Changes in catecholamine excretion after short-term tyrosine ingestion in normally fed human subjects

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ABSTRACT The effects of ingesting the aromatic amino acid L-tyrosine on excretion of unconjugated catecholamines (dopamine, norepinephrine, and epinephrine) and tyrosine were studied. (Tyrosine is the circulating precursor for the catecholamines, but only a small fraction of the tyrosine in the body is utilized for catecholamine synthesis.) In 10 of 11 normal volunteer subjects, ingestion of 100 mg/kg tyrosine (in three divided doses, preceding each meal, between 8 AM and 5 PM) for 1 day increased the 24-h excretions of total catecholamines by 25%. Only 0.42% of the tyrosine dose was excreted unchanged, but this was sufficient to increase urinary tyrosine by 138%. Both tyrosine and catecholamine excretions varied diurnally: 60% or more of the total output occurred during the day. Since urinary catecholamines reflect molecules synthesized outside the central nervous system, these findings indicate that tyrosine administration can accelerate catecholamine synthesis in the human sympathoadrenal system, probably by enhancing saturation of tyrosine hydroxylase. Therefore, tyrosine may be useful therapeutically in diseases characterized by peripheral catecholamine deficiencies.

KEY WORDS: Tyrosine ingestion, normal diets, catecholamine excretion in man

Catecholamine biosynthesis is initiated by uptake of circulating tyrosine (and, perhaps phenylalanine (1)) into neurons or chromaffin cells (2) and its conversion to DOPA by the enzyme tyrosine hydroxylase (3). Circulating tyrosine can derive from dietary amino acids, hydroxylation of dietary phenylalanine, or tissue protein breakdown; its uptake into brain varies directly with plasma tyrosine levels and inversely with plasma concentrations of other neutral amino acids carried into the central nervous system by a common blood-brain barrier transport system (4, 5). The rate at which tyrosine is converted to DOPA probably is influenced, in particular tissues, by allosteric activation of tyrosine hydroxylase (6), changes in the amount of enzyme protein (7), short-term variations in the extent to which the enzyme is subject to end-product inhibition (8), and changes in the enzyme's saturation with tyrosine, its amino acid substrate (9).

Studies in our laboratory have shown that synthesis rates of such neurotransmitters as acetylcholine, serotonin, dopamine, and norepinephrine in rat brain can be influenced greatly by brain levels of their circulating precursors: choline, tryptophan, and tyrosine (10). Studies with choline (11) and tryptophan (12), extended to humans, have provided the basis for new attempts at using neurotransmitter precursors to treat such disorders as tardive dyskinesia (13), Huntington's chorea (14), Alzheimer's disease (15), depression (16), and insomnia (17). In brains of experimental animals, exogenous tyrosine increases the DOPA accumulation after decarboxylase inhibition (9, 18), the accumulation of the dopamine metabolite homovanillic acid (HVA) in animals given a dopamine-receptor blocker (19), and the level of the norepinephrine metabolite MOPEG- SO4 (20). Tyrosine might similarly enhance brain catecholamine synthesis in humans, as sug-

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gested by the finding (21) that tyrosine administration raises both serum tyrosine levels and the ratio of serum tyrosine to the sum of the other large neutral amino acids (which compete with it for brain uptake (22)). If so, tyrosine, given alone or as part of an amino acid mixture, might be useful in treating brain disorders caused by deficient catecholaminergic transmission. The present study examines the possibility that tyrosine, taken with regular meals, might also enhance catecholamine synthesis in and release from peripheral cells (i.e., sympathetic neurons and adrenal chromaffin cells) and thus elevate urinary catecholamine levels.

Materials and methods

Subjects
Eleven healthy male Massachusetts Institute of Technology (MIT) students, aged 18 to 21, volunteered to participate in the study, according to the protocol approved by the MIT Subcommittee on the Use of Humans as Experimental Subjects. Before admission and throughout the study period, participants abstained from tea, coffee, vitamins, and drugs that interfere with catecholamine metabolism or catecholamine fluorescence (23); they also avoided stressful conditions that activate catecholamine release (24).

All subjects were admitted to the MIT Clinical Research Center at 7 AM after an overnight fast. On day 1 of the study, subjects ingested three equal portions of a specially prepared "house diet" at 8 AM, 12 noon, and 5 PM; each meal contained 38 g protein, 112 g carbohydrate, and 44 g fat. On day 2, the participants consumed, in addition to the "house diet", an aqueous suspension of L-tyrosine (100 mg/kg body weight) in three equally divided doses (i.e., 33 mg/kg) 1 h before each meal, thus consuming a total of 6.0 to 8.5 g/day of free tyrosine. (We have previously observed (25) that the daily rhythm in plasma tyrosine levels associated with consumption of this diet is the same after 2 days as after 1 day.) Pulse rates and blood pressure were monitored in all subjects throughout the day and night; these were not affected by the exogenous tyrosine.

