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Effects of L-Tyrosine on Mixed-Acting Sympathomimetic-Induced Pressor Actions

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HULL, K. M. AND T. J. MAHER. *Effects of* L-*tyrosine on mixed-acting sympathomimetic-induced pressor actions.* PHARMACOL BIOCHEM BEHAV **43**(4) 1047–1052, 1992.—We previously reported the ability of L-tyrosine (L-TYR) to potentiate the anorectic activity of various mixed-acting sympathomimetics including [*R*S**]-(±)-norephedrine [phenylpropanolamine (PPA)], [1*R*,2*S*]-(−)-ephedrine (EPH), and [*S*]-(+)-amphetamine (AMPH) in hyperphagic rats. Included in those studies was the attentuation of L-TYR's effect when coadministered with L-valine, a large neutral amino acid that competes with L-TYR for uptake into the brain, suggesting a central locus for the action of L-TYR. Additional studies demonstrated the inability of L-TYR to alter the peripherally mediated PPA-, EPH-, and AMPH-induced increases in gastrointestinal transit time and retention and intrascapular brown adipose tissue thermogenesis. Because the mixed-acting sympathomimetics are known to increase blood pressure, these studies examined the ability of L-TYR to influence the pressor responses to PPA, EPH, and AMPH (0.03–1 mg/kg) in urethane-anesthetized rats. Each of the mixed-acting sympathomimetics significantly increased mean arterial, systolic, and diastolic blood pressures when administered alone, but no potentiation by L-TYR was observed. These results demonstrate the inability of L-TYR to potentiate the peripheral vasopressor effects of PPA, EPH, and AMPH. These data, in conjunction with our previous findings, suggest that the potentiation by L-TYR of the mixed-acting sympathomimetics is largely restricted to centrally mediated responses.

Tyrosine Sympathomimetics Phenylpropanolamine Ephedrine Amphetamine Blood pressure

MIXED-acting sympathomimetics {e.g., [*R*S**]-(±)-norephedrine [phenylpropanolamine (PPA)], [1*R*,2*S*]-(−)-ephedrine (EPH), and [*S*]-(+)-amphetamine (AMPH)} derive at least a portion of their action by releasing newly synthesized, cytoplasmic, reserpine-insensitive pools of catecholamines (CAs) from presynaptic sites (15), as well as by inhibiting CA reuptake into presynaptic sites (2,13). One of these agents, AMPH, has been demonstrated to reduce tyrosine concentrations while increasing CA synthesis in dopamine-containing neurons (16). Our laboratory hypothesized that perhaps coadministration of the immediate amino acid precursor for the CAs, L-tyrosine (L-TYR), with the mixed-acting sympathomimetics might result in the potentiation of this drug class' responses, for example, suppression of food intake.

Soon after, we reported the ability of coadministered L-TYR to potentiate the anorectic activity of PPA, EPH, and AMPH in hyperphagic rats; L-TYR possessed no significant anorectic activity when administered alone (11). This effect was specific for L-TYR, as various other L-amino acids tested [alanine, aspartic acid, histidine, lysine, phenylalanine, threonine, tryptophan, valine (L-VAL)], as well as D-TYR, failed to mimic L-TYR. Other mixed-acting sympathomimetics—(1*S*,2*S*)-(+)-pseudoephedrine and (1*S*,2*S*)-(+)-norpseudoephedrine [(+)-NORP)]—but not direct-acting sympathomimetics [(+)-salbutamol and (+)-methoxyphenamine] were similarly potentiated by L-TYR. These data suggest the necessity for the presynaptic release of CAs by the anorexiant for L-TYR to potentiate such responses. In further support of this hypothesis, we demonstrated the ability of the competitive tyrosine hydroxylase (TH) inhibitor, α-methyl-para-tyrosine, to attenuate the previously observed potentiation by L-TYR. The L-TYR-mediated potentiation was time dependent and correlated with peak brain concentrations of L-TYR. Coadministration of the large neutral amino acid (LNAA) L-VAL concurrently with L-TYR and EPH negated the previously observed potentiation by L-TYR, presumably by competing with L-TYR for uptake into the brain (24).

The results of these experiments supported the hypothesis that as central CA-containing neurons increase the synthesis of CAs to replenish diminished stores, following the release and inhibition of reuptake caused by the mixed-acting sympathomimetics, endogenous levels of L-TYR available to the neuron may not be adequate to sustain enhanced rates of synthesis. In addition, these data also suggested that the potentiation by L-TYR occurred at a central loci.

