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COMMENTARY

CONTROL OF BRAIN NEUROTRANSMITTER SYNTHESIS BY PRECURSOR AVAILABILITY AND NUTRITIONAL STATE

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The body synthesizes two families of compounds that it uses to carry signals from one cell to another—the hormones and the neurotransmitters. Hormones are distributed ubiquitously by the circulation, but only cells containing specific receptors can "decode" them. In addition, hormones include a considerable variety of chemicals, covering a wide range of polarity and molecular weight. Neurotransmitters, in contrast, are distributed only to cells that are anatomically close to their neurons of origin. They are relatively homogeneous: all of the presently accepted group are low molecular weight, water-soluble compounds that are charged at physiological pH and are closely related to amino acids or to such other dietary constituents as choline. The studies that are summarized in this brief review provide evidence for another major difference between most neurotransmitters and all hormones, i.e. their different dependencies on precursor availability. The rates at which serotonin, acetylcholine, and perhaps other neurotransmitters are synthesized in the brain depend in part on "open-loop" control systems: synthesis increases or decreases as a consequence of diet-induced changes in the plasma concentrations of their precursors. The production of some hormones can be modified by imposing grossly unphysiologic limitations on the availability of their precursors (for example, the virtual absence of dietary iodine or lack of ultraviolet light exposure will limit production of thyroxine or vitamin D_3 respectively). To our knowledge, however, no evidence exists that normal variations in precursor levels ever control the rates at which glands produce or secrete steroids, peptides, or other hormones. In contrast, the simple choice between eating an apple or a chicken leg may determine how much serotonin the brain will make during the postprandial period, and a week of luncheon omelettes would probably elevate acetylcholine levels in the basal ganglia.

This susceptibility of neurotransmitter synthesis to the availability of precursors probably reflects an interplay of three biochemical mechanisms: (a) the propensity of rate-limiting biosynthetic enzymes (such as tryptophan hydroxylase and choline acetyltransferase) to require, for full saturation, higher concentrations of their substrates than are normally present in brain [1, 2]; (b) the inability of the brain to synthesize or store large amounts of these substrates and its consequent dependence on the circulation for obtaining them [3, 4]; and (c) the tendency for substrate levels in plasma to vary within a considerable dynamic range, responding primarily, but not exclusively, to food consumption [5–7]. The coupling of synthesis rates to precursor availability is a fairly widespread phenomenon in mammalian cells, especially in tissues like liver and skeletal muscle that function as reservoirs for dietary constituents. Protein consumption, for example, increases the proportion of hepatic tRNA molecules that are charged with tryptophan and other scarce amino acids, thereby facilitating the aggregation of polyribosomes and accelerating the production by liver of albumin, tyrosine transaminase, and probably many other proteins. The body's reliance on precursor availability as a control in the production of high molecular weight storage compounds differs considerably, however, from its use of this mechanism to produce trace quantities of highly potent chemical signals. Indeed, one is entitled to wonder why the evolutionary process "allowed" something so variable as food choice to influence something so important to the organism as neurotransmitter synthesis. This relationship does provide the brain with chemical "windows" for monitoring nutritional state and we speculate below as to the possible uses to which the brain puts this information.

The uses of these open-loop control mechanisms to the scientist and the physician are, happily, much more obvious: the fact that choline (or tryptophan) administration rapidly accelerates brain acetylcholine (or serotonin) synthesis provides the investigator with an enormously useful tool for manipulating cholinergic (or serotoninergic) synapses with a relatively high degree of specificity, and provides the clinician with a novel, and probably safe, tool for treating disease states thought to be related to inadequate cholinergic (or serotoninergic) transmission. Similarly, the fact that normally occurring changes in precursor levels create corresponding changes in neurotransmitter synthesis (and, probably, release) gives the investigator a rare opportunity to discover the real functions of the neurons that utilize these neurotransmitters: if cholinergic or serotoninergic neurons should happen to be essential to a system mediating a particular brain output (e.g. sleep, secretion of a pituitary hormone, blood pressure, or myoclonus), then diet-induced changes in the synthesis of their neurotransmitter may correspond to changes in that brain output.

The following discussion briefly summarizes the information currently available concerning the relationships between precursor availability and the syntheses

of brain serotonin, acetylcholine, and the catecholamines, dopamine and norepinephrine. It should be recognized that similar relationships may also exist for other neurotransmitters (for example, glycine in the spinal cord) and that these remain to be explored.

