Review

Aromatic amino acids and monoamine synthesis in the central nervous system: influence of the diet

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Introduction

There is little doubt that the brain is subject to the vagaries of the diet. In recent years, one of the clearest examples of this fact has been the impact of the diet on the synthesis of several low-molecular weight neurotransmitters. Neurotrasmitters are the central participants in interneuronal communications, and thus are critical to brain function. By being capable of influencing neurotransmitter synthesis, diet may thus have immediate and significant impact on brain function.

This article will primarily review the link between diet and the synthesis of one group of transmitters, the catecholamines (dopamine, norepinephrine, epinephrine), focusing in particular on dopamine. The catecholamines all derive from a common biosynthetic pathway, with the amino acid tyrosine as the initial substrate. As described below, the diet modifies dopamine production by changing the availability to the brain (and also other parts of the central nervous system [CNS], particularly the retina) of tyrosine. Under appropriate conditions, altering CNS tyrosine levels can influence directly the degree of saturation of the enzyme catalyzing the initial and rate-limiting step in the synthetic pathway (tyrosine hydroxylase). As a consequence, the rate of dopamine synthesis is affected. As will be seen, the most important condition appears to be high neuronal firing rate: dopamine synthesis is not sensitive to precursor supply when neurons are inactive. An additional regulatory wrinkle for the biosynthetic pathway (and the relationship of diet to dopamine synthesis) is that it appears to have not one, but two substrates: both tyrosine and phenylalanine are substrates for the initial, hydroxylating enzyme in the pathway (tyrosine hydroxylase), and some data suggest that variations in the concentrations of both should influence dopamine synthesis. Accordingly, the diet, by affecting the supply of both amino acids to the brain, might produce changes in dopamine production that are the sum effects of the alterations in both amino acids. These points will also be discussed, and comparisons will be made with the synthesis of another transmitter, serotonin [5HT], the rate of which is also influenced by the availability of its substrate, tryptophan.

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Tyrosine availability and dopamine synthesis

Tyrosine is considered the primary substrate for dopamine biosynthesis. It is converted to dihydroxyphenylalanine (DOPA) in a reaction mediated by the enzyme tyrosine hydroxylase. DOPA is then decarboxylated to dopamine by aromatic-L-amino acid decarboxylase. Dopamine is the end-product in neurons that utilize it as a neurotransmitter (*Figure 1*). In other neurons, however, it may subsequently be converted to norepinephrine by the action of dopamine-β-hydroxylase, while in still other neurons the norepinephrine can be N-methylated to epinephrine by phenylethanolamine-N-methyltransferase. All three catecholamines (dopamine, norepinephrine, epinephrine) are believed to be neurotransmitters in the CNS, though not in the same neuron. The enzyme complement of the neuron dictates which catecholamine(s) it will produce (e.g., a norepinephrine neuron can synthesize dopamine and norepinephrine, but not epinephrine).[1]

The initial step in the pathway, tyrosine hydroxylation, is rate-limiting and thus thought to be the site of the dominant controls on catecholamine synthesis rate. Tyrosine hydroxylase, which catalyzes the reaction, has multiple controls on its activity, including direct end-product inhibition and indirect phosphorylation-mediated effects.[2,3] The potential influence of tyrosine levels on hydroxylase activity has not generally been thought important, probably because kinetic studies using purified enzyme suggested early on that the hydroxylase must be saturated at normal CNS tyrosine levels. However, this view now appears to be too simplistic; indeed, tyrosine level can readily in-

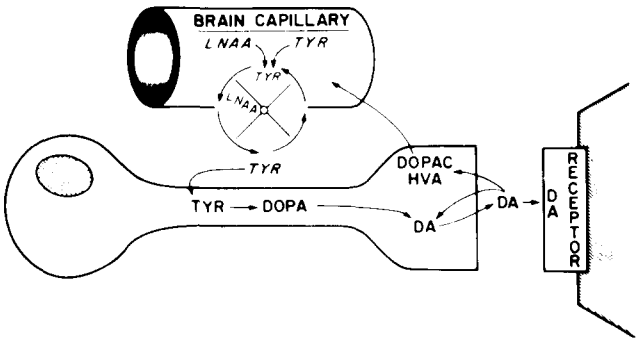

Figure 1 Tyrosine (TYR) availability and dopamine synthesis. Tyrosine is hydroxylated to dihydroxyphenylalanine (DOPA); DOPA is decarboxylated to dopamine (DA). Monoamine oxidase initiates the catabolism of DA to dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA), the principal DA metabolites in the CNS. Asterisk indicates rate-limiting step in DA formation, TYR hydroxylation; TYR competes with other LNAAs for uptake into brain.

fluence hydroxylation, but only in neurons that are active.[4]

