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L-Tyrosine Fails to Potentiate Several Peripheral Actions of the Sympathomimetics

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HULL, K. M. AND T. J. MAHER. *L-Tyrosine fails to potentiate several peripheral actions of the sympathomimetics*. PHARMACOL BIOCHEM BEHAV **39**(3) 755–759, 1991.—We have recently reported the ability of L-tyrosine (L-TYR) to potentiate the anorectic activity of various mixed-acting sympathomimetics including phenylpropanolamine (PPA), l-ephedrine (EPH) and d-amphetamine (AMP). Included in those studies was the attenuation of L-TYR's effect when coadministered with L-valine, a large neutral amino acid which competes with L-TYR for uptake into the brain, suggesting a central locus for the action of L-TYR. To determine to what extent L-TYR can potentiate peripheral actions, we investigated the effects of L-TYR with either PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg) on gastric transit, gastric retention and intrascapular brown adipose tissue thermogenesis. In each of the paradigms studied, PPA, EPH and AMP significantly increased the expected sympathomimetic-mediated response, but no potentiation of L-TYR was observed. These results are consistent with the hypothesis that L-TYR potentiates the anorectic activity of the mixed-acting sympathomimetics largely via an action at a central locus.

Tyrosine Sympathomimetics Phenylpropanolamine Amphetamine Ephedrine Gastric transit Thermogenesis

OUR laboratory has recently demonstrated the specific ability of the catecholamine precursor, L-tyrosine (L-TYR), to potentiate the anorectic activity of the mixed-acting sympathomimetics phenylpropanolamine (PPA), ephedrine (EPH) and amphetamine (AMP) (9). Included in these initial studies was the significant attenuation of the L-TYR enhancement when L-valine (L-VAL), a large neutral amino acid (LNAA) which competes with L-TYR for uptake into the brain (15), was coadministered. This suggested that the observed potentiation by L-TYR may be largely mediated at a central locus. Previous work by Chiel and Wurtman (4) in cold-stressed rats correlated the attenuation of amphetamine-induced hypothermia by the administration of the LNAA's L-VAL, L-leucine and L-isoleucine with a decrease in brain L-TYR concentrations. In contrast, the non-LNAAs L-serine, L-arginine and L-alanine failed to attenuate the amphetamine-induced hypothermia. Since the hypothermic response to AMP in these cold-stressed rats is believed to involve activation of hypothalamic centers, these results suggested the importance of endogenous L-TYR in sustaining neurotransmitter synthesis in centrally stimulated catecholamine-containing neurons.

In quiescent neurons, the rate of catecholamine synthesis is believed to be regulated by the rate-limiting enzyme, tyrosine hydroxylase (TH), which is itself dependent upon the availability of its cofactor, tetrahydrobiopterin (BH_4). Additionally, the enzyme is strongly influenced by end-product inhibition via its products: dopamine (DA), norepinephrine (NE) and epinephrine (EPI). However, when catecholamine-containing neurons are made to fire rapidly for sustained periods of time, TH undergoes a depolarization-dependent phosphorylation which activates the enzyme, greatly lowering the K_m for its cofactor, and resulting in saturation of the enzyme with BH_4 (12). Additionally, a loss of end-product inhibition is believed to occur (6). Thus, following activation, TH is no longer limited by the availability of BH_4, and catecholamine synthesis is believed to become dependent upon the concentration of its substrate, L-tyrosine (L-TYR). Indeed, it has been demonstrated that L-TYR supplementation is capable of increasing catecholamine synthesis and/or release from rapidly firing catecholamine-containing neurons, e.g., in electrically stimulated striatal slices (13), retinal cells exposed to light (7) and adrenal cortical cells activated during hemorrhage (5).