SEROTONIN

The uptake of the circulating essential amino acid L-tryptophan into raphe neurons initiates the synthesis of serotonin in the brain. The factors governing this uptake process apparently do not differ from those operating in other neurons or in glia, inasmuch as both the levels of tryptophan in whole-brain homogenates and the amounts of tryptophan available for conversion to serotonin within raphe neurons can be predicted from two parameters of the plasma [8, 9]: (a) its total concentration of tryptophan (free plus albumin-bound) and (b) the sum of the concentrations of the other neutral amino acids (chiefly tyrosine, phenylalanine, leucine, isoleucine, and valine) that compete with tryptophan for a common uptake system, which is probably localized at the blood–brain barrier [10, 11]. A high-affinity uptake system for tryptophan has been described in preparations of synaptosomes; however, this system probably bears little relation to the actual availability of tryptophan for serotonin synthesis *in vivo*. (It seems unlikely that tryptophan normally enters raphe neurons via the synaptic cleft.) The intraperitoneal administration of low doses of tryptophan (up to 25 mg/kg) causes linear increases in the concentrations of tryptophan and serotonin within whole-brain homogenates [6]. Although these doses are considerably less than the amounts of tryptophan that the animal would probably receive from a routine daily diet, their effects on brain tryptophan levels are much greater because they are not accompanied—as is normally the case—by the much larger quantities of other neutral amino acids present in all natural proteins. When tryptophan is administered alone, it must be thought of as a drug, not a food.

That the increases in brain serotonin (following treatments that raise brain tryptophan) reflect increased synthesis, and not decreased release or metabolism of the monoamine, is indicated by the fact that brain levels of 5-hydroxyindole acetic acid (5-HIAA), the major serotonin metabolite, also rise in parallel [6]. Investigators have also used an indirect approach to demonstrate the positive relationship between brain tryptophan levels and brain serotonin synthesis, i.e. by showing that the rate at which the serotonin precursor, 5-hydroxytryptophan (5-HTP), accumulates in brain after treatment with a centrally acting inhibitor of aromatic L-amino acid decarboxylase varies as a function of brain tryptophan concentration [12, 13]. Such studies have also allowed the calculation of a K_m *in vivo* for tryptophan hydroxylase, the enzyme that catalyzes the conversion of tryptophan to 5-HTP. This K_m value (6×10^{-5} M) [12] approximates that observed in studies *in vitro* and supports the notion that tryptophan hydroxylase is normally not saturated with its amino acid substrate *in vivo*. When brain tryptophan levels are elevated by administering the amino acid or, as described below, by feeding the animal a protein-free meal [14], serotonin and 5-HIAA levels rapidly increase in both the brainstem (which contains the perikarya of serotoninergic neurons) and the telencephalon (where the monoamine is largely confined to nerve terminals) [13]; the smallest per cent increases are noted in the hypothalamus and corpus striatum.

If a normal rat that has been fasted for 10–12 hr is allowed to consume a carbohydrate or carbohydrate-fat meal (i.e. one lacking protein), the levels of tryptophan, serotonin and 5-HIAA in brain increase significantly within 1 hr and peak after 2 hr [14]. These changes occur because insulin, which is secreted in response to the food, has peripheral effects on plasma amino acid concentrations: it lowers the plasma levels of all of the neutral amino acids other than tryptophan (largely by facilitating their uptake by skeletal muscle and their incorporation into muscle protein); in contrast, it raises plasma total tryptophan concentrations in laboratory rodents. (In humans, insulin has no effect on tryptophan levels, or else decreases them only slightly [15].)