The ability of tyrosine level to influence tyrosine hydroxylation in active neurons is amply illustrated by the results of studies in the retina. The retina contains a subpopulation of amacrine cells (interneurons) that utilize dopamine as their transmitter. Amacrine cell activity is low in the dark, and becomes very active when the lights are turned on. The dopamine-containing amacrine neurons show this light-activation biochemically via a remarkable stimulation of tyrosine hydroxylase activity.[5] And when light-activation occurs, tyrosine hydroxylation becomes responsive to changes in local tyrosine level.[6] This fact is illustrated by the data in *Table 1*. In this study, rats were maintained on a normal daily cycle of 12 hour light and 12 hour darkness. On the day of the experiment, the lights were turned on as usual, but three hours later groups of rats received an injection of either tyrosine or the vehicle. Shortly thereafter (90 minutes), they received a second injection, a drug that inhibits the enzyme aromatic L-amino acid decarboxylase (NSD-1015, or m-hydroxybenzylhydrazine), and were then killed 30 minutes later. The drug blocks the conversion of DOPA to dopamine (*Figure 1*), causing newly formed DOPA to accumulate linearly for 30 to 45 minutes. The simple quantitation of retinal DOPA content 30 minutes after drug injection therefore provides a direct measure of DOPA synthesis during this period, and thus of tyrosine hydroxylation rate in vivo.[6,7] In the light-exposed rats, tyrosine injection elevated retinal tyrosine levels, and caused a significant increase in DOPA production (tyrosine hydroxylation rate). If the identical experiment was then performed again, except that the lights were not turned on the morning of the study (the rats remained in darkness), tyrosine injection still increased retinal tyrosine levels, but no stimulation of tyrosine hydroxylation was observed (*Table 1*). Thus, an increase in retinal tyrosine level will stimulate tyrosine hydroxylation rate, but only following light exposure. Since light increases neuronal activity in retina, neuronal activation thus appears to be critical for hydroxylase activity to show sensitivity to tyrosine level.

Tyrosine injection also stimulates hydroxylation in dopamine-containing neurons in brain, but as for retina, the neurons must be activated. Thus, the mesocortical dopamine neurons, which are normally very active, readily increase hydroxylation rate following tyrosine administration,[8,9] while the striatal dopamine neurons, which fire slowly, do not.[6,10] In the case of the corpus striatum, if dopamine neurons are first exposed to a drug that increases firing rate (e.g., haloperidol), a subsequent injection of tyrosine will stimulate hydroxylation.[10]

Meal-induced stimulation of dopamine synthesis

If neuronal tyrosine levels can directly influence tyrosine hydroxylation when dopamine neurons are active, then metabolic phenomenon in the body that modify tyrosine access to these neurons should also indirectly influence catecholamine production. One such phenomenon is the act of eating: meal-associated insulin secretion, and the ingestion of dietary proteins, each of which alters the plasma amino acid pattern, might be expected indirectly to influence the CNS uptake of tyrosine and other amino acids. Such indeed is the case for both brain and retina, though the effect of a meal or insulin on the uptake of tyrosine depends not simply on the changes in blood tyrosine level, but also on the concentrations of several other amino acids.[11,12]

Tyrosine is taken up into the CNS (brain, retina) by

Table 1 Effect of tyrosine injection on DOPA accumulation in retinas from rats following exposure to light or darkness

Treatment	Serum tyrosine	Retinal tyrosine	Retinal DOPA
	(*nmol/ml*)	(*nmol/mg* protein)	(*ng/mg* protein)
Light–Vehicle	76 ± 2	2.8 ± 0.2	1.62 ± 0.10
Light–Tyrosine	167 ± 11[a]	7.4 ± 0.7[a]	2.60 ± 0.10[a]
Dark–Vehicle	120 ± 8	3.0 ± 0.2	0.81 ± 0.10
Dark–Tyrosine	339 ± 11[a]	6.2 ± 0.8[a]	0.81 ± 0.10

Groups of six rats received tyrosine methylester (250 mg/kg ip) or vehicle, followed 90 minutes later by an injection of NSD-1015 (100 mg/kg ip). They were killed 30 minutes after the second injection. The light-exposed rats received the first injection 3 hours after the lights were turned on in the morning; the dark-exposed rats received the first injection at the same time, but had not been exposed to light the prior three hours. Data are means ± SEM; [a] $P < 0.05$ versus vehicle values (t-test).[6]

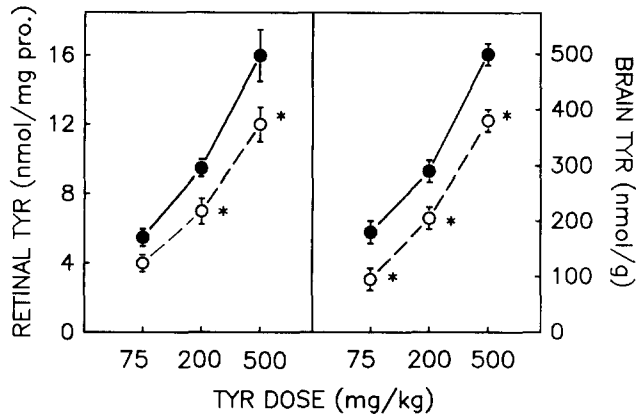

Figure 2 Dose-related effects of tyrosine injection, alone or in combination with branched-chain amino acids, on retinal (left panel) and brain (right panel) tyrosine levels. Groups of 6 rats received tyrosine ip (at indicated doses of free tyrosine) and 5 minutes later an ip injection of water (closed circles) or branched-chain amino acids (open circles; combined dose of 270 mg/kg). They were killed 60 minutes after first injection. Data are means ± SEM. By analysis of variance, significant effects of tyrosine dose and branched-chain amino acid injection were observed on retinal and brain tyrosine levels ($P < 0.01$). * Differs significantly from the value for group receiving tyrosine alone at same dose, $P < 0.01$ (t-test).[11]