Mixed-acting sympathomimetics, e.g., AMP, derive at least a portion of their pharmacologic action by releasing newly synthesized, cytoplasmic, reserpine-insensitive pools of catecholamines, and presumably to compensate for the cytoplasmic depletion, accelerate the synthesis of DA (10). Consistent with this mechanism, Lee and Mandell (11) demonstrated the ability of AMP to decrease brain tyrosine concentrations by 29% in rat caudate slices. Additionally, work by Pinto and Maher (16) demonstrated the ability of L-TYR to influence an indirect-acting sympathomimetic-mediated response. In these studies, L-TYR, in concentrations as low as 6 μM, was able to prevent the tyramine-induced tachyphylaxis in the isolated-perfused rat heart. The maintenance of the positive chronotropic response to tyramine was specific for L-TYR, as neither D-tyrosine nor any of the other nine L-amino acids tested were capable of preventing the observed tachyphylaxis. Pretreatment with the specific TH inhibitor alpha-methyl-p-tyrosine or the L-aromatic amino acid decarboxylase inhibitor, m-hydroxybenzylhydrazine, blocked the effect, indicating the dependence on catecholamine synthesis in mediat-

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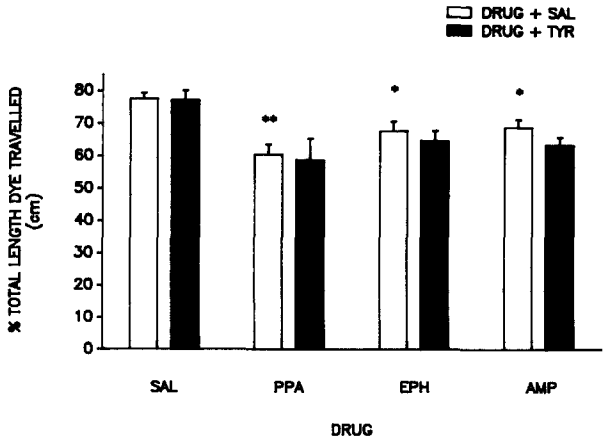

FIG. 1. Percent total length amaranth dye travelled through small intestine. Groups of eight rats were fasted for 24 h and administered either PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg) with SAL (□) or L-TYR (■; 200 mg/kg) IP sixty min prior to sacrificing. Twenty min following injections, rats received 1 ml of an amaranth "meal" via oral intubation. Percent total length dye travelled is expressed as the distance amaranth dye travelled per total length of the small intestine. Values represent the mean ± S.E.M. *$p<0.05$; and **$p<0.01$; significantly different from corresponding saline control as determined by ANOVA and Dunnett's test.

ing this response to L-TYR.

In addition to central mechanisms, it is believed that PPA, EPH and AMP may mediate a portion of their anorectic activity via the inhibition of gastrointestinal smooth muscle, thus resulting in increased gastric transport time (21) and gastric retention (3,22). Additionally, increases in intrascapular brown adipose tissue (IBAT) thermogenesis may be partly responsible for the weight loss associated with the use of such agents (23). Brown adipose tissue, which is regulated by the sympathetic nervous system, is a specialized form of adipose tissue that is involved with the oxidation of lipids and the production of heat. This tissue can be stimulated directly by β-adrenoceptor agonists, or indirectly by mixed-acting sympathomimetics such as PPA and EPH (25), which act partly to release norepinephrine from presynaptic terminals (14,25).

In light of our recent findings, we set out to determine if the observed potentiation by L-TYR of the anorectic activity of the mixed-acting sympathomimetics might also be observed when measuring their peripherally mediated actions on gastric function and IBAT status.

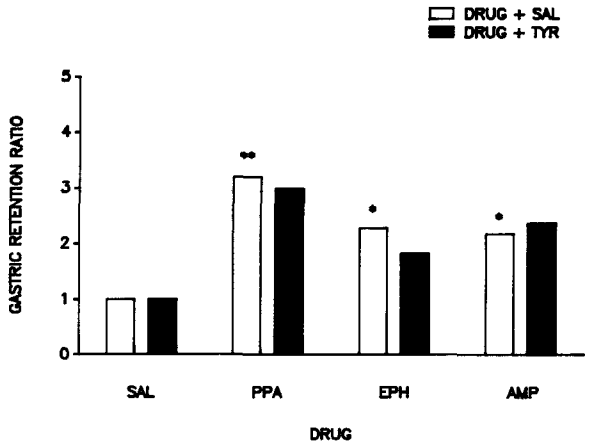

FIG. 2. Gastric retention ratio. Groups of eight rats were fasted for 24 h and administered either PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg) with SAL (□) or L-TYR (■; 200 mg/kg) sixty min prior to sacrificing. Twenty min following injections, rats received 1 ml of an amaranth "meal" via oral intubation. Gastric retention ratio is expressed as the ratio of amaranth remaining in the treated groups to that in the control group. The control group ratio is equal to 1.0. *$p<0.05$; and **$p<0.01$, significantly different from corresponding saline control as determined by ANOVA and Dunnett's test.