This unique response of circulating tryptophan to insulin derives from another unusual property of the amino acid, i.e. its propensity to bind to circulating albumin. The ability of albumin to bind tryptophan apparently bears an inverse relationship to the plasma concentration of nonesterified fatty acids (NEFA) [15, 16]. (Fasting rats have NEFA concentrations of about 1–2 mM, and more than 99 per cent of all circulating NEFA molecules are albumin-bound; in contrast, their concentrations of tryptophan in plasma reach only 20 μM, of which about 20 per cent is albumin-bound). Postprandial insulin secretion causes a marked fall in plasma NEFA, and, consequently, a major increase in the ability of albumin molecules to bind tryptophan. Hence, plasma levels of albumin-bound tryptophan may rise by as much as 50 per cent. Free (i.e. non-albumin-bound) tryptophan levels fall after the insulin secretion, as do the levels of other neutral amino acids. This fall, however, is of considerably less magnitude than the simultaneous rise in albumin-bound tryptophan; hence total plasma tryptophan levels rise. Fortunately, the binding of tryptophan to albumin does not seem to render it unavailable to the brain [9, 16]: the brain's affinity for tryptophan, *in vivo*, appears to be greater than that of albumin. Hence, brain tryptophan levels show a far better correlation with the ratio of plasma total tryptophan to the sum of the competing neutral amino acids than with the ratio of plasma-free tryptophan to the sum of its competitors.*

If the test meal consumed by the fasted rat happens to contain protein as well as carbohydrate fat, brain tryptophan and serotonin levels do not rise as much as they do when protein is lacking, even though plasma tryptophan concentrations exhibit a greater increase [17].* If the amount of protein in the meal is increased beyond the 18–22 per cent contained in most commercial rat chows, brain tryptophan and serotonin levels may actually decrease postprandially. This relationship is clearly counter-intuitive: one would think that the addition of tryptophan to the

* J. D. Fernstrom, M. J. Hirsch and D. V. Faller, submitted for publication.

food (i.e. as a constituent of the protein) would amplify, rather than suppress, the increase in brain tryptophan and serotonin. Its explanation arises from the fact, mentioned above, that the uptake of circulating tryptophan into the brain is competitive with the other neutral amino acids: virtually all natural proteins contain less than 1.5 per cent tryptophan (zein contains as little as 0.1 per cent), but more than 25 per cent of their total mass derives from the five competing amino acids listed above. Moreover, the enzyme tryptophan pyrrolase catabolizes a considerable fraction of the tryptophan entering the liver via the portal vein, while little or no metabolism of the branched-chain amino acids (leucine, isoleucine and valine) occurs in the liver. Hence, the consumption of any protein causes proportionately greater increases in the plasma concentrations of tryptophan's competitors than in that of tryptophan itself.

The precise effect of any meal on the plasma amino acid pattern (and, thereby, on brain tryptophan levels and serotonin synthesis) thus depends on a number of factors, including: (a) plasma insulin levels pre- and postprandially; (b) the amount of protein in the meal; and (c) the amino acid composition of the protein. The amount of fat in the meal also has a small effect on brain serotonin. If the fat content is very high, plasma NEFA may not fall postprandially; thus, less tryptophan will become bound to albumin, and total plasma tryptophan will not rise as much, or it may actually fall. The regular consumption of a diet especially rich or poor in this amino acid will, at steady state, cause the expected parallel changes in brain tryptophan. Thus, tryptophan (and serotonin) levels are chronically depressed in animals consuming a diet in which the only protein source is corn (5.6 per cent protein)[18]. However, even in this circumstance, each particular meal will transiently elevate brain tryptophan, as predicted by its effect on plasma amino acid patterns.

Just as brain tryptophan levels vary as a function of a plasma concentration ratio, so also can the brain levels of the other neutral amino acids be shown to vary as functions of their corresponding ratios. When plasma and brains are taken from a number of individual rats that have eaten a variety of different meals, the correlation coefficient (r) for brain tyrosine vs the ratio of plasma tyrosine to the sum of its five competitors is 0.91 [19]. This competition among neutral amino acids for brain uptake and the dependence of brain serotonin synthesis on brain tryptophan levels may allow the investigator to suppress serotonin synthesis by administering a new category of "drug" that is not known to interact with any of the enzymes involved in serotonin synthesis or metabolism: i.e. one of the branched-chain amino acids. As described below, this manipulation has already been used to suppress the synthesis of brain catecholamines from tyrosine.