a saturable, carrier-mediated transport mechanism.[13] The carrier is not specific for tyrosine, but is shared among a number of "large neutral amino acids" [LNAA], including tyrosine, phenylalanine, tryptophan, leucine isoleucine, and valine (*Figure 1*). These amino acids compete with each other for access to the carrier. Consequently, the CNS uptake of an amino acid like tyrosine can be reduced either by lowering serum tyrosine levels, or by raising the blood concentrations of one or more of the other LNAA. In the case of tyrosine uptake into retina, this competitive inhibition is easily demonstrated.[11] When tyrosine is injected into rats, a dose-related increase can be observed soon thereafter in retinal tyrosine levels (*Figure 2*). If the tyrosine is administered along with other LNAA, the rise in retinal tyrosine levels is attenuated. Such an inhibition is not observed if the tyrosine is co-administered with amino acids that utilize a different transport carrier (acidic amino acids, aspartate, and glutamate).[11] A similar effect is observed for brain.[11] This study indicates not only that variations in the blood levels of all LNAA impact on tyrosine uptake, but that conversely, the blood level of tyrosine alone cannot be viewed as a reliable predictor of CNS tyrosine uptake (because at any given dose of tyrosine, the inclusion of other LNAA produced a different rise in retinal tyrosine from that seen following tyrosine injection alone, even though the serum tyrosine levels were the same). In light of this fact, when considering the effects a treatment produces on CNS tyrosine levels by virtue of changes in plasma amino acid levels, we commonly report the plasma levels of all the aromatic and branched-chain amino acid competitors. This is most economically done as a serum ratio, of tyrosine to the sum of its LNAA competitors (TYR/ΣLNAA): when the ratio rises, CNS tyrosine uptake should increase; when it falls, CNS tyrosine uptake should fall.

Earlier, meal-induced changes in tryptophan uptake into brain were causally related to the effects the meal produces on the serum ratio of tryptophan to the sum of the other LNAA competitors (see below).[14] This same connection also holds between a meal, the attendant changes in the serum TYR/ΣLNAA ratio and retinal tyrosine uptake, and the resulting effects on retinal dopamine synthesis.[15] When a fasting rat consumes a protein-containing meal, large increases in serum tyrosine level occur, increases that greatly exceed those in the levels of its competitors (probably because the rise in serum tyrosine reflects not only the contribution of tyrosine from the dietary protein, but also from the phenylalanine contained in the protein, owing to its rapid hydroxylation to tyrosine in the liver). Consequently, the serum TYR/ΣLNAA ratio rises rapidly and substantially, leading to a large increase in retinal tyrosine levels (see *Table 2*), and in the rate of tyrosine hydroxylation (in light-exposed animals).[15] In fact, a stimulation of overall dopamine synthesis is seen.[16] Such increases in tyrosine ratio, retinal tyrosine levels and hydroxylation rate do not occur if the rats con-

Table 2 Effects of ingesting single meals on tyrosine levels and hydroxylation rate in the light-exposed rat retina

Treatment group	Serum tyrosine	Serum TYR/ΣLNAA	Retinal tyrosine	Retinal DOPA
Experiment I	*(nmol/ml)*		*(nmol/mg* protein)	*(ng/mg* protein)
Fasting	84 ± 6		1.60 ± 0.18	1.28 ± 0.04
Carbohydrate meal	72 ± 5		1.23 ± 0.04	1.63 ± 0.18
Protein meal	245 ± 33[a]		5.73 ± 0.75[a]	2.57 ± 0.24[a]
Experiment II				
Fasting	76 ± 5	0.24 ± 0.01	1.85 ± 0.10	1.27 ± 0.10
Carbohydrate meal	68 ± 4	0.23 ± 0.01	1.93 ± 0.16	1.86 ± 0.42
Protein meal	293 ± 20[a]	0.37 ± 0.02[a]	4.17 ± 0.13[a]	2.51 ± 0.32[b]

Groups of six rats were fasted overnight, and the next morning given free access to either nothing, a protein-containing meal, or a protein-free meal. Ninety minutes later, the food was removed, and all animals received NSD-1015 (100 mg/kg ip). They were then killed 30 minutes later. Data are means ± SEM; [a] $P < 0.01$ compared to fasting values; [b] $P < 0.05$ compared to fasting values (analysis of variance; Newman-Keuls test).[15]

sume a non-protein containing food, further indicating that the tyrosine changes are key, and that the effects are not produced by the simple act of eating.[15]

