhibited, and brain TRP concentration declines [27]. The changes in plasma LNAA concentrations that alter brain TRP uptake have been grouped into a single variable that predicts a meal’s effects on brain TRP uptake: the plasma TRP/ΣLNAA ratio (TRP divided by the sum of its transport competitors). Thus, this ratio rises when a carbohydrate meal is consumed, and declines when a meal is consumed that contains even moderate amounts of protein. This ratio has proved useful in numerous types of investigations, including studies in humans showing that mood, which is influenced by the functioning of brain 5-HT neurons, can be lowered by administering an amino acid cocktail that markedly lowers the plasma TRP/ΣLNAA ratio, and thus presumably brain TRP uptake and 5-HT synthesis [28].

The observation that brain TRP concentrations and 5-HT synthesis could be influenced by single meals, and in particular, could be elevated by carbohydrate ingestion, led to much speculation that carbohydrate intake might be regulated by 5-HT neurons. It also formed the basis for the suggestion that obesity in some subjects might be caused by a defect in this regulatory control system, leading to a ‘craving’ for carbohydrate-rich foods and an overconsumption of calories [19]. Drugs like fenfluramine, which release 5-HT from nerve terminals, were thus reported to reduce selectively the intake of carbohydrates, a finding that would be consistent with this viewpoint [29]. Data offered in support of this assertion, however, did not hold up to careful scrutiny [30], though to this day belief in this relationship continues. In addition, more recent data suggest there may be some validity to the notion that 5-HT neurons influence an appetite for carbohydrates, at least in rats: Leibowitz and Alexander [21] have reported that rats, when given the
Fig. 3. Hypothalamic tryptophan concentrations and serotonin synthesis rate in rats fed two sequential meals. Rats fasted overnight ingested the next morning either nothing or one or two small meals (4 g each, dry weight). Rats ingesting one meal received carbohydrates only, and were sacrificed 2 h later (gray bars), along with fasted controls (open bars). Rats ingesting two meals were sacrificed at 4 h, along with fasted controls (open bars), and consumed either carbohydrates alone at 0 and 2 hr (gray bars), or carbohydrates at 0 h and a protein-containing meal (4 g dry weight of 40% casein by weight) at 2 h (black bars). 30 min prior to sacrifice, all animals received an injection of NSD-1015, an inhibitor of aromatic L-amino acid decarboxylase. NSD-1015 causes the product of TRP hydroxylation, 5-HTP, to accumulate linearly for at least 30 min (since 5-HTP cannot be metabolized further to 5-HT), allowing it to serve as a reliable index of overall TRP hydroxylation rate (and thus 5-HT synthesis rate, since TRP hydroxylase is rate-limiting in the pathway). Data are means ± SEM (n = 7/group). *p < 0.05, **p < 0.01 vs. fasted group at 2 h (t-test); *p < 0.05, **p < 0.01 vs. fasted group at 4 h (Newman-Keuls test) [adapted from 27].
opportunity, select largely carbohydrates in their first meal of the day (which occurs for rats around the onset of darkness), and that this event is under the control of 5-HT neurons in the hypothalamus, which may serve to limit later carbohydrate ingestion. At the moment, we must wait to see how this issue resolves itself.

Another perspective on the diet, brain TRP, brain 5-HT relationship concerns chronic changes in dietary protein intake. Reductions in protein intake to or below the rat’s maintenance protein requirement (about 7% as a percent of calories) reduce plasma and brain TRP concentrations, and slows the rate of 5-HT synthesis in brain [31]. Such effects are related to the decline in brain TRP, and not to a deficiency in the enzymatic machinery for producing 5-HT, since injecting these animals with TRP causes an immediate increase in 5-HT production [32]. Not only are such effects seen with the intake of small amounts of protein, but also with the ingestion of TRP-deficient protein sources like corn. And, these effects appear also to occur in human populations that ingest corn as a major source of dietary protein [33]. Conceivably, this relationship might provide a biochemical conduit to the brain regarding the quality and quantity of protein ingested chronically in the diet.

Catecholamines: The initial step in the catecholamine (i.e., DA, NE) biosynthetic pathway, tyrosine hydroxylation, is rate-limiting, and the enzyme that catalyzes this reaction, tyrosine hydroxylase, is also unsaturated with substrate at normal brain TYR concentrations (see Fig. 2). As a result, a number of studies have found that TYR hydroxylation and catecholamine synthesis are rapidly influenced by local TYR concentrations [26]. This biochemical relationship appears to be sensitive to the firing rate of the neuron, since it is evident only when catecholamine neurons are active. The importance of the level of neuronal activity to this effect is most clearly demonstrated for the DA neurons in retina, which are active during daylight, but quiescent at night. Tyrosine administration stimulates DA synthesis in retina when rats are exposed to light, but not when they are kept in darkness [34].

