Life Sciences, Vol. 30, pp. 739-746
Printed in the U.S.A.

Pergamon Pres

EFFECT OF ACUTE ADMINISTRATION OF LARGE NEUTRAL AND OTHER AMINO ACIDS ON URINARY EXCRETION OF CATECHOLAMINES[1,2]

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(Received in final form December 21, 1981)

Summary

We examined the specificity of tyrosine's ability to increase catecholamine excretion by rats. Tyrosine alone among amino acids tested caused major increases in tissue and serum tyrosine, as well as urinary catecholamine levels. Large neutral amino acids (tryptophan, valine or isoleucine) and representatives of other classes of amino acids (glutamate, alanine, lysine or arginine) were unable to mimic tyrosine's action.

In the brain, the initial step in catecholamine biosynthesis--the uptake of tyrosine into the synthesizing tissues--is mediated by transport systems located within the blood-brain barrier (1) and nerve terminals (2). The blood-brain barrier system utilized by tyrosine is shared with such other large neutral amino acids (LNAA) as valine, leucine, isoleucine and tryptophan. At usual plasma amino acid concentrations, the uptake of tyrosine and other LNAA is competitive. Thus, tyrosine's uptake depends not only on its plasma concentration, but also inversely on the concentration of the competing LNAA (3). The calculated ratio of the tyrosine concentration to the sum of the concentrations of tryptophan, phenylalanine, and the branched-chain amino acids (leucine, valine and isoleucine) has been shown to be a sensitive predictor of brain tyrosine levels and thus of brain catechol synthesis (4,5).

In previous studies, we showed that tyrosine administration to rats (6) or humans (7) increases the output of catecholamines into urine. This effect was abolished in rats by pretreatment with carbidopa, a peripheral decarboxylase inhibitor that impairs catecholamine synthesis (8). This suggested that the effect resulted from enhanced catecholamine synthesis, perhaps due to increased substrate saturation of tyrosine hydroxylase, rather than to release of stored catecholamine. The present study was designed to characterize the specificity of tyrosine's effect on urinary catecholamines, determine the influence of other LNAA on the response to tyrosine, and explore the effect of the amino acids on catecholamines in peripheral tissues.

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[1] These studies were supported by grants from the National Institutes of Health and the National Aeronautics and Space Administration. Dr. Agharanya received a fellowship from the University of Nigeria.
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0024-3205/82/090739-08$03.00/0

Materials and Methods

L-tyrosine methyl ester hydrochloride (Aldrich Chemical Co. Inc., Cedar Knolls, NJ) (equivalent to free L-tyrosine) was dissolved in 0.9% saline and adjusted to pH 6.1. The other amino acids were prepared in concentrations equimolar to that of the free tyrosine and adjusted to pH 6.1. A suspension of tryptophan and other insoluble amino acids was made in saline and stirred constantly until the time of administration. All injections were made intraperitoneally (i.p.) in 0.5 ml volumes per rat (tryptophan was given in 1 ml volume).

The experiment used male Sprague Dawley rats (250 g; Charles River Laboratories, Wilmington, MA), housed and habituated in our animal facilities for at least 4 days before use. During this period, animals were allowed free access to regular laboratory chow (Charles River Rat-Mouse-Hamster Maintenance Formula) and water, and exposed to light (300 µW/cm; Vita Lite, Duro-Test Corp., Bergen, NJ) from 8 AM to 8 PM daily.

Rats were fasted overnight but allowed water to drink, then injected i.p. with tyrosine (200 mg/kg) or an equimolar concentration of another amino acid (tryptophan, valine, isoleucine, glutamate, alanine, lysine and arginine). Subsequently, 7 ml of water was given via stomach tube to promote diuresis. The animals were then placed in individual metabolic cages and urine was collected for 3 h. At the end of this interval they were decapitated and blood from the cervical wound was collected in centrifuge tubes placed on ice. Adrenals, heart and brain were quickly dissected free of surrounding tissues, and traces of blood were blotted out with soft absorbent gauze. The tissues were immediately placed on dry ice and later weighed and stored at -70°C until analyzed. The urines were preserved in 6 M HCl containing 5% sodium metabisulphite (bringing their pH from about 6.1 to 2.2) and stored at -20°C until analyzed.