It's not readily apparent what the utility is of tying retinal dopamine synthesis to meal-related changes in tyrosine supply. In fact, we did not adopt the retina to study the physiologic relevance of dietary habits to retinal function. This model was developed because it is quite easy to control the activity of the neurons of interest in retina, for the purpose of demonstrating sensitivity of dopamine synthesis to tyrosine supply (and to food intake). At this point, the important point about the meal studies is that the hydroxylase is sensitive to changes in tyrosine level produced by physiologic-sized changes in retinal tyrosine concentration (i.e., those produced by eating protein). These results make it more plausible that the synthesis of dopamine (and other catecholamines) in the brain is also sensitive to physiologic-sized changes in tyrosine level. However, it appears that such sensitivity is not a uniform property of all dopamine (or probably other catecholamine) neurons. For example, dopamine synthesis in the corpus striatum (site of the nerve terminals of the dopaminergic nigro-striatal tract) is unresponsive to changes in tyrosine level, unless the animal has been pretreated with a drug that greatly stimulates tyrosine hydroxylase activity (e.g., haloperidol or gamma-butyrolactone, drugs both thought to remove receptor-mediated, negative feedback controls on tyrosine hydroxylase activity).[6,10,17] These neurons cannot be thought of as normally responsive to physiologic variations in tyrosine supply. On the other hand, the dopamine neurons of the meso-cortical pathway, which project from the midbrain (mesencephalon) to the prefrontal cortex, are typically very active.[18] And, dopamine synthesis in the prefrontal cortex responds to tyrosine administration without pretreatment with any "activating" drugs.[8,9] The dopamine (and norepinephrine) terminal fields in the hypothalamus have not been studied in much detail. There is some evidence that prolactin-related dopamine neurons are sensitive to tyrosine supply under some conditions,[19] though the norepinephrine neurons are as yet unstudied. These latter neurons are perhaps the most interesting from a physiologic point of view, since the hypothalamus is a brain region important in regulating food intake, and the norepinephrine neurons that project into the hypothalamus represent a catecholamine group often linked to appetite control.[20] Future studies may show that at least one straightforward explanation for why catecholamine synthesis is responsive to meal-induced changes in tyrosine supply focuses on these hypothalamic norepinephrine neurons and their role in food-intake regulation.

Phenylalanine: substrate for or inhibitor of tyrosine hydroxylase

Tyrosine may not be the only amino acid important for catecholamine synthesis. Though it is well-known that phenylalanine can be hydroxylated to tyrosine in liver, and thus in this sense be considered an indirect substrate for tyrosine hydroxylase, phenylalanine also appears to interact directly with tyrosine hydroxylase in neurons. Two effects have been reported. First, phenylalanine is reputed to be a direct substrate for tyrosine hydroxylase. In studies using whole animal or synaptosomal preparations, DOPA and catecholamine synthesis have been demonstrated using phenylalanine as substrate.[21-26] And, catecholamine synthesis from phenylalanine may not be trivial: Two groups have shown that as much as one-third of all catecholamine synthesis in synaptosomes (pinched-off nerve endings formed during tissue homogenization, that can be isolated and studied metabolically in vitro) may derive from phenylalanine.[25,26] And second, in in vitro studies using purified preparations of tyrosine hydroxylase, phenylalanine has been reported to be a direct inhibitor of the enzyme.[27-29]

Despite these published reports, however, it is unclear to what extent phenylalanine variations in vivo might actually influence tyrosine hydroxylase. The only work conducted previously in vivo, which demonstrated phenylalanine conversion to catecholamine in brain, did not evaluate the effects of variations in endogenous phenylalanine levels on tyrosine hydroxylation and catecholamine synthesis.[21] Since phenylalanine can be a substrate for tyrosine hydroxylase, this effect is potentially very interesting: No other monoamine (e.g., serotonin, histamine) pathway appears to have more than a single substrate. Indeed, it would be of interest to know the extent to which normal variations in tyrosine and phenylalanine influence tyrosine hydroxylase and catecholamine production.

To this end, we have recently begun to examine the role of phenylalanine as a substrate for catecholamine production in vivo. Our initial studies have sought to determine if simply increasing neuronal phenylalanine levels (by phenylalanine injection) influences tyrosine hydroxylation rate, using a design like that employed for our earlier work with tyrosine (e.g., *Table 1*). We reasoned that if both tyrosine and phenylalanine are substrates for tyrosine hydroxylase, and injections of tyrosine can stimulate tyrosine hydroxylation rate in activated dopamine neurons, then perhaps the same result would occur following phenylalanine injection. However, the normal rat poses a problem in attempting this simple experiment: It is quite efficient and fast in hydroxylating a peripheral load of phenylalanine to tyrosine in liver, such that the post-injection rise in serum tyrosine level is much greater than that in phenylalanine.[30-32] Given that the effect of such increases in tyrosine might alone be sufficient to stimulate tyrosine hydroxylation rate, it would be impossible from this study to conclude whether phenylalanine injection stimulated tyrosine hydroxylation because of the rise in serum and tissue tyrosine level, phenylalanine level, or both.

To avoid this complication, we pretreated rats with a drug known to inhibit hepatic phenylalanine hydroxylase (para-chlorophenylalanine, PCPA). When rats were injected with PCPA two days earlier, an injection

Table 3 Effects of tyrosine or phenylalanine injection on in vivo tyrosine hydroxylation rate in light-exposed retinas of p-chlorophenylalanine-treated rats

Treatment group	Serum PHE	Serum TYR	Retinal PHE	Retinal TYR	Retinal DOPA
	(*nmol/ml*)		(*nmol/mg* protein)		(*ng/mg* protein)
Saline	112 ± 21	121 ± 10	2.96 ± 0.44	2.09 ± 0.15	1.26 ± 0.14
Phenylalanine	230 ± 56[c]	139 ± 16	8.92 ± 2.56[c]	2.36 ± 0.12	1.53 ± 0.19
Tyrosine	96 ± 25	363 ± 24[c]	1.99 ± 0.36	12.98 ± 0.73[c]	3.76 ± 0.48[c]
F-value	7.3[a]	58.3[a]	5.7[b]	189.0[a]	16.5[a]