Further experiments conducted in our laboratory were de-

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signed to examine the ability of L-TYR to influence several of the peripherally mediated actions that may also contribute to the observed mixed-acting sympathomimetic-induced effects, for example, gastrointestinal transit, gastric retention, and intrascapular brown adipose tissue (IBAT) thermogenesis (26, 27). In each case, the responses to PPA, EPH, and AMPH, were unaltered by L-TYR coadministration (12).

The observed dichotomy between the ability of L-TYR to potentiate the mixed-acting sympathomimetics under one set of circumstances and fail under another may best be explained by differences in the availability of L-TYR to the CA-synthesizing enzymes in their local environment. Peripheral concentrations of L-TYR are in general two-fold higher than those found in the CNS (1,9). Further, unlike central CA-containing neurons, those found in the periphery appear to have an abundant supply of L-TYR because the uptake of this amino acid in these noncentral tissues does not appear to be subject to competitive uptake with other LNAAs. However, during periods of intense challenge to homeostasis, the rate of CA synthesis in the periphery may be influenced by the concentration of L-TYR (23).

Conlay et al. demonstrated the ability of L-TYR to specifically enhance CA synthesis in rats made hypotensive by severe hemorrhage (3–5). Because this effect was abolished by adrenalectomy, it could be suggested that the peripheral synthesis of CAs (i.e., via the adrenal medulla) was influenced by the peripheral concentration of L-TYR during this time of physiological stress. Studies in cultured adrenal chromaffin cells further support the role of L-TYR at increasing CA synthesis (19). In contrast, when administered to spontaneously hypertensive rats L-TYR lowers blood pressure and increases the concentration of norepinephrine (NE) metabolites in the brainstem, suggesting that under these circumstances the amino acid affects CA-mediated physiological functions centrally (25,28).

In light of the involvement of L-TYR in blood pressure responses at peripheral and central loci, these studies were undertaken to determine if the acute pressor effects of PPA, EPH, and AMPH would be potentiated by pretreatment with L-TYR.

METHOD

Animals

Male, normotensive, Sprague-Dawley rats (N = 60; Charles River Breeding Laboratories, Wilmington, MA) were