Assay of urinary catecholamines. Preliminary purification of the acidified urine was carried out with one-tenth volume of 4 M perchloric acid, which precipitated the proteins. The urine was centrifuged at 25,000 x g for 10 min, the supernatant fluid transferred to glass vials, and an aliquot removed for creatinine assay. To the remaining supernatant fluid we added 200 µl of 10% EDTA and 10% sodium metabisulphite; the whole solution was diluted with 5 ml of 0.5 M tris buffer (pH 8.6) and then adjusted to pH 8.6. Samples were passed over prewashed alumina (400 mg) in glass columns. Catecholamines were extracted as described by Anton and Sayre (9), and assayed fluorimetrically by trihydroxyindole reaction. Dopamine was measured using the iodine oxidation procedure at pH 5.3 (10) and norepinephrine and epinephrine determined separately at pH 6.5 and 2.5 respectively (11).

Urinary creatinine was determined by a modification of the Jaffe reaction. Urines were diluted 10 times with water and the color developed with 4 ml alkaline picrate reagent (containing 5.5 ml of 2.5 N NaOH, 27.5 ml saturated aqueous picric acid in 100 ml solution). After standing for 20-25 min at room temperature, the resulting colored solution was measured at 520 nm.

Assay of serum and tissue tyrosine. Adrenals, heart and brain were homogenized in 10 vol of 0.4 M perchloric acid and centrifuged, and 1 ml of the resulting supernatant fluid was diluted with an equal volume of 12% trichloroacetic acid (TCA). Their tyrosine contents were then determined fluorometrically (12). Sera were separated from blood samples by centrifugation; the proteins were precipitated from 0.3 ml samples using 2.7 ml of 6% TCA. After centrifugation we determined the tyrosine contents of 2 ml of the resulting supernatant fluid

Amino acid analysis. Amino acid analysis was carried out using a Beckman model 119C Autoanalyzer (Beckman Instruments, Fullerton, CA). Serum proteins were precipitated with sulphosalicylic acid (50 mg/ml serum). After centrifugation, the supernatant fluids were filtered through a millipore filter (pore size 0.45 μm) before injection into the analyzer.

Statistical analysis. Results were analyzed by one-way analysis of variance and by Student's t test and presented as mean ± standard error of the mean (SEM).

Results

Tyrosine administration caused, after 3 h, highly significant increases in serum tyrosine and in tyrosine concentrations within the heart, adrenals and brain (Table I). Greatest proportionate increases were observed in the brain, where tyrosine rose more than twofold. Administration of valine or isoleucine alone significantly lowered serum, heart and brain tyrosine levels but failed to depress tyrosine levels in the adrenals. Tryptophan administered alone lowered serum tyrosine but failed to depress tyrosine concentrations in any of the tissues examined. Coadministration of valine or isoleucine with tyrosine suppressed the tyrosine-induced increases in serum tyrosine, as well as the tyrosine concentrations of the two tissues examined (adrenals and brain). In contrast, coadministration of tryptophan failed to block the tyrosine-induced elevations in tyrosine concentrations of serum or any of the tissues studied.

TABLE I

Effect of Administration of Large Neutral Amino Acids on Serum and Tissue Tyrosine Levels

	Control	TYR	VAL	TYR + VAL	ISOLEU	TYR + ISOLEU	TRP	TYR + TRP
Serum (μg/ml)	21.00 ±.83	37.31* ±1.82	16.92** ±.63	25.48† ±2.67	13.11* ±.83	30.83§ ±2.60	17.61** ±.43	45.06 ±3.31
Adrenal (μg/pair)	1.83 ±.13	2.79* ±.22	1.65 ±.23	2.08§ ±.19	1.33 ±.13	1.81‡ ±.23	1.68 ±.12	3.49 ±.22
Heart (μg/g)	25.41 ±1.07	35.09 ±2.00	19.79** ±.98	- -	21.22*** ±.94	- -	24.11 ±.81	39.79 ±2.90
Brain (μg/g)	13.53 ±.64	29.97* ±1.93	10.77* ±.33	17.26† ±.50	10.11* ±.50	17.27† ±1.90	15.41 ±.65	32.58 ±2.10

Groups of rats (n = 6) received tyrosine (TYR) (200 mg/kg), equivalent amounts of the other amino acids [valine (VAL), isoleucine (ISOLEU), or tryptophan (TRP)], or tyrosine concurrently with another amino acid. Animals were killed 3 h after injection. Control rats received saline.

* $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$, differs from control.
† $p < 0.001$; ‡ $p < 0.01$; § $p < 0.05$, differs from tyrosine alone.