The ability of the investigator to induce increases in brain serotonin synthesis—whether it be by administering tryptophan or by feeding rats meals that elevate brain tryptophan—is the basis of a useful paradigm for examining the relative importance of other factors that could control serotonin formation *in vivo*. For example, if brain serotonin levels are elevated pharmacologically [by pretreating animals with a low dose of a monoamine oxidase (MAO) inhibitor] and the animals are then allowed to consume a carbohydrate meal, the subsequent increase in brain 5-hydroxyindole levels is every bit as great as in control rats not given the MAO inhibitor [20]; this finding indicates that end-product inhibition is not a significant factor affecting brain serotonin synthesis *in vivo*. Similarly, a dose of chlorimipramine that is sufficient to suppress the flow of impulses along serotoninergic neurons (as shown by electrophysiological methods [21] and by a reduction in brain 5-HIAA) does not suppress the carbohydrate-induced rise in brain serotonin at all, and reduces the rise in brain 5-HIAA by only 18 per cent [20]. These results suggest that brain serotonin synthesis is not as dependent as that of, say, dopamine on the rate of impulse flow.

The above observations strongly suggest that serotoninergic brain neurons are highly specialized "variable ratio sensors," that is, they regulate their rate of neurotransmitter synthesis according to a plasma ratio that changes predictably in response to food consumption or hormone secretion. Unfortunately, data presently available do not prove that the flow of information from serotonin neurons also varies with brain tryptophan levels, or even that serotonin release exhibits this dependency. Ideally, one would choose to examine the relationships between plasma amino acids, serotonin synthesis, and serotonin release using a peripheral serotoninergic synapse, i.e. one from which the released serotonin and serotonin metabolites could be collected and the correlation between serotonin levels and the amounts of neurotransmitter released per nerve impulse examined. We are unaware of a suitable peripheral synapse, and hence are unable to measure serotonin release into brain synapses directly. Therefore, it seems necessary to approach indirectly the relationship between food-induced changes in serotonin synthesis and release of transmitter from serotoninergic neurons. Three strategies suggest themselves: (a) to measure the amounts of serotonin and 5-HIAA entering the cerebrospinal fluid in fed and fasted animals after peripheral stimuli known to activate serotoninergic neurons; (b) to monitor the spontaneous electrical activity of raphe neurons (and the neurons they innervate) in animals given meals that raise or lower brain serotonin; and (c) to examine the effects of such meals on brain outputs that are believed to depend on circuits with a serotoninergic synapse (examples of such outputs are food consumption, sleep, and secretion of anterior pituitary hormones).

The utility of the first strategy is limited by the unavailability of peripheral manipulations that reliably activate serotoninergic neurons. Preliminary uses of the second strategy have shown that the electrical activity of raphe neurons that happen to be active already (i.e. they are undergoing fairly frequent depolarizations prior to treatment) declines when rats are given pharmacologic doses of tryptophan (i.e. doses that elevate brain tryptophan levels beyond their normal dynamic range [22]). In other words, when precursor loading causes neuronal serotonin levels to increase, the accelerated release of transmitter activates the feedback mechanisms, which in turn suppress the further release that would accompany neuronal firing. Unfortunately, this finding with raphe neurons pro-

vides no insight into what happens in animals in which brain serotonin levels are varying physiologically. However, within this normal range of variation, changes in the quantity of serotonin released per nerve impulse do not appear to cause feedback modifications in the rate of impulse flow [23]; thus, the number of molecules impinging on post-synaptic receptors per unit of time may actually vary with brain serotonin levels.

Application of the third strategy is, of course, sorely compromised by the fact that any measurable brain output undoubtedly involves a very large number of synapses and many more neurotransmitters than just serotonin. Thus, for example, a dietary treatment that really did increase the flow of information across serotoninergic synapses might fail to modify sleep or food consumption because the outputs from other neurons in the synaptic chain underwent compensatory changes. Nevertheless, some evidence now exists that precursor-induced changes in brain serotonin levels can significantly modify brain outputs. Perhaps the most compelling data concern the mechanism by which the rat chooses how much protein to consume over a fairly long period (4 weeks): when animals were allowed to pick from several diets that varied both in protein content and in amino acid composition, their total protein intakes exhibited a very high, inverse correlation with the ratio of plasma tryptophan to the sum of its neutral amino acid competitors [24]. (Brain serotonin levels were not actually measured.) If one assumes that protein homeostasis exists and is important to the organism, it makes some sense teleologically that a family of neurons whose function depends on the scarcest amino acid should "inform" the rest of the brain about the state of the body's amino acid metabolism.

