4 Aromatic Amino Acids in the Brain

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Abstract: This chapter describes the aromatic L-amino acids tryptophan and tyrosine and the effects on tyrosine metabolism of phenylalanine. Tryptophan and phenylalanine are essential amino acids and must ultimately be derived from dietary proteins; tyrosine is obtained both from dietary proteins and from the hydroxylation of phenylalanine by phenylalanine hydroxylase (PAH). The proportions of dietary tryptophan, tyrosine, and phenylalanine that enter the systemic circulation are limited by three hepatic enzymes—tryptophan dioxygenase, tyrosine aminotransferase, and phenylalanine hydroxylase—that destroy them. These enzymes all have high substrate K_m's, hence they have little effect on their amino acid substrates present in systemic blood but major, concentration-dependent effects on the elevated concentrations, present postprandially, in portal venous blood.

All of the large, neutral amino acids (LNAA)—e.g., the three aromatic amino acids; the three branched-chain amino acids, leucine, isoleucine, and valine—across from the brain's capillaries into its substance through the action of a single transport molecule, LAT1. The kinetic properties of this molecule are such that it is saturated with LNAA at normal concentrations in systemic blood so that the individual LNAA compete with each other for blood–brain barrier transport. Hence the effect of any treatment on, for example, brain tryptophan, will depend not on plasma tryptophan, per se, but on the ratio of the plasma tryptophan concentration to the summed concentrations of the other, competing LNAA. Small quantities of LNAA molecules also enter the brain via choroid plexus transport into the cerebrospinal fluid.
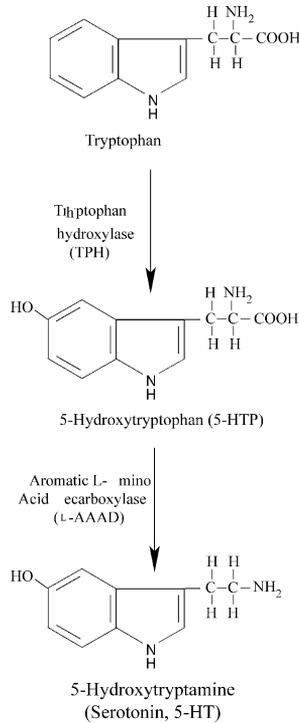
The levels of tryptophan in the brain determine the substrate-saturation of tryptophan hydroxylase, and thus the rate at which tryptophan is converted to 5-hydroxytryptophan and subsequently to serotonin or melatonin. Brain tyrosine levels may or may not affect the rate at which tyrosine is hydroxylated, and converted to the catecholamines dopamine and norepinephrine, depending on the firing frequency of the particular catecholaminergic neuron. If the neuron is firing with high frequency, the tyrosine hydroxylase enzyme becomes multiply phosphorylated; this markedly increases its affinity for its otherwise-limiting cofactor (tetrahydrobiopterin) so that local tyrosine concentrations become limiting (several groups of prefrontal dopaminergic neurons normally fire unusually frequently, and are thus always susceptible to precursor control by available tyrosine levels). The abilities of the precursor amino acids, tryptophan and tyrosine, to control the rates at which neurons can produce and release their neurotransmitter products underlie a number of physiological processes, and also constitute a potential tool for amplifying or decreasing synaptic neurotransmission.

List of Abbreviations: AMP, adenosine monophosphate; BBB, blood-brain barrier; BH_4, tetrahydrobiopterin; CSF, cerebrospinal fluid; DOCA, deoxycorticosterone; DOPA, dihydroxyphenylalanine; DOPAC, dihydrophenylacetic acid; EMS, Eosinophilia-Myalgia syndrome; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytriptamine; 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; IDO, indoleamine 2,3-dioxygenase; INF-gamma, interferon-gamma; L-AAAD, aromatic-L-amino acid decarboxylase; L-DOPA, L-dihydroxyphenylalanine; LAT1, Large Neutral Amino Acid Transporter 1; LNAA, Large Neutral Amino Acid; MAO, monoamine oxidase; MOPEG-SO4, 3-methoxy-4-hydroxyphenylethyleneglycol-Sulphate; NAD, nicotinamide adenine dinucleotide; NEFA, nonesterified fatty acids; NMDA, N-methyl D-aspartate; PAH, phenylalanine hydroxylase; PKU, phenylketonuria; SOD, Superoxide dismutase; TAT, tyrosine aminotransferase; TDO, tryptophan dioxygenase; TH, tyrosine hydroxylase; TNF-alpha, tumor necrosis factor-alpha; TPH, tryptophan hydroxylase

1   Introduction

This chapter describes the aromatic L-amino acids tryptophan and tyrosine, as well as the utilization of a third aromatic L-amino acid, phenylalanine, to produce tyrosine (the metabolism of phenylalanine in phenylketonuria [PKU] is described elsewhere in this volume). Like all dietary amino acids, each of these three compounds is used ubiquitously to synthesize proteins. But also, within some cell types, tryptophan is converted to the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) (❯ *Figure 4-1*), or tyrosine is converted to the catecholamines—the neurotransmitters dopamine and norepinephrine and the hormone epinephrine (❯ *Figure 4-2*) (tryptophan is also used in the pineal gland to make the hormone

◻ **Figure 4-1**
Biosynthesis of serotonin from tryptophan

Tryptophan

Tryptophan
hydroxylase
(TPH)

5-Hydroxytryptophan (5-HTP)

Aromatic L-amino
Acid decarboxylase
(L-AAAD)

5-Hydroxytryptamine
(Serotonin, 5-HT)

melatonin [▶ *Figure 4-3*], and tyrosine is used to make the thyroid gland's hormones, and the melanin in skin and brain).

The initial steps in producing these neurotransmitters (and in the process of converting phenylalanine to tyrosine) are catalyzed by specific but similar hydroxylase enzymes which, under certain conditions, are unsaturated with their amino acid substrates. Hence, physiologic increases in brain tryptophan or tyrosine levels can, by enhancing the saturation of their respective hydroxylases, control the rates at which serotoninergic or catecholaminergic cells form the intermediates 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA) and, ultimately, their neurotransmitter products. Similarly, changes in phenylalanine availability can affect tyrosine synthesis in the liver (▶ *Figure 4-4*) or DOPA formation in catecholaminergic neurons. This ability of precursor levels to control the syntheses of their biologically active products is unusual in the body: consumption of cholesterol, a precursor of testosterone or estrogens, in no way affects the syntheses of these gonadal steroids. This ability requires that plasma amino acid levels be allowed to vary (for example, in response to the macronutrient composition of the foods most recently consumed); that these variations be allowed to affect brain tryptophan or tyrosine levels; and that, as above, changes in these levels be sufficient to affect the rates at which the amino acids are hydroxylated.

2   Sources of Aromatic Amino Acids

Humans and other mammals are incapable of synthesizing tryptophan or phenylalanine de novo, and must ultimately obtain these essential amino acids by consuming proteins. The liver is able to make tyrosine from phenylalanine through the action of phenylalanine hydroxylase (PAH), hence mammals normally obtain

◘ Figure 4-2

Biosynthesis of the catecholamines dopamine, norepinephrine, and epinephrine from tyrosine
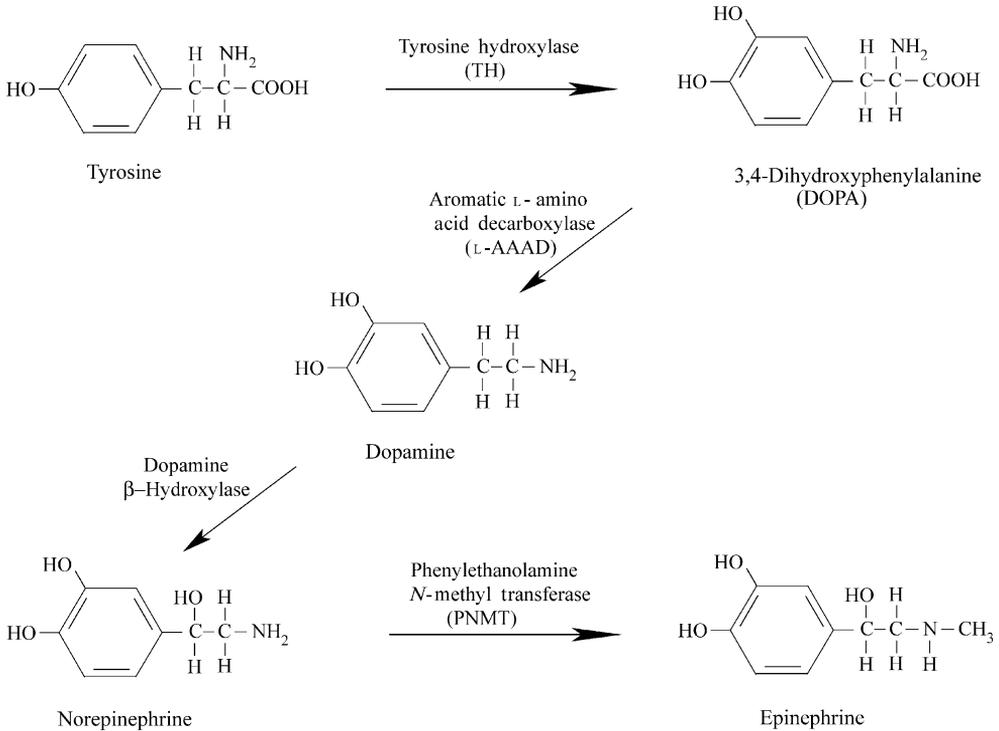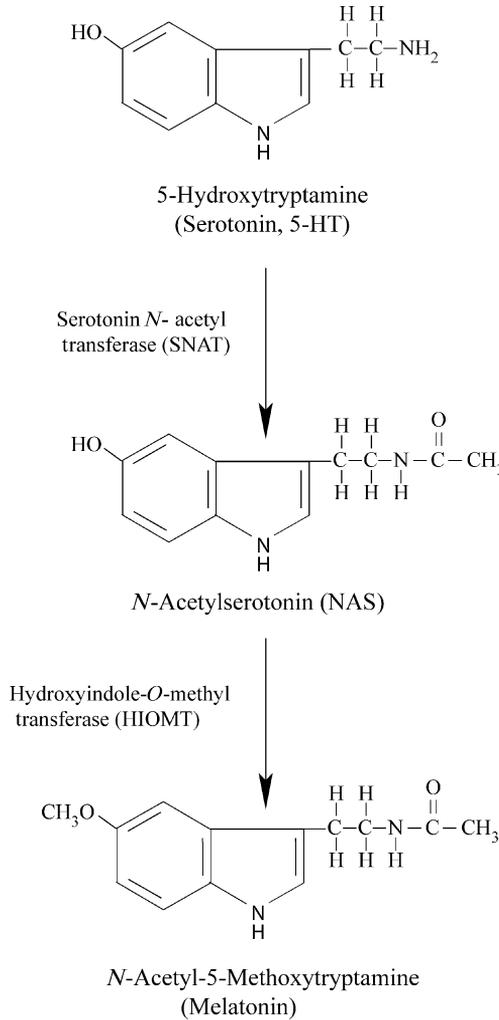

tyrosine from both an exogenous source, dietary protein, and endogenous synthesis (which provides about 15–20% of the tyrosine in human plasma [Barazzoni et al., 1998]). Tryptophan is usually the least abundant amino acid in most dietary proteins, constituting, for example, only 1–1.5% of the amino acids in casein, ovalbumin, and most meats (Orr and Watt, 1968), however, a few proteins—notably α-lactalbumin, a minor milk protein which is 6% tryptophan (Markus et al., 2002)—contain substantially more. Phenylalanine and tyrosine generally account for 3–4% of the amino acids in most dietary proteins. All three aromatic amino acids are primarily metabolized in the liver, by tryptophan dioxygenase (TDO), PAH, and tyrosine aminotransferase (TAT), respectively. Hence, only a portion of each actually enters the systemic circulation after a meal. The three aromatic amino acids can also be released from reservoirs in tissue or circulating proteins, however, this contribution is minor except in starvation.

3 Plasma Concentrations of the Aromatic Amino Acids

Aromatic amino acid concentrations in the systemic blood principally reflect the composition of the most recently consumed meal or snack (Fernstrom et al., 1979; Maher et al., 1984), and whether that food is still being digested and absorbed (❯ *Figure 4-5*). Consumption of carbohydrates with high glycemic indices (e.g., sucrose and starches, but not fructose) lowers plasma levels of most amino acids, principally via insulin-mediated facilitation of their uptake into skeletal muscle for conversion to protein (and, for the branched-chain amino acids leucine, isoleucine, and valine, for transamination and oxidation). In contrast, protein consumption raises plasma amino acid levels by directly contributing molecules which pass unmetabolized from the portal to the systemic circulations (i.e., virtually all of the leucine, isoleucine, and valine; a portion of each of the aromatic amino acids). In humans, a meal containing about 25-g protein

◘ Figure 4-3
Biosynthesis of melatonin from serotonin

5-Hydroxytryptamine
(Serotonin, 5-HT)

Serotonin *N*- acetyl
transferase (SNAT)

N-Acetylserotonin (NAS)

Hydroxyindole-*O*-methyl
transferase (HIOMT)

N-Acetyl-5-Methoxytryptamine
(Melatonin)

and 180-g carbohydrate will neither raise nor lower plasma levels of tryptophan, phenylalanine, or tyrosine (Fernstrom et al., 1979), because the insulin-mediated passage of these amino acids into tissues is compensated by their entry from the splanchnic system; in rats, the null-effect proportion of proteins to carbohydrates is somewhat less (Yokogoshi and Wurtman, 1986).

As described below, enzymes exist in the liver, which function as "gates" to control the proportions of dietary tryptophan, tyrosine, and phenylalanine that are allowed to gain access to the systemic circulation. These enzymes are characterized by having a substrate K_m that is appreciably higher than the concentrations of their amino acid substrates in systemic blood, but lower than the concentrations that may be present postprandially in portal venous blood. This kinetic property allows the enzymes to metabolize only small proportions of the aromatic amino acid molecules reaching the liver by the hepatic arteries, but half or more of those arriving via the portal venous blood—and to vary the rates at which they destroy these substrates depending on the amounts that were consumed in the most recent meal.