TABLE II

Serum Tyrosine Levels and Tyrosine Ratios following Administration of Competing Amino Acids

Amino Acid	Serum TYR (nmoles/ml)	∑ LNAA (nmoles/ml)	Tyrosine Ratio (TYR/∑LNAA)
Control	98 ± 12.8	598 ± 87.3	0.17 ± .01
TYR	173 ± 13.9*	555 ± 38.5	0.31 ± .02*
VAL	68 ± 6.9**	814 ± 64.6	0.08 ± .00*
TYR + VAL	98 ± 7.7	755 ± 46.5	0.13 ± .01†
ISOLEU	54 ± 5.0*	649 ± 48.1	0.09 ± .01*
TYR + ISOLEU	127 ± 14.1	661 ± 47.1	0.21 ± .02‡
TRP	69 ± 3.5**	654 ± 50.6	0.11 ± .01*
TYR + TRP	185 ± 29.1	645 ± 49.3	0.29 ± .03

Groups of six rats were injected with tyrosine (200 mg/kg), an equivalent concentration of the other LNAA or saline. Sera were analyzed for their amino acid content. Results are presented as mean ± SEM.

* $p < .001$; ** $p < .01$, differs from control.
† $p < .001$; ‡ $p < .01$, differs from tyrosine alone.

As expected, tyrosine administered alone raised the serum tyrosine ratio (TYR/ΣLNAA), while valine, isoleucine and tryptophan significantly decreased it (Table II). These amino acids tended both to raise the sum of the competin LNAA and, consequently, to depress the serum tyrosine concentration. Strong linear correlations were thus observed between serum or tissue tyrosine levels and either the serum tyrosine ratio or simply the tyrosine concentration (Table III).

Tyrosine administration also increased urinary dopamine, norepinephrine ar epinephrine levels [Table IV, as noted previously (6)]. The other LNAA all tended to reduce urinary catecholamine levels, but the only significant decrea --in urinary epinephrine--was observed after isoleucine administration. Co-administration of valine or isoleucine with tyrosine significantly suppressed the tyrosine-induced increases in urinary norepinephrine and epinephrine, wit out significantly affecting the rise in dopamine. Coadministration of tryptophan with tyrosine significantly diminished tyrosine-induced increases in urinary norepinephrine. None of the LNAA treatments significantly modified tissue catecholamine levels.

Administration of amino acids not in the LNAA group (glutamate, alanine, lysine or arginine) to rats failed to affect serum or tissue tyrosine levels, or to block the increases in these levels caused by tyrosine administration (Table V). Arginine and lysine both elevated urinary norepinephrine levels; glutamate and alanine caused no effect.

TABLE III

Correlation of Tissue Tyrosine Levels with Serum Tyrosine and Tyrosine Ratios

Tissue	Correlation Coefficient (r)	
	Tissue TYR vs Serum TYR	Tissue TYR vs TYR Ratio
Serum	-	0.94 (44)
Adrenal	0.82	0.81 (44)
Heart	0.95	0.96 (36)
Brain	0.90	0.94 (44)

Animals were treated as described in Table II. Correlation coefficients were calculated from individual tissues, the number of which is denoted in parenthesis.

Discussion

These findings confirm our previous observations that tyrosine administration to rats raises urinary catecholamines (Table IV) and demonstrate that this effect is not mimicked by related LNAA such as valine or isoleucine or by amino acids in other classes (e.g., glutamate or alanine). The basic amino acids--lysine and arginine--caused some elevation of urinary catecholamines (Table V). The reason for this effect is not clear, but may involve insulin secretion (13). The lack of correlation between serum tyrosine and urinary catecholamines following amino acid administration suggests that the amino acids are not acting solely by providing more substrate for peripheral catecholamine synthesis. The amino acids may have caused severe toxic efffects that led to catecholamine release; however, evidence for such toxicity was not observed.

When tyrosine is administered together with such other LNAA as valine or isoleucine, the elevation in urinary catecholamines is markedly suppressed. Tryptophan is an exception in this regard, since it fails to block tyrosine's effect on serum and tissue tyrosine, or on urinary dopamine or epinephrine (Tables I and IV). With regard to serum and tissue tyrosine levels, coadministration of tryptophan tends to raise these over and above the increases observed after tyrosine alone (Table I).

Strong linear correlations were observed between tissue or serum tyrosine levels and either the serum tyrosine concentration or the tyrosine ratio (Table III). Treatments which raised serum tyrosine (e.g., tyrosine administration) also raised the tyrosine ratio, whereas treatments which depressed the tyrosine concentration (e.g., administration of competing amino acids) also lowered the ratio and tended to raise LNAA concentrations. Hence, our data cannot be used to determine whether tyrosine levels in peripheral sympathoadrenal cells such as cardiac and chromaffin tissues, depend on serum tyrosine alone or on the competition between tyrosine and other large neutral amino acids.