Groups of six rats received p-chlorophenylalanine methylester-HCl (376 mg/kg ip) in the morning. Two days later, 3 hours after onset of the daily light period, they received an ip injection of either saline, phenylalanine methylester-HCl (262 mg/kg ip), or tyrosine methylester-HCl (512 mg/kg ip). Fifteen minutes later, all received an injection of NSD-1015 (100 mg/kp ip), and were killed 30 minutes later. Data are means ± SEM; [a] $P < 0.01$, [b] $P < 0.02$ (one-way analysis of variance, df = 15); [c] $P < 0.05$ compared to saline value (Newman-Keuls test).[31]

of phenylalanine was found to cause substantial increments in serum phenylalanine levels, and only modest increases in serum tyrosine.[31] More important, retinal phenylalanine levels rose, but tyrosine levels did not (*Table 3*). In this paradigm, when hydroxylation rate was measured in retina, we observed that even very large increases in retinal phenylalanine level did not stimulate hydroxylation rate. However, an injection of tyrosine did stimulate hydroxylation rate in PCPA-pretreated rats, suggesting that the drug (PCPA) did not somehow compromise hydroxylase responsivity to precursor (*Table 3*).[31]

The results of these experiments suggest that if phenylalanine is a substrate for tyrosine hydroxylase, raising its concentration does not stimulate hydroxylation rate in the same way a rise in tyrosine level does. (Parenthetically, these findings also suggest that phenylalanine is not an inhibitor of tyrosine hydroxylase, even at supraphysiologic concentrations.) A tentative conclusion might therefore be that physiologic variations in the local phenylalanine concentation (such as might be produced by food intake) are not important in determining catecholamine synthesis rates. However, additional work is required before such a conclusion can be accepted. Quantitative data are needed regarding the actual contribution in vivo of phenylalanine to the production of catecholamines. An appropriate paradigm would involve the use of radiolabeled phenylalanine and tyrosine to measure the relative rates of catecholamine production from each precursor. It would then be important to determine if and how the contribution to synthesis from each substrate changed in the presence of different concentrations of the other. Only then will it be possible to conclude definitively the role of phenylalanine in catecholamine production.

Finally, with regard to phenylalanine's effects on catecholamine synthesis, an argument has recently been made that the ingestion of the phenylalanine-containing dipeptide sweetener aspartame (NutraSweet) could so raise CNS phenylalanine levels as to inhibit catecholamine synthesis, and thereby promote deleterious nervous system effects (e.g., seizures).[32-35] This postulation is based largely on early reports that phenylalanine is an inhibitor of tyrosine hydroxylase in vitro.[29] However, it has been clear for 15 years that the early enzymologic studies of tyrosine hydroxylase employed a synthetic cofactor ($DMPH_4$; 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine) that yielded incorrect data regarding the enzyme's properties. Studies employing $DMPH_4$ suggested that phenylalanine was an inhibitor of tyrosine hydroxylase.[29] Such results were later corrected when the natural cofactor, tetrahydrobiopterin, was identified, and phenylalanine was observed to be a substrate, not an inhibitor of the enzyme.[24] Hence, these early enzymologic data do not serve as a valid base for promoting phenylalanine as an inhibitor of tyrosine hydroxylase activity or catecholamine synthesis.[32,36]

The findings in a recent in vitro study using brain slices have also been said to support indirectly an inhibitory role for phenylalanine in catecholamine synthesis.[37] In these experiments, high medium phenylalanine concentrations were reported to diminish stimulated dopamine release into the medium. However, our work with phenylalanine in vivo (above) finds no basis for suspecting that phenylalanine is a physiologically meaningful inhibitor of tyrosine hydroxylase.[31] And, more recent work involving the administration of very high doses of aspartame to rats (pretreated with PCPA, as above) extends this negative finding to the sweetener, showing no inhibitory effect on tyrosine hydroxylation rate, as estimated in vivo in rat retina.[38] The results of such studies therefore suggest that phenylalanine-induced inhibition of catecholamine synthesis cannot be invoked as a mechanism to explain putative CNS side-effects of aspartame.

Tryptophan availability and serotonin synthesis

Regulation of serotonin synthesis

The relationship of tyrosine availability to dopamine synthesis shares some interesting similarities and differences with another neurotransmitter precursor-product relationship: that between tryptophan and serotonin (5-hydroxytryptamine, 5HT). The rate of 5HT synthesis is also sensitive to the supply of its precursor (tryptophan). Serotonin synthesis (*Figure 3*)

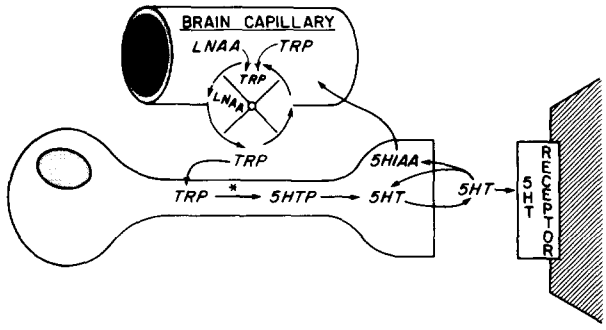

Figure 3 Tryptophan (TRP) availability and serotonin (5HT) synthesis. Tryptophan is hydroxylated to 5-hydroxytryptophan (5HTP); 5HTP is decarboxylated to 5HT. Monoamine oxidase initiates the catabolism of 5HT to 5-hydroxyindoleacetic acid (5HIAA), the principal 5HT metabolite in the CNS. Asterisk indicates rate-limiting step in 5HT formation, TRP hydroxylation; TRP competes with other LNAAs for uptake into brain.