METHOD

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were obtained at 125–150 grams and individually housed in suspended wire mesh cages with food (Purina #5001; 25% protein, 56% CHO and 5% fat) and tap water available ad lib. Animals were acclimated to our climate-controlled animal facility for at least one week prior to experimentation. All experimental protocols were approved by our Institutional Animal Use and Care Committee prior to commencement of the studies.

Drugs

Phenylpropanolamine HCl (PPA; Roehr), l-ephedrine HCl (EPH; Roehr), d-amphetamine SO_4 (AMP; Amend) and L-tyrosine (L-TYR: Ajinomoto) were injected in a volume of 1 ml/kg IP. A 1% amaranth (Sigma) in 1% gum arabic (Sigma) "meal" was administered by oral intubation in a volume of 1 ml/animal 40 min prior to sacrifice.

Gastric Transit and Gastric Retention

Rats, previously fasted for 24 h, received an IP injection of PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg) concurrently with either saline (SAL) or L-TYR (200 mg/kg) 60 min prior to sacrifice. These doses were chosen based on previous studies indicating moderate anorectic activity (9). Twenty minutes later, 1 ml of a standard "meal" containing 1% amaranth in a 1% aqueous gum arabic solution was administered intragastrically by oral intubation (17). Forty min following the "meal," animals were sacrificed by decapitation and the abdominal cavity exposed. Ligatures were placed at the esophageal and pyloric sphincters to confine the stomach contents, and the small intestine (duodenum to the ileocecal valve) was carefully removed and the omentum sufficiently separated to enable unfolding of the intestine. Special care was taken to avoid stretching. The total length of the intestine and the distance that the dye traversed were recorded to the nearest millimeter. Gastric transit is expressed as the mean percentage (± S.E.M.) of the distance that the dye traversed in relation to the total length of the small intestine (17). Data was analyzed via ANOVA and Dunnett's test.

To determine gastric retention, the tied-off stomach pouch was removed and the contents emptied into a 15-ml polypropylene test tube and brought up to a final volume of 5 ml with distilled water. Stomach and contents were then vortexed and centrifuged (Sorval-4) at 12,900 rpm for 10 min. One ml of the supernate was then analyzed for amaranth concentration using a