Urinary catecholamines
Twelve-hour urine samples (7 AM to 7 PM and 7 PM to 7 AM) were collected on both study days. Data were analyzed using paired t test and are given as means ± SEM, unless otherwise indicated.

Urinary tyrosine
Urinary tyrosine was extracted from the perchloric acid supernatants obtained from 10 ml acidified urine, by the method of Kehr et al. (30). The samples were diluted in 5 ml glycine buffer (pH 2.0) and the mixture adjusted to the same pH. Tyrosine was separated on a prewashed cation exchanger (Dowex grade 50 WX4, Na⁺ form), using a 4-cm column. The column was washed with 10 ml of 60% methanol to remove 5-hydroxyindole acetic acid and related interfering substances. After further washes with water and citrate phosphate buffer (pH 2.5), tyrosine was eluted with 3 ml of sodium citrate buffer (pH 4.5). Two hundred microliters of eluate were diluted to a volume of 2.0 ml with 6% trichloroacetic acid and analyzed fluorometrically by the method of Waalkes and Udenfriend (31).

Results

Urinary tyrosine
Urinary tyrosine exhibited a diurnal variation on both study days, peaking between 7 AM and 7 PM (Fig. 1) and paralleling the

FIG. 1. Diurnal rhythms in urinary excretion of tyrosine and catecholamines in normal humans before and after tyrosine ingestion. Subjects consumed a high-protein diet (38 g in each of three meals at 8 AM, 12 noon, and 5 PM on day 1; on day 2 they ingested, in addition, 33 mg/kg free tyrosine 1 h before each meal. Twelve-hour urine samples (7 AM to 7 PM and 7 PM to 7 AM) were collected on both days. Data are presented as total excretion per 12 h (means ± SEM).
fluctuations in urine volume. Daytime values in individual subjects ranged between 120 and 220% of nighttime values. Mean daytime values were 12.4 ± 4.1 on day 1 and 31.9 ± 8.7 mg/12 h on day 2 (mean ± SD), while nighttime values were 8.8 ± 3.3 and 18.9 ± 5.3 mg/12 h.

On day 1 of the study, when the subjects consumed only regular meals, urinary tyrosine levels averaged 21.1 ± 6.3 mg/24 h (or 23.0 ± 10.0 µg/mg creatinine), ranging between 8.8 and 31.2 mg/24 h (Table 1; Fig. 2). On day 2, when the subjects ingested regular meals plus free tyrosine, urinary tyrosine averaged 50.3 ± 12.7 mg/24 h excretion (or 46.5 ± 21.5 µg/mg creatinine; mean ± SD), representing an average increase of 138% (p < 0.001) over levels noted on day 1. The percentages of ingested tyrosine that were excreted unchanged ranged between 0.21 and 0.65, with a mean of 0.42%; 60% of this amount was excreted during the daytime. One subject showed no increase in tyrosine excretion after ingesting 7 g of the amino acid; as described below, he also failed to exhibit tyrosine-induced elevations in urinary catecholamines.

Urinary catecholamines

Urinary excretion of catecholamines, like that of tyrosine, exhibited diurnal variations (Fig. 1); this time-dependence was maintained on the day of tyrosine administration. The greatest increase in urinary NE after tyrosine administration occurred between 7 AM and 7 PM, when urinary tyrosine levels were also highest. In contrast, the greatest increase in urinary dopamine occurred during the subsequent 7 PM to 7 AM period.

![FIG. 2. Effect of tyrosine administration on urinary catecholamine excretion. Ten subjects consumed 33 mg/ kg of free tyrosine at 7 AM, 11 AM, and 4 PM, and also ingested a high-protein diet (38 g/meal in each of three meals) at 8 AM, noon, and 5 PM. The high-protein diet was also consumed on the day before the study. Urines were collected for 24 h starting at 7 AM on the day of, and the day before, tyrosine administration and assayed for tyrosine, dopamine, norepinephrine, and epinephrine. Data are presented as total excretion per 24 h (mean ± SEM) (analysis by paired t test, the subjects serving as their own controls).](image)

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<th>Dopamine After</th>
<th>Norepinephrine Before</th>
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* Base-line urine was collected for the first 24 h, after which the subjects consumed a total of approximately 100 mg/kg tyrosine along with regular meals between 8 AM and 5 PM.
neutral amino acids that compete with it for the ratio of tyrosine to the sum of other large and the serum tyrosine competitor ratio (i.e., (Fig. 1; Reference 32). Serum tyrosine levels cholamines exhibit similar diurnal rhythms at 9 PM and reaching their nadirs at 5 AM (E. Melamed, B. Glaeser, J.H. Growdon and R.J. Wurtman, unpublished observations). The present observation of a urinary tyrosine rhythm in subjects eating normally (Fig. 1) is consistent with previous findings (33). This serum tyrosine rhythm is probably produced primarily by food consumption per se (i.e., by amino acids from dietary protein) and by the food-elicted secretion of insulin which facilitates amino acid uptake into tissues; it tends to disappear with fasting (33). Serum tyrosine levels (and those of most other neutral amino acids) fall during the daytime when subjects ingest protein-free diets (probably because of insulin’s effect on tyrosine uptake into muscle), but rise during the daytime when high-protein diets like our “house diet” are consumed (25). Thus urinary tyrosine and catecholamine levels normally vary with time of day and parallel serum tyrosine levels. Exogenous tyrosine’s tendency to cause a delayed rise in urinary dopamine but not in norepinephrine (Fig. 1) is unexplained, but suggests that much of the circulating dopamine may be produced in sites other than sympathetic neurons.