◩ **Figure 4-4**

Conversion of phenylalanine to tyrosine

Phenylalanine

Phenylalanine
hydroxylase
(PAH)
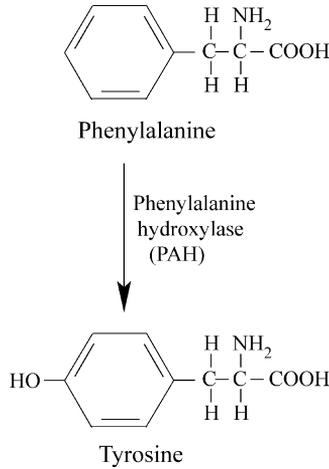
Tyrosine

The uptakes of plasma tryptophan, tyrosine, and phenylalanine into the brain depend not only on their own concentrations, but also on the plasma concentrations of other large neutral amino acids (LNAA) that compete with them for attachment to an LNAA carrier protein in brain capillaries. Hence, insulin—which profoundly lowers the plasma concentrations of the LNAA leucine, isoleucine, and valine—increases brain tryptophan uptake without increasing human plasma tryptophan levels because it decreases the competition for uptake generated by the other LNAA (Fernstrom and Wurtman, 1972b). Brain tyrosine and phenylalanine uptakes are not similarly increased by insulin, because insulin decreases their plasma levels by almost as much as it decreases those of the branched-chain amino acids (❯ *Figure 4-5*). The basis of plasma tryptophan's unique response to insulin, discussed below, is its also-unique ability to bind to circulating albumin: as much as 75–80% of the tryptophan in human plasma travels loosely bound to albumin (McMenamy and Oncley, 1958), but still largely able to enter the brain as shown by Pardridge (1977). Nonesterified fatty acids (NEFA)—which bind to a different site on the albumin molecule as shown by Goodman (1958)—inhibit this binding, hence insulin, which causes NEFA to strip off the albumin and enter adipocyes, increases albumin-bound tryptophan. This increase largely compensates for the reduction in "free" tryptophan that results from its insulin-mediated entry into skeletal muscle (❯ *Table 4-1*) (Lipsett et al., 1973; Madras et al., 1973).

Since people and most other mammals consume most of their food during either the day or the night depending on when their species sleeps, food consumption generates circadian rhythms in plasma amino acid levels (❯ *Figure 4-5*) (Wurtman et al., 1968b; Fernstrom et al., 1979), acting via insulin's effects and the passage of dietary amino acids from the portal to systemic circulations. These rhythms tend to disappear when people have been deprived of dietary carbohydrates and proteins for a day or two (Marliss et al., 1970).

When plasma glucose levels are above or below an "allowable" range, homeostatic feedback mechanisms are engaged to restore them to within that range, for example, insulin secretion in hyperglycemia, epinephrine secretion and glycogen breakdown in hypoglycemia. Similarly, when body temperature is above or below its allowable range, sweating or shivering are activated to restore it to normal. No such mechanisms regulate plasma amino acid levels: these levels are under "open-loop" control and, as described above, principally reflect the protein and carbohydrate contents of the meal or snack most recently consumed (❯ *Figure 4-5*). A behavioral feedback mechanism does exist through which a carbohydrate-rich, protein-poor snack can, by increasing brain serotonin, decrease the likelihood of continuing to eat carbohydrates (Wurtman et al., 1983). However, this mechanism does not "defend" allowable ranges for plasma

■ Figure 4-5

Diurnal variations in plasma aromatic amino acid concentrations (*top*) and ratios (*bottom*) in normal human subjects consuming different levels of dietary protein. Each diet was consumed for five consecutive days and blood samples were drawn on the 4th and 5th days of each period. Plasma amino acid concentrations are expressed in nmol/ml. Vertical bars represent SD. Abbreviations: P,L,I,V,M,T + T are phenylalanine, leucine, isoleucine, valine, methionine, and tyrosine and/or tryptophan, respectively. Data from Fernstrom et al. (1979)
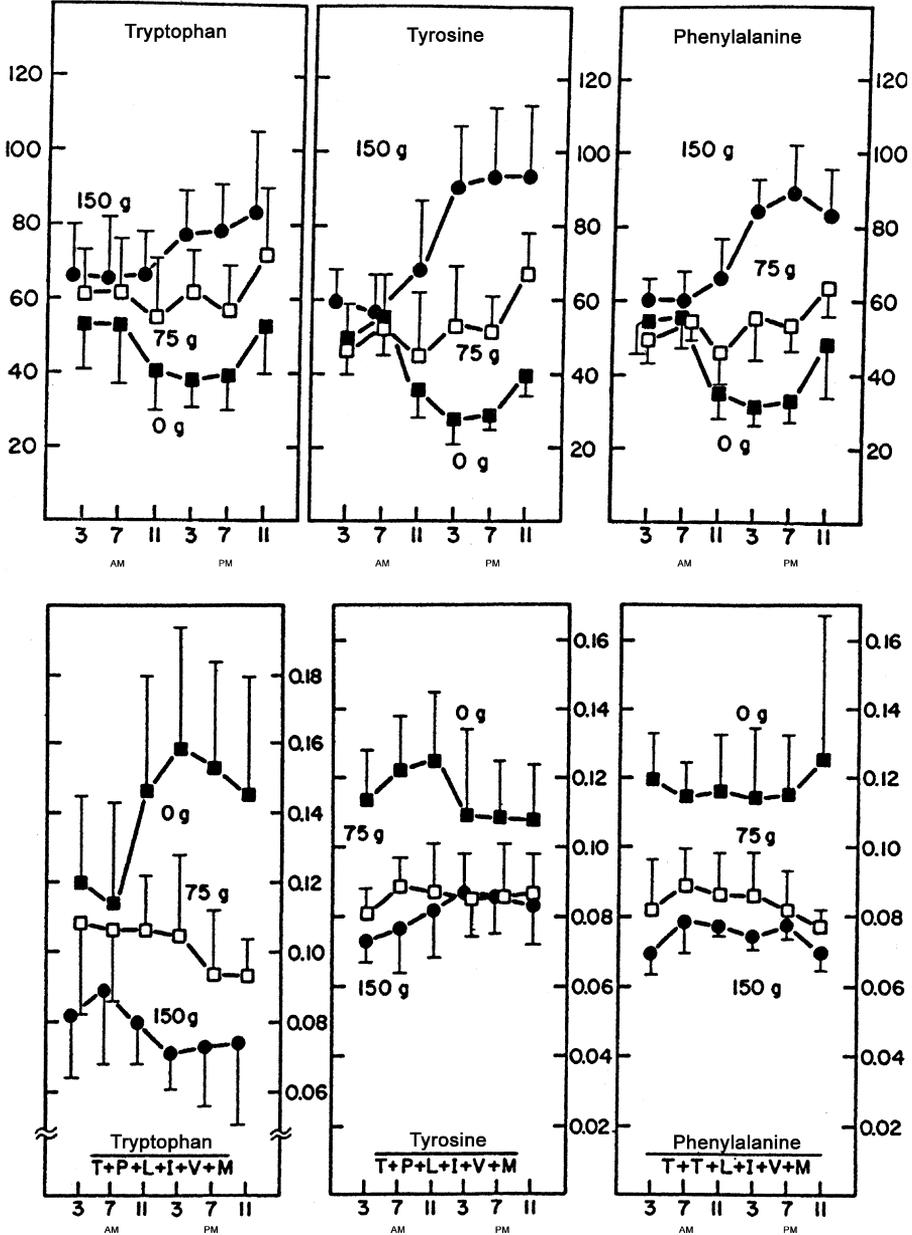
⬛ **Table 4-1**

Effects of glucose ingestion on brain tryptophan and on serum free and albumin-bound tryptophan

	Control	Glucose (1 h)	Glucose (2 h)
Serum total tryptophan (µg/ml)	16.2 ± 0.2	19.6 ± 0.6	19.9 ± 0.4***
Serum free tryptophan (µg/ml)	5.5 ± 0.1	4.8 ± 0.3*	4.2 ± 0.2***
Free (% of total)	34	25	21
Serum bound tryptophan (µg/ml)	10.7 ± 0.3	14.8 ± 0.6**	15.7 ± 0.5***
NEFA (meq/L)	1.147 ± 0.034	0.648 ± 0.077***	0.604 ± 0.044***
Brain typtophan (µg/ml)	4.16 ± 0.42	6.42 ± 0.56**	5.93 ± 0.72**

Rats received D-glucose (2 g/4 ml tap water) by stomach tube; control animals received tap water. Values in all tables are given as mean $\pm$ SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, differs from controls. Data from Madras et al. (1973)

amino acid levels in the same sense the body defends blood glucose levels or temperatures. Food-induced changes in the plasma amino acid pattern are thus able, as described below, to affect neurotransmitter synthesis, as well as appetitive and other behaviors mediated by affected neurotransmitters.

3.1 Plasma Tryptophan

Plasma tryptophan concentrations among fasting normal humans vary between 55 and 65 µM (❯ *Figure 4-5*), depending in part on the individual's prior protein intake (i.e., higher after consuming a high-protein diet for a few days) (Fernstrom et al., 1979); prior caloric intake (i.e., dieting [Goodwin et al., 1990]); age (lower in older men [Caballero et al., 1991]); gender (higher in males [Demling et al., 1996]); and body mass index (lower in obesity [Caballero et al., 1988]). In rats, fasting tryptophan concentrations reportedly vary between 80 and 150 µM (Fernstrom and Wurtman, 1971a; Madras et al., 1973). Maximal levels among people consuming three high-protein meals (50 g/meal) per day are about twice as high as minimal levels in people consuming three protein-free meals daily (❯ *Figure 4-5*) (Fernstrom et al., 1979); this defines the normal range for human plasma tryptophan concentrations. As mentioned above, about 75–80% of the tryptophan in human plasma is loosely bound (McMenamy and Oncley, 1958) to albumin. This binding is of low affinity: the Kd's for rats and rabbits under pentobarbital anesthesia are greater than 1 mM (as compared with in vitro estimates of 0.13 mM) (Pardridge and Fierer, 1990). The proportion of circulating tryptophan bound to albumin increases from 0.62 to 0.82 after rats consume carbohydrates (❯ *Table 4-1*) (Madras et al., 1973) because insulin causes "free" (i.e., nonalbumin-bound) tryptophan to decline (like the other LNAA) but, by decreasing the binding of NEFA to albumin (Madras et al., 1973), enhances the albumin's affinity for tryptophan (Madras et al., 1973).

Plasma tryptophan concentrations are readily increased by administering exogenous tryptophan. Since this treatment—unlike eating proteins—does not also raise plasma LNAA, it can cause proportionate increases in brain tryptophan (Fernstrom and Wurtman, 1971a). Similarly, administration to rats (Gessa et al., 1974) or humans (Moja et al., 1988) of a mixture containing other LNAA but not tryptophan decreases plasma and brain or CSF (❯ *Figure 4-6*) tryptophan by causing more to be used for tissue protein synthesis, and by competing with tryptophan for blood–brain barrier (BBB) transport as shown by Pardridge (1977). As described below, many investigators have used these techniques to implicate brain serotonin in particular physiologic processes or disease states (Delgado et al., 1991; Smith et al., 1997) (❯ *Figure 4-7*).

3.1.1 Tryptophan Dioxygenase and Indoleamine Dioxygenase

The proportion of dietary tryptophan able to pass from the portal to the systemic circulation is determined in large part by the activity of hepatic tryptophan 2,3-dioxygenase (TDO) (EC 1.13.11.11), a heme-containing enzyme that irreversibly cleaves tryptophan's indolic nucleus to form N-formylkynurenine. This enzyme, found only in the liver, probably has only minor effects on the breakdown of tryptophan

◘ Figure 4-6

Effect of consuming a tryptophan-free drink containing other LNAA on plasma and CSF tryptophan concentrations. Data on four or five subjects were obtained from Carpenter et al. (1998) and Delgado et al. (1991)
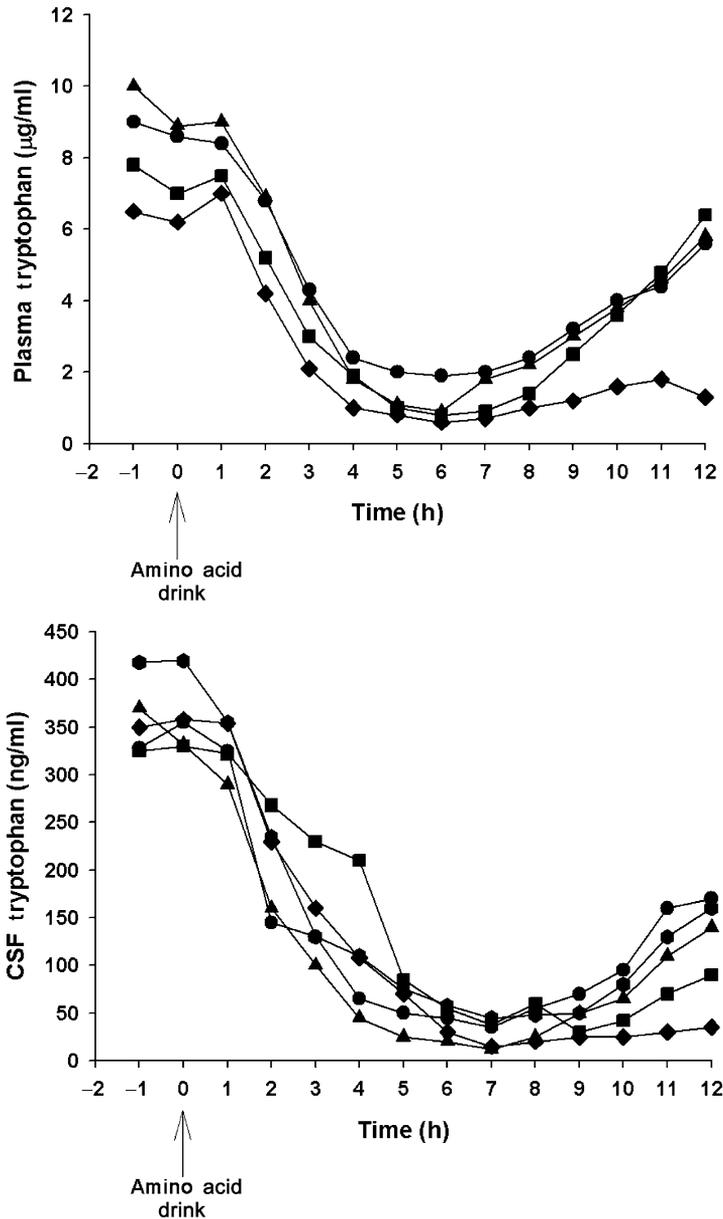
reaching the liver via the systemic circulation, because of its kinetic properties, i.e., a very high K_m—0.5×10^{-3} M (Schimke et al., 1965)—considerably greater than systemic, but not portal, venous tryptophan concentrations. In contrast, indoleamine 2,3-dioxygenase (IDO) (EC 1.13.11.17), the other enzyme that destroys tryptophan's indole nucleus, has a K_m for tryptophan at least an order of magnitude lower than