TABLE IV

Effect of Administration of Large Neutral Amino Acids on Tissue and Urinary Catecholamine Levels

	Control	TYR	VAL	TYR + VAL	ISOLEU	TYR + ISOLEU	TRP	TYR + TRP
Urinary DA (ng/mg creat.)	237.88 ± 20.05	522.89* ± 36.98	188.81 ± 17.28	416.71 32.46	209.35 ±13.95	428.53 ±24.01	204.42 ±11.81	639.92 ±52.86
Urinary NE (ng/mg creat.)	99.54 ± 5.22	240.49* ± 35.05	79.01 ±10.36	125.94† ±12.05	91.43 ± 8.74	104.39† ±4.38	84.27 ± 7.56	113.64† ±9.69
Urinary E (ng/mg creat.)	57.15 ± 4.03	177.45* ± 14.07	44.90 ± 8.68	76.83† ± 11.24	40.04** ± 6.79	64.54† ± 3.60	50.56 ± 3.34	163.33 ± 22.81
Adrenal NE (µg/pair)	3.09 ± .14	3.59 ± .31	3.68 ± .53	4.35 ±.41	3.73 ± .48	3.96 ± .39	3.43 ± .53	3.46 ± .52
Adrenal E (µg/pair)	15.90 ± 2.14	16.09 ± 1.13	17.40 ± 1.55	20.51 ± 1.27	19.35 ± 1.52	19.06 ± .83	16.97 ± 1.63	15.46 ± 1.35
Heart NE (µg/g)	475.07 ± 42.88	451.60 ± 40.72	442.77 ± 52.62	-- --	507.23 ± 50.07	-- --	556.47 ± 47.67	451.93 ± 43.04
Brain NE (ng/g)	189.40 ± 38.83	248.22 ± 38.66	239.37 ± 29.73	202.84 ± 27.41	222.92 ± 32.55	219.90 ± 20.20	214.40 ± 45.32	191.69 ± 53.42
Brain DA (ng/g)	616.60 ± 64.06	805.00 ± 27.44	693.88 ± 69.52	693.15 ± 77.93	701.76 ± 53.22	-- --	706.99 ± 56.31	675.51 ± 86.67

NE = norepinephrine; E = epinephrine; DA = dopamine; creat. = creatinine.

Animals were treated as described in Table I. Urine was collected for 3 h.

* $p < 0.001$; ** $p < 0.05$, differs from control.
† $p < 0.001$, differs from tyrosine alone.

TABLE V

Effect of Tyrosine and Other Amino Acids on Tissue Tyrosine and Urinary Catecholamine Levels

	Control	TYR	GLU	ALA	ARG	LYS	LYS + TYR
Serum TYR	11.90	20.95*	10.13	11.73	11.20	12.63	21.25
(µg/ml/100 g B.W.)	± .82	± 1.50	± .96	± .99	± .30	± .76	± 1.20
Urinary DA	241.28	562.04*	209.65	194.96	312.37	304.97	705.70
(ng/mg creat.)	± 17.89	± 74.63	± 23.43	± 26.03	± 28.57	± 24.89	± 34.00
Urinary NE	102.26	176.55*	102.23	93.99	162.77**	155.52**	226.03
(ng/mg creat.)	± 6.31	± 12.96	± 9.08	± 10.72	± 14.00	± 12.29	± 26.64
Brain TYR	8.75	13.30	8.20	9.80	8.50	9.65	14.95
(µg/g/100 g B.W.)	± .40	± 1.05	± .65	± .25	± .30	± .40	± .65

Groups of 6 rats weighing 110-195 g were injected i.p. with tyrosine (200 mg/kg) or the other amino acids at equimolar concentration with tyrosine. Control animals received saline. Urine was collected for 3 h after which the animals were sacrificed.

* $p < .01$; ** $p < .001$, differs from control.

It is interesting that tissue catecholamine levels remained relatively unchanged despite significant increases in tyrosine concentrations, following tyrosine loading. This is consistent with the original observations of von Euler and Hellner-Bjorkman (14) that neurotransmitter release from nerve terminals occurring during nerve stimulation is accompanied by rapid resynthesis, so that a constant steady-state transmitter level is maintained. We have observed that when the control of catecholamine synthesis is disturbed, (e.g., by blocking the biosynthetic pathway with carbidopa), the increase in urinary catecholamine after tyrosine loading is abolished (6).

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