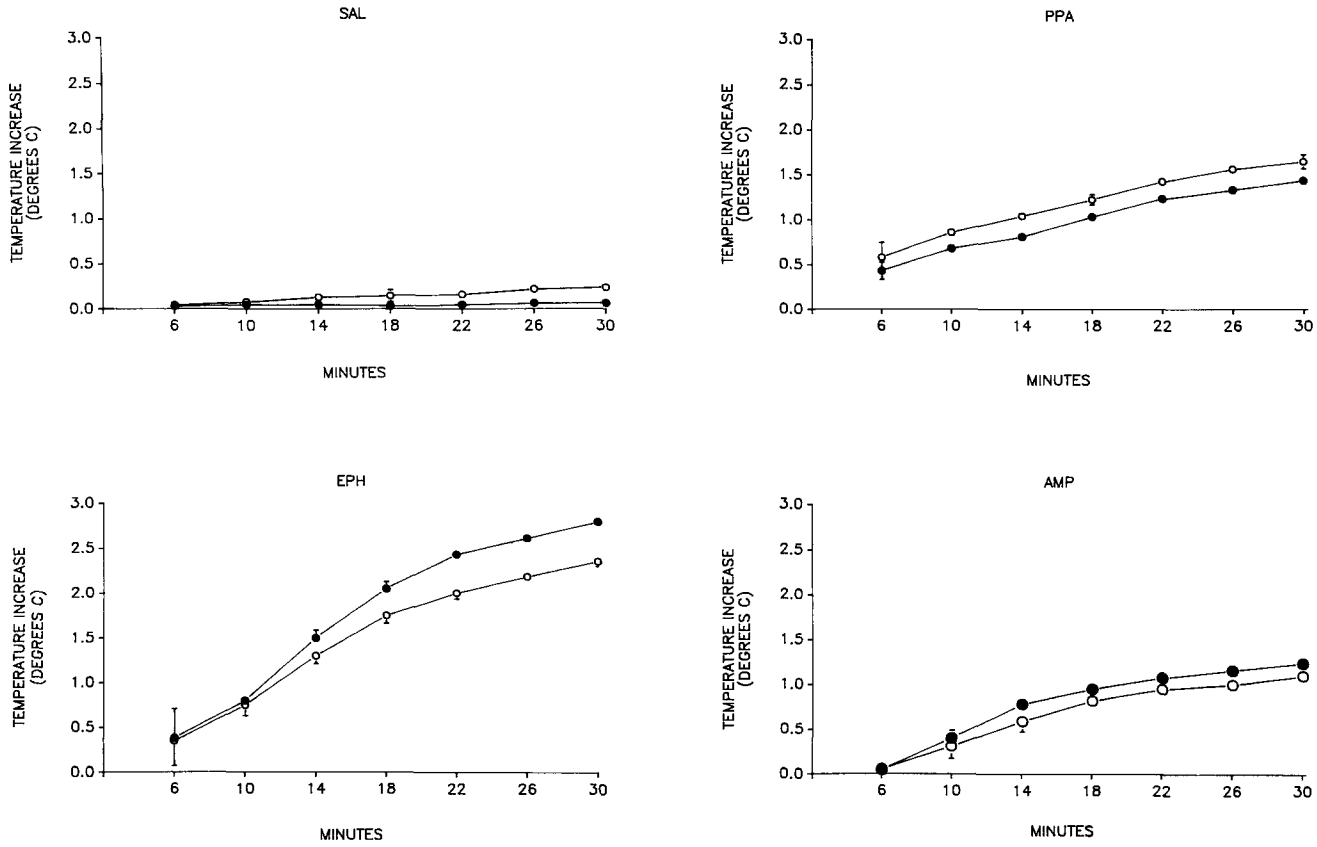

FIG. 3. Intrascapular brown adipose tissue (IBAT) thermogenesis. Groups of eight rats received an IP injection of either SAL or L-TYR (200 mg/kg) and thirty minutes later anesthetized with urethane. Sixty minutes following the L-TYR or SAL injections, rats received either PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg) and IBAT temperature quantitated for 30 min with a thermistor probe. All drugs significantly ($p<0.01$) increased IBAT thermogenesis compared to their SAL control as determined by ANOVA and Dunnett's. Values represent cumulative means ± S.E.M. ○ Drug + SAL, ● drug + TYR.

Bausch and Lomb Spectronic 20 spectrophotometer at 365 nm. When necessary, quantitative dilutions were made with distilled water. A standard curve for amaranth (0–16 μ/ml) was determined just prior to testing samples. This yielded a linear relationship between the concentration of amaranth and absorbance with a calculated linear regression coefficient of .99. Gastric retention is expressed as the ratio of amaranth remaining in the treated groups to that in the control group (control group equal to 1.0).

Thermogenesis

Thirty minutes following an IP injection of either SAL or L-TYR (200 mg/kg), animals were anesthetized with urethane (1.2 g/10 ml/kg) IP and the hair overlying the scapulae shaved with electric clippers. The rats were then placed on foam urethane padding to prevent heat loss from their ventral surface, and a 3-cm longitudinal incision was made in the skin between the scapulae to expose the connective tissue covering the lobes of brown fat. The tissues were then carefully separated, and a thermistor probe (#402, Yellow Springs Instruments Co., Inc.) was implanted such that the tip was entirely immersed within the brown adipose tissue. The thermistor probe was secured with tape, and the wound was closed using surgical staples. Sixty min following the injection of L-TYR (200 mg/kg) or SAL, rats received either SAL, PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg). Six min later, temperatures were recorded every 4 min for 30 min using a telethermometer (±0.1°C, Yellow Springs Instruments Co., Inc.). Thermogenesis is expressed as the mean cumulative increase in °C (±S.E.M.). Data was analyzed by ANOVA and Dunnett's test.