◘ **Figure 4-7**

Effect of consuming a tryptophan-free amino acid mixture on HAM-D (Hamilton rating scale for depression) in women with a prior history of depression. Data from Smith et al. (1997)
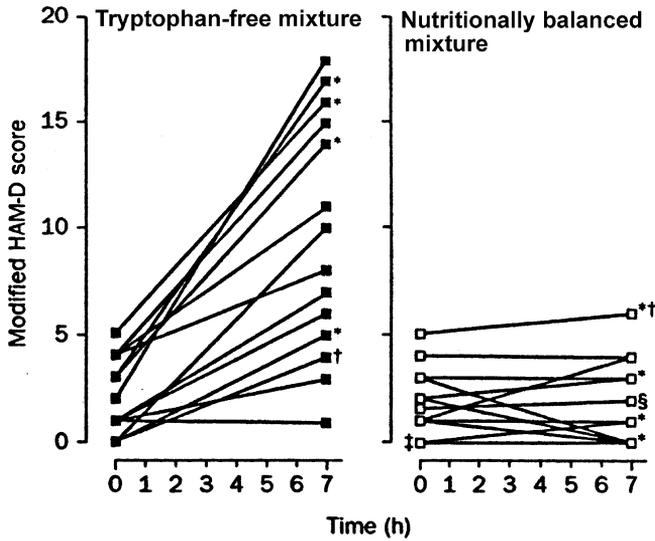
that of TDO (i.e., 26 μM [Yamazaki et al., 1985]; 45 μM [Shimizu et al., 1978]), and is active at the tryptophan concentrations found in systemic blood.

Tryptophan dioxygenase was initially called tryptophan pyrrolase (Kotake and Masayama, 1936), but renamed TDO after Hayaishi and coworkers (Hayaishi et al., 1957) showed that it incorporates two atoms of molecular oxygen into tryptophan to form the *N*-formylkynurenine. TDO, a tetrameric protein, is specific for the L-isomer of tryptophan (Tanaka and Knox, 1959), while IDO, a monomer, metabolizes a broad range of substrates (L-and D-tryptophan; serotonin; melatonin), and is found in all extrahepatic tissues (Yamazaki et al., 1985), including brain (Kwidzinski et al., 2005).

The human TDO gene is located on chromosome 4 (Comings et al., 1991). In rats, TDO activity exhibits a characteristic daily rhythm, peaking several hours after the onset of darkness, when the animals consume most of their food (Rapoport et al., 1966). This rhythm persists when animals consume protein-free diets (Ross et al., 1973), unlike the parallel rhythm in TAT activity (Wurtman et al., 1968b) described below. The mechanism causing the TDO rhythm remains unknown. High doses of glucocorticoid hormones can elevate TDO activity (Knox and Auerbach, 1955), and there is a daily rhythm in plasma glucocorticoid levels preceding the TDO rhythm. However, physiologic increases in plasma glucocorticoids have not been shown to increase TDO activity. Even major increases in TDO, produced by giving pharmacologic doses of glucocorticoids, do not affect plasma tryptophan levels (Kim and Miller, 1969) supporting the view that portal venous tryptophan, and not systemic tryptophan, is the normal substrate for this enzyme.

The hepatic *N*-formylkynurenine generated by TDO is further metabolized to L-kynurenine, kynurenic acid, xanthurenic acid, quinolinic acid, nicotinamide adenine dinucleotide (NAD), and, ultimately, to CO_2 and water. A kynurenine-producing pathway exists in rat brain (Guidetti et al., 1995) and several of its intermediates may have significant biological activity. Thus neurodegenerative effects have been attributed to quinolinic acid, which acts as an agonist for NMDA type glutamatergic receptors (Stone and Perkins, 1981); neuroprotective effects to the glutamate receptor antagonist kynurenic acid (Perkins and Stone, 1982); inhibition of striatal dopamine release by kynurenic acid (which blocks α-7 nicotinic receptors [Rassoulpour et al., 2005]); and an ability to stimulate neuronal growth and development to kynurenine

(which is conjectured to stimulate nerve growth factor production [Dong-Ruyl et al., 1998]). NAD formed from this pathway is, of course, a cofactor in various enzymatic reactions.

The gene for human IDO is located on chromosome 8 (Burkin et al., 1993). The enzyme uses reduced molecular oxygen and superoxide (O^-_2) as substrates; it is the only enzyme known to do so besides superoxide dismutase (SOD), suggesting a role for it as an antioxidant. Whereas TDO activity can be induced by tryptophan, tyrosine, phenylalanine, histidine, and kynurenine (Taylor and Feng, 1991), IDO activity is induced by viruses, lipopolysaccharides, TNF-alpha, and interferons such as INF-gamma and INF-alpha. Its induction leads to intracellular depletion of tryptophan and to inhibition of the proliferation of various cancer cells, viruses, bacteria (Carlin et al., 1989; MacKenzie et al., 1999) and parasites as shown by Pfefferkorn (1984). In agreement with the in vitro finding that T cell proliferation could be inhibited by IDO activation (Munn et al., 1999), IDO expression in placenta was shown to prevent fetal allograft rejection by depleting tryptophan and suppressing maternal T cell responses in pregnant mice (Munn et al., 1998).

3.1.2 Eosinophilia-Myalgia Syndrome

Prior to 1990, L-tryptophan was freely available as a dietary supplement within the USA, purchased principally for self-treatment of insomnia. Then, in 1989, a manufacturer, the Showa Denka Company, began to market a new tryptophan preparation, the synthesis of which involved fermentation using a newly engineered strain of Bacillus amyloliquefacien (Yamaoka et al., 1994). Soon thereafter a new disease, the "Eosinophilia-Myalgia Syndrome" (EMS) was identified (Centers for Disease Control, 1989), initially among users of this preparation (Slutsker et al., 1990), who lived in New Mexico (Eidson et al., 1990). The syndrome was characterized by muscle pain and weakness, striking eosinophilia, dyspnea, skin rash, and various abnormal laboratory findings. Subsequent chemical analysis of this preparation revealed that it contained a variety of novel impurities, including "Peak E," or 1,1-ethylidenebis[tryptophan]—a compound later shown to activate human eosinophils and to enhance cytokine production from T lymphocytes (Yamaoka et al., 1994).

Excellent medical detective work led to the rapid removal of tryptophan containing toxic impurities from the market. It has also led, in the USA, but not elsewhere, to the continuing unavailability of pure tryptophan for medical uses, other than as a constituent of enteral and parenteral preparations. Had tryptophan been regulated as a drug, any new preparation would have had to undergo Phase I safety testing, during which the propensity of the new Showa Denka preparation to cause severe eosinophilia would have been noted, and the product would probably not have been approved for medical use. Unfortunately, because of the Dietary Supplement Act of 1994—which exempts "amino acids and their products" (!) from having to undergo FDA-regulated testing prior to sale—other amino acid "dietary supplements" continue to be sold in the USA without prior Phase I testing.

3.2 Plasma Tyrosine

Plasma tyrosine concentrations among fasting normal humans vary between 50 and 80 μM (❯ *Figure 4-5*), (Fernstrom et al., 1979; Glaeser et al., 1979; Maher et al., 1984) depending in part on the individual's prior protein intake (Fernstrom et al., 1979), and age (higher in older than younger women [Caballero et al., 1991]). In rats, fasting tyrosine levels vary between 90 and 120 μM (Fernstrom and Faller, 1978; Agharanya and Wurtman, 1982a). Maximal concentrations among people consuming three high-protein meals (50 g/meal) per day are about 3.5 times as great as minimal levels observed in people consuming three protein-free meals daily (Fernstrom et al., 1979). Unlike tryptophan, neither tyrosine nor phenylalanine in blood is appreciably bound to albumin.

Plasma tyrosine levels are also readily increased by administering exogenous tyrosine (❯ *Figures 4-8* and ❯ *4-9*) (Glaeser et al., 1979; Melamed et al., 1980a), which also raises tyrosine levels in human CSF (Growdon et al., 1982) and rat brain (Morre et al., 1980). A single oral dose of 100 mg/kg increased human

□ **Figure 4-8**

Accumulation of MOPEG-SO4 in brains of cold-stressed rats treated with neutral amino acids. Rats received valine (200 mg/kg, i.p.) or tyrosine (125 mg/kg, i.p.), or saline; 30 min later they were placed in single cages in a cold (40 C) environment. After 1 h, all animals were killed, and their whole brains were analyzed for tyrosine and MOPEG-SO4. Each point represents the tyrosine and MOPEG-SO4 present in a single brain. Brain tyrosine and MOPEG-SO4 levels in animals kept at room temperature were 14.4 μg/g and 80 ng/g, respectively. Symbols: closed circles, animals pretreated with valine; open circles, animals pretreated with saline; closed squares, animals pretreated with tyrosine. Data from Gibson and Wurtman (1978)
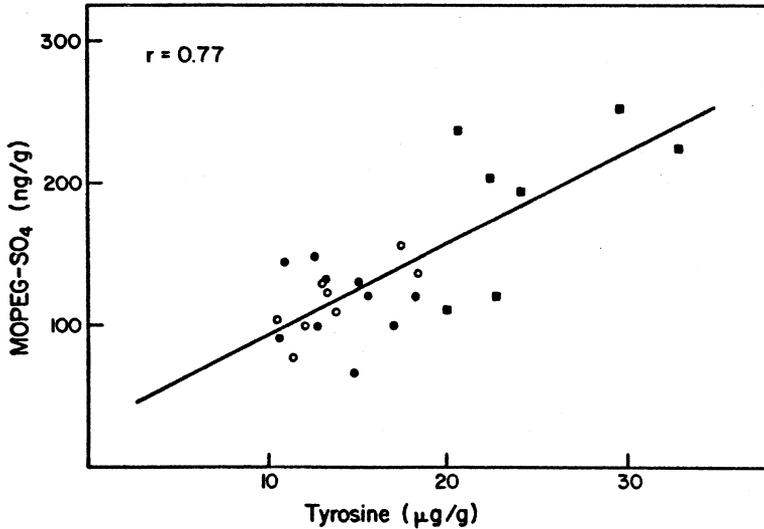
plasma tyrosine concentrations, after 2 h, from 69 to 154 μM, a 150-mg/kg dose increased this level to 203 μM. Both treatments reduced plasma levels of the other LNAA (Glaeser et al., 1979), probably by enhancing their utilization for tissue protein synthesis. A single intraperitoneal dose of 100 mg/kg given to rats increased tyrosine levels throughout the brain, but effects were greatest in hippocampus and cortex (Morre et al., 1980).

Similarly, administration to rats (Biggio et al., 1976) or humans (Sheehan et al., 1996; McTavish et al., 2005) of an LNAA mixture lacking tyrosine or its precursor phenylalanine lowers plasma tyrosine and, in rats, can be shown to deplete brain tyrosine as well (Biggio et al., 1976). Since brain tyrosine levels can, like those of tryptophan, control the rates of synthesis of its neurotransmitter products (the catecholamines dopamine and norepinephrine [Wurtman et al., 1974]), investigators are starting to use this technique to implicate brain catecholamines in particular physiologic processes or disease states (McTavish et al., 2005).

3.2.1 Tyrosine Aminotransferase

The proportion of dietary tyrosine, or tyrosine synthesized from phenylalanine by hepatic PAH, which can pass from the portal to the systemic circulations is determined in large part by the activity of hepatic TAT (EC 2.6.1.5). This enzyme catalyzes the conversion of tyrosine to p-hydroxyphenylpyruvate, the initial intermediate in tyrosine's complete degradation to fumarate and acetoacetate. Like TDO for tryptophan, TAT probably has only minor effects on the breakdown of the tyrosine that reaches the liver via the systemic circulation, because its K_m for tyrosine—1.7×10^{-3} M (Hayashi et al., 1967)—is even greater than TDO's for tryptophan, and also much greater than plasma tyrosine levels.

