I. INTRODUCTION

In the past the synthesis of brain neurotransmitters has generally been considered a tightly regulated phenomenon. This review demonstrates that this is not always so. For at least one transmitter, serotonin (5-HT), the controls on the rate of synthesis do not appear to involve appreciable end-product or feedback inhibition. Instead the key regulatory enzyme is unsaturated with its substrate, tryptophan, so that 5-HT synthesis varies readily with alterations in precursor supply. Studies are described showing that this precursor-product relationship holds physiologically as well as pharmacologically and pathophysiologically. Data are also summarized demonstrating that increments in 5-HT synthesis produced by tryptophan administration elicit changes in physiological functions believed to be partly controlled by 5-HT neurons in brain (suggesting enhancement of 5-HT release as well as synthesis). Given that variations in tryptophan supply influence 5-HT formation and release, the question is to what extent, if any, this relationship is employed by the brain as a channel to provide information for regulating physiological processes. Hypotheses have been offered based
on limited data. The discussion includes the possibility that 5-HT neurons participate in brain circuits that influence peripheral metabolic phenomena or regulate the intake of particular nutrients.

With the realization that 5-HT synthesis could so readily be influenced by precursor supply, subsequent investigations attempted to determine if the synthesis rates of other transmitters were also affected by precursor availability. Surprisingly, limited effects on the rate of catecholamine formation were found after tyrosine administration. This observation became decidedly interesting with subsequent findings that 1) precursor effects became more apparent when catecholamine neurons were activated (i.e., when rats received a treatment that led to enhanced neuronal firing rates) and 2) tyrosine administration produced clear effects on brain functions thought to be partly controlled by catecholamine neurons (e.g., tyrosine injection lowered blood pressure in hypertensive rats). Apparently tyrosine availability should be included in the group of factors governing the rate of catecholamine synthesis. However, it is less clear for tyrosine than for tryptophan how physiological variations in availability to brain participate in regulatory circuits controlling body functions. The catecholamine synthesis rate is not exquisitely sensitive to physiological variations in precursor supply. Further studies are necessary to learn why those brain functions commonly thought to be influenced by catecholamine neurons (e.g., blood pressure regulation) should be tied to day-to-day changes in tyrosine availability.

Finally, although the synthesis rates of other brain transmitters are reputed to vary with precursor supply, in this review I consider only 5-HT and the catecholamines. This limitation allows clarification of an additional important point. Tryptophan and tyrosine gain access to the brain via a competitive carrier that they share with other large neutral amino acids (LNAAs). The physiological, pharmacological, and pathophysiological studies described here illuminate the extraordinary extent to which this uptake carrier, located at the blood-brain barrier, determines the access of both of these amino acids to brain. In the process, the synthesis rates of the monoamines are clearly affected. Hence this line of investigation provides perhaps one of the most compelling arguments that the competitive transport system for the LNAAs itself normally influences biochemical events within the brain.

II. PHYSIOLOGICAL CONTROL OF BRAIN SEROTONIN (5-HT) FORMATION AND FUNCTION

A. Studies on Tryptophan Hydroxylase and 5-HT Formation

1. Synthesis of 5-HT from tryptophan

Serotonin (5-hydroxytryptamine; 5-HT) is synthesized in brain from the essential amino acid L-tryptophan (Fig. 1). The initial biosynthetic step in-
volves the hydroxylation of the amino acid to form 5-hydroxytryptophan (5-HTP). This reaction is catalyzed by tryptophan hydroxylase, an enzyme highly localized within 5-HT–producing cells (1). Tryptophan hydroxylase is an iron-containing enzyme that utilizes a reduced pteridine as a cofactor (probably biopterin) and requires molecular oxygen (148, 171).

5-Hydroxytryptophan is converted to 5-HT by the ubiquitous enzyme aromatic L-amino acid decarboxylase (172). This enzyme utilizes pyridoxal phosphate as a cofactor. The activity of this enzyme (measured in vitro) is much greater than that of tryptophan hydroxylase (132), and normally very little 5-THP is found within the mammalian brain. Thus the rate of 5-THP decarboxylation must be very rapid, even though the tissue concentration of the amino acid is probably well below the $K_m$ of the decarboxylase. The decarboxylase in brain can apparently also convert modest amounts of tryptophan directly to tryptamine; moreover the accumulation of tryptamine in brain is affected by drugs that also influence 5-HT formation from tryptophan, e.g., tryptophan and monoamine oxidase inhibitors (184, 238, 283, 299). These findings suggest to some investigators that tryptamine may well be a candidate neurotransmitter in the mammalian brain (e.g., 184); however, many more data are needed for a complete evaluation.

The predominant 5-HT metabolite in brain appears to be 5-hydroxyindoleacetic acid (5-HIAA). This compound is formed by a two-step reaction in which 5-HT is first converted to 5-hydroxyindoleacetaldehyde by monoamine oxidase (MAO) and then to 5-HIAA by aldehyde dehydrogenase. A small amount of 5-hydroxyindoleacetaldehyde in brain also appears to be reduced to 5-hydroxytryptophol via the action of alcohol reductase (47, 78). This latter compound has been reputed by some to be biologically active. For example, its administration has been reported to induce sleep in animals.
An additional pathway of 5-HT metabolism has recently been identified in which the amine is converted by the enzyme indolamine dioxygenase to 5-hydroxykynurenine (see 126). This enzyme is found in several organs of the body, including the brain, and can break the pyrrole ring of a number of indolamines, including 5-HT. The extent to which this pathway actually contributes to the normal degradation of 5-HT in brain and other tissues is currently a topic of active investigation.

2. Control of 5-HT synthesis in brain

Tryptophan hydroxylase catalyzes the rate-limiting step in 5-HT synthesis (148, 171) and is thus studied as the locus for controlling the overall rate of 5-HT formation. Central to this discussion is the repeated finding that changes in brain tryptophan level rapidly influence the rates of tryptophan hydroxylation and 5-HT formation. This effect suggests one general property of tryptophan hydroxylase; viz., under normal conditions the enzyme is not fully saturated with substrate. A clear implication is that the 5-HT pathway, at least in brain, can be influenced by substrate availability. Several laboratories have reported, however, that the rates of tryptophan hydroxylation and 5-HT synthesis are controlled by end-product inhibition. The data accumulated to date more strongly support substrate availability than end-product inhibition as the most important general mechanism governing the rate of tryptophan hydroxylation (and 5-HT synthesis), at least physiologically.

a) End-product inhibition of 5-HT synthesis. Evidence supporting end-product inhibition of tryptophan hydroxylation has been offered by several laboratories. Macon et al. (176) observed the rate of 5-HT synthesis in brain stem to be decreased in animals pretreated with an MAO inhibitor. A similar result was later obtained in vitro with slices of rat striatum (120). Carlsson et al. (39, 41, 42) studied the effect of administering MAO inhibitors on the in vivo rate of tryptophan hydroxylation. They noted that the brains of animals pretreated with an MAO inhibitor hydroxylated less tryptophan than those of animals receiving the vehicle. Only a modest suppression of hydroxylation was achieved (~30%), however, and tryptophan injection was still able to stimulate 5-HITP formation in animals pretreated with the MAO inhibitor (though the effect was not as great as that seen in animals receiving no MAO inhibitor). This latter finding indicates that even if some inhibition of 5-HT synthesis occurs when 5-HT levels are quite high (they averaged two- to threefold above normal after treatment with MAO inhibitor), this inhibition is insufficient to block the stimulation of 5-HT formation due to increases in brain tryptophan level. This view is strengthened by the observations that 1) brain 5-HT levels are higher in animals treated with tryptophan and an MAO inhibitor than in rats receiving the MAO inhibitor alone (115) and 2) brain 5-HT levels increase in rats due to the rise in brain
tryptophan accompanying the ingestion of a carbohydrate meal, despite prior elevation of brain 5-HT level by injection of an MAO inhibitor (137). The relative impotence of end-product inhibition as a normal mechanism governing tryptophan hydroxylation is further suggested by the finding of Carlsson and Lindqvist (41) that pretreatment with reserpine, which depleted the brain of 5-HT, had no significant impact on the rate of tryptophan hydroxylation. If endogenous 5-HT levels were important, one might expect the in vivo hydroxylation rate to increase after amine depletion.

Direct end-product inhibition has not been observed in studies of the properties of purified tryptophan hydroxylase (144). Groups demonstrating end-product inhibition have thus interpreted their findings as at least partly supporting an extraneuronal site for the inhibition (e.g., 120). This mechanism is supported by results showing that the administration of 5-HT agonists slows the rate of 5-HT synthesis and turnover (98) as well as the rate of raphe unit firing (see 2). Hamon et al. (120) speculated that the increments in brain 5-HT level produced by MAO inhibition lead to enhanced stimulation of inhibitory receptors, thereby turning down the rate of 5-HT synthesis. This view is taken by Saner and Pletscher (242) in a recent article involving the effects of reserpine on 5-HTP accumulation. Although a brief stimulatory effect of reserpine was noted, it disappeared long before 5-HT levels returned to normal, indicating to the investigators that some effect other than simple 5-HT depletion (and end-product inhibition) might be involved. They suggested that the effect on tryptophan hydroxylase might be indirectly mediated by a change in the interaction of 5-HT with its receptors, since injection of a 5-HT agonist blocked the effect of reserpine on 5-HTP accumulation. An extracellular site of action even seems supported by a portion of the data in the article by Hamon et al. (120). The injection of clomipramine, which blocks 5-HT reuptake, did not seem to sustain normal rates of 5-HT synthesis in the face of an intraventricular injection of 5-HT. If 5-HT directly affected tryptophan hydroxylase, clomipramine treatment should have completely blocked any 5-HT-induced reduction in 5-HT synthesis.

A receptor-mediated mechanism for explaining how 5-HT might inhibit its own rate of synthesis is not consistent with all the data in the literature. Pretreatment with clomipramine does not block the ability of a tryptophan injection or the ingestion of a carbohydrate meal (which itself elevates brain tryptophan) to stimulate 5-HT production in rat brain (137). To be consistent with this hypothesis, clomipramine should itself increase intrasynaptic levels of 5-HT, enhance the rate of 5-HT interaction with its receptors, and thereby reduce the ability of a tryptophan load to stimulate 5-HT synthesis. [The findings of Jacoby et al. (137) do not conflict with those of Neckers et al. (211), who observed that clomipramine administration enhanced tryptophan hydroxylase activity.] Hence, even if increments in extraneuronal 5-HT can cause some diminution of the steady-state rate of 5-HT synthesis, they certainly do not seem to inhibit the stimulation of 5-HT synthesis that accompanies increases in local precursor concentrations.
Finally, some data absolutely do not support the contention that increments in brain 5-HT, induced by MAO inhibition, inhibit the rate of 5-HT formation (167, 193, 201, 202). Therefore if some form of end-product inhibition of tryptophan hydroxylation exists, its physiological significance is not yet clear. Moreover available data indicate that the end-product inhibition thought to occur after pharmacological treatments does not significantly influence the ability of a change in precursor level to influence the rate of 5-HT synthesis.

b) Tryptophan availability and 5-HT synthesis. The notion that local tryptophan levels can directly affect 5-HT formation in brain has been extant for more than 20 years. Early studies in animals focused on the effects of chronic alterations in dietary tryptophan content on 5-HT levels in brain. In general, removal of the amino acid from food, either by feeding a diet containing a casein hydrolyzate (which lacks tryptophan) or a mixture of synthetic amino acids devoid of tryptophan, caused a reduction in brain 5-HT level over a several week period (32, 64, 99, 100, 308). Such effects were eliminated if normal amounts of the amino acid were supplied in these diets (about 0.2% of the amino acid content of casein, based on weight). Similarly, if supranormal amounts of tryptophan were added to these and similar diets, increments in brain 5-HT levels were observed (116, 282).

Around the same time, other investigators began experimenting with the use of tryptophan to treat depression and other mental diseases (58, 59, 155, 163, 219). Positive effects were often found, whether tryptophan was used alone (59) or in combination with an MAO inhibitor (e.g., 58, 219). In some cases measurements were made of the urinary excretion of 5-HIAA, which was elevated by tryptophan administration (155, 163); this effect was apparently not observed in dogs given large doses of tryptophan (270). Of course, changes in urinary 5-HIAA probably indicate very little about the turnover of 5-HT in the brain, but such data at least suggested that tryptophan loading stimulated 5-HT production somewhere in the body.

Interestingly, very scant rationales were given in these papers as to why such manipulations ought to produce the observed result. Tryptophan hydroxylase had not yet been characterized; the possibility that the reaction mediated by this enzyme might be "substrate-driven" certainly did not stand out as a clear hypothesis. (Perhaps at that time it was simply assumed that precursor loading could drive synthesis in the 5-HT pathway.) Nonetheless these studies provided early data very definitely consistent with the possibility that tryptophan availability (to brain) influences 5-HT formation.

In 1961, Hess and Doepfner (127) were the first to demonstrate an actual increase in brain 5-HT level soon after tryptophan injection. More complete evidence followed over the next decade (11, 40, 90, 284), which convincingly suggested that the rate of 5-HT formation varied directly with the availability of substrate. For example, injections of tryptophan into rats caused rapid dose-related increments in brain tryptophan level, in the brain concentration of 5-HT as well as 5-HIAA (Fig. 2; 90), and in the actual rate of
FIG. 2. Dose–response curve relating brain tryptophan and brain serotonin (5-HT). Groups of 10 rats received L-tryptophan (12.5, 25, 50, or 125 mg/kg ip) at noon and were killed 1 h later. Horizontal bars represent SEM for brain tryptophan; vertical bars represent SEM for brain 5-HT. All brain tryptophan levels were significantly higher than control values (P < 0.001). All brain 5-HT levels were significantly higher than control values (P < 0.01). [From Fernstrom and Wurtman (90).]

Brain Tryptophan (µg/g)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Brain Serotonin (µg/g)

0 5 10 15 20 25 30 35 40 45 50

25 mg/kg

12.5 mg/kg

50 mg/kg

125 mg/kg

Control

tryptophan hydroxylation (40, 43). The biochemical basis for this relationship depends on one property of tryptophan hydroxylase. The $K_m$ of this enzyme for tryptophan is surprisingly large ($\sim 2 \times 10^{-5}$ M when bioperin is the cofactor) compared to normal brain tryptophan concentrations ($\sim 1-5 \times 10^{-5}$ M in healthy rats) (90). This implies that the enzyme is normally not saturated in vivo with its substrate. Hence variations in the local tryptophan concentration should and do directly affect the degree of enzyme saturation, and thus the rate of tryptophan hydroxylation.

Interestingly, Figure 2 shows that the 25 mg/kg dose of tryptophan caused a 100% rise in brain tryptophan and a 50% increment in brain 5-HT level (90). This magnitude of variation in brain tryptophan (100%) is not in the pharmacological range; in fact, brain tryptophan levels in the normal, untreated, undisturbed rat undergo 24-h variations of about 100% (see 84). Hence tryptophan hydroxylase would appear particularly sensitive to the normal range of variation seen in brain tryptophan levels.