RESULTS

Gastrointestinal Transit

In SAL-pretreated animals, PPA (20 mg/kg), EPH (20 mg/kg) and AMP (1.75 mg/kg) produced a significant inhibition of gastrointestinal transit. Gastric transit in control animals was 77.6 ± 1.8%, while in PPA-, EPH- and AMP-treated animals, it was 60.6 ± 3.1% ($p<0.01$), 67.8 ± 3.0% ($p<0.05$) and 68.9 ± 2.5% ($p<0.05$), respectively, Fig. 1. This constituted a 22%, 13% and 11% inhibition of gastric transit for the three sympathomimetics compared to control, respectively.

The administration of L-TYR (200 mg/kg) alone failed to significantly alter gastric transit when compared to control animals (77.1 ± 3.0%). Additionally, the inhibitory effects of gastric transit on PPA, EPH and AMP were not significantly altered

by L-TYR [58.8±6.7%, 64.8±3.1% and 63.6±2.5%, respectively ($p>0.05$), when compared to appropriate drug plus SAL group], Fig. 1.

Gastric Retention

The gastric retention ratio (amount of amaranth remaining in stomach pouches of experimental animals divided by the amount in control animals, with controls equal to 1.00) was significantly increased by the three sympathomimetics tested. PPA, EPH and AMP produced gastric retention ratios of 3.20 ($p<0.01$), 2.28 ($p<0.05$) and 2.18 ($p<0.05$), respectively, Fig. 2. L-TYR failed to alter the gastric retention ratio in SAL-, PPA-, EPH- or AMP-treated animals (1.02, 2.99, 1.82 and 2.37, respectively; $p>0.05$ when compared to the corresponding SAL-pretreated group), Fig. 2.

Thermogenesis

While SAL administration failed to significantly alter IBAT temperatures during the 30-min test period (+0.1°C±0.1, $p>0.05$), the administration of PPA (20 mg/kg), EPH (20 mg/kg) or AMP (1.75 mg/kg) produced a significant ($p<0.01$) increase in IBAT temperature (+1.6°C±0.2, +2.2°C±0.1 and +1.2°C±0.2, respectively, at 30 min), Fig. 3.

L-Tyrosine failed to alter the IBAT temperature when administered alone (+0.1°C±0.1), Fig. 3. Additionally, the coadministration of L-TYR (200 mg/kg) with each of the three mixed-acting sympathomimetics failed to potentiate the observed increase in IBAT temperature (+1.4°C±0.2, +2.8°C±0.1 and +1.2°C±0.1, respectively, $p>0.05$, when compared to the corresponding SAL-pretreated group), Fig. 3.

DISCUSSION

We have recently reported the potentiation of PPA-, EPH- and AMP-induced anorexia by L-TYR in food-deprived hyperphagic rats (9). In that study, we also presented evidence which suggested the observed potentiation may be largely mediated at a central locus. To investigate the possibility that L-TYR's potentiation might extend to peripherally mediated mechanisms as well, we examined the effects of L-TYR coadministration with the three mixed-acting sympathomimetics on gastrointestinal transit, gastric retention and IBAT thermogenesis. The present results demonstrate no potentiation of these peripherally mediated events by L-TYR.

In quiescent neurons, the rate-limiting enzyme of catecholamine synthesis, TH, is believed to be approximately 88% saturated with its substrate, L-TYR (3). Previous experiments have demonstrated that the administration of additional L-TYR (20) to such neurons generally fails to significantly increase catecholamine synthesis and associated actions. On the other hand, when central neurons are made to fire frequently, as occurs in the brain stem during hypertension (2, 19, 26), in the retina when light-activated (7) or in the hypothalamus during stress (18), supplemental L-TYR via injection or in the diet has been shown to be effective in sustaining catecholamine synthesis and release.