■ **Figure 4-9**

Effect of tyrosine administration on the accumulation of HVA in corpora striata of rats given haloperidol or probenecid. Rats received tyrosine (100 mg/kg) or its diluent followed in 20 min by haloperidol (2 mg/kg) or probenecid (200 mg/kg); they were sacrificed 70 min after the second injection. Data from individual animals receiving haloperidol are indicated by open circles; data from rats receiving haloperidol plus tyrosine are indicated by closed circles. Striatal HVA levels were highly correlated with brain tyrosine levels in all animals receiving haloperidol ($r = 0.70$, $p < 0.01$). In contrast, the striatal HVA levels of animals receiving probenecid alone did not differ from those of rats receiving probenecid plus tyrosine. Brain tyrosine and striatal HVA concentrations in each group were (respectively): probenecid, 17.65 ± 1.33 and 1.30 ± 0.10 μg/g; probenecid plus tyrosine, 44.06 ± 3.91 and 1.31 ± 0.11 μg/g; haloperidol, 17.03 ± 0.97 and 2.00 ± 0.10 μg/g; and haloperidol plus tyrosine, 36.02 ± 2.50 and 3.19 ± 0.20 μg/g. Data from Scally et al. (1977)
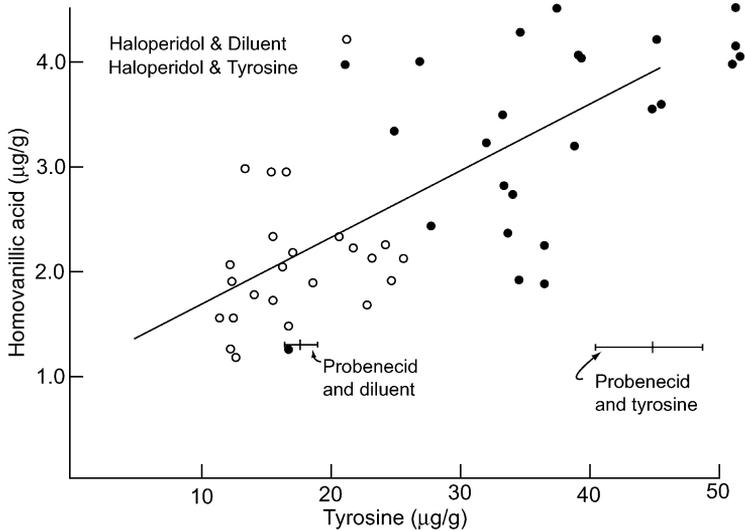
The gene for TAT is located on human chromosome 16 (Natt et al., 1986); mutations in this gene cause an inherited disorder, tyrosinemia type II (Richner-Hanhart syndrome). TAT was thought to have several chromatographically distinguishable forms (Johnson et al., 1973; Belarbi et al., 1979), however, these were subsequently shown to be generated by inappropriate purification methods: when the complete cDNA sequence coding for the rat gene was cloned (Grange et al., 1985), homogenous enzyme was obtained by Dietrich (1992). TAT is a relatively short-lived enzyme, with a half-life of less than 3 h in vivo and a rapid turnover rate as shown by Kenney (1967). It uses pyridoxal and pyridoxamine phosphates as cofactors and alpha-ketoglutarate as cosubstrate (Hayashi et al., 1967). Very low activities of an uninduceable form of TAT, about 1/50 of those present in liver, have been described in kidney and heart (Lin and Knox, 1958).

TAT activity in rats exhibits marked daily periodicity, increasing by fourfold or more in the evening, when the animal initiates rapid food consumption (Wurtman and Axelrod, 1967). This rhythm is, in fact, generated by the cyclic consumption of protein, because proteins contribute tryptophan, the limiting amino acid in hepatic protein synthesis (Wurtman et al., 1968b). Apparently, consumption of the tryptophan in protein allows the aggregation of long-lived messenger RNA coded for TAT into polyribosomes which synthesize TAT as shown by Munro (1968) and others (Fishman et al., 1969). The rhythm is rapidly extinguished in starved rats or in rats fed with a protein-free diet, and exhibits temporal shifts as soon as the feeding schedule is modified (Fuller and Snoddy, 1968). It is not generated by the daily rhythm in plasma glucocorticoids, even though high doses of these hormones can induce TAT activity, since it persists following adrenalectomy (Wurtman and Axelrod, 1967). Exogenous tyrosine, tryptophan, insulin, glucagon, dibutyrylcyclic AMP, and numerous other compounds can, in high concentrations, enhance TAT

synthesis (Lin and Knox, 1957; Kenney and Flora, 1961; Holten and Kenney, 1967; Wicks et al., 1969; Kroger and Gratz, 1980).

3.3   Plasma Phenylalanine

This chapter considers plasma phenylalanine only in relation to tyrosine and tryptophan, i.e., as a precursor for hepatic tyrosine and brain catechols; and as a competitor for their transport across the BBB. Phenylalanine's metabolism in PKU and related diseases is described elsewhere in this volume.

Plasma phenylalanine concentrations among fasting normal humans vary between 45 and 60 μM (❯ *Figure 4-5*) (Fernstrom et al., 1979; Maher et al., 1984), depending in part on the individual's prior protein intake (i.e., higher after consuming a high-protein meal for several days [Fernstrom et al., 1979]), age (higher in older than younger women [Caballero et al., 1991]), and body mass index (higher in obese subjects, with less of a fall in response to insulin [Caballero et al., 1988]). In rats, fasting phenylalanine concentrations vary between 75 and 100 μM (Fernstrom and Faller, 1978). Maximal levels among normal people consuming three high-protein meals (50 g/meal) per day are about three times as high as minimal levels in people consuming three protein-free meals daily (Fernstrom et al., 1979).

3.3.1 Phenylalanine Hydroxylase

Properties   PAH (E.C. 1.14.16.1), principally a hepatic enzyme, limits the proportion of dietary phenylalanine that is allowed to enter the systemic circulation. Its K_m for phenylalanine, estimated as 2.5–8.3×10^{-4} M (Ayling et al., 1974; Abita et al., 1976) is much higher than systemic plasma phenylalanine concentrations (0.8×10^{-4} M [Fernstrom and Faller, 1978]) suggesting that this enzyme has little role in metabolizing phenylalanine outside the portal vascular system, except perhaps in patients with untreated PKU. The enzyme catalyzes the initial step in the metabolism of phenylalanine, its hydroxylation at the 4-position of the benzene ring to generate tyrosine. This hydroxylation, in mammals, is the obligatory and rate-limiting step in the complete oxidation of phenylalanine to CO_2 and water; no other pathway exists which destroys phenylalanine's benzene ring (Milstein and Kaufman, 1975). In PKU, the lack of PAH or its natural cofactor BH_4 causes the accumulation of phenylalanine, which, in this circumstance, is decarboxylated to phenylethylamine, or transaminated to phenylpyruvic acid. Accumulation of these metabolites in brain, and the relative depletion of PAH's product, tyrosine, causes the clinical findings of this disease (Kim et al., 2004).

Like the tyrosine and tryptophan hydroxylases (TPHs), PAH uses ferrous iron as a cofactor, and molecular oxygen and tetrahydrobiopterin (BH_4) as cosubstrates with phenylalanine. Phenylalanine can also be a substrate for tyrosine hydroxylase (TH), which transforms it to tyrosine and then DOPA. This supplemental pathway for catechol synthesis has been demonstrated in preparations of bovine adrenal gland and guinea pig heart (Ikeda et al., 1967), rat brain synaptosomes (Katz et al., 1976), striatal slices (Milner et al., 1986), PC12 cells (DePietro and Fernstrom, 1998), and brains of rats receiving phenylalanine (During et al., 1988). At high concentrations, phenylalanine becomes an inhibitor of PAH; in vitro studies with PC12 cells showed that this effect represents "substrate inhibition" by phenylalanine itself (DePietro and Fernstrom, 1998). High concentrations of phenylalanine can also suppress DOPA synthesis and release in vivo (Wurtman et al., 1974; During et al., 1988) partly by competing with tyrosine for binding to the common LNAA transporter (Fernstrom and Faller, 1978) at the BBB.

The gene that encodes PAH has been located on human chromosome 12 (Lidsky et al., 1985). Rat liver PAH exists in two oligomeric forms, a tetramer which accounts for 75–80% and a dimer for the remainder (Parniak and Kaufman, 1985). Recombinant human and rat liver PAH's, and enzyme isolated from rat liver (Kowlessur et al., 1996), share similar oligomeric composition, whereas recombinant human PAH has different regulatory properties; the PAH's from livers of Sprague-Dawley rats are composed of identical subunits (Iwaki et al., 1985).

Most of the PAH in the body is in liver; small amounts are also found in kidney and pancreas (Tourian et al., 1969). However, the contribution of human kidney to total in vivo tyrosine synthesis may be substantial (Garibotto et al., 2002).

Regulation Evidence exists that PAH activity can be modulated at three sites; phenylalanine itself also attaches to a fourth "catalytic site" at which it is converted to tyrosine. The three noncatalytic sites include a serine residue (Wretborn et al., 1980) that becomes phosphorylated; an "activator" site to which physiologic concentrations of phenylalanine attach; and an additional site at which very high-phenylalanine concentrations (i.e., 2 mM or greater) inhibit enzyme activity (Dhondt et al., 1978). PAH's phosphorylation and the attachment of phenylalanine to its activator site both have the effect of increasing the hydroxylation of phenylalanine to tyrosine, phosphorylation increasing the enzyme's Vmax without changing its K_m for phenylalanine (Abita et al., 1976). The mechanism by which the activator site enhances phenylalanine's hydroxylation is not known. Phosphorylation of PAH doubles the affinity of the regulatory site for phenylalanine (Døskeland et al., 1984), thus further enhancing the enzyme's activity, and attachment of phenylalanine to the activator site similarly enhances the enzyme's susceptibility to phosphorylation. Hence, the two mechanisms manifest a positive feedback relationship. Activation of the rat hepatic enzyme by phosphorylation (i.e., after giving glucagon, in vivo [Kaufman, 1986]) reportedly increases plasma tyrosine and decreases phenylalanine, while activation of the regulatory site has been shown to increase tyrosine release from the isolated perfused liver (Shiman et al., 1982). It should be noted that the rat is considerably better at hydroxylating phenylalanine than the human. In fasted rats about 75 µmol/kg/h are converted to tyrosine, contributing about 20% of the tyrosine entering the circulation (Moldawer et al., 1983). If plasma phenylalanine concentrations are increased eightfold, the conversion of phenylalanine to tyrosine also increases, now contributing 70% of the tyrosine entering the circulation. In humans, PAH is much less responsive to a phenylalanine load: the basal hydroxylation rate is only 6 µmol/kg/h (Clarke and Bier, 1982), and a phenylalanine load preferentially elevates plasma phenylananine, not tyrosine (Caballero and Wurtman, 1988).

The proportion of PAH that is phosphorylated is diminished by a specific PAH phosphatase enzyme (Jedlicki et al., 1977). Moreover, phosphorylation of PAH can be inhibited by its own cosubstrate BH$_4$, which also stabilizes the low-activity, unphosphorylated form of the enzyme; this inhibition can be blocked by phenylalanine (Døskeland et al., 1984). A number of endogenous compounds, including lysolecithin and α-chymotrypsin, have been shown to modify PAH activity in vitro; moreover, numerous other amino acids, including methionine, norleucine, and tryptophan, can serve as substrates for PAH in vitro as shown by Kaufman (1986). None of these compounds has been shown to affect the enzyme, nor hydroxylated by it, in vivo.

4 Brain Tryptophan and Tyrosine

The levels of tryptophan and tyrosine in the brain can be of major importance in controlling the rates at which neurons synthesize and release their neurotransmitter products, serotonin, dopamine, and norepinephrine: all serotonin-producing neurons and some dopamine-producing neurons invariably synthesize more or less of their transmitter when tryptophan or tyrosine levels rise or fall; the other dopaminergic and noradrenergic neurons can become tyrosine dependent when they are firing frequently. Brain levels of tryptophan and tyrosine are controlled in part by their plasma concentrations (and share with these concentrations the property of not being subject to feedback control). However, they are even more dependent on plasma concentrations of other LNAA—particularly phenylalanine and the branched-chain compounds, leucine, isoleucine, and valine—which compete with tryptophan or tyrosine for transport across the BBB. Thus, for example, a treatment—consumption of a carbohydrate that elicits insulin secretion—which lowers plasma LNAA concentrations will raise brain tryptophan, even though it does not raise plasma tryptophan in humans. Or one that raises plasma tryptophan—consumption of a protein—can lower brain tryptophan by contributing larger amounts of the other LNAA than tryptophan to the blood ().

◨ Figure 4-10

Effect of consuming a protein-free (CARBO) or protein-containing (CHOW; 18%) meal on brain and plasma tryptophan levels in overnight-fasted rats. Rats were killed 2 h after diet presentation. Two-hour plasma tryptophan levels were significantly greater in rats consuming either diet than in fasting controls (CHOW: $p < 0.001$; CARBO: $p < 0.01$). Two-hour brain tryptophan levels were significantly elevated above control only in rats consuming the carbohydrate-plus-fat diet ($p < 0.001$). Data from Fernstrom et al. (1973)
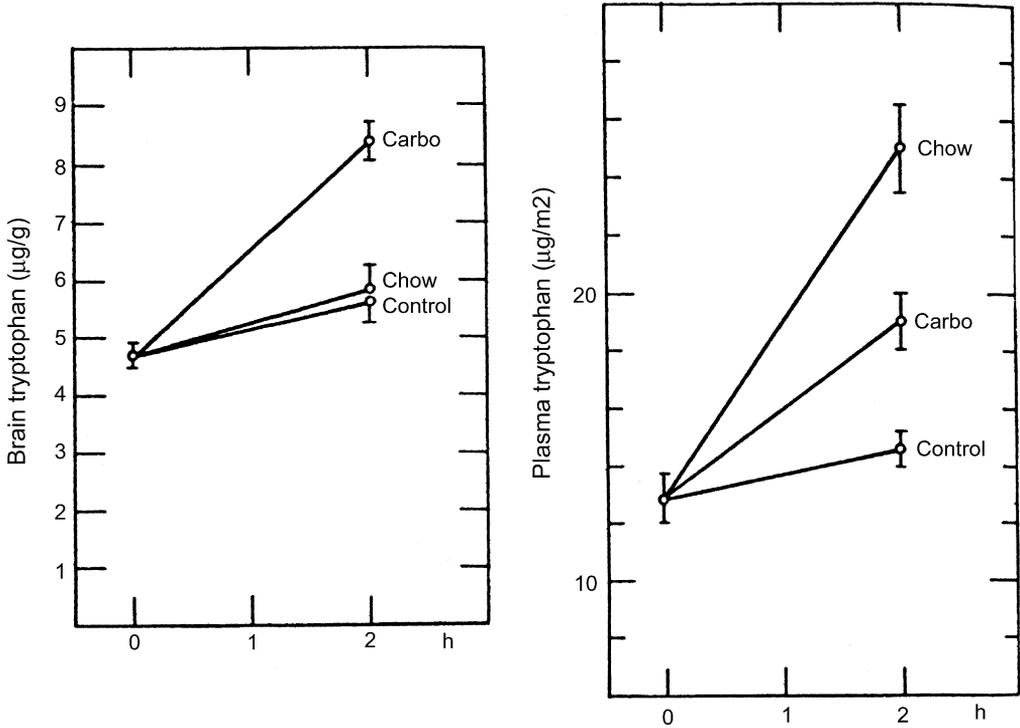
Brain tryptophan and tyrosine are utilized in all brain cells for protein synthesis, and serve as substrates for TPH or TH in monoaminergic neurons. Smaller quantities of brain tryptophan apparently are metabolized by IDO to form kynurenine and its products (Stone and Darlington, 2002) or, conceivably, by aromatic L-amino acid decarboxylase to form tryptamine (Saavedra and Axelrod, 1974). In the pineal organ serotonin formed from the hydroxylation and decarboxylation of tryptophan is further transformed to the hormone melatonin by N-acetylation followed by O-methylation (❯ *Figure 4-3*) (Axelrod et al., 1969). This process—which is accelerated each night (i.e., during the hours of darkness [Wurtman and Axelrod, 1965; Lynch et al., 1975])—is associated with increases in the activities of the enzymes (serotonin N-acetyltransferase and hydroxyindole-O-methyltransferase) that catalyze these two reactions, however, it probably depends more on the liberation of "bound" serotonin within pinealocytes, making the serotonin accessible both to serotonin N-acetyltransferase and to monoamine oxidase (MAO) (Wurtman, 2005).