TYR concentrations in brain rise rapidly following the ingestion of a protein-containing meal. As a consequence, catecholamine production in retina and brain increases [35, 36]. This precursor-product relationship also holds chronically: as the dietary protein content is stepped down below 10% to 2% (percent energy), brain and retinal TYR concentrations decline in parallel, as does catecholamine synthesis in retina (during daylight) and hypothalamus (Table 1) [37]. Preliminary data reveal that both DA and NE synthesis in hypothalamus decline with dietary protein content [38]. These latter findings suggest, at least for hypothalamus, that this biochemical relationship could provide a signal to appetite control circuitry in brain regarding the adequacy of protein in the diet. If so, it might constitute a portion of a mechanism governing protein seeking behavior. It is of interest that this relationship holds for both TRP and TYR and their respective transmitters, perhaps indicating some redundancy in this information pathway, if it exists.
Table 1. Effect of chronic protein ingestion on the TYR concentration and hydroxylation rate in hypothalamus

<table>
<thead>
<tr>
<th>Dietary protein content % energy</th>
<th>Serum tyrosine nmol/ml</th>
<th>Hypothalamic tyrosine nmol/mg protein</th>
<th>Hypothalamic DOPA ng/mg protein</th>
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<tbody>
<tr>
<td>2</td>
<td>42 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.04 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>5</td>
<td>86 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.82 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>153 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.14 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.51 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>183 ± 12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.08 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.85 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
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Male rats ingested for 14 days diets containing the indicated amounts of protein (casein). On the last day, they received NSD-1015 (100 mg/kg ip), an inhibitor of aromatic L-amino acid decarboxylase, and were killed 30 min later. NSD-1015 causes the product of TYR hydroxylation, dihydroxyphenylalanine (DOPA), to accumulate linearly for at least 30 min (since DOPA cannot be metabolized further to DA), allowing it to serve as an excellent index of overall TYR hydroxylation rate (and thus DA and NE synthesis rates, since TYR hydroxylase is rate-limiting in the pathway). Data are means ±SEM (n = 6/group). Values with different letters in the same column are significantly different, p < 0.05 (Newman-Keuls test) [adapted from 37].

Summary and Conclusions

This article has attempted to point out some of the relationships between 5-HT and catecholamine (NE, DA) neurons in brain and the control of appetite and food intake. At least two bodies of evidence support this connection. The first is pharmacologic, and demonstrates that drugs that stimulate transmission across 5-HT and/or catecholamine synapses suppress hunger and food intake. The second is physiologic and metabolic, and reveals that the ingestion of foods, on either an acute (single meal) or chronic basis, can reliably modify the uptake of TRP and TYR into brain (and hypothalamus), and directly alter the synthesis of their transmitters (5-HT and the catecholamines, respectively). The synthesis of these two bodies of information has led to models by which (1) changes in dietary carbohydrate ingestion, by modifying brain TRP uptake and 5-HT production, may cause like changes in 5-HT release, and in the stimulation of 5-HT receptors in brain circuits that control carbohydrate appetite, and (2) dietary protein intake, by altering brain TYR uptake, directly influences DA and NE synthesis (notably in hypothalamus), perhaps providing a signal to brain circuits monitoring dietary protein adequacy regarding protein intake. In this case, one might imagine that stimulating DA and/or NE receptors in such circuits might suppress protein intake, a possibility we are now examining in rats.

As indicated in the Introduction, the broader issue being touched upon in this article concerns the body’s need to acquire and maintain an optimal
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(or adequate) nutritional balance (for growth and ultimately, reproductive success). Rats and humans evolved in an environment that does not provide continuous access to all essential nutrients, and one that presents nutrients in a complex matrix (other animals, plants) that can also include toxic compounds. Together with the fact that animals and humans do not carry a guidebook to healthy eating, we must presume that the brain mechanisms that have evolved to optimize the acquisition of essential nutrients are ‘automatic’ (i.e., not conscious) and quite complex. In this context, the relationships described here must be viewed as rudimentary, touching only a small portion of this complex regulatory mechanism. The hope is, as further insights develop, that we will gain a better understanding of the workings of these mechanisms, and also be able to apply this knowledge to the development of better pharmacologic (and other) aids for controlling appetite and obesity in our modern, man-made environment.

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References


Discussion

Dr. Holm: What about tryptamine? As far as I know, when more tryptophan than usual enters the brain, more tryptamine than serotonin is formed. How could tryptamine act? Are there studies on that?

Dr. Fernstrom: Tryptamine has not been studied a great deal, but studies have been done, for example by Simon Young at McGill [1], looking at the synthesis of tryptamine vs. serotonin in relation to how much tryptophan is available. There is an ability to stimulate tryptamine biosynthesis with tryptophan. The relative amounts of tryptamine in the brain are a tiny fraction of the available serotonin. There’s no simple way to answer your question because the data aren’t available. There appear to be enough to say that there’s a connection biochemically between tryptophan and tryptamine, but there are not enough to say what the functional effects might be. Because of the limited studies on tryptamine, there’s no knowledge about whether there is a tryptamine receptor, whether tryptamine is present and/or released from neurons, whether it interacts with receptors, or whether it interacts with the serotonin receptor. There are amine receptors all over the brain in places where there are no serotonin or norepinephrine nerve terminals. Some of these may be tryptamine receptors.