In order for L-TYR to enter the brain, it must first traverse the blood-brain barrier (BBB). This is accomplished via the large neutral amino acid (LNAA) carrier system located within the BBB endothelium. The transport is mediated by a carrier protein with affinity for all of the LNAAs (e.g., branched chain amino acids, aromatic amino acids, methionine), and thus the uptake of a particular amino acid is dependent upon its concentration in relation to the sum of the other competing amino acids (15). Consequently, endogenous concentrations of L-TYR within the brain may be diminished during periods of prolonged catecholaminergic neuronal depolarization. It is under this premise that we have previously hypothesized that the mixed-acting sympathomimetics may be limited in their central nervous system actions/duration as a result of a decrease in L-TYR concentrations, thus resulting in a decreased synthesis of catecholamines (9).

Contrary to the situation in central catecholamine-containing neurons, in the periphery there appears to be available an abundant supply of L-TYR to neurons, since the uptake of this amino acid in noncentral tissues does not appear to be subject to competitive uptake with other LNAAs (1). Additionally, L-TYR concentrations in the periphery are normally 2-fold greater than those in the central nervous system (8). As a result, one would expect that moderate peripheral sympathomimetic-induced stimulation, as produced in the present study, would not be greatly influenced by an increase in L-TYR concentrations. On the other hand, the response to an intense activation of sympathetic neurons (e.g., via prolonged hemorrhage) might be expected to be influenced by the peripheral availability of L-TYR (5)

It is widely known that the gastrointestinal tract is sympathetically innervated, and, when stimulated, inhibition of gastric motility is observed. In our present study, we have confirmed (21,22) the ability of the mixed-acting sympathomimetics PPA, EPH and AMP to inhibit gastric transit by 22%, 13% and 11%, respectively, when compared to their SAL control. However, when coadministered with L-TYR, no potentiation was observed. Similarly, gastric retention, which was significantly increased by PPA, EPH and AMP, was not enhanced by L-TYR coadministration.

The induction of IBAT thermogenesis by PPA, EPH and AMP has been implicated as a possible mechanism by which they effect their associated weight loss (9, 10, 15,). As IBAT is sympathetically innervated, we extended our study to include the analysis of L-TYR administration with the above mixed-acting sympathomimetics. As indicated by our results, L-TYR failed to potentiate PPA, EPH and AMP, although, in both instances, IBAT thermogenesis was significantly increased by all three mixed-acting sympathomimetics ($p<0.05$).

The data from the experiments included in the present study demonstrate the inability of L-TYR to alter the peripherally mediated actions of the mixed-acting sympathomimetics. Experiments are currently in progress to determine if other peripheral actions (e.g., cardiovascular) of these agents are similarly unaffected by supplemental L-TYR. We conclude that the previously observed L-TYR potentiation of sympathomimetic-induced anorexia in hyperphagic rats most likely results from an action at a central locus.

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REFERENCES

1. Ablett, R. F.; MacMillan, M.; Sole, M. J.; Toal, C. B.; Anderson, G. H. Free tyrosine levels of rat brain and tissues with sympathetic innervation following administration of L-tyrosine in the presence and absence of large neutral amino acids. J. Nutr. 114:835–841; 1984.
2. Bossy, J.; Guidoux, R.; Milon, H.; Wurzner, H. P. Development