4.1 Transport of Plasma Tryptophan and Tyrosine into the Brain

All amino acids are ionized at physiological pH and would not be able to cross membrane bilayers to gain access to the brain were it not for two highly specialized sets of transport molecules.

The most important set is located within the endothelial cells that line brain capillaries. It includes three different types of macromolecules: those that allow LNAA to enter the brain, by facilitated diffusion;

those that do the same for basic amino acids (e.g., lysine; arginine); and those that actively transport acidic amino acids (e.g., glutamate, aspartate) in the opposite direction—from the brain's extracellular fluid to the intravascular space as shown by Pardridge (1977). It should be noted that many of the LNAA and the basic amino acid lysine are "essential," and cannot be made by brain tissue; the acidic amino acids, in contrast, are readily synthesized from glucose. The other set of macromolecules that allow some circulating amino acids to enter the brain are in the cells that line the choroid plexus as shown by Lorenzo (1974). Because the surface they cover is very much smaller than the brain's capillaries, they transport only about 1/1000 as many molecules per unit time as shown by Pardridge (2001).

Carrier-mediated transport of LNAA at the BBB and in other tissues is affected by a family of transport proteins called "L-System," which contain LAT1, a catalytic subunit (also known as the light chain) and a type II glycoprotein subunit (4F2hc, also known as heavy chain) (Kanai et al., 1998). The LAT1-4F2hc heterodimer is connected by a single cysteine residue (Mastroberardino et al., 1998) in a disulfide linkage. LAT1-4F2hc is selective for LNAA transport and is essential for BBB transport (Boado et al., 1999) of the LNAA as well as for these compounds to enter tissues with comparable anatomic barriers (e.g., placenta and testis as reviewed by Verrey [2003]). This transport is bidirectional, Na-independent, and nonenergy-requiring (facilitated diffusion). The affinity of BBB LAT1 for LNAA is extremely high compared with that of peripheral LNAA transporters and the carrier is highly saturated at physiological plasma LNAA concentrations (i.e., the K_m of BBB LNAA transport approximates the plasma concentration of these LNAA), which causes the competition among LNAA for entering the brain as shown by Pardridge (1977). Two alkylating agents: melphalan and DL-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphtoic acid (DL-NAM) inhibit BBB LNAA transport by damaging the disulfide bridge between LAT1 and 4F2hc in the heterodimer.

Once tryptophan, tyrosine, and the other LNAA molecules have passed from the plasma to the brain's extracellular fluid, they are able to enter all brain cells, to be used for synthesizing new protein molecules (chiefly in perikarya of neurons). Monoaminergic neurons also need large quantities of tryptophan and tyrosine in their nerve terminals to make serotonin and the catecholamines, and have additional mechanisms for obtaining such quantities. Amino acids are well transported into brain slices, by both saturable uptake and unidirectional influx (Vahvelainen and Oja, 1975), and it was using such slices that the competition for transport among LNAA was initially noted (Blasberg and Lajtha, 1966). Tryptophan (Grahame-Smith and Parfitt, 1970) and tyrosine (Morre and Wurtman, 1981) are also concentrated within synaptosomes, in competition with other LNAA. The proportions of tryptophan molecules used in serotoninergic neurons to synthesize serotonin versus proteins apparently are not known, however, it can safely be assumed that much more is used for the former purpose: among pineal cells, which also make both serotonin (and melatonin) and proteins, this ratio is greater than 100:1 (Wurtman et al., 1968a; Wurtman et al., 1969). Similarly, although acetylcholine-producing neurons constitute only a tiny fraction (about 1%) of brain cells, they are estimated to utilize at least 60% of the choline entering the brain to make acetylcholine (Farber et al., 1996), the rest being used in all brain cells to generate phospholipids.

4.2 Brain Tryptophan

The quantities of tryptophan that enter the brain, and sustain or elevate brain tryptophan levels, depend on three sets of nutrients in the plasma: tryptophan itself; the other LNAA; and NEFA, which, by binding loosely to albumin, diminish the binding of tryptophan and change the proportions of plasma tryptophan that are albumin-bound and free. (As described below, the actual effects of the fatty acids on brain tryptophan levels are minimal [Fernstrom et al., 1976; Pardridge and Fierer, 1990].) Brain tyrosine is similarly affected by plasma tyrosine and LNAA levels; however, tyrosine does not bind appreciably to albumin.

That giving high-tryptophan doses to rats could increase brain tryptophan (and serotonin) levels was first shown in 1962 for dietary tryptophan (Green et al., 1962; Wang et al., 1962) and in 1965 for injected tryptophan (800 mg/kg: Ashcroft et al., 1965). By 1971, it had been shown that brain tryptophan levels in rats normally vary within a twofold range, and that giving the animals as little as 12.5 mg/kg could

◨ **Figure 4-11**

Dose-response curve relating brain tryptophan and brain serotonin. Rats received tryptophan (12.5, 25, 50, or 125 mg/kg, i.p.) at noon and were killed 1 h later. Horizontal bars represent standard errors of the mean for brain tryptophan; vertical bars represent standard errors of the mean for brain serotonin. All brain tryptophan levels were significantly higher than control values ($p < 0.001$). All brain serotonin levels were significantly higher than control values ($p < 0.01$). Data from Fernstrom and Wurtman (1971a)
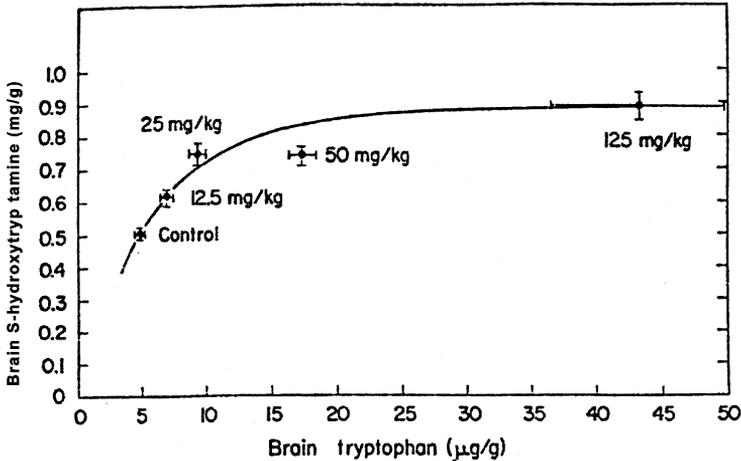
significantly increase brain tryptophan but keep it within this normal range (❯ *Figure 4-11*) (Fernstrom and Wurtman, 1971a). The rise in brain tryptophan occurs because it displaces other LNAA from the LAT1-4F2hc transport molecule in brain capillaries. The magnitude of this increase is predicted by the change in the "plasma tryptophan ratio"—the ratio of the plasma tryptophan concentration to the summed concentrations of other LNAA which exhibit high affinities for the transport carrier (usually taken as tyrosine, phenylalanine, and the branched-chain amino acids, but sometimes also methionine) (Fernstrom and Wurtman, 1971b). Since these affinities are not equal, one can theoretically improve the correlation between brain level and plasma ratio by correcting the summed LNAA concentrations for each amino acid's K_m, but operationally this correction is usually unnecessary (Fernstrom and Faller, 1978).

After giving tryptophan, the increase in brain tryptophan can also be predicted from plasma tryptophan levels alone, but more often this is not the case. Thus, a glucose-rich snack can increase brain tryptophan without increasing plasma tryptophan or increasing it only slightly in rats (❯ *Figure 4-10*), because the resulting insulin secretion lowers plasma levels of the other LNAA, while a protein-rich meal raises plasma tryptophan without raising brain tryptophan, because most proteins contain only 1.0–1.5% tryptophan, and thus contribute very little of this amino acid to the plasma, in comparison with the other LNAA (Fernstrom and Wurtman, 1972b). Individual LNAA (e.g., isoleucine) can be administered along with tryptophan, as experimental controls to block a tryptophan-induced rise in brain tryptophan, and groups of amino acids lacking tryptophan are often used to lower brain tryptophan levels (Gessa et al., 1974) (❯ *Figure 4-6*).

As noted above, about 75–80% of the tryptophan in the plasma travels loosely bound to albumin (McMenamy and Oncley, 1958). Initially it was anticipated that this binding would substantially retard the passage of tryptophan across the BBB, and scientists considered measuring plasma "free" (nonalbumin-bound) tryptophan, or the ratio of the "free tryptophan" to the other LNAA, as the best predictor of brain tryptophan levels (Knott and Curzon, 1972). However, subsequent studies often described treatment-induced changes in plasma "free" tryptophan which were opposite in direction to those in brain tryptophan. For example, rats consuming glucose (❯ *Table 4-1*) exhibited increases in brain tryptophan and albumin-bound plasma tryptophan (because the resulting secretion of insulin caused NEFA to dissociate

from albumin and enter adipocytes), but a decrease in "free" tryptophan (Madras et al., 1973) (because the uptake of the "free" amino acid into muscle—like that of all other LNAA—was enhanced by insulin). Or rats consuming high-carbohydrate or high-protein meals that also did or did not contain 40% fat exhibited no fat-dependent changes in brain tryptophan, even though "free" plasma tryptophan levels were markedly elevated by the fat (Fernstrom et al., 1976). At present few investigators differentiate between "free" and "total" (free plus albumin-bound) tryptophan in calculating plasma tryptophan ratios, nor does there seem any reason to do so. In actuality, albumin-bound tryptophan has been shown by Pardridge and his associates (Pardridge and Fierer, 1990) to be ". . . readily available for transport into the brain, secondary to enhanced dissociation within the cerebral microcirculation of amino acid from the albumin binding site, as represented by an increased Kd in vivo . . ." (i.e., an in vivo change in albumin's affinity for tryptophan rather than a "stripping" of tryptophan off the albumin molecule).

4.2.1 Tryptophan Hydroxylase

Properties TPH (E.C. 1.14.16.4) catalyzes the initial and rate-limiting step in serotonin biosynthesis, the hydroxylation of tryptophan at the 5-position to form 5-HTP (❯ *Figure 4-1*). This product, like the DOPA formed from tyrosine's hydroxylation, is readily decarboxylated to the corresponding monoamine, serotonin or dopamine, by the action of aromatic L-amino acid decarboxylase (L-AAAD), a widely distributed pyridoxine-dependent enzyme. TPH is in fact two distinct enzyme proteins, TPH1 and TPH2, which are encoded by genes on human chromosomes 11 and 12, respectively (Walther and Bader, 2003). TPH1 is localized within peripheral tissues (e.g., the enterochromaffin cells and certain intrinsic neurons of the gut) and the pineal organ, while TPH2 is a brain enzyme, concentrated within the perikarya and terminals of serotoninergic neurons (Walther and Bader, 2003; Patel et al., 2004). Both enzymes use ferrous iron as a cofactor and molecular oxygen and tetrahydrobiopterin (BH_4) as cosubstrates.

The kinetic properties of the two enzymes differ: the K_m of TPH2 for its major substrate, tryptophan, has been estimated to be several-fold (40.3 μM versus 22.8 μM [McKinney et al., 2005]) to tenfold (142 μM versus 13–22 μM [Kowlessur and Kaufman, 1999]) higher than that of TPH1, while its K_m for BH_4 is lower (20 μM versus 39 μM; McKinney et al., 2005). Since brain tryptophan and BH_4 concentrations in rats are around 5–10 μg/g brain tissue (Fernstrom and Wurtman, 1971a) and 3 μM (Nagatsu, 1983), respectively, it would be anticipated that—as is actually observed—the in vivo activity of TPH2 and the overall rate of serotonin synthesis in brain, both vary broadly with brain tryptophan levels (Fernstrom and Wurtman, 1971a), while peripheral serotonin synthesis, as reflected by blood serotonin, is much less responsive to changes in plasma tryptophan (Colmenares and Wurtman, 1979). And since, as described above, the principal factor that normally controls *brain* tryptophan levels is not plasma tryptophan, per se, but, rather, plasma concentrations of the other LNAA, the effects of any meal on brain serotonin synthesis will depend on the LNAA in that meal's protein and on the meal's ability to elicit insulin secretion (which lowers plasma LNAA), but only to a minor extent on its tryptophan content (Fernstrom and Wurtman, 1972b). On the other hand, the synthesis of serotonin in peripheral tissues, which lack a BBB, would be expected to depend principally on the meal's tryptophan content (Colmenares and Wurtman, 1979).

TPH enzymes are, along with PAH and TH, members of a protein superfamily (Hufton et al., 1995). They share considerable amino acid homology (71% for TPH1 and TPH2 [Walther and Bader, 2003]; 52% between TPH1 and PAH [Kappock and Caradonna, 1996]); utilize the same cofactors and cosubstrates; and to a variable extent, can hydroxylate all three amino acid substrates to variable extents (Kappock and Caradonna, 1996).