Tryptophan administration has also been shown to modify sleep latency in humans [25], the secretion of prolactin and gonadotropins in humans (cf. Ref. 26), and the pain sensitivity of rats previously given a corn diet [27]. Apparently, no data are available on how meal-induced changes in brain tryptophan affect these brain outputs. Unfortunately, changes observed after administration of large doses of tryptophan do not necessarily reflect alterations in serotonin release: as a neutral amino acid, tryptophan can lower brain tyrosine levels, and thus, in large doses, transiently suppress brain catecholamine synthesis [28]. Such changes in the plasma amino acid pattern and in brain tryptophan may underlie some of the neurologic findings in hepatic coma [29] and in numerous other metabolic diseases.

ACETYLCHOLINE

The K_m values of brain choline acetyltransferase for choline (400 μM) and acetyl CoA (18 μM) [2] are well above the actual brain concentrations of these acetylcholine precursors (approximately 37 μM [7, 30, 31] and 7–11 μM [32], respectively); hence, it should not be surprising that acetylcholine synthesis can be accelerated by treatments that elevate brain choline (or, presumably, acetyl CoA) levels. What is perhaps surprising is that this effect was not fully documented until 1975 [31], when our laboratory reported that whole-brain acetylcholine levels were significantly elevated (by 22 per cent) 40 min after rats received a single intraperitoneal dose of choline chloride (60 mg/kg). Technological problems account for this delay in exploring what now appears an obvious relationship: until the recent application of focused microwave beams for rapid inactivation of brain enzymes, no method existed by which brain samples could be obtained in which acetylcholine levels were not artifactually depressed (and those of choline elevated) as a result of postmortem hydrolysis of acetylcholine (and choline-containing phospholipids). More recent studies have affirmed that treatments which elevate brain choline levels subsequently increase those of acetylcholine and have further shown that this effect is especially marked within the caudate nucleus. The consumption of 129 mg of dietary choline/day for 11 days was associated with caudate acetylcholine concentrations that were 46 per cent greater than those found in control animals on choline-free diets, and 15 per cent greater than those in animals eating 20 mg choline/day [7]. These increases in brain acetylcholine produced by administering physostigmine were additive to those produced by adding choline to the diet, affirming that choline raises brain acetylcholine by accelerating its synthesis.

Although a controversy continues regarding the ability of the brain to synthesize choline *de novo*, most investigators seem to agree that the brain lacks this capacity and that its sole source of choline is the circulation; the circulation, in turn, receives choline from endogenous synthesis in the liver and from certain foods (especially eggs and meats). The extent to which the diet and the liver normally provide the brain with the choline molecules utilized for acetylcholine synthesis has not yet been characterized. The diet is probably a very important source, however, in view of the fact that changes in dietary choline can induce such rapid and major changes in brain acetylcholine levels. The range of daily choline intakes utilized in the above dietary studies was probably of the same order as the range occurring normally in the human diet.

Synaptosomal preparations from rat brain exhibit two uptake mechanisms for choline: (a) a sodium-dependent, high-affinity system specifically associated with the terminals of cholinergic neurons, and (b) a low-affinity system, not confined to cholinergic neurons, which is probably not saturated *in vivo* at normal plasma choline concentrations [33]. We suspect that these two uptake systems fulfill different functions: the high-affinity system may serve to allow choline molecules to be reutilized for acetylcholine synthesis, while the low-affinity system may regulate the uptake of circulating choline and, therefore, determine the total amounts of choline (plus acetylcholine) present at steady state in brain. (Compared to those in rats deprived of dietary choline for 11 days, free choline concentrations in plasma were twice as high among rats eating 20 mg choline daily and almost four times as high in animals eating 129 mg [7]. Hence, free choline levels do normally vary in plasma over a considerable dynamic range.) We do not yet known whether choline, like tryptophan, encounters competition from other dietary constituents or from endogenous choline analogs that can affect its uptake into brain. Another matter yet to be determined is

whether major changes in brain acetylcholine synthesis (produced by varying the availability of choline) ultimately cause compensatory feedback changes in the activity of choline acetyltransferase.

The physiological significance of diet- and precursor-induced changes in brain acetylcholine levels remains to be explored, as does the possibility that similar changes occur in the more accessible, peripheral cholinergic neurons. However, the speed with which choline adminstration elevates brain acetylcholine strongly suggests that the number of molecules in a "quantum" of acetylcholine need not be constant, and that it is subject to modification by nutritional and metabolic factors affecting plasma choline levels. Very soon after publication of the initial report on the choline effect in rats, oral choline supplements were tested as a therapeutic measure in a disease (tardive dyskinesia) thought by some psychopharmacologists to reflect inadequate central cholinergic tone. The choline treatment did ameliorate the clinical findings in the single patient studied; however, it also produced unwanted signs of peripheral cholinergic activation [34]. Both the therapeutic effect and the side effect provide evidence that choline-induced changes in acetylcholine synthesis do, in fact, modify the flow of information across cholinergic synapses. One anticipates that numerous additional uses for choline administration (or deprivation) will be discovered by physicians and research scientists wishing to alter the functional activities of cholinergic synapses.