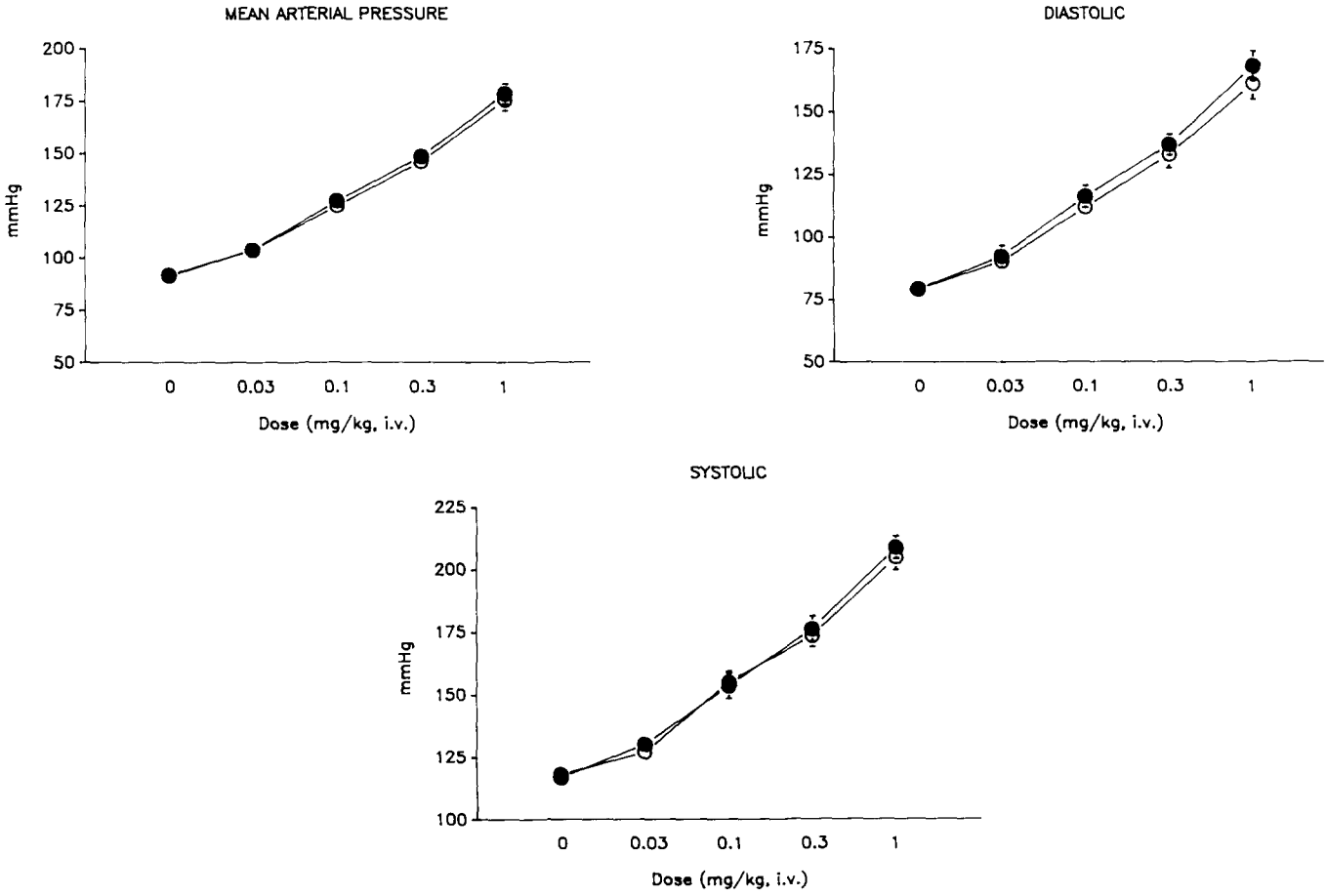

FIG. 1. Increases in blood pressure elicited by PPA with or without L-TYR. Rats weighing between 350–375 g were anesthetized using urethane (1.2 g/10 ml/kg) and their right carotid artery and left external jugular vein cannulated. Animals were allowed to equilibrate for 15 min prior to administration of L-TYR (200 mg/kg; ●-●) or SAL (1 ml/kg; ○-○). Sixty minutes later, animals received cumulative IV doses of PPA (0.03–1 mg/kg).

