Pharmacology Biochemistry & Behavior, Vol. 35, pp. 195–199. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Tyrosine Loading Increases Dopamine Metabolite Concentrations in the Brain[1]

WILLIAM T. CHANCE,*† TERI FOLEY-NELSON,* JEFFEREY L. NELSON* AND JOSEF E. FISCHER*

**Department of Surgery, University of Cincinnati Medical Center and †Veterans Affairs Medical Center, Cincinnati, OH*

Received 13 March 1989

CHANCE, W. T., T. FOLEY-NELSON, J. L. NELSON AND J. E. FISCHER. *Tyrosine loading increases dopamine metabolite concentrations in the brain.* PHARMACOL BIOCHEM BEHAV **35**(1) 195–199, 1990.—Administration of haloperidol (1.5 mg/kg) to rats increased concentrations of dopamine metabolites in the corpus striatum, nucleus accumbens, hypothalamus and amygdala. Treatment of similar rats with various doses (25, 100 or 400 mg/kg) of tyrosine methyl ester in combination with the haloperidol led to additional elevations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the corpus striatum and hypothalamus at the lowest dose tested. Therefore, it is concluded that during periods of increased neuronal activity, as induced by haloperidol, tyrosine availability may become a rate-limiting factor in dopamine metabolism.

Tyrosine Dopamine DOPAC HVA Amino acid Neurotransmitter Rat

THE conversion of the amino acid, tyrosine (TYR), to 3,4-dihydroxyphenyl-alanine (DOPA) is considered to be the rate-limiting step in the synthesis of catecholamines (11). Although the enzyme TYR-hydroxylase has traditionally been considered to be saturated at normal brain concentrations of TYR (3), more recent results suggest that the activity of this enzyme may be increased during periods of elevated dopamine (DA) neuronal activity (14). Thus, when DA neurons are firing rapidly, TYR availability should assume greater importance for the synthesis and metabolism of this neurotransmitter. Other researchers (22) have questioned the importance of brain TYR concentrations in DA metabolism, citing absence of changes in DA metabolite concentrations following TYR loading of the brain.

However, these authors did not report the effect of TYR on DA metabolism following a treatment that increased the firing rate of DA neurons and stimulated TYR-hydroxylase activity. To clarify whether increased TYR concentrations can lead to elevated DA metabolism, we investigated DA metabolism in several brain areas of rats treated with both TYR and the DA receptor blocker, haloperidol (HAL), which has been demonstrated to increase the release and metabolism of DA (20).

The results of this experiment should clarify whether elevated precursor availability can increase DA metabolism in several brain areas. The demonstration of TYR concentration as an additional control mechanism of DA synthesis and metabolism is important for deciding whether alterations in DA metabolism associated with feeding and satiety (6,9) or anorexia (5) may result from increased precursor availability.

METHOD

Twenty-eight adult, male, Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA) were treated with 1.5 mg/kg (IP) HAL (Sigma Chemical Co., St. Louis, MO) followed 30 min later by the administration (IP) of normal saline (SAL) (n=6), 25 mg/kg (n=8), 100 mg/kg (n=7) or 400 mg/kg (n=7) TYR methyl ester (Sigma Chemical Co., St. Louis, MO). An additional 6 rats served as controls and were treated with normal saline at each of these periods.

Sixty minutes after the second injections, all rats were decapitated to permit biochemical analyses. Blood was collected from the cervical wound for the analysis of plasma amino acid concentrations. The brains were rapidly removed, blotted and dissected into 4 specific brain areas over ice prior to freezing in liquid nitrogen. The hypothalamus, defined as tissue taken to a depth of approximately 2.5 mm extending from just anterior to the optic chiasm to the posterior mammillary area and bounded laterally by the choroid fissure, was removed from the ventral surface of the brain. The dorsal surface was next exposed and the cerebral cortex was separated at the midsagittal fissure with forceps and removed to the level of the rhinal sulcus. Corpus striatum, nucleus accumbens and amygdaloid tissue was next removed by vertical cuts along the internal capsule and separated medially from the septal area at the lateral ventricles. A horizontal cut extending from the anterior commissure to the rhinal sulcus separated nucleus accumbens and amygdaloid tissue from striatal tissue. The nucleus accumbens area was next separated from amygdaloid tissue by a vertical cut at the level of the anterior commissure. The

[1]Supported by USPHS NCI grant No. CA48057 and a research grant from the Department of Veterans Affairs to W.T.C.