Generally, when tryptophan is administered, it is converted to 5-HT only in those cells that normally contain it (i.e., that make it, due to the presence of tryptophan hydroxylase) (1). Hence physiological and pharmacological effects of tryptophan administration should be specific to 5-HT neurons (and other cells that normally synthesize the amine; e.g., 55). The one exception may be if tryptophan can also be decarboxylated directly to tryptamine when administered in large amounts. With the advent of sufficiently sensitive methods, several investigators have studied the effects of
tryptophan administration on tryptamine formation in rat brain. Some recent reports indicate that, in response to tryptophan injection, the brain will increase its rate of production of tryptamine (e.g., 238). The increase is quite small, however. According to other studies, the administration of an MAO inhibitor appears essential to the detection of a rise in tryptamine due to tryptophan administration. This suggests that the effect is pharmacological rather than physiological (283). Some investigators have even failed to find tryptophan-induced increments in brain tryptamine levels (184). However, this issue remains unresolved partly because of technical disagreements. Young et al. (299) recently reported that the administration of tryptophan to human subjects leads to increments in the concentration of indoleacetic acid (IAA) in cerebrospinal fluid (CSF). Indoleacetic acid is a metabolite of tryptamine, formed in a reaction catalyzed by MAO. This result was interpreted as indicating that the brain can normally form tryptamine from tryptophan. Note, however, that tryptamine can cross the blood-brain barrier (212). Hence the amine might have been formed peripherally and simply diffused into brain. Further work is needed to resolve this point. For now it seems a reasonably safe, if tentative, conclusion that after tryptophan administration the major metabolic effect is the stimulation of 5-HT formation in those cells that normally make it.

c) Other controls on 5-HT synthetic pathway. This review focuses on the availability of precursor as an important determinant of the rate of product formation (in this particular case, tryptophan as precursor and 5-HT as product). Although an impressive body of data supporting this connection exists, it would be naive to assume that this relationship constituted the only control mechanism governing 5-HT synthesis and release. As for catecholamines, it would be surprising if, for example, 5-HT neurons did not increase their rate of transmitter synthesis in response to significant increases in firing rate. Some data support this suggestion (e.g., 2). In addition one might also find the hormonal milieu of the extracellular fluid to have direct, selective, and significant effects on the 5-HT synthetic machinery. For example, corticosteroids have been reported to directly increase tryptophan hydroxylase activity, possibly by stimulating the production of tryptophan hydroxylase (e.g., 16). [Of course, others have suggested glucocorticoids alter brain 5-HT synthesis indirectly either by decreasing (e.g., 68, 253) or increasing (152) precursor availability to brain]. That hormones could directly influence the functioning of a 5-HT neuron is not unusual. Dopamine-containing neurons in the median eminence are also sensitive to the hormonal milieu: they increase their rate of transmitter production in response to exposure to prolactin (e.g., 6). Attempting to minimize the complexity of nervous system organization and function rarely meets with success. As more is learned about the functioning of 5-HT neurons, it should not be surprising to find that a number of mechanisms govern the rate of 5-HT formation and release.
B. Acute Effects of Diet on 5-HT Formation in Brain: Importance of Large Neutral Amino Acid (LNAA) Transport System

Because 5-HT levels (and synthesis) in brain seem readily influenced by acute changes in tryptophan level, an interesting question is to what extent do normal physiological processes modify 5-HT formation in brain by virtue of their ability to affect brain tryptophan levels? To date we have identified two processes, each related to food ingestion, that acutely dominate in their ability to influence brain tryptophan levels: insulin secretion (after the ingestion of almost any food) and the ingestion of proteins. Each appears to modify brain tryptophan level by altering tryptophan uptake into this organ. Such changes in uptake are effected indirectly and predictably, as described next, via meal-induced alterations in the blood levels of tryptophan and of several other amino acids.

1. Transport of LNAA s into brain

The basis for predictable diet-related changes in brain tryptophan level derives from the properties of the carrier mechanism for tryptophan transport into brain. An understanding of the dynamics of tryptophan transport has grown from more general studies, conducted over the past two decades, that have attempted to elucidate the mechanism(s) of entry of all amino acids into brain. Such studies of amino acid uptake have been undertaken in vivo, with both radiolabeled-tracer (212, 218, 301) and pharmacological-loading (49, 118) techniques, and in vitro, with brain slices (20, 26, 272) and synaptosomes (226). Such investigations have resulted in the now generally held views that 1) amino acids are transported into brain by saturable carrier-mediated processes located at the blood-brain barrier (presumably the capillary endothelial cells); 2) amino acids of similar size and charge (e.g., large neutral, small neutral, acidic, basic) share a common carrier; and 3) within a given transport group, the individual amino acids compete with each other for available carrier sites and thus brain uptake. Tryptophan is an LNAA and thus shares a competitive transport carrier with such other LNAA s as tyrosine, phenylalanine, leucine, isoleucine, valine, and possibly methionine and threonine. Consequently brain tryptophan levels should (and do) rise when either blood tryptophan levels rise or blood levels of one or more of the other LNAA competitors fall. They fall when either blood tryptophan level falls or the blood concentrations of the other LNAA s rise.

A number of studies report that brain neurons (and other cells) possess high-affinity transport mechanisms for amino acids (121, 179). They thus suggest that the neuronal membrane is probably the important site regulating amino acid supply to brain neurons, rather than the capillary endothelial cell. Pardridge (216) disputes this view. He points out that even if
such transport mechanisms exist at the neuronal membrane, they would be saturated at normal blood (and probably brain) amino acid levels and thus act at their $V_{\text{max}}$. Because such maximal velocities would exceed those at the blood-brain barrier by 200–300%, he concludes that the site of transport most likely to limit the access of tryptophan and other amino acids to brain neurons is at the blood-brain barrier, not at the cell membrane. Regardless of the ultimate site designated for these transport systems, it seems that 1) such carriers show competition with respect to substrates and 2), as next described, this competition is functionally important to neurons in the normal range of amino acid levels found in blood.

2. Acute effects of diet on brain tryptophan and 5-HT: mediation via LNAA transport system

The perception that competitive transport might be important in determining the changes in brain tryptophan that accompanied the ingestion of food occurred to us quite by accident. Several years ago we observed in fasting rats that blood tryptophan, brain tryptophan, and brain 5-HT levels all rose soon after the injection of insulin or the ingestion of a carbohydrate-fat meal that induced insulin secretion (91). The results appeared consistent with the notion that brain tryptophan uptake and levels simply reflected blood tryptophan levels. If true, then the ingestion of protein along with the carbohydrate-fat meal would cause larger increments in blood tryptophan, and thus brain tryptophan, than those seen after the consumption of just the carbohydrate-fat meal. The protein would presumably have this effect by contributing exogenous tryptophan molecules to the circulation (the average animal protein contains 0.1–0.3% tryptophan). In fact, when this study was performed, the data obtained were hardly consistent with the hypothesis (Fig. 3). Carbohydrate ingestion certainly elevated blood and brain tryptophan, as expected. Moreover the consumption of the protein-containing meal caused a greater elevation of blood tryptophan than did the ingestion of carbohydrate alone. However, the unanticipated finding was that no increase at all occurred in brain tryptophan among animals consuming the protein-containing meal. Clearly brain tryptophan levels were not simply a reflection of blood tryptophan concentrations.

This result prompted a reconsideration of the experimental design, including the treatment (i.e., a protein-containing meal). The meal provided not only tryptophan to the body, but also all of the other amino acids that would be expected to compete with tryptophan for brain uptake (i.e., the other LNAAAs). As a new hypothesis, it seemed a reasonable possibility that the inclusion of these amino acids in the meal so increased their levels in blood as to block tryptophan uptake into brain, despite the postprandial rise in blood tryptophan levels. This notion was most easily tested by looking for acute differences in brain indole levels in rats fed one of several special meals.
that differed in amino acid content (Fig. 4). One food contained a mixture of amino acids that approximated the amino acid composition of the standard dietary protein casein. When rats consumed this food, blood tryptophan increased but brain tryptophan did not, thus repeating the finding made with the natural dietary protein (92). A second diet contained the same amino acid mixture but lacked the major LNAAs (tyrosine, phenylalanine, leucine,
FIG. 4. Effect of ingestion of various amino acid-containing diets on plasma and brain tryptophan and brain 5-hydroxyindoles. Groups of 8 fasting rats were killed 1 or 2 h after diet presentation. Vertical bars represent SEM. ○ Fasting controls; □ rats consuming complete amino acid-mixture (i.e., equivalent to casein in amino acid composition) diet; and ● rats consuming amino acid-mixture diet lacking tyrosine, phenylalanine, leucine, isoleucine, and valine. Plasma tryptophan concentrations at 1 and 2 h were significantly greater in animals consuming either diet ($P < 0.001$) than in fasting controls. All brain tryptophan, serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were significantly greater in rats consuming diet lacking the 5 LNAAs than in fasting controls ($P < 0.01$). Among animals eating complete amino acid-mixture diet, only 2-h brain tryptophan concentration was significantly above that of corresponding fasting control group ($P < 0.001$). [From Fernstrom and Wurtman (92).]

isoleucine, and valine). When this food was ingested, both blood and brain tryptophan levels increased just as though the animals had consumed a carbohydrate-fat meal. The ingestion by rats of a third meal, which lacked either the acidic or basic amino acids (rather than the LNAAs), caused increments in blood but not brain tryptophan levels (i.e., it behaved as though all amino acids had been consumed). Hence the amounts of all of the LNAAs in the meal appeared to be important as determinants of the postprandial change in brain tryptophan level.

Based on these and other experiments, we formulated a general hypothesis to explain how diet-induced variations in brain tryptophan and 5-
HT depend on the changes induced by the meal in the blood concentrations of all the LNAAAs, including tryptophan (Fig. 5). For example, when the animal consumes a carbohydrate meal, not only does blood tryptophan increase, but the secretion of insulin elicits a reduction in the plasma concentrations of the other LNAAAs. Thus tryptophan gains a competitive advantage for uptake, and brain tryptophan and 5-hydroxyindole levels rise. The relevance of the reduction in blood LNAA levels to the increase in brain tryptophan uptake that follows carbohydrate intake is supported by at least two other observations: 1) when rats ingest LNAAAs along with carbohydrates, the reduction in their blood levels is attenuated, as is the increase in brain tryptophan (87); and 2) when diabetic rats ingest carbohydrates, blood competitor levels do not fall, and brain indole levels do not rise (61). Hence insulin is necessary for the carbohydrate effect on brain tryptophan, but this effect is indirect, occurring via the reductions in blood LNAA levels.

When the rat ingests a protein-containing meal, not only does blood
tryptophan increase but so do the blood concentrations of its LNAA competitors. For diets containing 12-40% casein, the relative increases in the blood concentration of tryptophan and its competitors are usually about equal, and thus no change occurs in the competition for brain uptake. Hence brain tryptophan levels and 5-HT synthesis do not change.

In Figure 5, these diet-induced changes in the blood neutral amino acid pattern are summarized as a ratio of tryptophan to the sum of its LNAA competitors. Thus carbohydrate ingestion raises this ratio and brain tryptophan; the ingestion of diets containing normal amounts of protein does not modify the ratio or brain tryptophan concentration.

Because this model for predicting the effects of food ingestion on brain tryptophan and 5-hydroxyindoles revolves around the competitive LNAA transport mechanism, it should apply to any of the LNAA. That is, after meal ingestion, the changes that occur in the brain level of any LNAA should be predictable from a ratio in blood of the concentration of that amino acid to the sum of the concentrations of its competitors. This appears to be the case in experiments performed to date. For example, in a typical experiment, LNAA levels in blood and brain samples were measured in normal animals that consumed either a 0, 18, or 40% casein meal. The effects on brain tryptophan and the serum tryptophan ratio were as anticipated, and the correlation was quite good (Fig. 6, left panel). For valine, another LNAA, brain levels also changed after food ingestion, falling with ingestion of the 0% casein diet, remaining at fasting values when 18% casein was consumed, and increasing with the ingestion of the 40% casein meal. The changes in the blood-valine ratio were also found to correlate extremely well with the alterations in brain valine levels (Fig. 7, left panel). Good correlations were also found for leucine, tyrosine, and phenylalanine (86). The ratio in this study was not as accurate for changes in brain isoleucine (but was so in other studies) but indeed was so when corrected for the differences in the affinities of each of the LNAA for the transport carrier (see 216, 218). For most of these amino acids, the postprandial changes in their brain levels, although correlating with the diet-induced changes in their blood ratios, did not correlate with the alterations in their blood levels alone. For example, the ingestion of 0% casein elevated brain phenylalanine and the plasma phenylalanine ratio but reduced serum phenylalanine concentrations (compared to fasting values). Consumption of the 40% casein diet raised serum phenylalanine but did not raise either the serum ratio or brain phenylalanine levels.

The blood ratios do not take into account the fact that each LNAA has a unique affinity ($K_m$) for the brain transport carrier. Pardridge (216) described an equation that utilizes the serum concentration of each LNAA and its $K_m$ and $V_{max}$ values (determined by Pardridge and Oldendorf; 218) to estimate the rate of uptake for each LNAA. We have used our data and these kinetic constants for the rat brain to calculate the brain influx parameter for each LNAA in the study described above (86). The results for tryptophan
FIG. 6. Relationship between brain tryptophan level and serum-tryptophan ratio or brain tryptophan influx in individual animals consuming single meals of differing protein content. Groups of overnight-fasted rats received one of the following diets and were killed 2 h later. ○ Rats consuming 0% protein (carbohydrate); □ rate consuming 18% casein diet; ▽ rate ingesting 40% casein diet; and ● fasting control rats. Data were analyzed by linear regression; values for correlation coefficient (r) are significantly different from 0, P < 0.01 (Student's t test). [From Fernstrom and Faller (86).]

and valine appear in the right-hand panels of Figures 6 and 7. They show an excellent correlation between the calculated brain influx parameters and the brain levels of tryptophan and valine after food consumption. Interestingly the data in these figures suggest that the serum ratio is as good a predictor of the brain amino acid level as the calculated influx. Hence in attempting to predict changes in competitive uptake in species such as humans, in which it is not possible to determine the kinetic constants for each amino acid (the brain must be removed to do so), the data imply that the serum ratio can be used.

Currently it is not possible to determine if the ingestion of foods by humans leads to predictable changes in the rate at which brain neurons take up tryptophan and synthesize 5-HT. However, it has been possible to obtain data showing the relationship between dietary protein intake and the serum tryptophan ratio (see Fig. 8). The results suggest this relationship in humans to be much like that in rats. That is, the ingestion of a nonprotein meal by humans (i.e., mostly carbohydrates, with some fat) elicits an increase in the serum tryptophan ratio (Fig. 8, 0-g line), just as it does in rats. This result thus suggests that the ingestion of carbohydrates by humans should elevate brain tryptophan levels just as it does in rats. The ingestion of protein-
containing meals also elicits similar effects on the serum tryptophan ratio in rats and humans. That is, the consumption of meals containing moderate amounts of protein (for the American diet, 75 g/day) is associated with no change in the ratio, whereas the ingestion of high levels of protein (150 g/day) elicits an overall reduction in the serum ratio (94). If the tryptophan ratio is a good predictor of brain tryptophan uptake in humans, then it seems likely that food ingestion will also influence 5-HT synthesis in the human brain. It is already known that the administration of tryptophan to humans stimulates 5-HT formation in the central nervous system (CNS) (e.g., 80). By such a mechanism, it is conceivable that diet might thereby ultimately influence brain functions in humans.