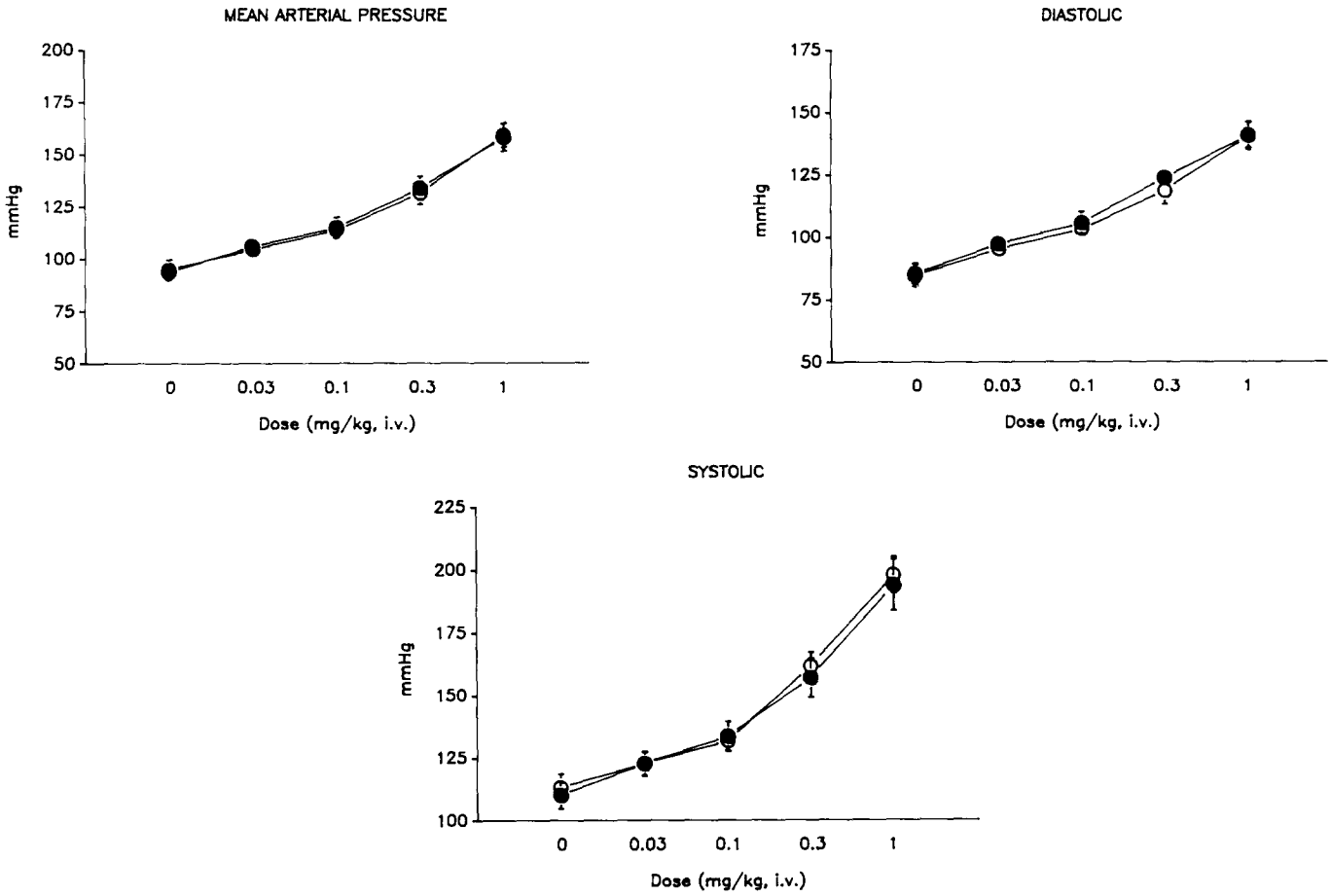

FIG. 2. Increases in blood pressure elicited by EPH with or without L-TYR. Rats weighing between 350–375 g were anesthetized using urethane (1.2 g/10 ml/kg) and their right carotid artery and left external jugular vein cannulated. Animals were allowed to equilibrate for 15 min prior to administration of L-TYR (200 mg/kg; ●-●) or SAL (1 ml/kg; ○-○). Sixty minutes later, animals received cumulative IV doses of EPH (0.03–1 mg/kg).

obtained at 350–375 g and housed in pairs in suspended wire mesh cages with food (Purina #5001; 25% protein, 56% carbohydrate, and 5% fat) and water available ad lib. Animals were acclimated to our facility for at least 1 week prior to experimentation. All experimental protocols were approved by our Institutional Animal Use and Care Committee prior to commencement of the studies.

Drugs

PPA HCl (Roehr, Long Island City, NY), EPH HCl (Roehr), and AMPH SO_4 (Amend, Irvington, NJ) were injected IV in a volume of 500 μl/kg. L-TYR (Ajinomoto, Tokyo, Japan) was injected IP in a volume of 1 ml/kg.

Direct Blood Pressure Measurements

On the day of the experiment, animals were anesthetized with an IP injection of urethane (1.2 g/10 ml/kg). The right carotid artery and left external jugular vein were cannulated using PE-50 tubing and blood pressure continually recorded via a pressure transducer coupled to a polygraph (Grass Instruments, Quincy, MA). Animals were allowed to equilibrate 15 min prior to administration of IP L-TYR [200 mg/kg, a dose known to increase both peripheral and central TYR concentrations (11,12)] or saline (SAL, 1 ml/kg). Sixty minutes later, animals received cumulative doses of PPA, EPH, and AMPH (0.03, 0.1, 0.3, or 1 mg/kg) IV via the jugular cannulae. Prior to the first dose of drug, animals received an equal volume of SAL (500 μl/kg). Each drug group, with or without L-TYR, contained 10 animals. Blood pressure was quantitated to the nearest 1 mm Hg and values presented represent the mean ± SEM.

Statistics

Individual treatment groups (i.e., PPA, EPH, AMPH, and SAL) coadministered with L-TYR were directly compared with their respective SAL controls utilizing analysis of variance (ANOVA). The minimum significance level was set at $p < 0.05$.

RESULTS

Starting mean arterial blood pressure (MAP) for SAL-pretreated control animals (n = 30) was 93 ± 3 mm Hg. Starting MAP in animals (n = 30) pretreated with L-TYR (200 mg/kg) was not significantly different from control ani-

mals (92 ± 3 mm Hg). Starting diastolic (DIA) and systolic (SYS) blood pressures were 82 ± 4 and 115 ± 4 mm Hg for control animals, respectively, and 82 ± 3 and 112 ± 4 mm Hg for animals pretreated with L-TYR (200 mg/kg), respectively, (n.s., $p > 0.05$).