As described earlier, the “house diet” consumed by our subjects contained relatively large amounts of protein, and thus could elevate serum tyrosine by itself (25). The present study does not allow us to ascertain the specific contribution of this high-protein diet to urinary catecholamines; however, our data show that administration of free tyrosine produces major increases in catecholamine excretion over and above those attributable to diet alone, without altering the daily pattern of urinary tyrosine (Fig. 1). This acute study also did not allow us to examine the long-term effects on catecholamine excretion of administering tyrosine to humans; however we have found (34) that, in laboratory animals chronic tyrosine administration (6 days) continues to produce significant elevations of urinary NE and E.

There is considerable evidence that most, if not all, of the catecholamine molecules in the urine originate from peripheral tissues (35). A blood-brain barrier blocks transport of NE (and probably other catecholamines) into or out of the brain (36-38). In unpublished experiments, we have found that bilateral adrenalectomy blocks the tyrosine-induced increase in urinary epinephrine but not the increase in dopamine or NE. This finding

Discussion

These data show that ingestion of 100 mg/kg/day of tyrosine in divided doses preceding three normal meals increased urinary tyrosine 2- to 4-fold and urinary catecholamines 25 to 33%. Such increases were observed in 10 of 11 subjects. In the single participant (no. 6) in whom exogenous free tyrosine did not affect tyrosine excretion, it also failed to increase urinary catecholamines; this result suggests that in this subject the amino acid also failed to increase plasma tyrosine or the amount of amino acid available to catecholamine-producing cells. Individual subjects differed considerably in the extent to which they metabolized exogenous tyrosine. Only 0.42% (average) of the ingested dose was excreted in the urine during the 23 h after the initial dose, however, this percentage varied among subjects over a 3-fold range. Since even large doses of exogenous tyrosine are cleared rapidly, i.e., within 6 to 8 h (21) from the bloodstream, our present data are compatible with the view that the bulk of ingested tyrosine is metabolized in the liver or diverted to the tissues, so that only a small fraction of it is handled by the renal tubules. Thus the amounts of tyrosine that may be used therapeutically probably would not have deleterious effects by saturating the nephron and forming renal calculi.

Both urinary tyrosine and urinary catecholamines exhibit similar diurnal rhythms (Fig. 1; Reference 32). Serum tyrosine levels and the serum tyrosine competitor ratio (i.e., ratio of tyrosine to the sum of other large neutral amino acids that compete with it for brain uptake) also exhibit characteristic diurnal variations in subjects given exogenous tyrosine with meals, peaking between 1 and 9 PM and reaching their nadirs at 5 AM (E. Melamed, B. Glaeser, J.H. Growdon and R.J. Wurtman, unpublished observations).
suggests that the adrenal medulla is relatively unimportant in mediating tyrosine-induced increases in NE and dopamine excretion, but is very important in producing the epinephrine increase.

Results obtained from these studies indicate that, as noted also in experimental animals (34), tyrosine administration to humans accelerates catecholamine synthesis in, and release from, peripheral tissues. Our findings in rats suggest that tyrosine hydroxylase in these tissues may, like the brain enzyme, become more saturated with its substrate after administration of exogenous tyrosine. Sved et al. (39) recently observed that tyrosine administration causes blood pressure to fall in hypertensive rats and attributed this effect to a central mechanism, i.e., release of more norepinephrine in the brainstem and consequent activation of brainstem adrenergic receptors that suppress sympathetically outflow. Tyrosine had only a minor effect on blood pressure in normotensive animals (and no discernable effect on blood pressure in the normal subjects discussed here), and did not have a large effect on norepinephrine release within their brains (as indicated by MOPEG-SO₄ levels). Hence we cannot draw any conclusions as to whether tyrosine's ability to increase urinary norepinephrine in normotensive subjects is associated with a reduction, an increase, or no change in the firing rates of sympathetic neurons. Most likely, the tyrosine-induced rises in urinary catecholamines observed in our normotensive subjects reflect increases in the amount of catecholamines released during each discharge from some sympathetic neurons and adrenal chromaffin cells. The sympathetic neurons thus affected by tyrosine in normal individuals may not be those responsible for maintaining blood pressure.

The finding that tyrosine administration or ingestion raises urinary catecholamines in human subjects has obvious clinical implications for improving therapeutic agents for shock, orthostatic hypotension, and other disorders involving the autonomic nervous system.

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References