[2]Requests for reprints should be addressed to William T. Chance, Ph.D., Department of Surgery, University of Cincinnati Medical Center, 231 Bethesda Avenue (ML#558), Cincinnati, OH 45267-0558.

TABLE 1

MEAN (±SEM) CONCENTRATIONS OF AMINE NEUROTRANSMITTERS, METABOLITES AND AMINO ACID PRECURSORS IN VARIOUS BRAIN AREAS OF SIX SALINE-TREATED CONTROL RATS

Brain Area	TYR (μg/g)	DA (ng/g)	3-MT (ng/g)	DOPAC (ng/g)	HVA (ng/g)
Hypothalamus	16.2 ± 1.8	619 ± 31	—	206 ± 12	—
Corpus Striatum	15.7 ± 1.1	7707 ± 249	184 ± 11	881 ± 35	686 ± 67
Nucleus Accumbens	17.6 ± 1.2	4177 ± 379	77 ± 8	699 ± 43	486 ± 47
Amygdala	14.2 ± 1.4	1535 ± 93	32 ± 7	304 ± 16	161 ± 6

remaining brain was combined with the cerebral cortex for the analysis of amino acid concentrations, while the 4 specific regions outlined above were processed for amine neurotransmitter levels using HPLC with electrochemical detection.

Concentrations of TYR, DA and DA metabolites [3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA)] were determined in each brain sample according to previously published procedures (6). Each sample was homogenized in 3 ml of 1 N formic acid/acetone (15:85) containing N-methyl dopamine as an internal standard. Following centrifugation (10,000 × g, 10 min, 4°C), 7.5 ml of heptane/chloroform (8:1) was added to 2.5 ml of the supernatants. The samples were shaken, centrifuged (2,500 × g, 10 min, 4°C) and the organic phase discarded. The aqueous phase of each sample was next dried under a stream of N_2 (36°C) after which the sample was reconstituted in 0.8 ml of the mobile phase HPLC buffer (8% acetonitrile and 92% of 0.1 M KH_2PO_4, 0.185 mM sodium octylsulfate and 0.195 mM EDTA, pH = 2.94). Fifty μl of each sample was injected onto the HPLC column (Altex, reverse phase, C-18, ultrasphere), with the amines being quantified by amperometric detection (Bioanalytical Systems Model LC-4, potential = 0.89 V) at a flow rate of 1.2 ml/min and sensitivity of 50 nA/V.

Concentrations of free amino acids were determined according to our previously published methods (5) in brain and plasma following deproteinization with 3 volumes of 5% sulfosalicylic acid, containing thienylalanine as an internal standard. After vortexing, the samples were centrifuged (30,000 × g, 4°C) and filtered (0.45 μm). Amino acid levels were quantified on this filtrate (50 μl) using an automated Beckman 121-MB amino acid analyzer employing a three-buffer, single column, lithium citrate system.

The data were evaluated statistically using analysis of variance (ANOVA) techniques with individual means being compared by Newman-Keuls post hoc *t*-tests (23).

RESULTS

Since the comparisons of treatment groups are illustrated as percentages of saline-treated control values, Table 1 presents the actual mean concentrations of each of these compounds for each brain region of these control rats. These values agree reasonably well with published reports demonstrating appropriately concentrated levels of DA and metabolites in the corpus striatum and nucleus accumbens area.

As may be observed in Fig. 1, treatment with HAL significantly increased concentrations of the DA metabolite DOPAC in each brain area and HVA in all areas except the hypothalamus, where HVA was not reliably detected. Levels of DA and the intermediary metabolite 3-MT were decreased in the corpus striatum and nucleus accumbens area following treatment with HAL. The administration of TYR methyl ester led to dose-related increases in brain TYR concentrations and to additional elevation of DOPAC levels in the corpus striatum, hypothalamus and amygdaloid areas. The other major metabolite of DA, HVA, was not reliably affected by the TYR injections.