Administration of the 0-mg/kg dose (SAL) failed to significantly alter blood pressure (MAP, DIA, or SYS; <1 mm Hg change). Cumulative IV doses of PPA (0.03–1 mg/kg) in control animals significantly increased MAP, DIA, and SYS blood pressures ($p < 0.05$; Fig. 1). The maximum increases in MAP were 64 ± 5 mm Hg over baseline, while DIA and SYS increased 56 ± 5 and 85 ± 6 mm Hg, respectively. L-TYR pretreatment failed to significantly alter the cumulative dose-response curves to PPA (Fig. 1).

Cumulative IV doses of EPH (0.03–1 mg/kg) in control animals significantly increased MAP, DIA, and SYS blood pressures ($p < 0.05$; Fig. 2). The maximum increases in MAP were 85 ± 3 mm Hg over baseline, while DIA and SYS increased 82 ± 4 and 87 ± 4 mm Hg, respectively. L-TYR pretreatment similarly failed to significantly alter the EPH cumulative dose-response curves (Fig. 2).

Administration of cumulative IV doses of AMPH (0.03–1 mg/kg) in control animals resulted in a significant increase in MAP, DIA, and SYS blood pressures ($p < 0.05$; Fig. 3). The maximum increases in MAP were 59 ± 4 mm Hg over baseline, while DIA and SYS increased 60 ± 4 and 46 ± 3 mm Hg, respectively. Again, pretreatment with L-TYR failed to significantly alter the cumulative dose-response curves to AMPH (Fig. 3).

DISCUSSION

The CAs NE and epinephrine (EPI) are capable of altering blood pressure via actions at both central and peripheral loci. Released within the brainstem, NE typically results in a decrease of blood pressure, while NE and EPI released from sympathetic nerves or the adrenal medulla typically increase blood pressure. Under quiescent conditions, CA synthesis is believed to be regulated by TH, itself dependent upon the availability of its cofactor, tetrahydrobiopterin (17), and influenced by end-product inhibition (8). However, when CA-containing cells are made to fire rapidly, such as occurs during hypertension or hemorrhagic shock, the rate-limiting enzyme of CA synthesis, TH, is believed to undergo a depolarization-dependent phosphorylation that renders the enzyme responsive to its substrate L-TYR. Thus, by increasing the availabil-