Regulation Both TPH and TH are readily phosphorylated on specific residues (e.g., serine-58 and 260 for TPH1 [Walther and Bader, 2003]; these plus serine-19 for TPH2 [McKinney et al., 2005]). This phosphorylation causes major changes in TH's kinetic properties, enhancing its saturation with BH_4 and making its net activity more dependent on available tyrosine levels. However, phosphorylation does not appear to cause major changes in the properties of TPH, whether the phosphorylated enzyme also subsequently binds

to a 14-3-3 protein (McKinney et al., 2005). Experimental procedures that block serotonin autoreceptors or activate neuronal firing can increase brain serotonin synthesis (Stenfors and Ross, 2002), however, such procedures have not been shown to affect the enzyme's Vmax or its K_m's for tryptophan or BH_4. A major reduction in TPH2 activity does diminish brain serotonin synthesis, as shown in studies comparing this rate in a wild mouse strain and one containing a TPH2 allele with a single nucleotide polymorphism (C1473G). The 5-HTP synthesis and levels were markedly reduced in the animals with the polymorphism (Zhang et al., 2005).

4.2.2 5-Hydroxytryptophan and L-DOPA

The biosynthesis of serotonin can also be accelerated by administering 5-HTP, the amino acid intermediate in its physiologic synthesis from tryptophan (❯ *Figure 4-1*). Given orally, this compound readily enters the blood stream of humans and, via the LNAA transport carrier, the brains of rats (Amer et al., 2004). It can be decarboxylated to serotonin in any of the numerous CNS and peripheral cell types that contain the enzyme aromatic-L-amino acid decarboxylase (L-AAAD), for example, monoaminergic brain neurons, kidney, and gut. Presumably, only authentic serotoninergic cells have the capacity to store the serotonin thus formed— which protects that serotonin from immediate destruction by MAO—and to recapture intrasynaptic serotonin by serotonin-uptake molecules. But for a period of time after 5HTP's administration, many nonserotoninergic neurons and other cells produce and release serotonin as a "false neurotransmitter."

Similar caveats affect the use of oral L-DOPA, e.g., in treating Parkinson's disease: this catechol amino acid, an intermediate in dopamine's synthesis from tyrosine (❯ *Figure 4-2*), does enter the brain via the LNAA transport system—hence its efficacy can be enhanced by dietary carbohydrates (Berry et al., 1991) or suppressed by concurrently eating proteins (Mena and Cotzias, 1975). However, in both the brain and periphery the L-DOPA is decarboxylated to dopamine in many cell types besides its targeted nigrostriatal neurons. Both L-DOPA and 5-HTP (Van Woert and Rosenbaum, 1979) are usually given along with peripheral decarboxylase inhibitors; since only the brain dopamine or serotonin is desired. This lowers the required therapeutic dose. 5-HTP has been used experimentally to treat depression by van Praag (1981) and to suppress stress-induced eating (Amer et al., 2004); it not uncommonly causes sleepiness as a side effect. The brain uptakes of amino acid drugs like the antihypertensive agent α-methyldopa similarly depend on corresponding plasma ratios (to concentrations of the LNAA in dietary proteins [Zavisca and Wurtman, 1978; Pardridge et al., 1986]).

4.3   Brain Tyrosine

Brain tyrosine levels in fasted rats vary between about 60–80 micromolar (Milner and Wurtman, 1986). Administration of tyrosine increases these levels, a 100-mg/kg intraperitoneal dose causing peak elevations of about 150–200% (Morre et al., 1980). Consumption of a protein-free, carbohydrate-rich meal may or may not change rat brain tyrosine levels significantly (Gibson and Wurtman, 1977), probably because the insulin-induced fall in plasma tyrosine is comparable to that in the other LNAA (Fernstrom and Fernstrom, 1995). However, if the meal contains 8–40% protein, brain tyrosine rises, roughly in proportion to the protein content (Gibson and Wurtman, 1977). Chronic consumption (14 days) of meals containing 10% protein was associated with cortical tyrosine levels approximately double those seen among rats consuming 2% protein (Fernstrom and Fernstrom, 1995). As with tryptophan, brain tyrosine levels vary not with plasma tyrosine, per se but with the ratio of the tyrosine concentration to the summed concentrations of the other principal LNAA (Fernstrom and Faller, 1978).

CSF tyrosine levels were significantly elevated, by about 70%, among Parkinsonian patients receiving tyrosine (100 mg/kg/day, in 6 divided doses; the last dose was administered 2 h before the second lumbar puncture [Growdon et al., 1982]). CSF levels of the dopamine metabolite homovanillic acid (HVA) were also significantly elevated (by 36%), while those of the serotonin metabolite 5HIAA were, as expected, unchanged.

4.3.1 Tyrosine Hydroxylase

Properties   TH (E.C. 1.14.16.2) catalyzes the initial and rate-limiting step in dopamine biosynthesis (Levitt et al., 1965), and thereby also affects the rates of formation of dopamine's biologically active products: norepinephrine and epinephrine (❯ *Figure 4-2*). This step involves the hydroxylation of p-tyrosine at the m-position to generate the catechol amino acid 3,4-dihydroxyphenylalanine, or DOPA. Like the tryptophan and PAHs, TH uses ferrous iron as a cofactor, and molecular oxygen and tetrahydrobiopterin (BH_4) as well as tyrosine as substrates.

In humans, there are four distinct TH enzyme proteins, exhibiting perhaps twofold variations in catalytic activity (Kappock and Caradonna, 1996); all are products of mRNAs arising from a single gene on chromosome 11 (Kaneda et al., 1987). In the rat (Grima et al., 1985) and mouse (Ichikawa et al., 1991), this gene encodes a single TH protein. TH is present in all of the cells that synthesize catecholamines, e.g., dopaminergic nigrostriatal neurons, mesocortical and mesolimbic tracts, tuberohypophyseal neurons, and neurons in the retina and olfactory bulbs; noradrenergic neurons originating in the locus coeruleus and lateral tegmentum; epinephrine-containing neurons in the brainstem; postganglionic noradrenergic sympathetic neurons; and adrenomedullary chromaffin cells.

Regulation   It has been recognized for decades that the rate at which catecholamine-producing cells produce catecholamines from tyrosine is highly regulated, and coupled to the rates at which the cells are releasing these compounds. Thus, even major increases in release caused by prolonged neuronal firing tend not to lower the quantities of catecholamine remaining in the cells, as first shown by Elliott (1912). At least three regulatory processes can enhance tyrosine's hydroxylation when the need for additional catecholamine molecules arises: the phosphorylation of TH on specific serine residues—which activates the enzyme and markedly increases its affinity for its BH_4 cosubstrate; enhanced de novo synthesis of the enzyme protein; and development of the ability to respond to increased tyrosine levels, once the TH has been phosphorylated. A fourth regulatory process—end-product inhibition by cytoplasmic catecholamines—inhibits the enzyme's activity and catecholamine synthesis.

Like TPH, TH is readily phosphorylated. But unlike TPH, TH's phosphorylation leads to major changes in its kinetic properties, increasing its affinity for BH_4 (Lloyd and Kaufman, 1975; Wang et al., 1991) and thereby increasing the extent to which its net activity is regulated by local levels of tyrosine, its primary substrate (Wurtman et al., 1974). Four of the enzyme's serine residues (Ser8, Ser19, Ser31, and Ser40) are substrates for phosphorylation reactions, which are catalyzed, in human cells, by cAMP- and Calmodulin-dependent protein kinases (Harris et al., 1974). Phosphorylation at the Ser19 position sensitizes the TH to the subsequent phosphorylation of its Ser40 moiety, which increases the enzyme's net activity, both in vitro and in vivo (Dunkley et al., 2004). The phosphorylated enzyme, like TPH, is able to combine with a 14–3–3 "activator" protein, however, this step is not required in order for the TH to be activated by the phosphorylation reactions (Kleppe et al., 2001). The increase in TH's affinity for BH_4 as a consequence of its phosphorylation can be considerable: In one study, the K_m for various BH_4 analogs fell from 300 μM in unphosphorylated enzyme to 0.8–14.0 μM (Bailey et al., 1989). Other investigators, using BH_4, described decreases in its K_m of 2- to 12-fold (Kappock and Caradonna, 1996).

TH's Vmax apparently is not affected by its phosphorylation (Le Bourdelles et al., 1991), however, major increases in the rate at which it produces catechols do occur in vivo, possibly caused by the increases in the enzyme's saturation with BH4 (Zigmond et al., 1989) and by consequent decreases in its susceptibility to end-product inhibition by cytoplasmic catecholamines (Ames et al., 1978). Moreover, once the enzyme has been phosphorylated the rate at which it produces DOPA can readily be enhanced by administering tyrosine, as described below. Phosphorylated TH is rapidly dephosphorylated, with an initial half-life approximating 5 min (Yamauchi and Fujisawa, 1979); this suggests that one or more phosphatase enzymes are colocalized in the cell with TH (Yamauchi and Fujisawa, 1979).

TH is inhibited by catechols, particularly by its catecholamine end-products dopamine, norepinephrine, and epinephrine (Nagatsu et al., 1964). Most of the catecholamine molecules in neurons and adrenomedullary cells are sequestered within synaptic vesicles and thus unable to interact with TH and affect its activity. However not all of them are "free": cytoplasmic dopamine continues to be formed from

the decarboxylation of DOPA, and dopamine also enters presynaptic terminals from the synaptic cleft via high-affinity catecholamine uptake system. And until such molecules are sequestered in vesicles, or destroyed by the mitochondrial enzyme MAO, they are able to bind to the TH enzyme protein and to diminish its activity—probably by inhibiting its phosphorylation (Almas et al., 1992) and by competing with BH_4 for binding to the ferric iron in the TH (Andersson et al., 1988). The levels of cytoplasmic dopamine (or norepinephrine, or epinephrine) in cells are, as might be expected, increased by drugs that inhibit MAO activity, hence such drugs can also act as potent inhibitors of tyrosine's hydroxylation in vivo (Spector et al., 1967).

The major changes in TH's kinetic properties caused by its phosphorylation allow its net activity to increase rapidly when neurons or adrenomedullary cells are physiologically activated and are releasing catecholamines at a more rapid rate (Weiner and Rabadjija, 1968). This has been shown by in vivo studies using electrical stimulation or potassium-induced depolarization of neurons. Moreover, sustained increases in in vivo TH activity, caused by enhanced synthesis of the enzyme protein (Silberstein et al., 1972) occur when the accelerated firing of catecholaminergic neurons (or of the cholinergic neurons that innervate the adrenal medulla) is sustained, for example, in sympathetic neurons of animals exposed to hemorrhagic shock (Conlay et al., 1981); or in adrenal medullas of rats receiving drugs that cause hypotension (Thoenen et al., 1969) or hypoglycemia (Viveros et al., 1969), or that destroy postganglionic terminals (Thoenen et al., 1969); or in animals exposed to cold or to immobilization stress (Kvetnansky et al., 1992); or in retinas of rats exposed to light (Witkovsky et al., 2004). TH synthesis can also be induced in vitro by culturing adrenals in a medium containing depolarizing concentrations of potassium (Silberstein et al., 1972). As described below, in most of these experimental systems, elevation of tissue tyrosine has been shown to further increase the synthesis and release of the catecholamines. "If the Km of neuronal tyrosine hydroxylase is, as described (Wurtman et al., 1974) 100–140 micromolar, then the enzyme may be only 25–50% saturated with this substrate under basal conditions, in as much as basal brain tyrosine levels reportedly are about 60–80 micromolar (Milner and Wurtman, 1986). Giving exogenous tyrosine could raise neuronal tyrosine levels substantially, however dopamine synthesis would still be limited by the poor saturation of tyrosine hydroxylase with its cofactor, BH_4, until the neuron began firing. Then tyrosine levels would affect the rate of dopamine synthesis."

Certain neurons, for example, the mesocortical dopaminergic neurons projecting to the rat's medial prefrontal and cingulate cortices, produce and release more catecholamine whenever tyrosine levels have been increased, even in the absence of an additional treatment to accelerate their firing (and, presumably, TH's phosphorylation) (Tam et al., 1990). This ability may derive from their lack of somato-dendritic autoreceptors which would modulate impulse flow, and of nerve-terminal autoreceptors which would modulate dopamine synthesis (Chiodo et al., 1984). This lack causes the neurons to exhibit faster dopamine turnover, faster basal firing rates, and more bursting activity than other midbrain dopamine neurons. Insofar as these neurons couple dopamine synthesis to tyrosine levels under basal conditions, they are similar to brain serotoninergic neurons, in which changes in substrate (i.e., tryptophan) levels will always affect the net activity of the hydroxylase enzyme (TPH). Presumably, most of the TH in these mesocortical neurons is phosphorylated under basal conditions; however this has not yet been demonstrated.

5 Consequences of Changing Brain Tryptophan and Tyrosine Levels

5.1 Precursor Availability and Neurotransmission

The total amount of information that a group of neurons can transmit during any particular interval depends, in large part, on the number of neurotransmitter molecules that their presynaptic terminals release during that interval. This, in turn, depends on the total number of synapses that the neurons make, the average frequency with which the neurons happen to be firing, and the average amount of transmitter released at each synapse per firing. If the changes in neurotransmitter *synthesis* caused by elevating brain tryptophan or tyrosine levels are to be of physiologic relevance, they must be associated

with increases in the amount of transmitter *released*, per depolarization and per unit time. This may or may not modify postsynaptic responses depending on, for example, whether unoccupied postsynaptic receptors are available to respond to the additional neurotransmitter molecules, i.e., increased transmitter release is necessary but not yet sufficient in order for the precursor effect to have functional significance. If the firing rates of most brain neurons are, as is generally believed, constrained by mechanisms, involving presynaptic receptors or multisynaptic reflex arcs, programed to keep total neurotransmitter release constant despite fluctuations in transmitter levels within presynaptic terminals, then when does precursor availability actually affect neurotransmission? There are a number of situations in which this seems probable.

5.1.1 Neurons That Lack Multisynaptic or Autoreceptor-Based Feedback Loops

Peripheral sympathetic neurons and chromaffin cells in humans (Agharanya et al., 1981) and experimental animals (Alonso et al., 1980) release more catecholamines after tyrosine has been administered or a protein-rich meal has been consumed. That the resulting increase in urinary catecholamine levels (❯ *Figure 4-12*) represents increased catecholamine release and not, for example, alterations in catecholamine metabolism, is indicated by the fact that levels of catecholamine metabolites in the urine also rise; that this reflects accelerated catecholamine synthesis and not simply release of stored material is indicated by the failure of tissue catecholamine levels to decline.