Dr. Holm: My second point is about glutamine. You showed us that the simultaneous inhibition of tyrosine and serotonin uptake results in a decrease in meal size. If we were to give ammonia, which is metabolized to glutamine, which in turn is exchanged for tyrosine and tryptophan at the blood-brain barrier, it should increase the appetite.

Dr. Fernstrom: Glutamine is an interesting issue. The blood-brain barrier takes up tryptophan and several other large neutral amino acids, one of which is glutamine. The brain is a net exporter of glutamine, and it’s generally thought that this is a type of facilitated transport, where glutamine is pushed out of the brain and the counter transport is a large neutral amino acid of particular interest. I don’t know how high you would have to raise blood glutamine to actually push it into the brain. The ammonia point is a good one. The only studies I know of that have investigated the ammonia-glutamine connection have involved the glutamate–glutamine cycle in glia and neurons. To my knowledge no-one has looked in much detail at the relation with tryptophan or tyrosine.

Dr. Holm: Are there studies using NMR spectroscopy that could provide further information?

Dr. Fernstrom: Well, there are some very interesting amino acid analogs that have been used to look at transport into brain, α-methyltryptophan for example, which has been advertised as a good marker for use with MRI to look at serotonin production. However, there are now papers that show that α-methyltryptophan is unacceptable as an index of serotonin synthesis and I was sorry to hear that. What is accepted is that α-methyltryptophan is a good marker for MRI spectroscopy for tryptophan uptake in the brain. So at least one has a signal that one can use to look at transport, if not the conversion to serotonin. I would guess that α-methyltyrosine might prove to be just as useful for tyrosine. No-one has really looked at these compounds using this technology from the standpoint of amino acid transport, which amazes me because you could take
the animal studies that have been available for several years and apply this technology to humans immediately. With just one amino acid one could straight away make some very interesting observations about whether the transport systems in the rat or the dog, or whatever, are also present in the human.

*Dr. Uauy:* In your study on monkeys, you definitely showed a dose response, both in CSF and in plasma. Were there any changes in food behavior in these animals?

*Dr. Fernstrom:* The study couldn’t do that. These animals were wearing jackets with tethers, and they had cannulas for sampling CSF and blood. Also the data were obtained over 24-hour periods only. We didn’t design the study to be a feeding study so we didn’t have the set up to collect any remaining food to see if there might have been an effect.

*Dr. Kaye:* I would assume that you are going to get compensatory downregulation of postsynaptic receptors and ultimately the signal that’s being sent has probably not changed very much. I think the reason why we don’t see big changes in diet and behavior in humans is that these buffering systems are probably fairly robust and compensate for these kinds of changes. That may be the difference between people with eating disorders and people without eating disorder – the former don’t compensate so well for these kinds of changes.

*Dr. Fernstrom:* However, the animal in its environment is always working to achieve adequate intakes. Some Japanese investigators working with monkeys on Japan’s small islands have shown that there are times of the year when the animal is above its requirements for protein and energy, and other times of the year – 2 or 3 months – when it’s below. So under normal circumstances for a major part of the year the animal is challenged to meet its requirements. I don’t think a receptor is going to be downregulated under those circumstances – in other words, where you don’t normally get to the 25% protein level. Studies from round the world confirm that in the wild, species such as the rat – which can eat slugs, nuts, and all sorts of high protein foods – do not achieve an intake greater than about 12% protein. This means that the animal is not working at a level where you’d assume there might be receptor downregulation. I believe that animals in the wild do not have the luxury of having a downregulated receptor.

*Dr. Møller:* Years ago, when the lower limits of essential amino acids were determined, it appeared that the longer the period over which you reduced the intake, the lower you could go, probably because of an associated decrease in activity of the enzymes concerned. How do you think that enzymes influence the protein requirements of rats in the studies you have presented?

*Dr. Fernstrom:* Indeed, it is interesting that when a rat exceeds its protein requirement, the activities of amino acid catabolic enzymes rise. For example, a group of Japanese investigators used radiolabeled amino acids (including tryptophan and tyrosine) in rats to examine their metabolism at different levels of chronic protein intake [2]. They observed that at chronic protein intakes above about 10%, the amino acids were catabolized. To me, this suggests that above 10% protein intake, the rat is not using the amino acids (e.g., tryptophan) for anything other than fuel (i.e., energy generation). This makes sense, if at this level of intake the rat has consumed all the amino acids needed for the synthesis of protein. In this light, it is curious that in the laboratory (though not in nature), when given the choice, rats will chronically consume protein levels vastly in excess of their requirement (about 35% of calories). Metabolically, it is not at all obvious why they do this, since almost all of the excess amino acids are broken down and used for energy generation. (Presumably, the rat is responding appropriately to an imbalance in some nutrient we have unknowingly imposed upon it).
Reference