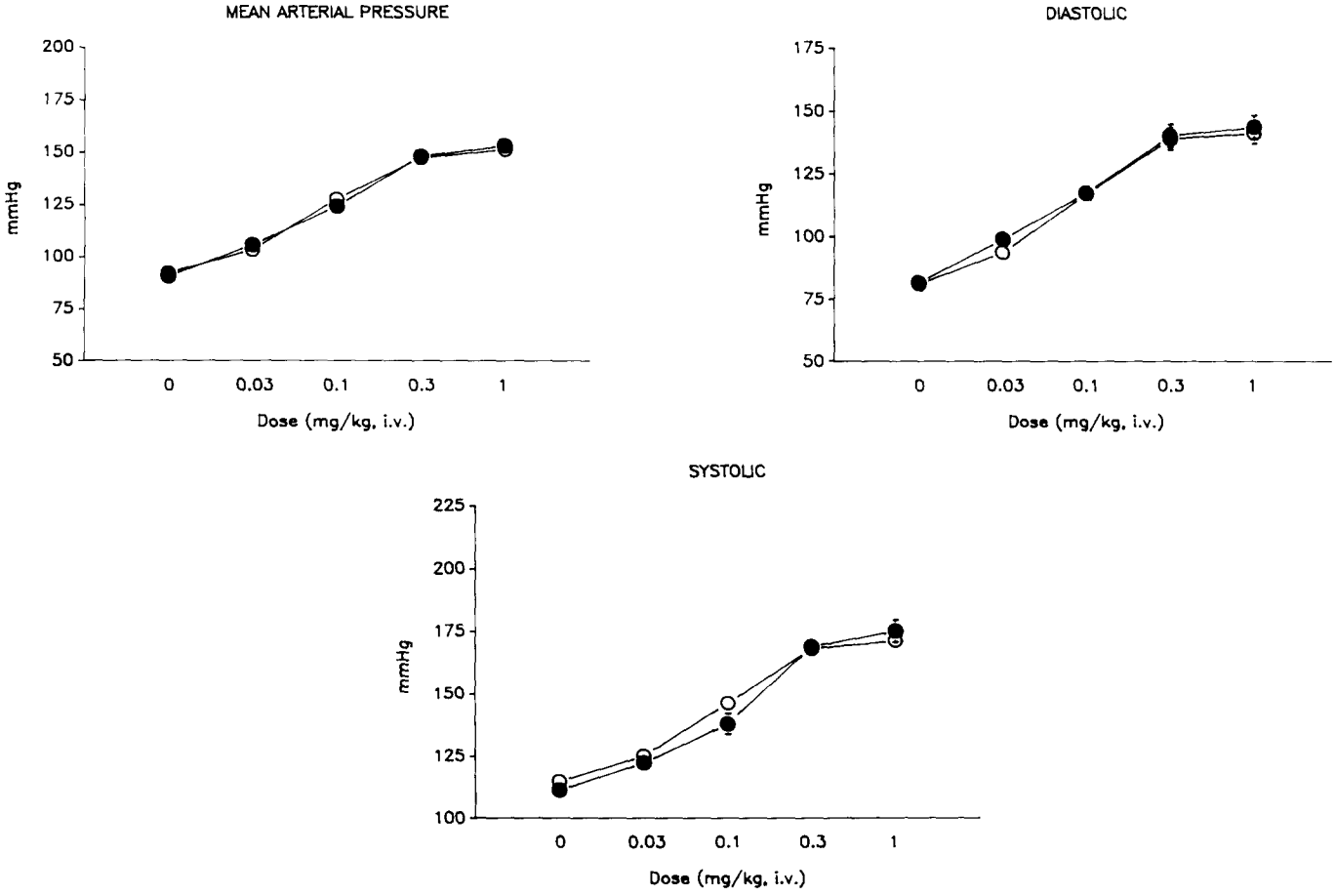

FIG. 3. Increases in blood pressure elicited by AMPH with or without L-TYR. Rats weighing between 350–375 g were anesthetized using urethane (1.2 g/10 ml/kg) and their right carotid artery and left external jugular vein cannulated. Animals were allowed to equilibrate for 15 min prior to administration of L-TYR (200 mg/kg; ●-●) or SAL (1 ml/kg; ○-○). Sixty minutes later, animals received cumulative IV doses of AMPH (0.03–1 mg/kg).

ity of L-TYR to the rapidly firing CA-containing cell, CA synthesis is enhanced. In both hemodynamic paradigms described above, L-TYR administration is capable of increasing the synthesis and release of CAs, thus contributing to the attempt by the animal to normalize blood pressure.

Previous reports in the literature describe severe hypertension associated with the ingestion of an over-the-counter anorectic preparation called Trimolets® (10), which was alleged to contain one of the agents utilized in this study, PPA. PPA is a racemate consisting of equal proportions of the two enantiomers of norephedrine (NOR). Our laboratory previously compared the cardiovascular activities of both enantiomers of each stereoisomer (14,20–22) and found PPA and its component enantiomer, (-)-NOR, to increase blood pressure largely via peripheral α_1-adrenoceptor activation. In addition, (+)-NORP increased blood pressure only slightly, and then most likely via the release of presynaptic CAs from peripheral sympathetic nerves (20). Behavioral testing (open-field locomotion), as expected, demonstrated a stimulatory effect for (+)-NORP and AMPH; PPA was without stimulatory actions (7). In addition, the (-)-NOR enantiomer of PPA was more potent than (+)-NOR as an anorexiant; (+)-NORP was intermediate in action (6,7). Together, the data from those experiments demonstrate the differences between the stereoisomers NOR and NORP, as well as the differences between the enantiomers of PPA, (-)- and (+)-NOR. In light of those findings, we previously suggested that the active ingredient in Trimolets was not PPA but rather one of its enantiomeric components, (-)-NOR; others suggest (+)-NORP (18).

These data presented here demonstrate the inability of L-TYR to potentiate the vasopressor effects of the mixed-acting sympathomimetics, PPA, EPH, and AMPH. Thus, any increase in TH activity that could render CA synthesis dependent upon L-TYR would most likely not be limited by this substrate's availability to the enzyme because previous experiments demonstrated peripheral TYR concentrations to be approximately two-fold greater than those in the CNS (1). Further, the apparent lack of competitive uptake of L-TYR with other LNAAs distinguishes the influence of L-TYR upon peripherally mediated cardiovascular responses vs. those that are mediated centrally.

In addition to our previous studies, this work suggests that coadministration of L-TYR with the mixed-acting sympathomimetics may constitute a beneficial therapeutic combination when used in the control of appetite. These combinations could allow for a reduction in the dose of the anorectic agent while maintaining efficacy and decreasing the potential for adverse affects associated with high doses of mixed-acting sympathomimetics. Further studies are required to examine the interactions between L-TYR and the mixed-acting sympathomimetics in humans.

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