◘ **Figure 4-12**
Urinary levels of catecholamines in normal humans after tyrosine administration. Thirteen subjects fasted overnight; the next morning eight received a single oral dose (100 or 150 mg/kg) of tyrosine mixed in water; while five controls received only water. Urinary samples were obtained 0, 2, 4, and 8 h after tyrosine or water injection. Data are given as μg excreted/h (mean ± SEM). •$p < 0.01$ by ANOVA for repeated measurements; ••$p < 0.005$. Data from Alonso et al. (1982)
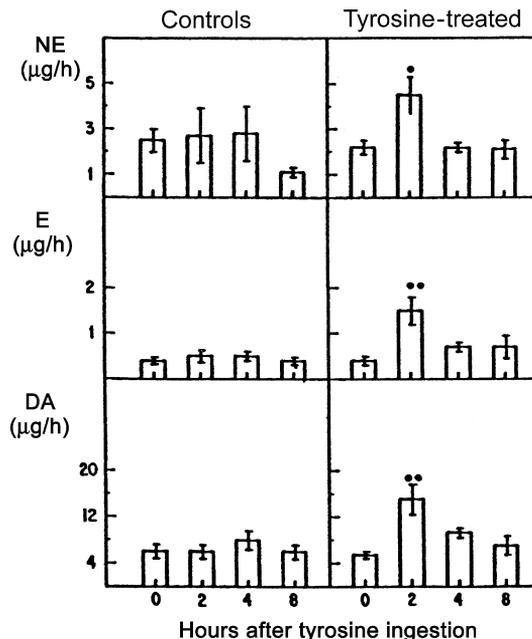
Similarly, rat mesocortical dopaminergic neurons lacking impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors (White and Wang, 1984; Tam et al., 1990) respond without additional treatment to physiological tyrosine doses by synthesizing more dopamine (Tam et al., 1990) (❯ *Table 4-2*).

■ Table 4-2

Effect of tyrosine administration on prefrontal cortex DOPA and dopamine levels after a decarboxylase inhibitor

	Time after tyrosine treatment (min)			
	0	30	40	60
DOPA accumulation (% control)	100 ± 5	117 ± 13	$124 \pm 7*$	113 ± 5
Dopamine (% control)	100 ± 10	104 ± 12	115 ± 16	$160 \pm 15*$

Rats received tyrosine (50 mg/kg, i.p.) 30, 40, or 60 min before sacrifice, and to measure DOPA accumulation, a decarboxylase inhibitor, NSD-1015 (100 mg/kg, i.p.), 30 min before sacrifice. Values are expressed as percentages of levels in saline-treated control animals at each respective time point. *Differs significantly from saline controls ($p < 0.05$). Data from Tam et al. (1990)

5.1.2 Neurons That Are Components of Positive Feedback Loops

If a neuron releases a precursor-dependent *excitatory* neurotransmitter directly onto its own receptors, or if its depolarization, acting transsynaptically, causes it to receive greater quantities of excitatory transmitters from other neurons and thus to fire more frequently, then the initial increase in transmitter release after precursor administration could enhance subsequent responses to the precursor.

5.1.3 Neurons That Normally Release Variable Quantities of Neurotransmitter Per Firing Without Engaging Feedback Responses

If the mechanisms controlling a neuron's firing frequencies allow it to release widely varying amounts of neurotransmitter per unit time without undergoing feedback changes in firing, then precursor availability might be expected to exert undampened effects on neurotransmitter output within this broad range.

One group of neurons that exhibits such control is the serotonin-releasing cells of the raphe nucleus. The rate at which they synthesize and release (❯ *Figure 4-13*) their neurotransmitter apparently varies directly with brain tryptophan levels, which normally vary within at least a twofold physiologic range (Fernstrom and Wurtman, 1971a; Schaechter and Wurtman, 1989). Raphe firing does decrease when animals are given *very large* doses of tryptophan, which cause the release of supraphysiologic amounts of serotonin (Gallager and Aghajanian, 1976), indicating that there *is* an upper limit to serotonin release beyond which the neurons are subject to feedback control as shown by Bramwell (1974). The ability of serotoninergic neurons to serve as "variable ratio sensors," releasing more or less of their transmitter when the plasma tryptophan ratio rises or falls within its normal range, allows these neurons to provide the rest of the brain with useful information about peripheral metabolic state, which might then be used to formulate behavioral strategies. Serotoninergic neurons apparently do participate in a complex neural-behavioral mechanism controlling appetite for carbohydrates. If animals are pretreated with a drug that, like carbohydrate consumption (Fernstrom and Wurtman, 1971a), increases serotonin release, and if they are then given a choice between various diets, they selectively reduce their consumption of carbohydrates while sustaining protein intake (Wurtman and Wurtman, 1977; Wurtman and Wurtman, 1979). This effect is independent of whether the carbohydrates in the test foods happen to be sweet (Wurtman and Wurtman, 1979).

□ Figure 4-13

Effect of tryptophan availability on serotonin content of, and release from, rat brain slices. The superfusion media contained the tryptophan concentrations indicated. Both spontaneous and electrically evoked release were measured. Data from Schaechter and Wurtman (1989)
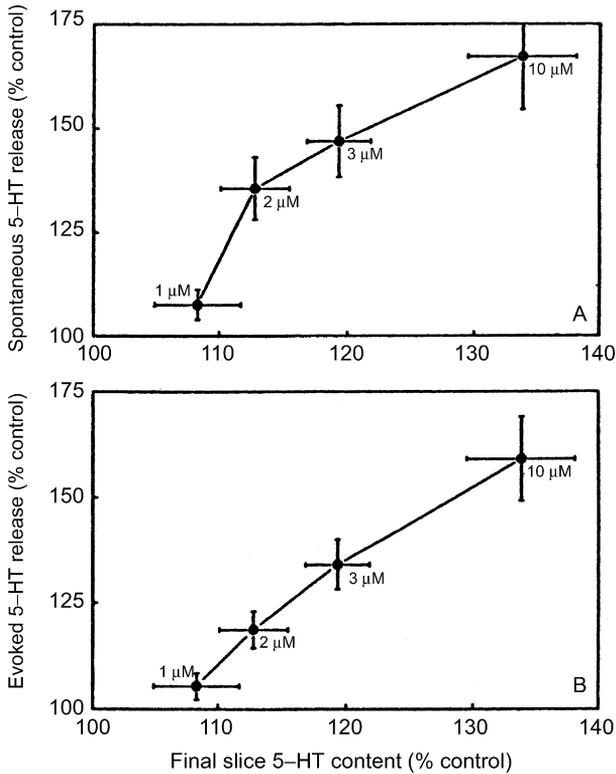

5.1.4 Physiologic Situations in Which Neurons Undergo Sustained Increases in Firing Frequency

The relation between sustained neuronal firing and precursor responsiveness is well illustrated by the ability of exogenous tyrosine to raise *or* lower blood pressure when the starting blood pressure is too low or too high, depending on which of the animal's noradrenergic neurons happen to be most active at the time of its administration. Noradrenergic neurons at several loci participate in the control of blood pressure (Palkovits and Zaborszky, 1977): Norepinephrine release from peripheral sympathetic nerves tends to elevate blood pressure, while its application to or release from certain brainstem sites tends to lower blood pressure (presumably by diminishing sympathetic outflow [DeJong et al., 1975]). If normotensive rats (mean systolic blood pressure = 120–130 mm Hg) receive a given dose of tyrosine (100–200 mg/kg, i.p.), blood pressure changes only slightly or not all (Sved et al., 1979a); blood pressure also fails to change in normotensive human subjects (Glaeser et al., 1979; Melamed et al., 1980b). If the same tyrosine dose is given to a spontaneously *hypertensive* rat (mean systolic blood pressure = 170–210 mm Hg), blood pressure *falls* by 28–46 mm Hg for several hours (❯ *Figure 4-14*) (Sved et al., 1979a); however, if that dose is given to a *hypotensive* animal (mean blood pressure = 63 mm Hg, 45 min after a hemorrhage of 20% of its calculated blood volume), systolic pressure *rises* by 31 mm Hg (Conlay et al., 1981). Treatments that cause hypertension accelerate norepinephrine release from brainstem neurons terminating in the anterior hypothalamus (presumably activating compensatory mechanisms), while those that cause hypotension

◨ Figure 4-14

Effect of tyrosine on blood pressure in spontaneously hypertensive rats (SHR). The amino acid was given intraperitoneally (i.p.) and blood pressure was measured at 0 time and 90 min after. Data from Sved et al. (1979a)
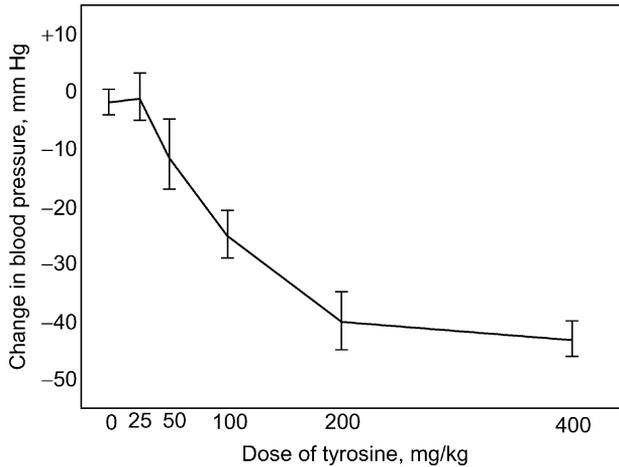
activate sympathoadrenal structures and catecholaminergic terminals in the posterior hypothalamus (Philippu et al., 1980). Hence, the most economical explanation for the paradoxical ability of tyrosine to lower or raise blood pressure, depending on whether it is chronically elevated or depressed, is that in the hypertensive animal, the precursor enhances norepinephrine release selectively within one set of brainstem neurons (because these happen to be firing frequently), while in shock peripheral sympathetic neurons and adrenomedullary cells, and posterior hypothalamic neurons, are activated and thus become tyrosine-sensitive.

Intravenous tyrosine markedly reduces blood pressure in animals with renovascular or DOCA-salt hypertension. These changes are associated with reductions in heart rate as shown by Bramwell (1974). Tryptophan has about half the blood-pressure-lowering activity of tyrosine, probably acting by increasing serotonin release from bulbospinal neurons, while branched-chain LNAA lack any effect on blood pressure and, when coadministered with tyrosine, block its effect (Sved et al., 1979a).

Similar relationships between precursor-dependence and chronic changes in firing frequency have also been described in nigrostriatal dopaminergic neurons (Melamed et al., 1980b), where unilateral destruction of most of the neurons renders the surviving ipsilateral neurons, but not the contralateral dopaminergic neurons, tyrosine-sensitive. Thus, physiologic conditions that accelerate the firing of precursor-depending neurons may overcome the feedback mechanisms that would otherwise maintain the constancy of neurotransmitter *release*, and allow the neuron to couple precursor availability to transmitter synthesis.

5.1.5 Neurologic Diseases That Cause Either a Decreased Number of Synapses of Decreased Transmitter Release Per Unit Time

Neurodegenerative disorders that diminish the number of presynaptic terminals issuing from a precursor-dependent brain nucleus may be associated with accelerations in the average firing rates of the surviving neurons (Bernheimer et al., 1973), enhancing their sensitivity to precursor control. This formulation provides a theoretical basis for testing neurotransmitter precursors in, for example, Parkinson's disease (Growdon et al., 1982). It also offers an explanation for the physiological specificity that seems to be associated with the therapeutic use of neurotransmitter precursors. If the brain contains, for example, ten groups of catecholaminergic neurons, and if all but one of these groups are intact and functioning normally,

it might be anticipated that only this one group would be substantially affected by giving tyrosine. An elevation in brain tyrosine levels would initially enhance dopamine or norepinephrine release from all ten groups, but in the normal nuclei, this effect would rapidly be dampened by presynaptic or multisynaptic feedback mechanisms.

Brains of normal women produce significantly less serotonin than those of men (Nishizawa et al., 1997). This might explain why, as described below, women not infrequently develop "carbohydrate craving" in serotonin-related disorders like the premenstrual syndrome (or "late-luteal phase dysphoric syndrome"), the behavioral syndrome that can follow nicotine withdrawal, and the seasonal affective disorder. Eating the insulin-secreting carbohydrates will increase brain serotonin release, as described above, and improve serotonin-dependent feelings (Wurtman and Wurtman, 1989).

5.2 Brain Tryptophan and Serotonin

Basal brain serotonin levels in fasting rats vary between 0.47 and 0.70 μg/g tissue (Fernstrom and Wurtman, 1971a, b). In such animals, brain tryptophan levels are 3.3–6.8 μg/g. Administration of large doses of tryptophan via the diet (Green et al., 1962; Wang et al., 1962) or by injection (800 mg/kg: Ashcroft et al., 1965) were first shown in 1962 and 1965, respectively, to increase brain serotonin levels. A few years later (Fernstrom and Wurtman, 1971a), it was demonstrated that even much lower doses (12.5 mg/kg)—which increased brain tryptophan but kept it within its normal range—could elevate brain serotonin significantly. Doubling that dose approximately doubled the increment in brain serotonin (Fernstrom and Wurtman, 1971a), however, increasing the dose further caused only minor increments in serotonin levels, instead increasing those of serotonin's deaminated metabolite 5-hydroxyindole acetic acid (5-HIAA). This suggested that a perhaps-twofold variation in brain serotonin levels, resulting from parallel changes in the rate of serotonin synthesis, is "allowed" to exist in serotoninergic neurons, but that beyond that range the excess serotonin cannot be stored (and protected from intracellular MAO), nor, probably, released.