3. Albumin binding of tryptophan in blood

In mammals about 80–90% of all tryptophan molecules in blood are loosely bound to serum albumin. The remainder circulates in the free form. Tryptophan appears to be the only amino acid to distribute itself between a bound and free form in blood (179). This phenomenon, and its uniqueness among amino acids, suggested to a number of investigators that, like certain drugs and hormones, perhaps only free-tryptophan molecules were available
for transport into tissues. Accordingly they conjectured that the size of the free-tryptophan pool in blood might be the important determinant of tryptophan uptake into brain (152, 262).

Early studies attempted to show positive correlations between the sizes of the serum free-tryptophan pool and the brain tryptophan pool. For example, the administration of such drugs as salicylate, probenecid, or clofibrate was found to elevate serum free-tryptophan and brain tryptophan levels acutely while reducing serum bound-tryptophan concentrations (see 104). Fasting rats for ~24 h or immobilizing them (as a model for stress)
was reported to have a similar set of effects (152, 263). Such effects of stress and food deprivation were suggested by Knott and Curzon (152) to be the result of the increment in serum nonesterified fatty acid (NEFA) levels produced by these treatments. Nonesterified fatty acids were known to bind reversibly to albumin, and appeared to compete with tryptophan for binding sites (192). Hence elevations in serum NEFA levels presumably enhanced competition with tryptophan for albumin binding, leading to a reduction in the size of the bound-tryptophan pool. The liberated tryptophan molecules would enter the free pool, thereby increasing its size, and ultimately lead to enhanced tryptophan uptake into brain.

These notions were subsequently tested with a more physiological paradigm and found to be wanting. For example, the simple act of giving an oral glucose load to an overnight-fasting rat rapidly elicited (via insulin secretion and action on blood amino acid levels; see above) an increment in brain tryptophan (182). However, this treatment also reduced serum NEFA levels, thereby enhancing tryptophan binding to albumin and reducing the size of the free-tryptophan pool (168). Hence, in this experimental situation, the two variables in question, the brain tryptophan and serum free-tryptophan levels, moved in opposite directions—implying that these two variables are independent of each other. In other experiments (178), groups of overnight-fasting (about 12 h) rats were fed protein-containing diets to which different amounts of fat had been added. Animals consuming the diets of low fat content experienced reductions in postprandial serum NEFA levels, and consequently reductions in serum free-tryptophan levels. Animals consuming the high-fat diets showed increases in serum NEFA and free tryptophan levels. Brain tryptophan levels, however, did not differ among the various diet groups because the meals also contained 20% protein (Fig. 9). As discussed above, brain tryptophan levels do not change after the consumption of a meal containing moderate amounts of protein (92). Therefore, again, sizable variations in the serum free-tryptophan pool were not accompanied by parallel alterations in brain tryptophan levels (178). Still other experiments, with natural (89) or synthetic (88) diets, only affirmed the apparent lack of connection between serum free-tryptophan and brain tryptophan levels. These data were not confounded by difficulties in measuring free tryptophan accurately, despite one a posteriori suggestion (130), as was attested by a compulsive comparison in advance of all available methodologies (see 178). The method least likely to introduce errors in free-tryptophan separation was always employed.

Such results tended to make the serum free-tryptophan pool uninteresting as an indicator of brain tryptophan uptake and levels. However, data have continued to accumulate, usually of a pharmacological or pathophysiological nature. For example, Knott and Curzon (153) reported acute carbon tetrachloride poisoning of rats to elevate serum NEFA, free-tryptophan, and brain tryptophan (and 5-hydroxyindole) levels, providing a further correlative connection between these tryptophan pools. The same laboratory later
FIG. 9. Effect of dietary fat on serum free-tryptophan and brain indole levels. Groups of 12 animals, fasted overnight, were fed diets containing 0, 15, 30, or 45% fat (all contained 20% protein) and were killed 2 h later. NEFA, nonesterified fatty acid. Data are means ± SE. [From Madras et al. (178).]

noted that chronic hepatic failure produced a much less consistent correlation (69); the investigators speculated that more fundamental changes in the blood-brain carrier for tryptophan might have occurred. This was indeed verified by James et al. (138) and Zanchin et al. (307). Most recently, Gillman et al. (109) reported that correlations between serum free-tryptophan and brain tryptophan levels were excellent in depressed subjects undergoing psychosurgery, whereas those between serum total-tryptophan levels or the serum ratio of total tryptophan to competitors and brain tryptophan were not.

Because of the controversy, several laboratories undertook kinetic studies of the effects of tryptophan binding to albumin on tryptophan transport into brain, with the brain uptake index (BUI) technique described by Oldendorf (212). The BUI technique involves administering a bolus of solution containing [14C]labeled tryptophan and tritiated water into the rat carotid artery. The animals are killed 5–15 s later, the brains are removed, and carbon-14 and tritium in forebrain samples are counted. The tritium counts
provide a correction (readily diffusible water) for the percentage of the bolus that actually reached the sampled tissue; the carbon-14 counts give an index of the uptake of the material of interest. The actual BUI equation is

$$\frac{^{14}C(\text{sample})}{^{14}C(\text{injectate})} \div \frac{^{3}H(\text{sample})}{^{3}H(\text{injectate})}$$

This technique, which is clearly simple and rapid, allows collection of data in vivo concerning the unidirectional uptake of a substance across the blood-brain barrier. It also allows the study of factors that might influence the uptake process.

This technique was used in several laboratories to assess the importance of albumin binding of tryptophan in blood to the amino acid's uptake into brain. As a corollary, these groups compared the relative abilities of albumin and the competing LNAAs to inhibit tryptophan access to brain. All groups seemed to agree on one point: the addition of LNAAs to the injection solution, or an increase in their levels (if already included), led to quite significant inhibition of single-pass tryptophan extraction by brain (e.g., 28, 81, 305). Less agreement was apparent concerning albumin binding. Etienne et al. (81) studied the effect on tryptophan BUI of adding albumin to an injectate consisting of tryptophan dissolved in the injection buffer. A clear inhibition of tryptophan uptake was observed and was attributed to the binding of the amino acid to albumin. Yuwiler et al. (305) used an injectate solution containing albumin and labeled tryptophan to which palmitate was added to dissociate the amino acid from the protein. The effect of the palmitate addition on tryptophan BUI was then tested. A fourfold increase in the free-tryptophan level of the injectate could be obtained by the addition of 2 mM palmitate, which, when injected into the carotid, produced a 40% rise in tryptophan uptake (compared to an injectate containing no palmitate). Yuwiler et al. (305) compared these data to those showing typical fluctuations in serum palmitate levels, and concluded that acute variations in free-tryptophan produced by even the largest physiological variations (0–0.5 mM) in blood palmitate levels would probably elicit only a few percent change in brain tryptophan uptake. To these investigators, tryptophan binding to albumin thus did not appear to be terribly significant physiologically. Further calculations from their results led to the suggestion that well over 60% of the tryptophan in blood is readily available for transport into brain (i.e., most of the tryptophan molecules are weakly associated with albumin, thus they can readily dissociate from the protein molecules and be available to the transport carrier for brain uptake.)

An additional impact of these studies (305) and those of Pardridge (217) was that flux finally became an important quantity. The commonly held view that the size of the free-tryptophan pool predicted brain tryptophan uptake could be set aside (the size of this pool certainly bears no simple relationship to its input and output fluxes, as was commonly held).
However, with the same BUI technique, Bloxam et al. (28) came to a different conclusion. Their paradigm involved varying the size of the free- and total-tryptophan pools in the injectate, which consisted of plasma and not a buffer. This was accomplished with clofibrate, which when given in vivo caused moderate reductions in serum total but not free tryptophan, and in vitro (i.e., added directly to the injectate) increased free-tryptophan but not total-tryptophan levels. In the former case the injectate led to a normal tryptophan BUI, while in the latter the tryptophan BUI was increased. The conclusion was that albumin binding is physiologically an important determinant of brain tryptophan uptake.

One possibly appropriate comment in considering this conclusion comes from Yuwiler et al. (305). Based on their results, these investigators suggested that the changes in brain tryptophan level caused by the administration of such drugs as clofibrate, probenecid, and salicylate could not be the indirect consequence of changes in serum tryptophan binding. The effects in serum were too small. Perhaps other, obscure effects of these drugs led to a false impression of a causal connection between serum free and brain tryptophan. Only future studies will tell.

The studies of Yuwiler et al. (305) and Partridge (217) seem the most complete kinetically, and they clearly suggest that if an effect of serum tryptophan binding does exist, it is physiologically small. Such a conclusion coincides with the data collected and conclusions drawn from animals given diets or hormones. One final comment seems appropriate, however. In the one clinical study available on the subject (223), CSF levels of tryptophan and 5-HIAA in patients who had just ingested a normal meal seemed to follow the change in serum free tryptophan (relative to the changes in the other LNAAs) rather than in serum total tryptophan (relative to the changes in these same amino acids). Results such as these suggest that it may be a bit premature to fully discard the notion that tryptophan binding to albumin is unimportant. As Yuwiler et al. (305) suggested, there are physiological paradigms that have not yet been tested in which tryptophan binding might conceivably influence brain tryptophan uptake (e.g., with long-term changes in plasma NEFA levels caused by changes in dietary fat intake). Such studies should be pursued, for the results could be of considerable significance.

C. Pathophysiological Correlates Involving Brain Tryptophan, 5-HT, and LNAA Transport

One major aim of this review is to illuminate the substantial amount of recent data supporting the importance of the LNAA transport system in determining amino acid entry into brain. So far the discussion has focused on physiological and pharmacological changes in brain LNAA and a neurotransmitter (5-HT) that are predictable from the nature of the competitive LNAA transport system. The focus now turns to pathophysiological states
in which alterations in brain LNAA and 5-HT levels have also been linked to the competitive LNAA transport system.

The notion that disease states associated with considerable changes in the blood levels of the LNAA should cause predictable, possibly abnormal changes in their brain levels was recognized and initially studied some 20 years ago for phenylketonuria (PKU). It has more recently been explored for cirrhosis and diabetes (and other diseases). The changes in brain tryptophan and 5-HT noted in PKU and diabetes seem related to the alterations in the competitive uptake of tryptophan into brain due to the abnormal blood levels of other LNAAAs (i.e., a simple effect due to altered competition). The changes noted in these brain variables in cirrhosis, however, do not appear simply to come from an indirect effect on competitive LNAA transport. Instead there appears to be a fundamental change in the characteristics of the transport system itself. This latter finding suggests that the properties of the blood-brain barrier transport carriers should not be viewed as immutable and that each time a new paradigm (e.g., disease state and pharmacological treatment) is explored for changes in amino acid pattern in blood and uptake into brain, the kinetics of the uptake process itself ($K_m, V_{max}$) should also be studied.

1. Diabetes

Diabetes is most commonly associated with an imbalance in glucose homeostasis. The absence of insulin in blood (or its actions at receptors) retards the normal removal of glucose into the tissues, and blood glucose levels become abnormally high. However, insulin is also important in controlling amino acid fluxes and utilization in the body. Diabetes therefore produces aberrations in the plasma concentrations of amino acids as well. In particular, blood levels of the branched-chain amino acids (leucine, isoleucine, and valine) become quite high (22), a reflection of the fact that these amino acids are the most aggressively removed from blood by insulin action (63, 207). The relevance of diabetes to a discussion of tryptophan transport into brain (and 5-HT synthesis) follows from these changes in branched-chain amino acid levels. Because their blood concentrations are elevated, tryptophan uptake into the diabetic brain should be retarded indirectly due to increased competition. [Diabetes does not appear to be associated with reduced tryptophan uptake due to a direct effect on the transport carrier (see 174).] Accordingly brain tryptophan levels and 5-HT synthesis should also be reduced.

A number of neuropharmacologists have considered this possibility. MacKenzie and Trulson (174) and Curzon and Fernando (67) observed brain tryptophan and 5-HIAA levels to be below normal in rats made diabetic with streptozotocin. MacKenzie and Trulson (175) also noted that above-normal doses of tryptophan were required to raise brain tryptophan and 5-HT levels
in diabetic rats. They suggested that the latter result was consistent with
the presence of increased serum levels of other LNAAs, and thus increased
competition with tryptophan for brain transport.

We have also found brain tryptophan levels to be below normal in the
streptozotocin-diabetic rat. This reduction is associated with a diminished
rate of 5-HT synthesis in the brain (Fig. 10; 62). Our finding is not consistent
with those of Trulson and MacKenzie (269), who reported in vitro increased
tryptophan hydroxylase activity in the diabetic rat brain. They interpreted
their finding as supporting a normal 5-HT synthesis rate in the diabetic
brain; the effect of increased hydroxylase activity was said to be offset by
that of diminished precursor supply. Because in vivo hydroxylation rate is
below normal, the significance of their finding is functionally unclear.

In addition to a general reduction in the rate of 5-HT synthesis, we also
noted that physiological phenomenon that should elevate brain tryptophan
and stimulate 5-HT formation (by stimulating insulin secretion) fail to do

![Graph showing in vivo rate of tryptophan hydroxylation in normal and streptozotocin-diabetic rats.](image-url)
so in the diabetic rat (61). For example, Table 1 shows that the ingestion of a carbohydrate meal by diabetic rats does not increase brain indole levels soon after its ingestion, as it would if consumed by normal animals. The serum tryptophan ratio is also not increased, presumably because of the absence of insulin secretion. It is this absence of change that probably accounts for the lack of an increase in brain tryptophan. If insulin is injected soon after diabetic rats begin to consume the carbohydrate meal, the serum tryptophan ratio increases, and is associated with increments in brain tryptophan and 5-HIAA levels.

To support further the notion that diabetes-related changes in brain tryptophan follow from indirect alterations in the competitive transport of tryptophan into brain, we have observed that this phenomenon can be generalized to the other LNAAs. For example, because the blood level of tyrosine in the diabetic rat is about normal, but blood concentrations of the branched-chain amino acids are quite high, it might be anticipated that brain tyrosine levels would be lower than normal. In fact, they are. As shown in Figure 11, for example, brain tyrosine levels are unusually low throughout the 24-h period in diabetic rats (60; E. A. Crandall and J. D. Fernstrom, unpublished observations). The concentration of tyrosine in blood is not a good predictor of brain tyrosine, because this parameter is alternately above and below normal during the day. However, the serum tyrosine ratio reflects brain tyrosine faithfully throughout the 24-h period (Fig. 11).

Two additional points seem appropriate concerning experimental diabetes. 1) All of the above studies were performed on animals diabetic for a relatively brief period (4–14 days). The competitive amino acid transport mechanism seems to be intact in the brains of such animals. However, the blood–brain barrier transport carriers reside in the capillary endothelial cells (see 216). Eventually CNS capillary and microvascular complications occur.