from tryptophan occurs in a two-step reaction sequence: The amino acid is first hydroxylated to 5-hydroxytryptophan (5HTP), in a reaction catalyzed by tryptophan hydroxylase; 5HTP is then decarboxylated to 5HT, in a reaction mediated by aromatic-L-amino acid decarboxylase, the same enzme involved in the decarboxylation of DOPA to dopamine. Tryptophan hydroxylation is the rate-limiting step in 5HT synthesis (as tyrosine hydroxylation is in dopamine production), and the activity of tryptophan hydroxylase therefore governs 5HT synthesis rate.[39]

The primary similarity between the serotonin and dopamine pathways of relevance to the present discussion is that the rates of synthesis of both are sensitive to precursor supply.[4] In fact, the observation that changes in tryptophan level readily influence serotonin formation was made long before the effects of tyrosine on catecholamine synthesis was identified.[40,41] And, at the time that effects of tryptophan administration on serotonin formation were being characterized,[42] it appears that the focus of studies of the controls on catecholamine synthesis was on direct end-product inhibition. End-product inhibition was indeed found for the catecholamine pathway.[2] Interestingly enough, though this mechanism was later reputed also to operate in the 5HT pathway,[43] subsequent work could not confirm the findings.[44,45] Consequently, prior to about 1975, the serotonin pathway seemed primarily sensitive to precursor variations, while the catecholamine pathway appeared to be governed by feedback mechanisms, and not precursor supply.

Since then, knowledge of the controls governing catecholamine production has expanded to include not just (a) direct, end-product inhibition,[2] but also (b) indirect, receptor mediated feedback inhibition of synthesis,[18,46] (c) neuronal activity-related positive effects on tyrosine hydroxylase (both short-term and long-term effects, mediated by different mechanisms),[47-49] and (d) precursor effects on tyrosine hydroxylation/catecholamine synthesis.[4] Concerning the 5HT pathway, it now appears that the controls governing synthesis rate have much in common with those controlling the catecholamine pathway. Certainly, a difference exists regarding direct, end-product inhibition, as mentioned above. However, indirect, receptor mediated effects on 5HT synthesis have been identified,[50] as have neuronal-activity related effects on tryptophan hydroxylase,[51] and the precursor effects discussed above are also held in common.

Recently, we have noted another similarity between the serotonin and catecholamine pathways, which concerns the relationship of neuronal activity to precursor sensitivity.[52] The sensitivity of dopamine synthesis to local tyrosine levels highlights as a regulatory feature the fact that neurons will not make more dopamine when more tyrosine is supplied unless the neurons are active (firing). A connection between neuronal activity and tryptophan effects on 5HT synthesis has not heretofore been widely embraced. However, this relationship can readily be demonstrated using a pharmacologic paradigm: Rats are pretreated with a drug known to slow the firing rate of 5HT neurons (8-hydroxy-2-(di-n-propylamino)tetralin; 8-OH-DPAT). They then receive an injection of tryptophan, and tryptophan hydroxylation rate (an in vivo estimate of 5HT synthesis) is examined, to determine if increasing tryptophan levels produces similar increments in 5HT synthesis in both control and 8-OH-DPAT-treated rats. The results clearly show that 8-OH-DPAT pretreatment substantially suppresses the tryptophan-induced stimulation of hydroxylation rate (*Figure 4*). Since this drug is known not to have a direct effect on tryptophan hydroxylase activity,[53] the most likely interpretation is that the slowing of firing rate, a prominent action of this drug mediated by somatodendritic 5HT autoreceptors,[54-56] is indirectly responsible for the diminished responsivity of the hy-

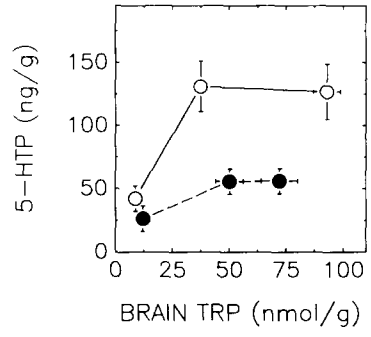

Figure 4 Effect of 8-hydroxy-2-(di-n-propylamino)tetralin on the tryptophan-induced increase in tryptophan hydroxylation rate in rat cerebral cortex. Groups of five rats received saline (open circles) or 8-OH-DPAT (0.64 mg/kg ip; closed circles), followed 15 minutes later by TRP (0, 50, or 125 mg/kg ip). NSD-1015 (100 mg/kg ip) was then injected 30 minutes later, and the rats were killed after another 30 minutes. Mean cerebral cortical 5HTP accumulation values are plotted against mean brain TRP levels for each TRP dose (left-most points, 0 mg/kg; middle points, 50 mg/kg; right-most points, 125 mg/kg). Bars are SEM for both TRP (horizontal) and 5HTP (vertical). By two-way analysis of variance, the effects of 8-OH-DPAT and TRP on 5HTP accumulation were both highly significant ($P < 0.01$). And, at each TRP dose (0, 50, and 125 mg/kg), the saline-treated value differed significantly from the 8-OH-DPAT-treated value ($P < 0.01$, t-test). (Fernstrom, M.H., Massoudi, M.S. and Fernstrom, J.D., unpublished observations.)

droxylase to tryptophan loading. Recently, Schaechter and Wurtman[57] reported that 5HT formation in vitro in hypothalamic slices was sensitive to local tryptophan concentration, regardless of whether the slices were stimulated electrically. The interpretation given was that 5HT synthesis is sensitve to tryptophan supply regardless of neuronal activity. However, results obtained in vitro must be validated in vivo, before they can be accepted as physiologically meaningful. Our findings with tryptophan and 8-OH-DPAT indicate that the in vitro findings may not accurately reflect the true in vivo regulatory environment.