Follow-up studies on rats attempted to determine whether a short-term treatment that lowered brain tryptophan levels would similarly decrease those of serotonin. Toward this end, rats received insulin—a treatment known to lower plasma levels of most amino acids; surprisingly, insulin failed to lower plasma or brain tryptophan, actually raising them in rats (Fernstrom and Wurtman, 1972a) (insulin secreted in response to carbohydrate consumption does lower plasma tryptophan in people but by less than it lowers plasma concentrations of other LNAA [Martin-Du-Pan et al., 1982]). This response—shown later to result from tryptophan's unique propensity, described above, to bind to circulating albumin (▶ *Table 4-1*)—raised the question of whether insulin, secreted physiologically in response to an insulin-releasing mix of dextrose, sucrose, and dextrin would similarly affect plasma and brain tryptophan (Fernstrom and Wurtman, 1971b). Insulin did, and also elevated brain serotonin, providing the first evidence that a particular macronutrient, certain carbohydrates, could cause characteristic changes in a brain neurotransmitter.

If, indeed, plasma tryptophan concentrations determined brain tryptophan, then giving animals a high-protein meal would be expected to raise brain tryptophan and serotonin levels by even more than the carbohydrate, since even though tryptophan is scarce in proteins (1–1.5%), protein consumption still would elevate plasma tryptophan concentrations. But surprisingly, though plasma tryptophan concentrations did rise, this change was not accompanied by parallel elevations in brain tryptophan—or serotonin (Fernstrom and Wurtman, 1972b). Prior studies had shown that other large, neutral amino acids can suppress the transport of tryptophan into perfused slices (Blasberg and Lajtha, 1966), and that the LNAA compete with each other for transport into brain (Guroff and Udenfriend, 1962). These findings raised the possibility that the failure of dietary proteins to elevate brain tryptophan or serotonin levels resulted from the larger increases the proteins would produce in plasma concentrations of these other, more abundant LNAA. Thus an experiment was performed comparing the changes in brain tryptophan and serotonin occurring after animals received either tryptophan alone, or an amino acid mixture containing tryptophan plus the other two aromatic amino acids and the three branched-chain amino acids. As anticipated, brain levels of the indoles rose after tryptophan alone but not after animals consumed the mixture. This led

to recognition of the paradoxical effects of macronutrients on brain serotonin: a meal that lacks tryptophan (but contains carbohydrates and perhaps fats) maximally *elevates* brain tryptophan (because it lowers plasma levels of the LNAA competitors), while one that contains the most tryptophan (i.e., a high-protein meal) *fails to elevate* brain tryptophan (❯ *Figure 4-10*). Hence, serotoninergic brain neurons are "variable ratio sensors" of the plasma amino acid pattern—specifically the ratio of tryptophan to other LNAA—producing more serotonin when protein-poor carbohydrate-rich foods are eaten, and less after consumption of protein-rich foods.

That such changes in serotonin production and tissue levels following carbohydrate consumption can be sufficient to affect serotonin *release* was initially shown in studies using rat brain slices (❯ *Figure 4-13*) (Schaechter and Wurtman, 1989). Superfusion of the slices with a physiologic medium containing 1–10 μM tryptophan caused dose-dependent elevations in tissue tryptophan levels which, below 5 μM tryptophan, were in the physiologic range for brain tryptophan in rats. Serotonin and 5-HIAA levels also rose significantly, proportionately greatest effects being observed with the lowest tryptophan concentrations. Both the spontaneous release of serotonin from the slices and the release caused by neuronal depolarization were also significantly increased (Schaechter and Wurtman, 1989). Moreover, the effects of providing tryptophan and of increasing the frequency of field stimulation were additive, and tryptophan's effects were blocked by adding leucine, another LNAA, to the medium (Schaechter and Wurtman, 1990).

That such changes in brain serotonin release also occur in response to meals which are, for Americans, normal, was demonstrated indirectly in a study on the effects of a high-carbohydrate versus a high-protein breakfast on plasma tryptophan ratios (Wurtman et al., 2003). The carbohydrate-rich breakfast (waffles, maple syrup, orange juice, coffee with sugar—containing 69.9 g of carbohydrate and 5.2 g of protein) generated plasma tryptophan ratios that were 54% higher than the protein-rich meal (turkey ham, eggs, cheese, butter) (Wurtman et al., 2003). Since a 50% increase in the plasma tryptophan ratio in rats can elevate brain tryptophan concentrations by 39% (Fernstrom and Faller, 1978; Fernstom et al., 1973), this increase, in turn, would be expected to elevate brain serotonin levels by 25% (Schaechter and Wurtman, 1989) and cause significant increases in the spontaneous and evoked release of serotonin (by 28 and 14%, respectively) (Schaechter and Wurtman, 1989). It seems probable that the 50% difference generated by the two breakfast meals also affects brain serotonin release in humans. The two meals also caused smaller (30%) but significant differences in the plasma tyrosine ratio.

A number of diseases and disorders are characterized by both mood disturbances—sadness, anger, anxiety—and weight gain associated with "carbohydrate craving," i.e., a strong desire, usually not associated with subjective hunger, to consume carbohydrate-rich foods which may or may not be sweet, usually at a characteristic time of day (Wurtman et al., 1993). The weight gain and the excess caloric load thus consumed relate not so much to the carbohydrates eaten but to the fats, which usually accompany them in the foods that the patients tend to select. Examples of such disorders include the seasonal affective disorder syndrome ("winter blues": Rosenthal et al., 1984), the premenstrual syndrome (Brzezinski et al., 1990), the behavioral syndrome sometimes following nicotine withdrawal (Spring et al., 1991), and, in some cases, obesity itself (Wurtman et al., 1981; Lieberman et al., 1986). Inasmuch as the consumption of carbohydrate-rich, protein-poor meals or, especially, snacks can, like antidepressant drugs, increase brain serotonin, and since many such patients volunteer that they choose these snacks because they have learned that the snacks make them "feel better—less tense, more motivated, less socially withdrawn, less sad"—it can be conjectured that the carbohydrate-craving represents a usually unrecognized attempt at self-medication directed at increasing intrasynaptic serotonin levels (Wurtman and Wurtman, 1989). All of these syndromes can be treated with serotoninergic drugs; some apparently also can be treated by giving patients a mixture of insulin-secreting carbohydrates (Sayegh et al., 1995) formulated for rapid absorption and sustained release.

When oral tryptophan was available in the USA for human use it was widely used to diminish symptoms of depression (Coppen et al., 1972) (a use which has continued in Canada, the UK, and elsewhere) and to treat insomnia (Demisch et al., 1987). Its administration can also decrease appetite (Wurtman et al., 1981) and caloric intake (Hrboticky et al., 1985); decrease sleep latency (Hartmann and Greenwald, 1984) and increase subjective drowsiness and fatigue (Lieberman et al., 1985); and decrease pain sensitivity (Lieberman et al., 1982/1983; Seltzer et al., 1982). Conversely, administration of amino acid mixtures

containing LNAA but lacking tryptophan reportedly can bring on depressive symptoms and even full-scale depressive episodes in patients with histories of depression (❯ *Figure 4-7*) (Delgado et al., 1991; Smith et al., 1997), and can exacerbate aggressive tendencies in subjects with histories of aggressive behavior (Bjork et al., 2000). Interestingly, the presence of a less-active allele of the gene for hTPH2, the brain enzyme that initiates the conversion of tryptophan to serotonin, is also highly associated with unipolar major depression (Zhang et al., 2005).

5.3 Brain Tyrosine and the Catecholamines

Basal brain catecholamine levels in fasting rats vary widely from region to region; indeed, it was this variation that initially suggested to Marthe Vogt (1954) that norepinephrine, concentrated within the hypothalamus, and to Arvid Carlsson (Carlsson et al., 1958) that dopamine, concentrated within the corpus striatum, might function as neurotransmitters. Administration of even large doses of tyrosine generally does not affect brain norepinephrine or dopamine levels (although it does elevate urinary catecholamines in humans [❯ *Figure 4-12*; Alonso et al., 1982] and rats [Agharanya and Wurtman, 1982a], and does correct the depletion of brain norepinephrine observed in stressed rats [Reinstein et al., 1984]). But if the conversion of tyrosine's hydroxylated product DOPA to a brain catecholamine is suppressed pharmacologically, and catecholamine release is thus diminished, then giving tyrosine will enhance DOPA synthesis (Wurtman et al., 1974; Carlsson and Lindqvist, 1978; Badawy and Williams, 1982) and giving the LNAA leucine has the opposite effect (Wurtman et al., 1974)—observations that first suggested that, under particular circumstances, tyrosine availability might affect catecholamine production, just as tryptophan availability affects that of serotonin.

Those circumstances relate to the physiological activity of the neuron (or adrenomedullary chromaffin cell [Agharanya and Wurtman, 1982b]) that is producing the catecholamine. If the neuron's firing rate is sufficiently high, much of its TH enzyme protein is polyphosphorylated, causing conformational changes which increase its affinity for its BH_4 cofactor and allowing its activity to become limited by the extent to which it is saturated with its amino acid substrate (Lloyd and Kaufman, 1975). In general—as described above—this happens, for example, when the amounts of dopamine or norepinephrine released into brain synapses are diminished (e.g., in Parkinson's Disease), or when physiologic circumstances require a greater-than-usual release of the transmitter (as in hemorrhagic hypotension), or among neurons that normally fire frequently (mesocortical dopaminergic neurons that lack impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors [❯ *Table 4-2*: Tam et al., 1990]), or in peripheral sympathoadrenal cells that are not components of polysynaptic feedback systems.

These relationships are well illustrated in rats, described above, that have been subjected to a unilateral lesion which destroys about 80% of a dopaminergic nigrostriatal tract, causing the surviving neurons to sustain rapid firing frequencies (Hefti et al., 1980): If such animals receive tyrosine systemically, no changes are noted in markers of dopamine release on the intact side of the brain, but major increases (which can be blocked by the LNAA valine) are observed on the lesioned side (Melamed et al., 1980b). The relationship between tyrosine availability and catecholamine synthesis is also evident when dopaminergic transmission has been blocked pharmacologically with haloperidol (Scally and Wurtman, 1977) or chronically administered reserpine (Sved et al., 1979a); when dopaminergic firing is enhanced by giving gamma-buytrolactone (Sved and Fernstrom, 1981) or amfonelic acid (Fuller and Snoddy, 1982), or when brain norepinephrine release is enhanced by cold stress (Gibson and Wurtman, 1978).

That brain tyrosine levels can also control catecholamine release has been shown directly using the technique of in vivo microdialysis: Giving tyrosine caused a short-term increase in striatal dopamine release (Acworth et al., 1988), which was enhanced in animals previously subjected to partial destruction of a nigrostriatal tract by 6-hydroxydopamine (During et al., 1989), and was prolonged if animals that also received the dopamine receptor antagonist haloperidol (During et al., 1989). Giving rats 250 mg/kg of phenylalanine enhanced striatal dopamine release by 59%, peaking after 75 min; a 500-mg/kg dose had no effect and a larger (1,000 mg/kg) dose reduced dopamine release by 26%—suggesting inhibition of TH (During et al., 1988). In none of these situations were changes noted in the release of dopamine's

metabolites homovanillic acid (HVA) or dihydrophenylacetic acid (DOPAC), suggesting that the metabolites are a less sensitive marker of dopamine release than the transmitter itself. In patients with Parkinson's disease given tyrosine chronically, however, significant increases have been noted in CSF levels of HVA (Growdon et al., 1982). Tyrosine also increased the levels of dopamine metabolites in retinas of dark-adapted rats exposed to light (❯ *Table 4-3*) (Gibson et al., 1983).

■ Table 4-3

Effect of tyrosine on increase in retinal catechols caused by light exposure

		Dopamine (ng/pair)	DOPAC (ng/pair)
Dark	Saline	2.9 ± 0.2	1.5 ± 0.3
	Tyrosine	2.6 ± 0.3	1.6 ± 0.3
Light	Saline	4.7 ± 0.4*	2.8 ± 0.1*
	Tyrosine	4.3 ± 0.5*	4.0 ± 0.5**

Rats were dark-adapted for 11 h and then exposed to light (1 h; 350 lux) or continued in darkness before killing. Thirty minutes before decapitation animals received tyrosine (100 mg/kg, i.p.) or its vehicle. *$p < 0.05$, differs significantly from either dark group. **$p < 0.01$, differs significantly from dark groups and from light-exposed controls. Data from Gibson et al. (1983)

The changes in catecholamine release caused by giving tyrosine are associated with some of the known physiological and behavioral effects of dopamine and norepinephrine. Thus, tyrosine can restore blood pressure in hemorrhage-induced hypotension (Conlay et al., 1981); this effect is not mediated by tyramine (Conlay et al., 1984) and is diminished if animals have been deprived of their adrenal medullas (Conlay et al., 1981). Tyrosine also lowers blood pressure in spontaneously hypertensive rats (Sved et al., 1979a)—an effect which also is blocked by other LNAA and is associated with an increase in brain levels of norepinephrine metabolites. Moreover, tyrosine diminishes serum prolactin levels in rats following their elevation by chronic treatment with reserpine (Sved et al., 1979b), and suppresses the rise in plasma corticosterone that follows acute stress in rats (Reinstein et al., 1985). In contrast, amino acid mixtures lacking tyrosine and phenylalanine increased plasma prolactin and decreased performance on a neurophysiological task sensitive to impaired dopaminergic function in humans (Gijsman et al., 2002). Giving tyrosine potentiated the brain-mediated anorexic effect of sympathomimetic drugs (phenylpropanolamine; ephedrine; amphetamine) (Hull and Maher, 1990), but not peripheral, sympathoadrenal-mediated as changes in gastric transit, thermogenesis (Hull and Maher, 1991), or blood pressure (Hull and Maher, 1992). Tyrosine administration also prevented hypoxia-induced decrements in learning and memory in rats (Shukitt-Hale et al., 1996).

Of potential significance for the development of treatments for psychiatric disturbances, tyrosine enhanced the release of dopamine from medial prefrontal cortical neurons of rats given clozapine, an atypical antipsychotic drug, but had no effect on striatal dopamine release in clozapine-treated animals. In contrast, tyrosine did potentiate the increase on striatal dopamine released caused by haloperidol, a "typical" antipsychotic agent, but had no effect on mesocortical dopamine release after haloperidol (Jaskiw et al., 2001). In otherwise-untreated animals, a low dose of tyrosine (25 mg/kg) enhanced dopamine synthesis in rapidly firing mesoprefrontal neurons. Higher doses initially also enhanced dopamine synthesis, but then stopped doing so, suggesting the operation of feedback processes (Tam et al., 1990).

Acknowledgements

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