CATECHOLAMINES

When rats received a tyrosine dose considerably larger than the tryptophan doses needed to elevate brain serotonin, their brains, assayed several hours later, did not contain increased quantities of dopamine or norepinephrine. In coupling this negative finding with the broad repertory of alternate mechanisms by which the enzyme tyrosine hydroxylase can, by itself, control catecholamine synthesis, most observers have concluded that precursor availability does not significantly determine the rates at which brain neurons produce dopamine or norepinephrine. For tyrosine levels not to influence catecholamine synthesis would be somewhat surprising, however, inasmuch as (a) tyrosine hydroxylase, like tryptophan hydroxylase, is probably not fully saturated *in vivo*, and (b) plasma and brain tyrosine levels normally vary within a broad dynamic range, rising considerably after animals consume high-protein meals [4] (probably because both the tyrosine and the phenylalanine in the protein contribute to plasma tyrosine).

Because relatively large quantities of brain catecholamines are present within pools with slow turnovers and may thus obscure a tyrosine-induced rise in brain catecholamines, we explored the relationship between brain tyrosine levels and catechol synthesis using the decarboxylase inhibition method: rats received RO4-4602 intraperitoneally and, 15 min later, a second injection of an amino acid that would either raise, lower, or have no effect on brain tyrosine. Animals were killed 1 hr after the first injection, and their brains were assayed for tyrosine and for the dopa that had accumulated subsequent to decarboxylase inhibition. Treatments that elevated whole-brain tyrosine levels were found to have increased the synthesis of dopa; those that lowered or failed to affect tyrosine produced parallel changes in brain dopa levels [28]. We next examined the relationship between brain tyrosine levels and dopamine synthesis by following the accumulation of brain dopamine after inhibition of MAO, or of homovanillic acid (HVA), after the administration of probenecid. With these paradigms, the correlation between brain tyrosine and catecholamine synthesis was poor. In contrast, an excellent correlation appeared when caudate HVA and whole-brain tyrosine levels were examined in rats pretreated with haloperidol (a dopamine receptor agonist).*

We currently interpret these findings as follows: catecholamine synthesis within dopaminergic neurons (i.e. of the nigro-neostriatal tract) does, indeed, depend on both tyrosine hydroxylase activity and precursor (tyrosine) availability. When tyrosine levels are elevated, a transient acceleration occurs in dopamine synthesis, which, in turn, increases the amounts of the neurotransmitter that are released into synapses. The increase in dopamine release activates feedback mechanisms (which might be mediated either by multi-synaptic pathways or by pre-synaptic receptors, or both), and these mechanisms cause a compensatory decrease in tyrosine hydroxylase activity; this decrease restores dopamine synthesis to normal. When animals received the decarboxylase inhibitor or the dopamine receptor-blocking agent, precursor-induced changes in dopa (or dopamine) synthesis were blocked from causing corresponding changes in the activation of dopamine receptors; hence, these feedback mechanisms did not operate. However, when animals received tyrosine or other amino acids plus an MAO inhibitor or probenecid, the hypothetical feedback mechanism could still operate; hence, brain tyrosine levels correlated poorly with dopamine synthesis. If this explanation is indeed correct, it will be of interest to determine whether similar feedback mechanisms also prevent brain tyrosine levels from influencing the rates of catecholamine synthesis in other dopaminergic neurons, or in noradrenergic neurons. A recent observation suggests that amino acid treatments that lower brain tyrosine can depress dopamine synthesis, at least transiently: pretreatment of rats with one of the branched-chain amino acids suppressed the hypothermic effect of *d*-amphetamine, which is mediated by dopamine release [35].

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REFERENCES

1. S. Kaufman, in *Aromatic Amino Acids in the Brain*, CIBA Foundation Symposium 22, p. 85. Elsevier, Amsterdam (1974).
2. H. L. White and J. C. Wu, *J. Neurochem.* **20**, 297 (1973).