**TABLE 1. Effect of insulin injection in diabetic rats consuming a carbohydrate meal**

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Saline</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan, nmol/g</td>
<td>14.1 ± 0.6</td>
<td>17.1 ± 0.6</td>
<td>27.3 ± 1.3*</td>
</tr>
<tr>
<td>5-HT</td>
<td>3.16 ± 0.15</td>
<td>3.15 ± 0.06</td>
<td>3.27 ± 0.10</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>1.96 ± 0.07</td>
<td>2.04 ± 0.05</td>
<td>2.83 ± 0.12*</td>
</tr>
<tr>
<td>Serum tryptophan ratio</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.46 ± 0.04*</td>
</tr>
<tr>
<td>Serum glucose, mg/100 ml</td>
<td>102 ± 9</td>
<td>966 ± 28</td>
<td>832 ± 50*</td>
</tr>
<tr>
<td>Serum insulin, μU/ml</td>
<td>13 ± 2</td>
<td>7 ± 2</td>
<td>276 ± 64*</td>
</tr>
</tbody>
</table>

Values are means ± SE. After an overnight fast, groups of 7 diabetic rats were either given free access to a carbohydrate meal or continued on their fast. Just after food presentation, animals receiving the meal were injected with either saline or insulin (4 IU/kg ip). All animals were killed 2 h after food was presented. *P < 0.01 compared with saline or fasted group. [From Crandall and Fernstrom (61)]
in diabetes (see 230 and 233). It thus seems a reasonable hypothesis that when capillary degeneration occurs, the properties of the amino acid carriers might also deteriorate. If so, then the nice correlations between the blood ratio of a given LNAA and its brain level observed in normal and newly made diabetic rats (61) might no longer occur and would be interesting to study. The data may provide new information concerning metabolic changes forced on the diabetic brain by the process of capillary degeneration.

2) The diabetic rat should be an interesting model to study for tryptophan binding in blood. Does the absence of insulin secretion lead to increased blood levels of the nonesterified fatty acids in the streptozotocin-diabetic rat with reduced brain tryptophan levels? If so, is the proportion of tryptophan bound to albumin reduced and the free pool increased? If so, such steady-state data will tend to support the notion that variations in the size of the free-tryptophan pool do not bear on the access of tryptophan to brain. Limited data concerning this question are already available. But in one report (67), the animals were studied only 72 h poststreptozotocin treat-
ment, and plasma NEFA levels had not changed significantly. Nonetheless serum free-tryptophan level had increased and brain tryptophan decreased, suggesting the probable outcome of further experimentation (i.e., again to dissociate these two variables).

2. Phenylketonuria

Phenylketonuria (PKU) constitutes another group of diseases of amino acid metabolism that leads to alterations in LNAA transport into brain. Phenylketonuria is an inherited disorder characterized by an inability to hydroxylate phenylalanine to tyrosine. One clearly distinguished characteristic of the disease is high circulating levels of phenylalanine. Phenylketonuria is characterized by extraordinary mental retardation developing early in infancy. However, if blood phenylalanine levels are lowered immediately after birth for an extended period (by careful control of diet), normal development of intelligence occurs. This latter finding has led to the suggestion that the high blood levels of phenylalanine cause or contribute to the development of the brain dysfunction (see 117, 154).

In the search for mechanisms by which PKU might damage the brain (many have been proposed, e.g., 8, 117, 235), one line of investigation led to abnormal 5-HT synthesis. Subjects with PKU have low blood 5-HT levels and subnormal amounts of 5-HIAA in their urine (220). These effects could be partly reversed by placing the subjects on a low phenylalanine diet (221). Reduced formation of 5-IIT in brain was a postulate from these data and was suggested to contribute to the development of mental retardation. However, changes in blood and urinary levels of 5-IIT and 5-HIAA do not usually reflect accurately the state of 5-HT synthesis and turnover in brain. Hence the postulated effect of PKU on brain 5-IIT could not be accepted until data were obtained concerning this pool of the amine. Such data were ultimately obtained, first in rats and then in humans. In animals a model for chronic hyperphenylalanemia was developed, which was associated with a reduced brain level and synthesis rate of 5-HT (30, 100, 302-304). In a similar experimental preparation, Wang et al. (282) showed that the reduction in brain 5-HT could be prevented by adding tryptophan to the diet. Then, McKean (189) showed in autopsy material from PKU patients that brain stem and caudate levels of 5-HT were abnormally low. He also observed in PKU subjects given probenecid that the accumulation of 5-HIAA in cerebrospinal fluid (CSF) was low. Thus data from both humans and animals suggested that 5-HT formation in the CNS might indeed be reduced in this disease state.

Concerning the mechanism by which PKU reduced 5-HT formation in brain, one possibility was thought to involve tryptophan hydroxylation, inasmuch as large amounts of 5-HT could be synthesized in PKU patients when 5-HTP, but not tryptophan, was administered (244). Certainly in vitro
enzymatic data supported such a mechanism by showing that phenylalanine could act at high concentrations as an inhibitor of tryptophan hydroxylase (171). However, some investigators did achieve increments in urinary 5-HIAA levels after tryptophan loading (19). There was thus much disagreement that the defect was at the hydroxylation step.

Another hypothesis suggested that the high circulating levels of phenylalanine competitively blocked tryptophan uptake into brain and thereby limited 5-HT formation. Human data supported this view. For example, the studies of McKean (189) in autopsy material from PKU patients showed that cortical levels of tryptophan were below normal, while phenylalanine levels were high. Rat data also supported such an hypothesis. In the adult rat, for example, phenylalanine injection (1,000 mg/kg) rapidly reduced brain tryptophan concentrations (190). Larger reductions in brain tryptophan could also be produced by the ingestion over several weeks of a diet containing excess amounts of phenylalanine. Both treatments produced large increments in blood and brain phenylalanine levels. Similar acute effects were also noted in the infant rat (8, 190). This latter finding is consistent with evidence that the competitive blood-brain barrier transport systems for amino acids are in place and functional in the newborn mammal (33, 119).

The demonstration of competitive effects early in life and reductions in brain tryptophan and 5-HT in infant rats after phenylalanine injection are necessary data for an hypothesis of brain dysfunction involving 5-HT, because the major damage incurred to the CNS by PKU is thought to occur early in life (see 117, 154).

However, it should be considered that there is still no definitive proof that reduced levels of 5-HT early in life produce brain damage such as that seen in PKU. Additionally, in one early study, Pare et al. (222) reported that the administration of 5-HTP to affected children does not improve IQ. The devastating mental effects of high blood phenylalanine levels early in life may thus not be related to the reduced 5-HT synthesis associated with the disease. Of course the reduction in 5-HT might ultimately lead to deficits in brain functions unrelated to intelligence (e.g., control of blood pressure and sensitivity to painful stimuli), but this possibility seems as yet unexplored.

Consider also that if brain tryptophan (and 5-HT) levels are reduced in the PKU brain secondary to reduced tryptophan uptake from the circulation, the uptake of other LNAAs should also be affected. In fact, McKean (189) reported reduced cortical levels of tyrosine in PKU subjects at autopsy, as well as low levels of dopamine (DA) in the corpus striatum (a brain region particularly rich in DA nerve terminals) and norepinephrine (NE) in the brain stem and caudate. The accumulation in CSF of a major DA metabolite, dihydroxyphenylacetic acid, after probenecid administration was also observed to be subnormal in PKU subjects ingesting a high phenylalanine diet. This finding suggests that DA synthesis in the PKU brain is reduced in association with low brain tyrosine levels. Experimental models of PKU-related hyperphenylalanemia, by their very design, usually do not allow
meaningful studies of alterations in brain tyrosine levels (in most of these models phenylalanine can be hydroxylated to tyrosine). However, in one study in infant rats, McKean et al. (190) noted brain tyrosine levels to fall after a single injection of phenylalanine. Apparently phenylalanine hydroxylase activity was low enough in these young animals to prevent substantial conversion of phenylalanine to tyrosine (see 190). The brain levels of other LNAAs also fell in both adult and infant rats after phenylalanine injection. Most noteworthy were reductions in the branched-chain amino acids and in histidine. The significance of these changes to brain function in the PKU subject has not yet been studied.

3. Cirrhosis

Cirrhosis serves as a final example of a metabolic disease reputed to influence brain amino acids (and thus monoamine transmitters) indirectly by altering their competitive uptake into brain. No doubt a number of alterations in brain chemistry occur in cirrhosis and hepatic encephalopathy, which may predispose to coma. In this context, however, those dealing mainly with aromatic amino acids and brain 5-HT are discussed. This line of investigation has grown more interesting with reports in the past two years that the LNAA transport carrier is directly altered in advanced stages of experimental cirrhosis. This change is reputed to account for many of the modifications seen in brain amino acid levels (see below; 138, 307).

A number of early studies report changes in the amino acid pattern in plasma associated with cirrhosis (e.g., 131, 135, 292). Around 1970–1975, however, investigators became especially interested in the changes often found in blood levels of the aromatic and branched-chain amino acids. Specifically, the blood levels of tyrosine and phenylalanine were elevated and those of the branched-chain amino acids were depressed, whereas the blood tryptophan (total) level was usually unchanged both in human cirrhotics (e.g., 96, 151) and animals with experimental liver failure (e.g., 66, 138, 140, 248). The reductions in the branched-chain amino acids were hypothesized to be due to very high circulating levels of insulin, which promoted their uptake into peripheral tissues (presumably muscle) (e.g., 85), whereas the increments in amino acids like phenylalanine and tyrosine were caused by reduced liver catabolism (206). However, Soeters and Fischer (252) conjectured that this view was probably incomplete. If increased insulin levels were the only important factor, the increase in amino acid uptake into muscle should be accompanied by enhanced protein synthesis and accretion of muscle mass. This was clearly not the case; in fact, significant muscle wasting occurred. These investigators noted that serum glucagon levels were substantially elevated in the cirrhotic and that the insulin-glucagon ratio was actually below normal. Such findings would imply a hormonal milieu conducive to amino acid catabolism, and they offered data suggesting that branched-chain amino
acid metabolism is significantly enhanced in adipose tissue of cirrhotic subjects (see 134, 252). This catabolic mechanism, which could account for reduced plasma branched-chain amino acid levels, would also be consistent with the large increases in ammonia production seen in cirrhosis.

Regardless of mechanism, the data showing altered plasma levels of aromatic and branched-chain amino acids suggested that the uptake of aromatic amino acids into brain (via the competitive transport carrier) might indirectly be increased in the cirrhotic subject. Data soon followed supporting this view: substantial increases were found in the levels of tryptophan, tyrosine, and phenylalanine in the brains of experimental animals bearing a chronic portocaval anastomosis (69, 138-140, 307) or experiencing acute hepatic failure (portocaval anastomosis and hepatic artery ligation) (180, 181, 187). Similar alterations were noted in CSF samples obtained from cirrhotic patients (300), and in brain samples of individuals dying in hepatic coma (142, 143).

Such changes were of interest to investigators searching for metabolic etiologies of hepatic coma. In this case, for example, it seemed possible that the increased brain levels of tryptophan might account for the elevated levels of 5-HT and 5-HIAA seen in the brains of humans and animals dying of hepatic coma (e.g., 17, 65, 142, 151). The notion was that high rates of 5-HT formation and release in the comatose brain were consistent with contemporary knowledge concerning the role of 5-HT neurons in the modulation of sleep. If so, it then seemed possible that one might be able to rescue the cirrhotic patient in deep coma by providing a treatment that would reduce 5-HT formation in brain. Because brain 5-HT formation was believed to be enhanced because of increased availability of tryptophan, and the increase in brain tryptophan was thought to follow from an abnormal plasma LNAA pattern, the treatment proposed was one that would normalize the plasma LNAA pattern (and thereby reduce brain tryptophan and 5-HT). This notion was tested in cirrhotic animals and humans while comatose, apparently with a good degree of success; i.e., the depth of the coma often (but not always) decreased, and subjects awoke within ~24 h (see 95, 234). The treatment consisted of an infusion of either a mixture high in branched-chain amino acids and low in aromatic amino acids or simply a branched-chain amino acid. In the latter case human autopsy material showed that this treatment did lower brain levels of tryptophan, 5-HT, and 5-HIAA, a finding consistent with the presumed action (142, 143). [One obvious alternative therapy to amino acid infusions that should have the same effect might be to administer a 5-HT antagonist. If it worked, the results would add important correlative support to the notion that 5-HT is involved in the etiology of coma. The treatment might also be preferable to amino acid infusion.]

Despite these positive results, belief was not uniform in the hypothesis that the increased rate of tryptophan uptake by the cirrhotic brain was due to reduced competition by other LNAAAs in blood. The results of some experiments showed that the increments in brain and CSF tryptophan were associated with considerable increases in serum free-tryptophan levels (65,
This finding suggested that a reduction in tryptophan binding to serum albumin in cirrhosis might be another (or the) mechanism producing increased brain tryptophan transport and levels in cirrhosis. Moreover, some investigators apparently failed to find clear or consistent reductions in the serum levels of the branched-chain amino acids in liver disease, implying that the serum (total) tryptophan ratios were not elevated (27, 94, 180, 181). Still other studies failed to reveal large enough increments in serum free-tryptophan levels to account for the very sizable increases in brain tryptophan levels (27, 300).

Enough doubt was apparently generated by such conflicting data concerning plasma amino acid levels that a new explanation was sought for the clear increases in tryptophan levels in the cirrhotic brain. If the brain uptake of tryptophan was not influenced indirectly by changes in free tryptophan or competing amino acids in plasma, perhaps cirrhosis caused a direct effect on tryptophan transport into brain. Two laboratories undertook studies to explore this new hypothesis by attempting to determine the actual in vivo rate of tryptophan transport into the cirrhotic rat brain. With the single-pass carotid artery injection technique (218), both groups found a selective increase in the BUI for tryptophan and other LNAAs (138, 307). Zanchin et al. (306) also found no increases in the uptake of tryptophan or other amino acids into brain slices from rats bearing portocaval anastomoses. This suggested that the effects seen in vivo reflected altered exchange processes across the blood-brain barrier (where the competitive transport mechanisms are thought to reside; 216) rather than across brain cell membranes. This conclusion seems amply supported by the results of more recent studies with microvessel preparations from the brains of normal rats and rats with portocaval anastomoses (38).

James et al. (141) have hypothesized a comprehensive mechanism to account for the apparent induction of the neutral amino acid carrier in brain. With cirrhosis comes elevated blood levels of ammonia, the result of increased production (a by-product of enhanced amino acid catabolism) and decreased elimination (reduced conversion to urea in the liver). Because ammonia diffuses readily across biological membranes, considerable amounts enter brain and are ultimately incorporated into glutamine (glutamine levels in brain are quite high in cirrhotic subjects). Glutamine can be transported across the blood-brain barrier via the LNAA carrier. Since the carrier is thought to be bidirectional (exchange transport: one amino acid enters as one leaves the brain), increased glutamine efflux from brain could promote the influx of LNAA from blood. High levels of brain glutamine would additionally tend to elevate brain levels of the other LNAAs by preventing their access to the transport carrier once inside the brain, thereby diminishing their rate of efflux from this pool. James et al. (141) partly supported this hypothesis by showing that the administration of ammonium acetate to rats itself reduced plasma levels of the branched-chain amino acids and elevated the brain-to-plasma ratio for each of the LNAAs.