Table 2 presents the plasma and brain amino acid concentrations for each treatment group of this experiment. As in the HPLC analysis, plasma and brain TYR concentrations were decreased significantly following treatment with HAL. Plasma alanine and brain threonine levels were also decreased following the HAL treatment. Following the administration of the higher doses of TYR methyl ester, plasma concentrations of glycine, valine, leucine, isoleucine and arginine were decreased. At the highest TYR dose, brain levels of threonine, serine, glutamate, proline, methionine, isoleucine, leucine, phenylalanine, TRP, ornithine and histidine were decreased, while glutamine concentrations were increased.

DISCUSSION

The results of this experiment demonstrate that under conditions of increased neuronal DA metabolism, elevation of TYR availability can lead to further increases in DA metabolism in certain brain areas. The corpus striatum was the most sensitive of the four brain areas examined to the stimulating effects of TYR, exhibiting increased concentration of DOPAC at the lowest TYR dose. Hypothalamic DA metabolism was elevated above the HAL baseline by the 100 mg/kg dose of TYR, while significant effects were observed in the amygdala only following the highest dose of TYR. Nucleus accumbens tissue did not respond significantly to any dose of TYR.

The results of the present experiment support and extend several reports demonstrating that brain TYR concentration can influence DA metabolism within a variety of experimental paradigms. Thus, either TYR administration (24) or feeding rats a 40% protein diet (8) increased DOPA accumulation following inhibition of L-aromatic amino acid decarboxylase. A lower dose of TYR (20 mg/kg), but not higher doses (100 to 500 mg/kg), was reported to increase whole brain concentration of DOPAC (1). Following HAL treatment, TYR injection was also reported to increase HVA levels in the corpus striatum of rats (17). In another report (4), TYR administration to rats pretreated with HAL resulted in significant increases in DOPA accumulation in the limbic forebrain, corpus striatum and cerebral hemispheres. These authors concluded that under normal conditions, TYR-hydroxy-

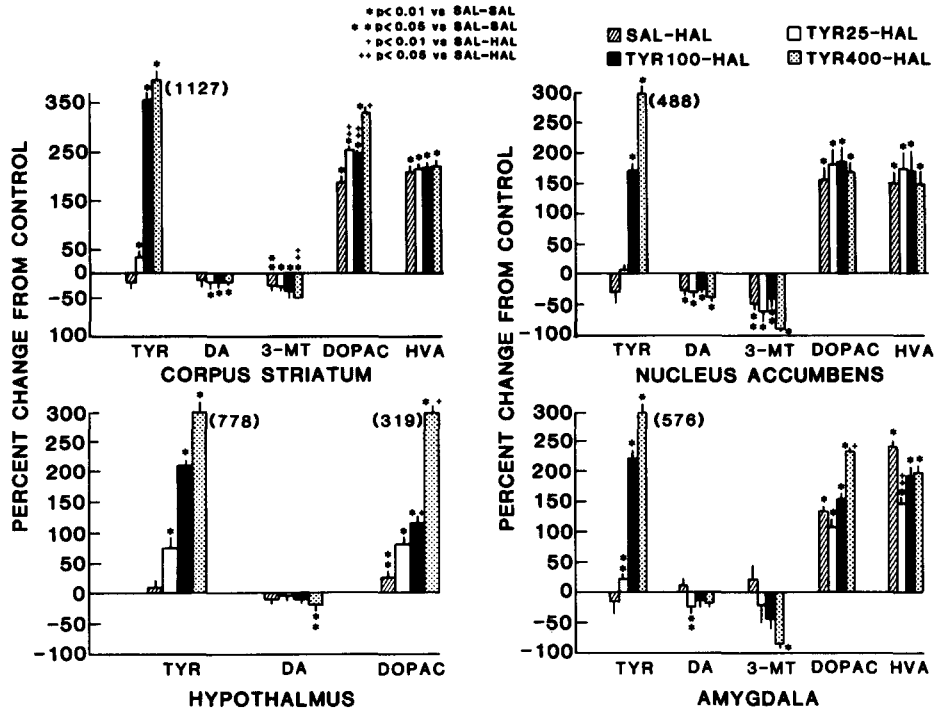

FIG. 1. Mean (±SEM) concentrations of tyrosine (TYR), dopamine (DA), 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in various brain regions of rats treated with haloperidol (Hal) or various doses of tyrosine methyl ester (25, 100, 400 mg/kg) in combination with haloperidol. Values are presented as percent increase or decrease from control means.