of hypertension in SH rats fed L-tyrosine supplemented diets. Z. Ernahrungswiss. 22:45–47; 1983.
3. Bridges, J. W.; Dent, J. G.; Johnson, P. The effects of some pharmacologically active amines on the rate of gastric emptying in rats. Life Sci. 18:97–108; 1976.
4. Chiel, H. H.; Wurtman, R. J. Suppression of amphetamine-induced hypothermia by the neutral amino acid valine. Psychopharmacol. Commun. 2:207–217; 1976.
5. Conlay, L. A.; Maher, T. J.; Wurtman, R. J. Tyrosine increases blood pressure in hypotensive rats. Science 212:559–560; 1981.
6. El Mestikawy, S.; Gozlan, H.; Glowinski, J.; Hamon, M. Characteristics of tyrosine hydroxylase activation by K^+-induced depolarization and/or forskolin in rat striatal slices. J. Neurochem. 45:173–184; 1985.
7. Gibson, C. J.; Watkins, C. J.; Wurtman, R. J. Tyrosine administration enhances dopamine synthesis and release in light-activated rat retina. J. Neural Transm. 56:153–160; 1983.
8. Glaeser, B. S.; Maher, T. J.; Wurtman, R. J. Changes in brain levels of acidic, basic, and neutral amino acids after consumption of single meals containing various proportions of protein. J. Neurochem. 41:1016–1021; 1983.
9. Hull, K. M.; Maher, T. J. L-Tyrosine potentiates the anorexia induced by mixed-acting sympathomimetic drugs in hyperphagic rats. J. Pharmacol. Exp. Ther. 255:403–409; 1990.
10. Kuczenski, R. Effects of catecholamine releasing agents on synaptosomal dopamine biosynthesis: Multiple pools of dopamine or multiple forms of tyrosine hydroxylase? Neuropharmacology 14:1–10; 1975.
11. Lee, E. H. Y.; Mandell, A. J. Relationships between drug-induced changes in tetrahydrobiopterin and biogenic amine concentrations in rat brain. J. Pharmacol. Exp. Ther. 234:141–146; 1985.
12. Levine, R. A.; Miller, L. P.; Lovenberg, W. Tetrahydrobiopterin in the striatum: Localization in dopamine nerve terminals and role in catecholamine synthesis. Science 214:919–921; 1981.
13. Milner, J. D.; Wurtman, R. J. Tyrosine availability determines stimulus-evoked dopamine release from rat striatal slices. Neurosci. Lett. 59:215–220; 1985.
14. Moya-Huff, F. A.; Kiritsy, P. J.; Maher, T. J. Cardiovascular differences between phenylpropanolamine and its related norephedrine isomers in the rat. J. Pharm. Sci. 76:114–116; 1987.
15. Pardridge, W. M.; Oldendorf, W. H. Kinetic analysis of blood-brain barrier transport of amino acids. Biochim. Biophys. Acta 401:128–136; 1975.
16. Pinto, J. M. B.; Maher, T. J. Tyrosine availability prevents tyramine-induced tachyphylaxis in the isolated rat heart. Neurochem. Int. 9:533–537; 1986.
17. Pober, Z.; Johnson, D. A.; Moya-Huff, F. A.; Maher, T. J. Effect of diazoxide and hydralazine on intestinal transit and blood pressure in the rat. Pharmacol. Toxicol. 64:51–54; 1989.
18. Reinstein, D. K.; Hendrik, L.; Scott, N. A.; Wurtman, R. J. Tyrosine prevents behavioral and neurochemical correlates of an acute stress in rats. Life Sci. 34:2225–2231; 1984.
19. Shalita, B.; Dikstein, S. Central tyrosine prevents hypertension in uninephrectomized DOCA-saline treated rats. Experientia 33:1430–1431; 1977.
20. Sved, A. F. Precursor control of the function of monoaminergic neurons. In: Wurtman, R. J.; Wurtman, J. J., eds. Nutrition and the brain. New York: Raven Press; 1983:223–275.
21. Tainter, M. L. Actions of benzedrine and propadrine in the control of obesity. J. Nutr. 27:89–105; 1944.
22. Wellman, P. J.; Arasteh, K.; Ruddle, J. L.; Strickland, M. D. Effects of phenylpropanolamine on gastric retention in the adult rat. Brain Res. Bull. 17:127–128; 1986.
23. Wellman, P. J. Influence of dl-phenylpropanolamine on brown adipose tissue thermogenesis in the adult rat. Physiol. Psychol. 12:307–310.
24. Wellman, P. J.; Marmon, M. M. Comparison of brown adipose tissue thermogenesis induced by congeners and isomers of phenyl-propanolamine. Life Sci. 37:1023–1028; 1985.
25. Wellman, P. J.; Marmon, M. M. Effects of reserpine and adrenal demedullation on brown adipose tissue thermogenesis induced by dl-phenylpropanolamine. Res. Commun. Chem. Pathol. Pharmacol. 47:211–220; 1985.
26. Yamori, Y. Fujiwara, M. Horie, K.; Lovenberg, W. The hypotensive effect of centrally administered tyrosine. Eur. J. Pharmacol. 68:201–204; 1980.