Further data are needed to validate this interesting hypothesis. How-
ever, the concept of alterable transport properties is appealing, and it now finds support as a general phenomenon from studies of other disease states (e.g., the glucose transport carrier in brain is reduced in diabetic hyperglycemia; see 110, 188). If the findings hold up, this line of investigation will have been the first to show that the characteristics of a specific amino acid transport carrier in brain can change and that such changes can have a clear impact on the levels of the appropriate amino acids in brain. These new findings add yet a further and quite intriguing complexity to the control of amino acid transport into brain (and perhaps other tissues).

Finally, other possible mechanisms have been proposed by which altered neutral amino acid pattern might lead to coma. One involves the increased brain levels of octopamine (β-hydroxylated tyramine) seen in hepatic coma (18, 79, 139, 248). It seems unlikely that the octopamine in brain is derived from blood, as such amines do not cross the blood-brain barrier to any appreciable extent (218), and the rise in brain octopamine seen in animals with chronic portocaval anastomoses precedes that in blood (139). The octopamine is thus presumably formed in brain from tyrosine, the reaction possibly driven by the high levels of tyrosine in the cirrhotic brain (e.g., 139). This view is supported further by the observation that the infusion of an amino acid solution high in branched-chain amino acids and low in aromatic amino acids dissipates coma in cirrhotic dogs and lowers CSF levels of tyrosine and octopamine (248). Further studies should elucidate the mechanisms by which elevated brain levels of such presumed false transmitters contribute to the etiology of hepatic coma.

D. Effects of Tryptophan and Diet on Brain Functions Influenced by Central 5-HT Neurons

Interest in the relationships among circulating LNAAs, brain tryptophan uptake, and 5-HT synthesis that have been observed in the physiological, pharmacological, and pathophysiological situations already discussed derives from the anticipation that tryptophan-induced alterations in 5-HT synthesis will ultimately influence brain function. Is this possibility unreasonable? The issue at the synaptic level is whether a rise in brain tryptophan concentration should enhance not just 5-HT synthesis and levels but also release. An increment in release is presumably essential to an ultimate effect on brain function. This expectation does not seem radical. In the past, for example, investigators have administered blockers of amine metabolism (e.g., MAO inhibitors) to elevate brain levels of 5-HT (or catecholamines) and interpreted observed changes in brain functions as reflecting increased transmitter release secondary to increased transmitter levels. Certainly, stimulating synthesis, rather than inhibiting catabolism, to achieve the same end seems reasonable (and more likely to yield more specific effects). In fact, as far back as 20 years ago investigators were attempting to cure depression (a disease thought by some to be related to undersecretion of 5-HT by brain
neurons) by combining these two notions into one treatment. They administered tryptophan and an MAO inhibitor to cure depression (58).

Clear effects of tryptophan on brain functions have been difficult to identify in the past. Only now, as discussed below, are some clear actions emerging. This difficulty does not indicate that tryptophan administration will not enhance 5-HT synthesis and release. Instead it should be recognized that the amino acid is, above all, a nutrient normally provided to the body (and brain) in modest amounts, and thus not likely to induce large-scale changes in 5-HT formation and release. In addition, the amino acid is converted to 5-HT only in those cells that normally make it, and probably at a rate that is not pharmacologically rapid (as opposed to 5-HTP, which is rapidly decarboxylated to 5-HT by any cell in the body after its administration). Hence the expectation that tryptophan should induce effects on a scale similar to those produced by an MAO inhibitor, 5-HTP, or a receptor agonist is inflated. The effects of tryptophan are no doubt there, but most likely they are small (physiological, not pharmacological). In fact, Chouinard et al. (50) reported that attempts to push tryptophan loading to maximize 5-HT production and release cause a shutting-off effect. That is, the amino acid appears efficacious at lower doses but becomes less potent as the dose is increased. This observation is perhaps explained by the electrophysiological finding that moderate-to-high doses (but not low doses) of tryptophan turn off raphe neurons (102, 268). It does not, of course, explain why tryptophan and an MAO inhibitor should be efficacious in treating depression (58).

Much information is now available showing effects of tryptophan, or its lack, on brain functions and behaviors thought to be related to the release of 5-HT by brain neurons. Behaviorally, for example, the administration of tryptophan to animals, either by injection or addition of the pure amino acid to the diet, has been shown to improve sleep (124), reduce locomotor activity (185), diminish the response to acoustic startle (277) and electroshock (173), suppress male homosexual behavior (44), attenuate muricide behavior (106), and modify appetite and food intake (162, 290, 293). In humans tryptophan administration has also been reported to act as a hypnotic (124) and an antidepressant (58, 203, 276).

We have also looked for pharmacological and behavioral effects of L-tryptophan over the past several years. We sought to identify brain outputs based on published pharmacological data that seemed most convincingly tied to the activity of central 5-HT neurons. Although results to date involve only tryptophan, the ultimate aim is to investigate the effects of diet- and hormone-induced changes in brain tryptophan and 5-HT on these brain outputs.

1. Growth hormone

The secretion of growth hormone (GH) appears to be partly controlled by 5-HT neurons. In humans plasma GH levels increase after administration
of 5-HTP (161, 210), an effect that can be blocked by cyproheptadine (a 5-HT antagonist; 210). Cyproheptadine administration also blocks increases in plasma GH levels caused by such stimuli as hypoglycemia (25, 251) and sleep (48, 71). [Effects with other 5-HT receptor antagonists have not always yielded consistent findings (e.g., 75, 197); such compounds, however, have multiple actions, which may often indicate alternative explanations for their effects.] A number of recent articles have shown that tryptophan administration to humans has a small but consistent stimulatory effect on blood GH levels (111, 158, 205, 291). All of these effects combined suggest that 5-HT release by brain neurons ultimately stimulates GH secretion.

The conclusion in rats is basically the same, but the animal model has been somewhat more difficult to use. Unlike humans, nonstressed rats secrete GH episodically; blood hormone levels oscillate between 10 and 500 ng/ml, with a frequency of about once each 3.5 h. Hence over a 24-h period the rat experiences about seven peaks and troughs in blood GH levels (264). Thus to make meaningful measurements it has been necessary to collect blood samples frequently (e.g., every 15 min), which led to the use of rats bearing chronic, right atrial cannulae (see Fig. 12, which shows blood GH variations over a 4- to 5-h period). Although this technique is laborious, it is powerful. Effects of drugs on serum GH observed continually over a 4-h period are much clearer than when observed at a single time point. For example, in Figure 12, metergoline clearly suppressed blood GH; but if only a single time

![Fig. 12. Effect of 5-HT antagonist on plasma growth hormone (GH) profiles of rats at onset of daily dark period (1900 h). Vehicle-injected rats (○) showed typical elevations in GH between 1745 and 2100 h, whereas rats injected with metergoline (●), 1 mg/kg at 1700 h, had a 43% reduction in mean plasma GH levels during this period. Data are means ± SE; n = 8/group. [From Arnold and Fernstrom (9).]
point had been studied, such as 1945 h or 2000 h, a different conclusion would have been drawn (9). Moreover a common difficulty is avoided: in single time-point studies, large standard errors in the control group (generated by rats being unsynchronized in their secretory cycles) have made it difficult to demonstrate significant effects of drugs on blood GH levels. In the past, some investigators attempted to circumvent this problem with nongentled (i.e., stressed) rats (see 249). Stressing rats suppresses GH secretion (186) and gives consistently low control values for serum hormone levels, with small standard deviations for grouped data (249). A stimulatory effect of 5-HTP was observed, which could be antagonized by cyproheptadine (249, 250). However, the problem with this approach is that one cannot determine whether a 5-HT antagonist by itself will reduce serum GH levels (i.e., in the absence of any other treatment); it is also difficult to apply a mild stimulus, like tryptophan, and hope to obtain an increase in serum GH—the animals are too suppressed by the strong negative stimulus of stress.

In the cannulated rat (not stressed), we have shown that 1) metergoline, a 5-HT antagonist, suppresses episodic GH secretion (Fig. 12); 2) MK-212, a 5-HT agonist, stimulates GH secretion at a time of day when serum GH levels are normally low; and 3) tryptophan injection significantly enhances episodic GH secretion (Fig. 13; 9, 10). The increase in plasma GH produced

![Graph](image-url)

**FIG. 13.** Effect of tryptophan on plasma growth hormone (GH) profiles of cannulated rats at onset of daily dark period (1200 h in these experiments). Tryptophan (100 mg/kg ip) or its vehicle was injected at 0945 h; each rat received each treatment, in randomized order, with at least 2 days intervening between experiments. When animals received vehicle (○), typical GH profiles occurred between 1015 and 1345 h; when animals received tryptophan (●), a 97% increase in mean GH levels over vehicle values was observed (vehicle, 63 ± 11 ng/ml; tryptophan, 124 ± 20 ng/ml; mean values, *P* < 0.05). Data are means ± SE; *n* = 8. [From Arnold and Fernstrom (10).]
by tryptophan can be blocked by coadministering another LNAA (valine), which attenuates the tryptophan-induced increments in brain tryptophan and 5-hydroxyindole levels (10). The ability of tryptophan to increase serum GII levels in rats further supports the notion that enhancing 5-HT neurotransmission stimulates GH secretion (9, 10, 289). It will be interesting to determine whether an effect similar to that seen with tryptophan can be obtained when such animals consume a carbohydrate meal.

2. Blood pressure

The 5-HT neurons also participate in the central control of blood pressure (presumably by modulating sympathetic outflow). Although this area of research has its unique controversies (as do all such areas of blood pressure and hypertension research), one hypothesis suggests that 5-IIT neurons lower blood pressure. Consistent observations include: 1) the administration of 5-IITP to animals reduces sympathetic outflow and blood pressure (e.g., 261); 2) the administration of parachlorophenylalanine (PCPA) to rats raises blood pressure (136); 3) the administration of 5-HTP or tryptophan to dogs pretreated with carbidopa and phenylzine (an MAO inhibitor) increases the threshold for cardiac excitability (i.e., reduces sympathetic neural activity; 232); and 4) the injection of fluoxetine, an inhibitor of 5-HT reuptake, modestly reduces blood pressure in hypertensive rats and potentiates the antihypertensive potency of 5-HTP (97).

Recently we have obtained data with tryptophan supporting the participation of 5-HT neurons in neural circuits that lower blood pressure. In initial studies, we observed that tryptophan had a significant antihypertensive effect when administered to spontaneously hypertensive rats (SHRs) at a dose of 225 mg/kg (258). This dose is quite large; recent dose-response experiments indicate 100 mg/kg to be equally potent, and doses below 100 mg/kg are less effective. However, the antihypertensive efficacy of smaller doses (e.g., 50 mg/kg) is substantially enhanced by coadministration of fluoxetine, a 5-HT reuptake blocker that also lowers blood pressure (see 260). This latter effect is particularly interesting, assuming fluoxetine cannot itself release 5-HT. That is, if tryptophan injection potentiates the antihypertensive action of fluoxetine, the amino acid must have alone led to enhanced 5-HT release after conversion to the amine. Otherwise it would not have amplified the effect of fluoxetine, because this compound blocks 5-HT reuptake and does not affect synthesis or release. Such a possibility leads to the reasonable suggestion that tryptophan-induced increments in brain 5-HT level probably do not turn off 5-HT neurons, at least not under these conditions, as has been suggested by others (e.g., 268).

Also, the ability of tryptophan to reduce blood pressure in hypertensive rats can be antagonized by the administration of metergoline, a 5-IIT antagonist, or by valine, which competes with tryptophan for brain uptake.
The ability of valine to block the antihypertensive action of tryptophan probably does point to a central site of action for tryptophan, despite the fact that competitive uptake systems for amino acids occur in a variety of tissues. Guroff and Udenfriend (118) showed in pharmacological studies that the uptake of another LNAA (tyrosine) into brain, but not heart or striated muscle, was antagonized by the coadministration of tryptophan or isoleucine. We have also obtained a similar kind of data showing that LNAA administration blocks methyldopa access to brain but not spleen (259); methyldopa is transported into brain by the LNAA carrier (183). These results suggest that pharmacological competition experiments, such as those with tryptophan and valine, are likely to provide specific information about actions of tryptophan within the CNS. Thus, overall, these studies support the notion that tryptophan must gain access to the brain and ultimately lead to a stimulation of 5-HT receptors (presumably by being converted to the amine) in order to lower blood pressure.

3. Pain

The perception of pain may be influenced by tryptophan availability to brain. The notion that tryptophan administration may alter pain perception stems from pharmacological data on the role of 5-HT neurons in this process. For example, early studies on 5-HT and pain included the demonstration that PCPA injection (which depletes the brain of 5-HT) or the placement of lesions that partly destroyed central projections of 5-HT neurons produced hyperalgesia (e.g., 125, 199, 266). Such effects were reversible by injecting 5-HP, the immediate (if unphysiological) precursor of 5-HT. Later studies showed that the administration of drugs that increase transmission at 5-HT synapses [e.g., reuptake blockers fluoxetine or zimelidine (146, 200), 5-HT agonists quipazine or MK-212 (241, 297)] produced analgesia. These studies also showed that the analgesic action of morphine could be enhanced by drugs such as fluoxetine (200) and antagonized by compounds such as PCPA (267), 5,6-dihydroxytryptamine (a 5-HT neurotoxin; 274), and cinanserin (a putative 5-HT antagonist; 77). Such data support the notion that increased activity of 5-HT neurons elicits analgesia or at least enhances the analgesic action of compounds like morphine (see 199, 296).

A resulting hypothesis is that tryptophan administration might produce analgesia. Although not everyone has obtained supportive data (e.g., 129), a number of groups have noted some mild analgesic effects with L-tryptophan. For example, Telner et al. (265) observed that tryptophan (in high doses) raised the pain and escape thresholds of animals. In addition, Lytle et al. (173, 198) noted that jump thresholds were sizably reduced (i.e., apparent hyperalgesia) in animals with low brain levels of 5-HT due to the chronic ingestion of a corn-based diet. In these animals a single injection of L-tryptophan (125 mg/kg) had a sizable, short-term analgesic effect. Long-
term analgesia could also be produced in corn-fed rats by adding small amounts of L-tryptophan to the diet; in animals consuming this supplemented diet, shock thresholds (and brain 5-HT) became statistically indistinguishable from those of rats consuming a high-quality protein diet. These changes occurred even though the corn-fed rats remained very small (and malnourished) compared to their well-nourished counterparts. With these experimental conditions, the apparent analgesic effect of tryptophan was certainly not modest and was probably related to its ability to restore 5-HT and 5-HIAA levels to normal (see 198).

Although tryptophan is not a potent analgesic, 1) the amino acid would appear to have analgesic action under certain conditions, and 2) the analgesia can be fairly long-lived with continued exposure to tryptophan. Consequently it would be interesting to determine in humans whether tryptophan would be efficacious in the treatment of mild chronic pain. Perhaps if the amino acid were administered to humans (or animals) experiencing chronic pain (as opposed to subjects with no complaints of pain or animals provided with a brief, intensely painful episode), an analgesic action would be more readily observed. This idea certainly is not novel in humans: in the study of zimelidine, a 5-HT reuptake blocker, the compound was found to be efficacious in certain patients experiencing chronic pain (146). L-Tryptophan seems specific in its action on 5-HT neurons. If effective, its use to treat pain could be expected to produce many fewer side effects than more potent pharmacological agents.