TABLE 2

MEAN (±SEM) PLASMA AND BRAIN AMINO ACID CONCENTRATIONS (nmol/ml or nmol/g) IN RATS TREATED WITH NORMAL SALINE (SAL) OR 1.5 mg/kg HALOPERIDOL (HAL) FOLLOWED 30 MIN LATER BY VARIOUS DOSES (0, 25, 100 or 400 mg/kg) OF TYROSINE METHYL ESTER (TYR)

	SAL-SAL		HAL-SAL		HAL-TYR 25		HAL-TYR 100		HAL-TYR 400	
	Plasma	Brain	Plasma	Brain	Plasma	Brain	Plasma	Brain	Plasma	Brain
ASP	24± 2	3,823± 88	40±10	3,354±194	22± 3	3,918±161	22± 2	3,771± 55	22± 4	3,795±112
HPR	56± 2	18± 3	51± 3	21± 4	64± 3	23± 3	58± 5	21± 2	56± 4	25± 1
THR	289±17	535± 22	244±16	438± 34†	245±12	454± 20†	227±13	485± 20	275±13	449± 17†
SER	283±19	991± 23	250±16	869± 67	258±13	911± 41	236±12	907± 24†	294±13	901± 25†
GLU	100± 4	10,981±165	138±17	9,581±628	114± 9	9,909±490	109± 8	10,480±304	124± 8	8,997± 85*
GLN	709±35	4,590±181	606±42	3,982±204	638±23	4,721±239	660±34	4,832±152	719±23	5,319± 79*
PRO	143± 7	87± 4	120± 6	78± 4	130± 4	80± 6	118± 4	79± 4	137± 4	70± 2*
GLY	437±33	723± 18	392±22	630± 42	363±18	638± 30†	334±24†	668± 11†	300±21*	689± 18
ALA	360±13	525± 7	247±20*	454± 36	282±10*	491± 26	277±15*	491± 12	403±25	522± 9
VAL	172± 7	77± 3	168±12	70± 7	155± 6	71± 5	121± 7*	70± 2	120± 9*	74± 4
MET	59± 2	48± 3	51± 2	37± 4	53± 4	41± 2	48± 1	41± 1	54± 5	30± 3*
ILE	81± 3	34± 1	80± 3	28± 3	79± 5	32± 2	54± 3*	28± 1*	47± 8*	23± 1*
LEU	135± 5	65± 2	133± 8	54± 5	125± 7	60± 4	89± 7*	51± 2*	75± 8*	41± 4*
TYR	89± 6	79± 5	65± 5†	56± 5†	113± 5*	105± 7*	230±11*	251± 14*	1,057±98*	663± 49*
PHE	60± 1	53± 7	56± 2	41± 5	60± 4	48± 5	51± 2	49± 6	81± 3	34± 2†
TRP	90± 5	78± 1	84± 5	63± 5†	76± 2†	73± 3	73± 6	73± 2	73± 4†	69± 2†
ORN	44± 3	34± 6	41± 3	24± 3	38± 3	23± 2	48± 6	31± 4	100±18*	21± 2†
LYS	486±45	194± 15	437±25	170± 12	505±29	187± 17	410±24	186± 7	435±23	169± 13
HIS	51± 3	60± 3	48± 2	52± 4	50± 2	54± 3	45± 3	55± 2	59± 2	45± 2*
ARG	177±10	98± 4	159± 8	85± 9	155± 5	82± 4	128±13†	89± 5	104±18*	88± 4

*$p<0.01$ vs. SAL-SAL; †$p<0.05$ vs. SAL-SAL.

lase is approximately 75% saturated. In a study designed specifically to assess the importance of TYR loading on DA metabolism during periods of increased TYR-hydroxylase activity, Westerink and Wirix (22) reported significantly elevated DOPA accumulation in the rat corpus striatum following decarboxylase inhibition. However, since this increase was small (17%), and since in another experiment TYR loading had no effect on DA metabolites, they concluded that increased brain TYR was not an important factor in the control of catecholamine synthesis, even during periods of increased TYR-hydroxylase activity. However, in the experiment assessing the effect of TYR loading on DA metabolite concentration, no treatment to increase TYR-hydroxylase, such as HAL or gamma-butyrolactone, was administered. Therefore, in the absence of stimulated TYR-hydroxylase activity, one may not expect an effect of TYR administration on DA metabolism.