The issue of neuronal activity as a modulator of tryptophan's effects on 5HT synthesis may be physiologically important. The activity of 5HT neurons is known to be tied to arousal state: These neurons are most active when the animal is awake; electrical activity falls to a low level when the animal goes to sleep, and stops during a particular stage of sleep, rapid-eye movement sleep.[58] Consequently, effects of tryptophan on 5HT production might well be considerable when the animal is awake, and minimal during sleep.

Food effects on serotonin synthesis

Because the 5HT and dopamine pathways are both susceptible to precursor supply, both are responsive to physiologic/metabolic factors that influence the CNS uptake of each precursor. One such metabolic phenomenon is food intake. Both precursors and their transmitters are affected by food intake, with some interesting differences occurring in their responses.

First, tryptophan (like tyrosine) is an LNAA. Its uptake into brain therefore involves the competitive LNAA transporter. Consequently, meal effects on brain tryptophan uptake depend on how the meal alters the serum level of tryptophan relative to those of its LNAA competitors. (A serum ratio of tryptophan/ΣLNAA has been found to predict meal effects on CNS tryptophan levels, much as has been the case for the serum tryosine/ΣLNAA ratio and CNS tyrosine uptake[4]; see above discussion.)

The effects of single meals on CNS tryptophan levels and 5HT synthesis show some interesting differences from those on CNS tyrosine levels and dopamine synthesis. In *Table 2*, it can be seen that the ingestion of a protein-containing meal by fasting rats produces a large increase in retinal tyrosine level (and hydroxylation rate), while retinal tyrosine levels are unchanged when rats consume a non-protein, carbohydrate meal. This result is similar to that observed earlier for brain.[12] In contrast, the same meals produce different effects on brain tryptophan levels and 5HT synthesis: Ingestion of a protein meal produces no change in brain tryptophan level, while the consumption of carbohydrates (no protein) increases brain tryptophan (and stimulates 5HT formation).[4] These different effects can all be accounted for by the effects of the meals on the serum LNAA pattern, and thus indirectly on the competitive transport of tryptophan and tyrosine into the CNS: The ingestion of carbohydrates increases the serum tryptophan/ΣLNAA ratio, but does not modify the tyrosine/ΣLNAA ratio; the consumption of a protein-containing meal does not alter the serum tryptophan/ΣLNAA ratio, but greatly increases the serum tyrosine/ΣLNAA ratio.

The changes in serum LNAA pattern and CNS tryptophan and tyrosine uptake that occur following the ingestion of single meals cannot be extrapolated to a chronic dietary setting. In particular, the chronic ingestion of different levels of protein in the diet leads to changes in CNS tryptophan and tyrosine levels that cannot be anticipated from the results of single-meal studies. For example, when rats ingest for ten days diets containing different levels of protein, no remarkable diet-related variation in the serum tryptophan/ΣLNAA ratio or in brain tryptophan level is observed, even though dietary protein levels ranged from 5% to 75%.[59,60] In contrast, the serum tyrosine/ΣLNAA ratio and brain tyrosine levels do show a substantial correlation with dietary protein content, but one opposite to that which might be predicted from single-meal studies: The serum tyrosine/ΣLNAA ratio and brain tyrosine levels vary inversely with dietary protein content in adults rats, with the variation being as large as three-fold, depending on the dietary protein content[59] and the time-of-day the samples are taken.[60]

Data such as these, tying dietary protein and carbohydrate consumption to the production of CNS neurotransmitters, have an obvious appeal to investigators exploring for food-driven metabolic signals that inform the brain about recent dietary history. The brain is presumed to use such information to plot dietary strategy (which it implements through hunger/directed appetites). Some interesting models have been constructed, which bear mention. One attempted to link the chronic level of protein electively consumed by young rats to the serum tryptophan/ΣLNAA ratio, and thus brain tryptophan levels and 5HT synthesis. However, the experimental design involved providing rats with diets containing proteins deficient in one or more amino acids, or with these diets supplemented with the deficient amino acids.[61] Though good correlations were obtained between the level of protein consumed and the serum tryptophan/ΣLNAA ratios, when the relationship was examined some years later, looking simply for a "dose-response" relationship between dietary protein content and the serum tryptophan/ΣLNAA ratio, using normal protein sources, none was found.[59,60] The conclusion drawn was that though the brain may regulate protein intake via a metabolic cue, that cue is unlikely to be the serum tryptophan/ΣLNAA ratio.