4. Appetite

Tryptophan, 5-HTP, and a number of drugs that alter transmission across 5-HT synapses influence food consumption. For example, injections of 5-HTP or fenfluramine, which enhance 5-HT formation or release, respectively, suppress food intake in animals (29, 147, 247). Conversely the administration of PCPA or cyproheptadine increases food intake and weight gains (34, 105, 245). In this context, where animals have access only to a single food source, the administration of L-tryptophan (50 mg/kg) has been observed by one group to cause a diminution of food intake. When administered to freely feeding rats just prior to the onset of the daily dark period, the amino acid reduced food intake and meal size for a 4-h period (162). Moreover no rebound increase in food intake followed, such that the total 24-h postinjection intake remained reduced compared to control values. This finding contrasts with that of Weinberger et al. (285). These investigators found no effect of tryptophan administered to rats previously fasted for 24 h and studied for a subsequent 2-h period. However, Latham and Blundell (162) also performed studies in fasted rats and observed both a significant (though small) reduction in postinjection food intake and a sizable increase in the postmeal interval. They speculated that acute experiments in fasted animals are less likely to provide significant results, when a nutrient such
as tryptophan is the treatment. The nutrient's effect will be small, unlike that of a drug, and thus obscured by the animal's drive to consume food after a significant fast. More sensitive paradigms for measuring biological responses are thus required. The investigators support this contention by their data showing a greater effect of tryptophan in free-feeding rats than in fasted rats at the onset of the major daily feeding period. This conclusion is similar to that reached in studies of the effects of tryptophan on GH secretion: a sensitive measure of GH secretion in undisturbed animals was required to observe clear effects of tryptophan administration (10). Such is likely to be the case with other indices of brain function as well as with other nutrients suspected of influencing such functions.

Such studies of the biological effects of tryptophan would also be greatly strengthened if accompanied by corroborating pharmacological data to assess whether the effect of tryptophan was mediated by an action on 5-HT production and release in brain. For example, the effects of tryptophan on food intake observed by Latham and Blundell (162) would be more compelling if they had also shown that pretreatment with a 5-HT antagonist blocked the action of tryptophan and/or that coadministration of a 5-HT reuptake blocker (e.g., fluoxetine) enhanced the effect of the amino acid. Otherwise it is not possible to conclude convincingly that an effect of tryptophan is mediated by an action on 5-HT neurons anywhere in the body, much less in the brain.

Nonetheless the observed acute effect of tryptophan on food intake is intriguing. It would be interesting to determine whether the repeated administration of tryptophan over a period of weeks continues to suppress food intake. If so, then such a cumulative, though modest, effect might ultimately produce rather sizable decreases in food consumption and perhaps also weight. In humans, at least, such gradual alterations in weight (and apparent appetite) might be more likely to produce an enduring weight loss even after discontinuation of therapy.

Another approach to the study of tryptophan and appetite control has recently been developed by Anderson et al. (see 4). In 1975, Ashley and Anderson (12, 13) reported that when rats were allowed to select their own daily intake of protein and calories, a clear relationship emerged between the level of protein consumed in the diet and the serum ratio of tryptophan to the sum of its competitors, a predictor of brain tryptophan uptake and 5-HT formation (see Fig. 5). This effect was particularly notable when zein or partially corrected zein diets were supplemented with tryptophan. As the amount of tryptophan added to the diets increased (from 0 to 1.2 g tryptophan/100 g protein or equivalent), the animals reduced the amount of protein selected in the diet over a 4-wk period. (No dose-related effect was noted, however, on total food intake; 12, 13.) A dose-related increase in the serum-tryptophan ratio (and thus presumably in brain tryptophan and 5-HT) occurred with the addition of tryptophan to these diets.

These and other studies, both in rats (e.g., 200) and most recently in humans (5), have led Anderson (4) to hypothesize that 5-HT neurons par-
participate in a brain circuit that regulates the intake of dietary protein. He conjectured that an increase in the serum ratio of tryptophan to its competitors (and the presumed rise in brain tryptophan and 5-HT) should reduce protein intake, while a decrease in the ratio should cause the opposite effect. Of course, without further evidence, it is impossible to state whether a diet-induced increase in ratio and brain indoles produces a reduction in protein intake or whether the increase in protein intake simply leads to reductions in plasma ratio and brain indoles as one of many side effects. These investigators thus designed a study to test their hypothesis, based on the idea that treatments that reduce brain 5-HT formation (and presumably release) should lead to enhanced protein intake (14). In this experiment, weanling rats were tested for their protein selection after pharmacological depletion of brain 5-HT levels with PCPA, 5,7-dihydroxytryptamine, or median raphe lesions. Over a period of several weeks after treatment, rats with sizable depletions of brain 5-HT reduced their protein intake while maintaining total caloric intake. If the hypothesis were to be affirmed, one would have expected an increase in protein intake, not a reduction. Hence this result does not support the original notion. One therefore wonders if the changes observed in the correlative studies really support a causal relationship between brain 5-HT and protein intake.

Of course all of the findings and conclusions on 5-HT and protein self-selection should not be rejected based on this result alone. Other experiments should also be performed, and in fact are needed, to allow a full evaluation of the hypothesis. For example, in published studies from the Toronto group (4, 5, 12-14, 290), no kinetic measurements relating to 5-HT have ever been made, whereas the conclusions drawn assume that when 5-HT levels are chronically high or low, the change in level is reflected in a similar change in the rate of 5-HT synthesis or release. Such might be true 1 or 2 h after an acute perturbation of brain tryptophan (e.g., after injection of the amino acid); it may not be true in chronic studies wherein a single measurement is made. (In chronic studies, for example, a reduction in level could reflect either a reduction in synthesis rate, or an increase in release or catabolic rate.)

Moreover, pharmacological studies should also be pursued that do not involve treatments as disruptive as brain stem lesions or neurotoxin administration. For example, suppose the hypothesis is that increased brain 5-HT release reduces protein selection. The administration of a 5-HT antagonist to animals consuming low levels of protein in their diets (i.e., that presumably have high brain 5-HT) might have a much greater impact on protein selection over a 4- to 12-h postinjection period (perhaps to increase it) than if the drug were administered to animals already consuming a high proportion of dietary protein. Effects of chronic drug administration could also be studied. Similarly, administration of a 5-HT agonist to rats electively consuming a high-protein diet should reduce protein consumption much more effectively than in rats already selecting low protein.

One group (293, 294) has actually pursued pharmacological studies, but
with a modification of the free-feeding selection paradigm. The model involves rats that experience an enforced fast for 16 h each day, and have access to food only during the remaining 8-h period. In a typical study, a drug or amino acid is injected just prior to the onset of the daily feeding period, the food is presented, and the effect of the treatment on the selection of protein and carbohydrates is measured for 8 h. With this model, these investigators have reported that the short-term effect of an injection of fenfluramine, fluoxetine, or MK-212 (a 5-HT agonist; 51) was to reduce total caloric intake but not to alter protein intake (293, 294). They concluded that increasing transmission across 5-HT synapses sustains normal protein intake while reducing carbohydrate consumption. These authors also noted a small stimulatory effect of tryptophan on protein intake when combined with a subanorectic dose of fenfluramine. This latter result seems to contradict directly that predicted by some (12, 13), but not all (14), of the data obtained in Anderson's laboratory. Wurtman and Wurtman (294) suggest that their results indicate that the body and brain may interact via 5-HT neurons to regulate carbohydrate (not protein) intake. For example, if a rat consumes a largely carbohydrate meal, the serum tryptophan ratio, brain tryptophan, and brain 5-HT will all increase. The rise in 5-HT level should cause more transmitter to be released, which (like fenfluramine or MK-212) should elicit a reduction in carbohydrate intake. Reduced carbohydrate ingestion should ultimately cause brain tryptophan and 5-HT levels to fall, thereby restoring the animal's appetite for carbohydrates.

Presently it is not possible to reconcile the views put forth by Ashley and Anderson with those by Wurtman and Wurtman. But difficulties in design and interpretation of results are not unique to Anderson's studies. First, Latham and Blundell (162) noted the problems in discerning small effects of amino acids such as tryptophan (administered alone) when starved animals are studied. Hence some of the potentially high sensitivity of the free-feeding approach is compromised when a short-term fasting approach is pursued. Clearly, such a compromised approach is unnecessary, as Latham and Blundell (162) showed. Perhaps if the selection of nutrients and the effect of tryptophan alone were explored acutely in nonstarved animals, different effects would be observed.

Second, in studying the effects of such drugs as fluoxetine, fenfluramine, and MK-212, an implicit assumption is that the observed effects are mediated via actions of the drugs within the brain. Such need not be the case at all. Compounds like 5-HT itself and fenfluramine exert effects on appetite peripherally (7, 229), perhaps partly by slowing gastric emptying (see 31). In fact, the use of at least one of the drugs (fenfluramine) employed in the above studies is known to produce a taste aversion to saccharin, suggesting that it may cause discomfort to the animal (169), and thereby affect food intake and choice. The fact that fenfluramine is known to induce gastric distress in some species (255) may partly explain this effect. Clearly, future studies of the effects of such drugs on appetite should attempt to differentiate a peripheral from a central site of action.
Chee et al. (46) recently obtained data in genetically obese and normal mice that tend to support the correlations obtained by Ashley and Anderson (13) in rats. However, Peters and Harper (225) attempted to reproduce the original findings of Ashley and Anderson (12, 13). They utilized essentially the same diets, animals at the same age, and performed the same correlational analyses. They could not reproduce the basic correlational effects: i.e., that the serum tryptophan ratio correlates negatively and significantly with protein intake. They also failed to observe a number of other reported correlations. Because to date such a small number of laboratories have attempted to reproduce and capitalize on this line of investigation, it is probably premature to conclude whether the basic findings are correct. Certainly, additional studies like those of Chee et al. (46) give some confidence that the effects are real. But more data are needed. This general approach to the investigation of appetite control is important, as it more closely models the human condition. Moreover the dietary self-selection paradigm has been extant for decades and has produced some extremely interesting information on the extent to which the rat separately regulates intakes of protein and energy (e.g., 54, 164, 214, 237).

Finally, one of the original rationales for undertaking studies on the relationship of nutrient self-selection to the plasma tryptophan ratio and brain indoles follows from the long-held belief that animals somehow sense amino acid throughput or blood levels and use this information to regulate their intake of protein (e.g., 4, 122, 165, 215). If so, perhaps such effects of the diet on brain 5-HT form the basis for the brain’s ability to sense amino acid throughput. The ultimate outcome is that appetite for protein changes according to the diet’s (or possibly other metabolic) effects on brain 5-HT. Of course this is just one hypothesis currently being explored that might explain how amino acid balance is regulated. Whether it is ultimately validated, this line of investigation has rekindled interest in the dietary self-selection paradigm. One positive consequence may be that more individuals will use it in their studies of the control of appetite, because it more closely approximates the dietary conditions to which humans (and animals in the wild) are exposed.

III. EFFECTS OF PRECURSOR AVAILABILITY ON CATECHOLAMINE FORMATION AND FUNCTION IN BRAIN

A. Studies on Tyrosine Hydroxylation and Catecholamine Formation

1. Catecholamine synthesis from tyrosine

Brain neurons synthesize catecholamines (DA, NE, epinephrine) from tyrosine (see Fig. 14; 57). The initial biosynthetic step involves the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa). This reaction is catalyzed by tyrosine hydroxylase, an iron-requiring enzyme that utilizes molecular oxygen as cosubstrate and a reduced pteridine as cofactor (209). Some
FIG. 14. Tyrosine (TYR) availability and catecholamine synthesis. Tyrosine is hydroxylated to dihydroxyphenylalanine (DOPA); dopa is decarboxylated to dopamine (DA), which is then β-hydroxylated to norepinephrine (NE). β-Hydroxylation of DA does not occur in neurons that use DA as their transmitter. Monoamine oxidase initiates catabolism of NE to 3-methoxy-4-hydroxyphenylethylglycol sulfate (MOPEG-SO₄), one of the principal NE metabolites in brain. Asterisk indicates rate-limiting step in catecholamine formation, TYR hydroxylation; TYR competes with other LNAAs for uptake into brain.

data suggest that phenylalanine is also a substrate for tyrosine hydroxylase, and that sizable amounts of dopa may normally be formed in brain from this substrate (see 148).

Dopa is converted to DA by the ubiquitous enzyme aromatic-L-amino acid decarboxylase, the same enzyme that catalyzes the decarboxylation of 5-HTP (172). Tyrosine is not normally an important substrate for the decarboxylase enzyme (83), despite earlier reports (see 72). Norepinephrine (NE) is formed from DA by β-hydroxylation in a reaction mediated by dopamine β-hydroxylase (DBH). Like tyrosine hydroxylase, DBH (a copper-requiring enzyme) employs molecular oxygen as a cosubstrate, but it utilizes ascorbate as cofactor. Finally, small amounts of epinephrine and the necessary enzyme, phenylethanolamine-N-methyltransferase (PNMT), have been measured in brain (157, 228, 239), suggesting that some brain neurons may synthesize and release this catecholamine. S-adenosylmethionine is used by PNMT as a methyl donor (15).

Various metabolites arise from each of the catecholamines. These are formed in reactions mediated principally by two enzymes: MAO and catechol O-methyltransferase. Several of these metabolites can also be conjugated to form sulfate (or other) derivatives (see 57). A number of the catecholamine metabolites are of interest functionally, because acute changes in their brain levels may be indicative of alterations in transmitter release (see below).

2. Control of catecholamine synthesis in brain

Much evidence shows end-product inhibition to be a major factor governing the rate of catecholamine formation. Despite the presence of this
control, however, precursor availability seems to influence the rate of catalysis at the rate-limiting step in the biosynthetic pathway (tyrosine hydroxylation). The following discussion considers current information on the mechanism(s) of end-product inhibition, and how precursor effects fit into this broader control mechanism.

a) Tyrosine hydroxylase: end-product inhibition and enzyme activation. Tyrosine hydroxylase catalyzes the rate-limiting step in catecholamine biosynthesis (166), and thus has been studied as the principal site for regulation of this pathway in brain. Early studies demonstrated that tyrosine hydroxylase activity in vitro was inhibited by catechols and catecholamines; this inhibition was competitive with the pteridine cofactor (e.g., 133, 271). Later experiments in vivo confirmed that catecholamine synthesis rate in brain was subject to end-product inhibition (254). This notion was expanded in studies where increases in tyrosine hydroxylase activity after peripheral nerve stimulation were blocked by NE (e.g., 287). The effect of NE was antagonized by cocaine (cocaine acts as a catecholamine reuptake blocker), suggesting that the catecholamine effect was mediated intracellularly, not via an external receptor. A similar set of findings was made with brain slices (123). From such studies the hypothesis emerged that acute nerve stimulation increases tyrosine hydroxylation, because an important transmitter pool in the nerve terminal has become depleted, thereby releasing the enzyme from end-product inhibition. Reduced inhibition was thought to take the form of diminished competition with the pteridine cofactor (227, 286, 287), though not all studies uniformly affirmed this notion (52).