Tyrosine-hydroxylase activity in DA neurons is regulated by several factors including substrate availability, end-product inhibition, impulse flow, and DA autoreceptor activity (12). The administration of HAL increases the firing rate of DA neurons and blocks terminal DA autoreceptors (2). Thus, HAL treatment leads to increased DA release and metabolism as well as to increased TYR-hydroxylase activity. Therefore, treatment with HAL has been reported to increase DOPAC and HVA levels in the corpus striatum, nucleus accumbens and hypothalamus, while decreasing DA concentrations in these same brain areas (19). In another report, the concentration of TYR was also reduced in DA-rich brain areas by HAL treatment (22). Waldmeir *et al.* (18) reported decreased 3-MT and increased DOPAC and HVA concentrations following the injection of HAL. In the present experiment, levels of 3-MT and DA were reduced significantly by the HAL treatment in DA-rich brain areas. A possible reason for this reduction in DA may be that at this dose of HAL the release and metabolism of DA is proceeding at a faster rate than is its synthesis. Thus, in both striatal and mesolimbic (accumbens) tissue, the maximum stimulation of TYR-hydroxylase by HAL treatment was approximately 100%, while HVA and DOPAC levels were increased by considerably greater amounts (10). The decrease in 3-MT also supports this interpretation, since lowered levels of this intermediary metabolite have been suggested to be a useful indicator of decreases in newly-released DA (18,21). However, 3-MT concentration may not be a reliable indicator of newly released DA in decapitated rats, since the metabolic pathway of DA metabolism switches from the O_2-requiring monoamine oxidase mediated formation of DOPAC to the formation of 3-MT via catechol-O-methyl transferase during anoxia (18,21).

The large neutral amino acids (LNAA), Tyr, tryptophan, valine, leucine, isoleucine, phenylalanine, methionine and histidine share a common carrier system for transport across the blood-brain barrier (15). In the present study, however, it was only at the highest dose of Tyr that transport of LNAA across the blood-brain barrier appeared to be affected. Although brain concentrations of leucine and isoleucine were reduced at the 100 mg/kg dose of Tyr, this effect was confounded by reduced plasma levels of these amino acids. The reduction in plasma levels of leucine and isoleucine may have been due to Tyr-induced release of insulin and increased uptake of these branched-chain amino acids by muscle (13).

An additional question raised by these demonstrations of a role of TYR availability on DA metabolism concerns the physiological circumstances under which TYR-hydroxylase activity is increased and DA metabolism affected. Increased activity in DA neurons has been associated with satiety, and the anorexia-producing drug, amphetamine, induces the release of DA (16). Experiments have also associated increased DA metabolism in DA-rich brain areas with feeding and satiety (6,9). Therefore, induction of TYR-hydroxylase and increased DA metabolism may be associated with the onset of satiety (5). We have also observed increased concentrations of TYR, DOPAC and HVA in several brain regions of anorectic tumor-bearing rats (5,7). Since the elevation of brain Tyr in anorectic tumor-bearing rats is approximately three-fold, it is possible that the anorexia in this experimental cancer model may be mediated in part by elevated DA metabolism which is secondary to an increase in TYR-hydroxylase activity coupled with elevated precursor availability.