A second model of interest also involved the 5HT neuron. In this hypothesis, carbohydrate intake was thought to be regulated. It was based on the observations that (a) the ingestion of carbohydrates, but not protein, raises the serum tryptophan/ΣLNAA ratio, brain tryptophan level, and 5HT synthesis and release; and (b) the release of 5HT by brain neurons suppresses appetite for carbohydrates. (This latter postulation was based on data suggesting that drugs that stimu-

lated 5HT receptors in brain selectively suppressed appetite for carbohydrates.[62] These observations were employed to construct the following control loop: When a rat consumes carbohydrates, the serum tryptophan/ΣLNAA rises, and brain tryptophan level and 5HT synthesis increase, leading to enhanced 5HT release from nerve terminals, which then feeds back to suppress subsequent appetite for carbohydrates. The animal then shifts more to protein consumption. The ingestion of proteins then causes the serum tryptophan/ΣLNAA ratio to fall (there are data showing this does occur), and thus also brain tryptophan levels, 5HT synthesis, and 5HT release. Because of this, the inhibitory effect of 5HT release on carbohydrate appetite is moderated, and the animal subsequently begins to consume more carbohydrates. As the loop continues to operate, a fixed level of carbohydrate intake is presumably maintained. Though this has been a provocative model, it has not withstood the test of time for a number of experimental reasons. A few reasons follow, and a fuller discussion can be found elsewhere.[63,64] First, and most important, the negative feedback aspect of the model depends on the pharmacologic data purporting to show that 5HT drugs selectively suppress carbohydrate appetite. Sufficient studies have now been performed to show that such is rarely the case, particularly with the drug most often studied, fenfluramine (an indirect 5HT agaonist).[65,66] It appears that drugs like fenfluramine nonselectively suppress food intake. In order for the model to work, these drugs must be selective in suppressing carbohydrate intake.[63,64] Second, studies that have examined the normal pattern of carbohydrate intake by rats allowed to select their level of intake show there to be no obvious common level of carbohydrate selected: Animals show widely differing levels of carbohydrate intake at any point in time; the same animal shows very different levels of carbohydrate intake at different times; and when an animal's diet is modified slightly, it changes its intake of protein and carbohydrates, establishing new (and different) levels of intake of each macronutrient.[67-69] If there were a system governing carbohydrate intake, one would think it would have a set-point. Finally, if rats have a regulatory system to maintain a given level of carbohydrate intake, one would think the rat would defend this level of intake. However, behavioral studies show that when the rat must work to obtain protein, carbohydrates, or total calories, it will work to maintain caloric and protein intakes, but not carbohydrate intake.[70] Taken together, these and other arguments make it unlikely that animals (and probably humans) regulate their level of carbohydrate intake, or that they regulate it via a metabolic mechanism involving meal induced changes in the serum tryptophan/ΣLNAA ratio, and thus brain tryptophan uptake and 5HT synthesis. As an aside, in the absence of such a regulatory loop (and for other important reasons[63,64]), which has been used to postulate the existence of "carbohydrate-craving" obese individuals whose loops are defective, it is unlikely that such individuals exist.

No appetite models exist at present that involve the relationship of protein intake to CNS tyrosine levels and catecholamine synthesis. This probably reflects the fact that insufficient information is presently available to evaluate if the subpopulations of catecholamine neurons in brain involved in food intake regulation are sensitive to diet-related changes in tyrosine supply. If this connection is ultimately made, then it will be interesting to examine the potential relationship of dietary protein effects on CNS catecholamine synthesis, and on protein appetite. Catecholamine neurons definitely influence appetite[20]; and some of the most potent appetite-suppressing agents are active at catecholamine synapses (e.g., amphetamine). Consequently, a connection of diet-induced changes in CNS catecholamine synthesis to appetite is not far-fetched.

Summary and conclusions

This article reviews briefly the influence of precursor availability on the synthesis of two CNS neurotransmitters, dopamine (a catecholamine) and serotonin. Normally, the enzyme catalyzing the initial and rate-limiting reaction in each pathway in the CNS is not fully saturated with substrate. Consequently, transmitter synthesis rate can be directly influenced by variations in the local precursor pool size. Single meals can modify the competitive transport of the dopamine and serotonin precursor amino acids into the CNS (tyrosine and tryptophan, respectively), and thus also the rate at which they are converted to transmitter. Recent data suggest that tryptophan and tyrosine levels in the CNS respond to different levels of protein versus carbohydrate intake in the diet (and not fat intake). For serotonin, such diet-related effects appear limited to single meals; chronic variations in dietary protein and carbohydrate intake have little impact on CNS tryptophan levels or 5HT synthesis (except in extreme nutritional cases, such as protein-calorie malnutrition[71]). For the catecholamines, diet-related effects have been demonstrated following single meals, and are likely to be present in a chronic dietary context as well, since chronic differences in protein intake are associated with a large range in CNS tyrosine levels. The potential role of such diet-related changes in CNS transmitter synthesis on CNS functions has thus far been examined primarily in relation to the brain's control of macronutrient appetite. The results to date have been less than totally satisfying. It seems logical that meal- and diet-related changes in CNS transmitter synthesis should provide a useful signal to brain regarding the ingestion of particular macronutrients. The question is whether such a signal connects directly to appetite for that specific macronutrient, as has been the assumption in previous studies, or whether it provides one of many metabolic signals that the brain uses to manage the overall energy and nitrogen economies of the body. This broader view intuitively seems more likely, since the physiologic focal point of the tryptophan-tyrosine effects on CNS transmitter synthesis resides at the blood-brain (-retina) barrier (i.e., the competi-

tive LNAA transport carrier). Though the competitive transport of tryptophan and tyrosine into the CNS is subject to diet-induced changes in the LNAA pattern, it is also subject to any other metabolic phenomenon that influences the serum LNAA pattern. Hopefully, future studies will examine the connection of the serum LNAA pattern to the CNS uptake of tryptophan and tyrosine, and subsequently to the synthesis and release of the monoamine neurotransmitters in this broader metabolic context.

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