Another hypothesis explaining end-product inhibition in relation to nerve activity posited that catecholamines inhibit hydroxylation indirectly via receptors. This notion was thought to explain, for example, why the transection of DA fibers acutely increased both the DA level and the rate of tyrosine hydroxylation in severed nerve terminals (see 40). In the absence of catecholamine release, inhibitory receptors were no longer occupied by transmitter, end-product inhibition was therefore lost, and both DA formation and levels rose. Similar results were found from studies with γ-hydroxybutyrate (GHB), a drug that acutely diminishes the firing rate of nigrostriatal DA neurons (278, 281). After GHB administration, both DA levels and the rate of tyrosine hydroxylation increased in the striatum. The effect on hydroxylation was blocked by apomorphine or amphetamine, and the action of these drugs was blocked by haloperidol or chlorpromazine (280). These findings implied that stimulation of an external DA receptor was required to elicit end-product inhibition. Additionally these same investigators observed that 1 h after GHB treatment, when striatal DA levels were still elevated but tyrosine hydroxylation rate had returned to normal, administration of pargyline (to raise DA levels very high) caused no inhibition of hydroxylation rate. The authors concluded that in the absence of impulse flow, a treatment that elevates intracellular (but not extracellular) DA levels does not cause end-product inhibition. However, not all data fit with this
hypothesis of end-product inhibition (e.g., the pargyline-induced reduction in tyrosine hydroxylation is not blocked by haloperidol; see 280), and perhaps aspects of both hypothesized mechanisms (intracellular and extracellular) function together normally to control the rate of catecholamine synthesis.

The above studies of end-product inhibition were accompanied by other, related experiments. These explored for specific changes in the kinetic properties of tyrosine hydroxylase after treatments that altered catecholamine synthesis rate (see Table 2). One experimental paradigm involved acute electrical stimulation of catecholamine fibers. This treatment caused significant increases in the rates of tyrosine hydroxylation and catecholamine synthesis (e.g., 52, 240, 287). However, while all investigators found enhanced hydroxylation rates, they disagreed about the specific kinetic changes. In peripheral noradrenergic nerves an increase in tyrosine hydroxylase $V_{\text{max}}$, with no changes in $K_m$ for substrate or cofactor, was observed (52). In CNS noradrenergic fibers, Roth et al. (236) reported a reduction in $K_m$ for tyrosine with an increase in $K_1$ for NE. These investigators also reported a similar finding for CNS DA neurons, with the additional result that cofactor $K_m$ was reduced (208). A reduction in cofactor $K_m$ has also been observed in striatum after

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kinetic Constant</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical stimulation of sympathetic nerves</td>
<td>Increase in $V_{\text{max}}$</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>No change in substrate $K_m$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No change in cofactor $K_m$</td>
<td></td>
</tr>
<tr>
<td>Electrical stimulation of locus ceruleus</td>
<td>Increase in end-product $K_i$</td>
<td>236</td>
</tr>
<tr>
<td>(assay in hippocampus)</td>
<td>Decrease in substrate $K_m$</td>
<td></td>
</tr>
<tr>
<td>Electrical stimulation of nigrostriatal bundle</td>
<td>Increase in end-product $K_i$</td>
<td>208</td>
</tr>
<tr>
<td>(assay in striatum)</td>
<td>Decrease in substrate $K_m$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decrease in cofactor $K_m$</td>
<td></td>
</tr>
<tr>
<td>Injection of haloperidol (assay in striatum)</td>
<td>No change in end-product $K_i$</td>
<td>309</td>
</tr>
<tr>
<td></td>
<td>No change in substrate $K_m$</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>Decrease in cofactor $K_m$</td>
<td>311</td>
</tr>
<tr>
<td>Phosphorylation conditions in vitro</td>
<td>Increase in end-product $K_i$</td>
<td>113, 128</td>
</tr>
<tr>
<td></td>
<td>No change in substrate $K_m$</td>
<td>170, 182</td>
</tr>
<tr>
<td></td>
<td>Decrease in cofactor $K_m$</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>No change in substrate $K_m$</td>
<td>145</td>
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<tr>
<td></td>
<td>No change in cofactor $K_m$</td>
<td></td>
</tr>
<tr>
<td>Injection of $\gamma$-hydroxybutyrate</td>
<td>Increase in end-product $K_i$</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>Decrease in substrate $K_m$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decrease in cofactor $K_m$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No change in substrate $K_m$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No change in cofactor $K_m$</td>
<td>311</td>
</tr>
</tbody>
</table>
haloperidol administration (which should enhance the rate of firing of DA neurons), but no changes in substrate $K_m$ or DA $K_i$ were found (309–311).

Tyrosine hydroxylase activity has also been studied in vitro under conditions thought to mediate the increase in enzyme activity after nerve stimulation. The enzyme has been most characterized under phosphorylating conditions, and the usual changes observed are reduced $K_m$ for cofactor (but not substrate) and increased $K_i$ for end product (113, 128, 170, 182, 275). One group, however, found no changes in cofactor affinity under phosphorylating conditions (145).

Kinetic parameters of tyrosine hydroxylase have also been obtained in studies designed to reduce nerve impulse flow. In such experiments, GHB has been the treatment of choice. One group observed that this treatment increased the $K_i$ for DA and reduced substrate and cofactor $K_m$ values in striatal preparations of tyrosine hydroxylase (204). Others, however, observed no changes in $K_m$ values for substrate or cofactor (311).

Apparently, considering all of the above results (see Table 2), the property of tyrosine hydroxylase that changes most consistently in experiments from various laboratories is the affinity for cofactor. Several groups also report that activated tyrosine hydroxylase shows reduced affinity for end product. Apparently only one laboratory found changes in tyrosine affinity. If the findings concerning cofactor and end-product affinities hold up, they support at least one possible mechanism whereby nerve stimulation could turn on tyrosine hydroxylase. Increased nerve activity would cause greater amounts of cofactor to associate with the enzyme (secondary to the increase in affinity), thereby enhancing enzyme activity, because cofactor normally seems to limit the rate of hydroxylation (see 150). At the same time, affinity for the end product would be reduced. Even if local concentrations of transmitter did not change, this latter effect alone should cause fewer interactions between transmitter and enzyme and thereby diminish end-product inhibition (by whatever mechanism). The result would again be an apparent increase in enzyme activity. Of course future studies will determine the validity of these notions.

Finally, such changes in enzyme activity, regardless of ultimate mechanism, seem to make the enzyme more responsive to alterations in substrate availability. If activation is not associated with a change in substrate affinity, what might the mechanism for this apparent relationship be?

b) Tyrosine availability and catecholamine synthesis in brain. Interest in the possibility that variations in tyrosine availability might influence the rate of catecholamine synthesis appeared only in the middle of the last decade. Lack of enthusiasm for this potential relationship may have been related to the intense interest in feedback mechanisms for controlling tyrosine hydroxylase, the report that high concentrations of tyrosine could inhibit tyrosine hydroxylase (148), and the belief that at normal brain tyrosine concentrations tyrosine hydroxylase was probably saturated with substrate (e.g., 57). Roth et al. (236), for example, who reported that the $K_m$ of
tyrosine for the hydroxylase was reduced when the enzyme became activated, concluded that this effect could not be of functional significance inasmuch as the hydroxylase was probably already saturated with tyrosine.

The fact is, tyrosine hydroxylase in brain should not be fully saturated with tyrosine. Brain tyrosine levels normally vary between 100–200 μM (e.g., 86, 295), whereas the $K_m$ of tyrosine hydroxylase for tyrosine is about 50–90 μM (e.g., 170, 204, 311). Brain tyrosine levels are thus above the $K_m$, but not remarkably so. This suggests that elevating brain tyrosine levels might more fully saturate the enzyme and thereby enhance the rate of tyrosine hydroxylation. Such a result was first obtained by Wurtman et al. (295), who showed that the injection of tyrosine (50 mg/kg ip) raised brain tyrosine levels and caused a small but significant increase in the rate of dopa accumulation (see Table 3). If tryptophan was injected instead of tyrosine, a small but significant reduction in brain tyrosine ensued, which was accompanied by a sizable fall in dopa accumulation (Table 3). This in vivo finding suggested that the hydroxylase was not fully saturated with substrate. However, the enzyme probably was at greater than half-saturation with respect to tyrosine: a 15% reduction in brain tyrosine level after tryptophan injection caused a much larger change in hydroxylation rate than the twofold increase in brain tyrosine that accompanied tyrosine injection. Tryptophan was employed in these studies because it, like tyrosine, is an LNAA. Both are thus taken up into brain via the competitive LNAA transport carrier. Hence a rise in blood tryptophan level competitively inhibits tyrosine binding to the carrier, and thus its uptake into brain. The finding that other LAAs, such as leucine (see Table 3), also lowered brain tyrosine level and the rate of dopa accumulation (295) suggests that the effect of tryptophan was due to its role as an uptake competitor. Subsequently it was shown that dopa accumulation also increased in the brains of rats who ingested a meal that raised brain tyrosine levels (107). It thus appeared that physiologically sized changes in brain tyrosine level were capable of modifying tyrosine hydroxylation rate.

If the above studies established that tyrosine hydroxylase is not fully saturated in vivo, they did not show whether the overall rate of catecholamine synthesis is normally influenced by tyrosine availability. Hence, in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, mg/kg ip</th>
<th>Tyrosine, %</th>
<th>Dopa, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>50</td>
<td>181 ± 3*</td>
<td>113 ± 3*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>50</td>
<td>82 ± 3*</td>
<td>68 ± 10*</td>
</tr>
<tr>
<td>Leucine</td>
<td>100</td>
<td>78 ± 7*</td>
<td>75 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SE, % of controls. Groups of 7-9 rats received amino acid 15 min after Ro 4-4602 (800 mg/kg ip) and 45 min before they were killed. Control rats received saline instead of amino acid and then decarboxylase inhibitor. * Differs from control means (saline), $P < 0.01$. [From Wurtman et al. (295).]
later studies, tyrosine administration was reported to stimulate NE synthesis in whole brain [reflected as an increase in the brain level of 3-methoxy-4-hydroxyphenylethylglycol sulfate (MOPEG-SO₄), a major metabolite of NE; see 108]. However, tyrosine injection would not stimulate the accumulation of homovanillic acid (HVA, a major DA metabolite) in striatum after probenecid administration (243), indicating that the overall rate of DA synthesis was unaffected by tyrosine. The observation that tyrosine administration to rats pretreated with haloperidol would enhance HVA accumulation then suggested that DA neurons had to fire rapidly to be sensitive to tyrosine (recall that haloperidol administration enhances the firing rate of nigrostriatal neurons; 37).

Other data corroborate the necessity that DA (and NE) neurons be active for tyrosine hydroxylase to be responsive to precursor administration. For example, we (257) have observed that the administration of tyrosine increases dihydroxyphenylacetic acid (DOPAC) and HVA levels in striatum and hypothalamus of rats treated chronically (but not acutely) with reserpine. Others have noted tyrosine administration to increase striatal DA turnover in rats bearing partial lesions of the nigrostriatal tract (195), and brain MOPEG-SO₄ accumulation in rats placed at 4°C for 1 h (108). Each of these treatments is reported to increase transmitter turnover in brain (76, 114, 194, 195), presumably by enhancing neuronal firing rate. In addition, similar effects of tyrosine have been observed in rats after injection of γ-butyrolactone (GBL; the in vivo form of GHB). This drug reduces impulse flow in nigrostriatal neurons (278, 281) but activates striatal tyrosine hydroxylase as though neuronal firing rate was increased rather than decreased (204, 279). If animals treated with GBL also receive an injection of tyrosine, striatal levels of DA and the rate of dopa accumulation increase significantly over those values achieved after GHB injection alone (Table 4; 256). This

| TABLE 4. Effect of γ-butyrolactone and tyrosine on striatal dopa accumulation and dopamine levels in rats |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Treatment                                      | Dopa, μg/g                                      | Tyrosine, μg/g                                  | Dopa, μg/g                                      | Tyrosine, μg/g                                  |
| Vehicle-vehicle                                | 1.51 ± 0.08a                                    | 26.6 ± 0.9a                                    | 10.6 ± 0.6a                                     | 23.2 ± 0.5a                                     |
| Tyrosine-vehicle                               | 1.52 ± 0.09a                                    | 38.5 ± 1.3b                                    | 10.3 ± 0.4b                                     | 39.7 ± 2.2b                                     |
| Vehicle-GBL                                    | 4.73 ± 0.46b                                    | 25.4 ± 1.0b                                    | 20.0 ± 0.7b                                     | 21.5 ± 0.8b                                     |
| Tyrosine-GBL                                   | 5.96 ± 0.34b                                    | 37.5 ± 2.2b                                    | 23.6 ± 0.9b                                     | 31.4 ± 1.6b                                     |

Groups of 8 rats were injected with tyrosine (200 mg/kg ip) or vehicle (4 ml/kg) and then γ-butyrolactone (GBL) (750 mg/kg ip) or vehicle (2 ml/kg) 15 min later. In experiment 1, rats received NSD 1015 (100 mg/kg) 5 min after GBL and were killed 30 min thereafter. In experiment 2, rats were killed 45 min after injection of GBL. Values in each column followed by different superscript letters are significantly different (P < 0.05) by analysis of variance and Newman-Keuls test. [From Sved and Fernstrom (256).]
result indicates that increasing the substrate levels accelerates tyrosine hydroxylation and DA synthesis in this population of DA neurons when activated.

Why should tyrosine be most effective in stimulating catecholamine synthesis in rats treated so as to activate tyrosine hydroxylase? If the $K_m$ for substrate is reduced in such animals, this alone should lead to further saturation of the enzyme. One should then expect tyrosine injection to have less effect than in untreated rats: the enzyme is presumably almost fully (or fully) saturated with precursor. But tyrosine's effect is enhanced by treatments that activate the hydroxylase, perhaps suggesting that a reduction in substrate $K_m$ does not occur. If only the $K_m$ for cofactor were reduced, the result would appear (at least to the tyrosine molecules) to be a functional increase in the amount of active tyrosine hydroxylase. Hence the $V_{max}$ with tyrosine as variable should be increased (113, but cf. 208). Could this account for the increase in dopa accumulation and DA synthesis seen after tyrosine injection in animals treated with GHB, haloperidol, or reserpine? It seems at least partly possible. If the enzyme were normally, say, 70% saturated with substrate, and saturation increased to 100% after tyrosine injection, the rise in the actual number of catecholamine molecules formed per unit time would be greater in rats pretreated with one of the above drugs, compared to rats receiving vehicle. Of course this possibility requires further experimentation to be evaluated fully.

But even if a change in cofactor affinity would allow enhanced hydroxylation after tyrosine administration, it seems likely that some form of end-product inhibition would quickly limit any increase in transmitter formation. Hence, finally, the reputed change in $K_i$ for DA (in striatal studies) may be important. If the $K_i$ for DA was increased by any of the previously mentioned pharmacological treatments, this change might release tyrosine hydroxylase from end-product inhibition and allow larger amounts of catecholamine to be synthesized after a rise in tyrosine levels. Thus, for tyrosine to stimulate catecholamine formation in the activated neuron, it may well be that two conditions are necessary: the $K_i$ for the end product is increased, and the $K_m$ for the cofactor is decreased. However, although most laboratories observe a reduction in cofactor $K_m$, not all have obtained an increase in DA $K_i$. Hence the above possibilities remain speculation but are important topics for future investigation.