REFERENCES

1. Badawy, A. A. B.; Williams, D. L. Enhancement of rat-brain catecholamine synthesis by administration of small doses of tyrosine and evidence for substrate inhibition of tyrosine-hydroxylase activity by large doses of the amino acid. Biochem. J. 206:165–168; 1982.
2. Bannon, M. J.; Roth, R. H. Pharmacology of mesocortical dopamine neurons. Pharmacol. Rev. 35:53–68; 1983.
3. Carlsson, A.; Davis, J. N.; Kehr, W.; Lindqvist, M.; Atack, C. V. Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. Naunyn Schmiedebergs Arch. Pharmacol. 275:153–168; 1972.
4. Carlsson, A.; Lindqvist, M. Dependence of 5-HT and catecholamine synthesis on concentrations of precursor amino-acids in rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 303:157–164; 1978.
5. Chance, W. T.; Cao, L.; Nelson, J. L.; Foley-Nelson, T.; Fischer, J. E. Reversal of neurochemical aberrations after tumor resection in rats. Am. J. Surg. 155:124–130; 1987.
6. Chance, W. T.; Foley-Nelson, T.; Nelson, J. L.; Fischer, J. E. Neurotransmitter alterations associated with feeding and satiety. Brain Res. 416:228–234; 1987.
7. Chance, W. T.; Cao, L.; Foley-Nelson, T.; Nelson, J. L.; Fischer, J. E. Possible role of ammonia in experimental cancer anorexia. Brain Res. 486:316–324; 1989.
8. Gibson, C. J.; Wurtman, R. J. Physiological control of brain catechol synthesis by brain tyrosine concentration. Biochem. Pharmacol. 26:1137–1142; 1977.
9. Heffner, T. G.; Hartman, J. A.; Seiden, L. S. Feeding increases dopamine metabolism in the rat brain. Science 208:1168–1170; 1980.
10. Kuczenski, R. Amphetamine-haloperidol interactions on striatal and mesolimbic dopamine metabolism. J. Pharmacol. Exp. Ther. 215: 135–142; 1980.
11. Levitt, M.; Spector, S.; Sjoerdsma, A.; Udenfriend, S. Elucidation of the rat limiting step of norepinephrine biosynthesis in the perfused guinea-pig heart. J. Pharmacol. Exp. Ther. 148:1–8; 1965.
12. McMillen, B. A.; German, D. C.; Shore, P. A. Functional and pharmacological significance of brain dopamine and norepinephrine storage pools. Biochem. Pharmacol. 29:3045–3050; 1980.
13. Maroni, B. J.; Karapanos, G.; Mitch, W. E. System A amino acid transport in incubated muscle: effects of insulin and acute uremia. Am. J. Physiol. 251:F74–F80; 1986.
14. Melamed, E.; Hefti, F.; Wurtman, R. J. Tyrosine administration increases striatal dopamine release in rats with partial nigrostriatal lesions. Proc. Natl. Acad. Sci. USA 77:4305–4309; 1980.
15. Pardridge, W. M. Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. J. Neurochem. 28: 103–108; 1977.
16. Samanin, R.; Bendotti, C.; Bernasconi, S.; Pataccini, R. Differential role of brain monoamines in the activity of anorectic drugs. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:233–242.
17. Scally, M. C.; Ulus, I.; Wurtman, R. J. Brain tyrosine level controls striatal dopamine synthesis in haloperidol-treated rats. J. Neural Transm. 41:1–6; 1977.
18. Waldmeier, P. C.; Lauber, J.; Blum, W.; Richter, W. J. 3-Methoxytyramine: its suitability as an indicator of synaptic dopamine release. Naunyn Schmiedebergs Arch. Pharmacol. 315:219–225; 1981.

19. Westerink, B. H. C.; Bosker, F. J.; Wirix, E. Formation and metabolism of dopamine in nine areas of the rat brain: Modification by haloperidol. J. Neurochem. 42:1321–1327; 1984.
20. Westerink, B. H. C.; Korf, J. Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain. Eur. J. Pharmacol. 37:249–255; 1976.
21. Westerink, B. H. C.; Spaan, S. J. Estimation of the turnover of 3-methoxy-tyramine in the rat striatum by HPLC with electrochemical detection: Implications for the sequence in the cerebral metabolism of dopamine. J. Neurochem. 38:342–347; 1982.
22. Westerink, B. H. C.; Wirix, E. On the significance of tyrosine for the synthesis of dopamine in rat brain: evaluation by HPLC with electrochemical detection. J. Neurochem. 40:758–764; 1983.
23. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971:210–219.
24. Wurtman, R. J.; Larin, F.; Mostafapour, S.; Fernstrom, J. D. Brain catechol synthesis: control by brain tyrosine concentration. Science 185:183–184; 1974.