* M. D. Scally and R. J. Wurtman, abstract to be presented at 1976 Meeting of the American Society of Neurochemistry, Vancouver, Canada.

3. R. J. Wurtman and J. D. Fernstrom. *Am. J. clin. Nutr.* **28,** 638 (1975).
4. G. B. Ansell and S. Spanner, *Biochem. J.* **122,** 741 (1971).
5. R. J. Wurtman, C. M. Rose, C. Chou and F. Larin, *New Engl. J. Med.* **279,** 171 (1968).
6. J. D. Fernstrom and R. J. Wurtman, *Science, N.Y.* **173,** 149 (1971).
7. E. L. Cohen and R. J. Wurtman, *Science, N.Y.* **191,** 561 (1976).
8. J. D. Fernstrom and R. J. Wurtman, *Science, N.Y.* **178,** 414 (1972).
9. J. D. Fernstrom and R. J. Wurtman, *Scient. Am.* **230,** 84 (1974).
10. R. Blasberg and A. Lajtha, *Archs Biochem. Biophys.* **112,** 361 (1965).
11. W. H. Oldendorf, *Am J. Physiol.* **221,** 1629 (1971).
12. A. Carlsson, W. Kehr, M. Lindqvist, T. Magnusson and C. V. Atack, *Pharmac. Rev.* **24,** 371 (1972).
13. J. L. Colmenares, R. J. Wurtman and J. D. Fernstrom, *J. Neurochem.* **25,** 825 (1975).
14. J. D. Fernstrom and R. J. Wurtman, *Science, N.Y.* **174,** 1023 (1971).
15. D. Lipsett, B. Madras, R. J. Wurtman and H. N. Munro, *Life Sci.* **12,** 57 (1973).
16. B. K. Madras, E. L. Cohen, R. Messing, H. N. Munro and R. J. Wurtman, *Metabolism* **23,** 1107 (1974).
17. J. D. Fernstrom, F. Larin and R. J. Wurtman, *Life Sci.* **13,** 517 (1973).
18. J. D. Fernstrom and R. J. Wurtman, *Nature, Lond.* **234,** 62 (1971).
19. J. D. Fernstrom and D. V. Faller, *Fedn Proc.* **34,** 243 (1975).
20. J. Jacoby, J. L. Colmenares and R. J. Wurtman, *J. neural Trans.* **37,** 25 (1975).
21. D. W. Gallager and G. K. Aghajanian, *J. Pharmac. exp. Ther.* **193,** 785 (1975).
22. G. K. Aghajanian, *Fedn Proc.* **31,** 91 (1972).
23. G. J. Bramwell, *Brain Res.* **79,** 515 (1974).
24. D. V. M. Ashley and G. H. Anderson, *J. Nutr.* **105,** 1412 (1975).
25. E. Hartmann, R. Chung and C.-P. Chien, *Psychopharmacologia* **19,** 114 (1971).
26. J. H. MacIndoe and R. W. Turkington, *J. clin. Invest.* **52,** 1972 (1973).
27. L. D. Lytle, R. B. Messing, L. Fisher and L. Phebus, *Science, N.Y.* **190,** 692 (1975).
28. R. J. Wurtman, F. Larin, S. Mostafapour and J. D. Fernstrom, *Science, N.Y.* **185,** 183 (1974).
29. H. N. Munro, J. D. Fernstrom and R. J. Wurtman, *Lancet* **I,** 722 (1975).
30. W. B. Stavinoha and S. T. Weintraub, *Science, N.Y.* **183,** 964 (1974).
31. E. L. Cohen and R. J. Wurtman, *Life Sci.* **16,** 1095 (1975).
32. J. Sollenberg, in *Drugs and Cholinergic Mechanisms in the Central Nervous System* (Eds. E. Heilbronn and A. Winter) p. 27. Försvarets Forskningsanstalt, Stockholm (1970).
33. M. J. Kuhar, V. H. Sethy, R. H. Roth and G. K. Aghajanian, *J. Neurochem.* **20,** 581 (1973).
34. K. L. Davis, P. A. Berger and L. E. Hollister, *New Engl. J. Med.* **293,** 152 (1975).
35. H. J. Chiel and R. J. Wurtman, *Fedn Proc.* **35,** 405 (1976).