The precise mechanism by which pharmacological treatments that activate tyrosine hydroxylase and promote tyrosine-induced increases in hydroxylation rate remains only poorly understood, whereas the results with tyrosine seem clear enough. Catecholamine synthesis can be stimulated under certain conditions by providing increased amounts of substrate. The enzyme is thus not fully saturated with substrate. Of course much more work is required before a complete understanding of these relationships can emerge.

Parenthetically, tyrosine is not the only reactant in the catalysis of
tyrosine to dopa whose local concentration can influence reaction rate. A number of studies indicate that reductions in atmospheric oxygen content can decrease the rate of tyrosine hydroxylation (73, 74). The enzyme is probably saturated with oxygen under normal atmospheric conditions, however, because increasing oxygen tension does not stimulate reaction rate (73). The physiological significance of these findings is unclear, because the oxygen content of the atmosphere must be reduced to 9.4% before hydroxylation rate is seriously compromised. This roughly corresponds to an atmospheric oxygen content encountered at an altitude of 20,000 ft. However, the effect might be important pathophysiologically, for example, during the ischemia/anoxia associated with stroke.

Several groups also believe the hydroxylase to be unsaturated with pteridine cofactor (101, 148, 287). One group has even shown that the injection of tetrahydropterin into the cerebral ventricles increases catecholamine synthesis (150). However, it is presently unclear under what conditions cofactor supply might normally vary, and this line of investigation thus remains an interesting area for future work. Certainly the absence of pteridine cofactor is potentially catastrophic; for example, Kaufman et al. (149) reported a variation of PKU in which phenylalanine hydroxylation deficiency results from cofactor deficiency secondary to a deficiency in pteridine reductase rather than from an absence of the hydroxylase enzyme.

B. Effects of Tyrosine on Brain Functions Influenced by Central Catecholamine Neurons

Presently there is little information concerning effects of tyrosine on brain functions. This presumably reflects the heretofore widely held belief that only end-product inhibition and similar mechanisms are significant factors controlling catecholamine synthesis rate. In the past few years, however, a number of laboratories have begun to look for effects of tyrosine on brain outputs influenced by CNS catecholamine neurons. Interesting results have been obtained.

For example, the acute injection of tyrosine into spontaneously hypertensive rats (SHRs) causes a sizable lowering of blood pressure (Fig. 15; 258). This effect is blocked by concurrent administration of another LNAA, valine, which limits tyrosine access to brain. This finding suggests that the amino acid must gain access to brain to exert its antihypertensive effects. [Early pharmacological studies with tyrosine support the interpretation of a CNS (rather than a peripheral) site of action based on competition studies (e.g., 118).] The tyrosine-induced fall in blood pressure was also accompanied by an increase in MOPEG-SO₄ levels in the brain stem but not elsewhere in the CNS. Similar results were obtained by Yamori et al. (298), who noted that intraventricular injection of tyrosine reduces blood pressure in SHRs. Increments in MOPEG-SO₄ levels in brain stem and other regions were also
noted. Reportedly MOPEG-SO₄ is a good index of NE release (156). Hence the tyrosine-induced increase in its levels may indicate an enhanced rate of synthesis and release of NE in brain stem and possibly other brain regions. These findings are at least compatible with the general belief that stimulation of brain stem NE receptors by a variety of drugs (e.g., clonidine, methyldopa) lowers blood pressure (see 273).

Bresnahan et al. (35) studied the effect of intravenous tyrosine administration on blood pressure. They found that tyrosine injection lowered blood pressure in rats made hypertensive by treatment with deoxycorticosterone acetate (DOCA) and salt, or by renal artery clamping. These changes were accompanied by increments in blood levels of DA and epinephrine (but not NE), but the cause of these alterations is presently unknown. Some data in the literature suggest that the acute administration of tyrosine can enhance peripheral catecholamine biosynthesis (e.g., 3), and it can even raise blood pressure in rats with reduced blood volumes (56). However, further data are needed.

![Figure 15](image)

**Figure 15.** Effect of valine on antihypertensive action of tyrosine. Tyrosine (100 mg/kg, 0.55 mmol/kg), valine (0.55 mmol/kg), tyrosine plus valine (0.55 mmol/kg of each), or vehicle was administered to groups of 4 spontaneously hypertensive rats. Blood pressures were measured just before treatment and then at 1-h intervals thereafter. Data are means ± SE for change in blood pressure from base-line values. Tyrosine injection caused a significant fall in blood pressure (P < 0.01); treatment with tyrosine plus valine produced a slight decrease in blood pressure (P < 0.05), but this effect was not as great as that produced by tyrosine alone. Valine injection had no effect. [From Sved, Fernstrom, and Wurtman (258).]
necessary before such a mechanism can be invoked to account for the results of Bresnahan et al. (35).

Finally, studies of the antihypertensive action of tyrosine are not new. Laborit et al. (159, 160) observed that repeated injections of tyrosine could prevent the development of hypertension in rats treated with DOCA and salt and could also reverse hypertension once established. No clear rationale was presented for these studies, much less a chemical mechanism of action involving catecholamines. Nonetheless the observation stands. In addition Osumi et al. (213) showed blood pressure to fall in SHRs consuming a diet containing a 1% tyrosine supplement. The authors, however, believed the tyrosine effect could not be due to NE formed from the amino acid. And finally, Shalita and Dikstein (246) observed in DOCA- and salt-treated rats that the chronic administration of high doses of tyrosine would attenuate the development of hypertension. They advanced a false transmitter hypothesis involving the conversion of tyrosine to tyramine in brain, with tyramine acting to release NE to lower blood pressure. No measurements of tyramine supported these conjectures. In fact, Fellman et al. (83) reported that the conversion of tyrosine to tyramine is not a major route of tyrosine catabolism in mammals. Hence all of these reported effects of tyrosine on blood pressure may be explicable by the hypothesis offered earlier; i.e., that the amino acid accelerates the rate of NE synthesis, and ultimately, release by brain neurons involved in blood pressure regulatory circuits.

Tyrosine administration has also been reported effective in the treatment of depression. Though only limited data are presently available, two groups noted positive effects in very small, open trials (103, 112). Presumably tyrosine would exert its antidepressant effect by enhancing NE synthesis and release by brain neurons. Such a mechanism of action would be consistent with that proposed for the antidepressant actions of a wide variety of drugs (e.g., 23, 24), and also with findings that the brains (or CSF or urine) of some depressed patients often contain subnormal amounts of NE metabolites (e.g., 244). Also Beckman et al. (21) reported that phenylalanine has antidepressant actions. They suggested that the amino acid may be converted to a false transmitter (phenylethylamine) that releases NE. It seems just as likely, however, that the amino acid is hydroxylated to tyrosine in liver and brain, thereby providing the brain with a significant source of precursor with which to synthesize NE. Kaufman (148) notes that the brain is capable of using phenylalanine as a substrate for catecholamine synthesis; this finding is supported by the work of others (e.g., 295). With these preliminary data, it would now be interesting to test the efficacy of tyrosine as an antidepressant in a large, double-blind, cross-over study.

Finally, the injection of tyrosine into rats treated chronically with reserpine rapidly reduces serum prolactin levels (257). The mechanism of this effect presumably involves the tuberoinfundibular neurons that synthesize and release DA into the hypothalamohypophysial portal circulation. Released in this way DA is thought to function at the pituitary as a prolactin...
release-inhibiting factor (see 93). Reserpine depletes the hypothalamus (and elsewhere) of DA and may thereby elevate serum prolactin indirectly by causing the hypothalamus to release less of this inhibitory amine. Reserpine also stimulates the rate of catecholamine synthesis in brain (76) and appears to activate tyrosine hydroxylase (310). These latter effects may render hypothalamic DA neurons sensitive to an injection of tyrosine, in response to which they may enhance their synthesis (and release) of DA, and thus ultimately inhibit prolactin secretion. If true, then animals receiving other pharmacological treatments that lead to enhanced DA synthesis (i.e., activation of the neurons) and increased serum prolactin (e.g., the administration of a DA antagonist; 53) may also respond to tyrosine injection by reducing their serum prolactin levels. If so, tyrosine might become a useful adjunct, when such drugs are used, to attempt to minimize elevations in serum prolactin. It may be desirable to keep serum prolactin low in such situations, because high circulating levels of the hormone are reputed to be involved in the etiology of certain disease states (e.g., 36, 45, 231, 288).

C. Diet, Methyldopa Uptake Into Brain, and Blood Pressure

One major point developed in this review is the physiological importance of the LNAA transport system in determining the uptake into brain of amino acids such as tryptophan and tyrosine. For example, meal ingestion normally seems to influence the brain levels of these amino acids; the changes observed are predicted by the alterations in the plasma LNAA pattern and in the indirect modifications they induce in the competitive uptake of the LNAA into brain. But diet and the competitive transport system are also pharmacologically relevant, as tryptophan and tyrosine are used clinically for their central actions. Note in this case that the properties of the competitive transport system, in conjunction with the diet, could compromise (as well as promote) the therapeutic efficacy of tryptophan or tyrosine (or, for that matter, of any LNAA employed for its central effects).

This potential problem is illustrated by the effect diet composition has on the antihypertensive potency of methyldopa in SHRs. Methyldopa is an LNAA analog that is transported into brain by the same competitive carrier system used by the natural LNAAAs (183). Consequently it might be anticipated that increased blood levels of the LNAA, caused by any phenomenon, would inhibit methyldopa uptake into brain. This is indeed the case (183, 259). The most common natural inducer of an increase in blood LNAA levels is the ingestion of a protein-containing meal. Not surprisingly, therefore, the ingestion of protein prior to methyldopa injection inhibits the drug's access to brain (259). Methyldopa must enter brain to lower blood pressure (see 273). Hence it was predicted and later confirmed that the ingestion of a protein-containing meal would antagonize the antihypertensive action of methyldopa. This occurs when SHRs consume single meals or when for long
periods they ingest diets containing sizable quantities of protein. As the dietary protein content is increased, the antihypertensive efficacy of methyldopa is diminished (see Fig. 16). Dietary protein also reportedly antagonizes the therapeutic actions of another LNAA used to treat a CNS disease; viz., it diminishes the antiparkinsonian effects of L-dopa (196).

The therapeutic implications are straightforward for LNAAAs having CNS sites of action, and should apply regardless of the LNAA used. The ingestion of a single protein-containing meal prior to LNAA administration should reduce the acute effects of the treatment. Chronic ingestion of high levels of protein should also reduce LNAA efficacy at most times of administration. Hence, to maximize drug effect, the amino acid should be consumed between meals or with carbohydrates (which would cause insulin secretion and lower blood LNAA levels); a general reduction in dietary protein intake would probably also improve efficacy, if subjects normally consumed substantial amounts daily.

If the LNAAAs are to be tested and used in humans for their CNS effects, it is important to keep such dietary considerations in mind. Overlooking the relationship of dietary protein content to LNAA efficacy may lead to marginal results, whereas otherwise a positive effect might be obtained.

IV. SUMMARY AND CONCLUSIONS

The discussion and data presented here support the view that tryptophan availability to the brain readily influences the rate of 5-HT formation physiologically, pharmacologically, and pathophysiologically. The pool of tryptophan in brain available for 5-HT synthesis apparently depends considerably on the supply of the amino acid from the blood. Because tryptophan uptake into brain occurs via a saturable, competitive transport carrier shared with several other LNAAAs, the brain tryptophan pool is influenced not only

![FIG. 16. Effect of acute methyldopa administration on blood pressure in spontaneously hypertensive rats consuming protein diets for several weeks. Groups of 6 rats consumed either a 12, 22, or 36% casein diet for 5 wk. They then received methyldopa (25 mg/kg ip) in the morning, and blood pressures were measured at indicated intervals thereafter. By analysis of variance, dietary protein content significantly affected the antihypertensive potency of methyldopa ($P < 0.05$). Mean blood pressures at 0 h were $208 \pm 3$ mmHg. [From Sved, Goldberg, and Fernstrom (259).]
by changes in the blood level of tryptophan but also by alterations in the blood concentrations of the other LNAAs. It is the ability of the diet (and hormones) to modify the plasma levels of tryptophan and these other LNAAs, and thereby indirectly the competitive uptake of tryptophan into brain, that allows it to alter CNS 5-HT synthesis predictably.

Although tryptophan binds to albumin in serum (only a small portion of the amino acid normally circulates free), the importance of this phenomenon to the uptake of tryptophan into brain appears modest. A variety of studies indicate that albumin binding does not influence tryptophan access to brain, at least physiologically. It is mainly a selection of pharmacological studies that supports a relationship between albumin binding and brain tryptophan uptake. Pharmacological results do not always predict physiological relationships. Only additional work can determine if there is physiological relevance to these findings.

Tryptophan administration influences brain functions thought to be at least partly controlled by 5-HT neurons (e.g., GH secretion, blood pressure control). Such effects can be blocked by coadministration of 5-HT antagonists, and enhanced by coinjection of 5-HT reuptake blockers. These and other findings thus strongly support the notion that tryptophan-induced increments in 5-HT synthesis enhance transmitter release and interaction with postsynaptic receptors. The effects of diet-related changes in brain tryptophan and 5-HT on these brain functions are still unstudied.

Tyrosine availability to brain, under selected circumstances, influences the rate of DA and NE synthesis. Generally tyrosine appears effective under physiological and pharmacological conditions wherein the activity of catecholamine neurons is above normal. Why this should be the case is not entirely clear. However, the current speculation is that increased neuronal activity leads to an activation of tyrosine hydroxylase. The activated form of the enzyme is more vulnerable to variations in brain tyrosine level, due to changes in its kinetic properties.

Tyrosine administration appears to influence brain functions partly controlled by catecholamine neurons. These include the control of prolactin secretion and of blood pressure. Hence tyrosine-induced increments in the synthesis of DA or NE are apparently associated with increased transmitter release. At present the most interesting effects of tyrosine are pharmacological. It is not known if the physiological variations in brain tyrosine level and catecholamine formation that have been observed also influence transmitter release and thereby modify appropriate brain functions. This remains an interesting and probably difficult area for future investigation.

The most difficult question concerning these findings is: where lies the broader physiological significance of the relationship between amino acid availability and transmitter biosynthesis? So far the answer is unclear for tyrosine and the catecholamines, because in normal animals tyrosine hydroxylation rate is not remarkably sensitive to precursor supply. For tryptophan and 5-HT, the hydroxylation rate is normally quite sensitive to pre-
cursor supply. Diet and hormones have clear, predictable effects on 5-HT synthesis, elicited by changes in the plasma LNAA pattern. Because the 5-HT content of neurons rapidly reflects plasma LNAA pattern, and this pattern is readily influenced by any number of hormonal and metabolic phenomena (as well as the diet), perhaps these neurons monitor and control aspects of body amino acid metabolism. The 5-HT neurons might also participate in the regulation of nutrient intake, as several investigators have suggested. But these (and other) notions have not yet been fully evaluated. The resolution of this issue will probably require a great deal of thought and effort for years to come.

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