Elevation of Urinary Catecholamines and Their Metabolites Following Tyrosine Administration in Humans

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A single oral dose of tyrosine (100 or 150 mg/kg) caused significant increases in urinary levels of norepinephrine (NE), epinephrine (E), dopamine (DA), 3-methoxy-4-hydroxyphenylglycol (MHPG), vanilmandelic acid (VMA), and homovanillic acid (HVA) during the first 2 hr after its ingestion; water administration failed to produce such changes. The temporal patterns of these increases paralleled those of previously described increases in plasma tyrosine. Since urinary catecholamines derive from peripheral sources, while the catecholamine metabolites in urine may reflect both CNS and peripheral catecholamine turnover, these findings indicate that tyrosine administration may accelerate catecholamine synthesis in and release from cells throughout the human body. Tyrosine may thus constitute a useful agent for treating central or peripheral disorders associated with insufficient release of catecholamines.

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INTRODUCTION

Circulating tyrosine is the physiological precursor for catecholamine molecules synthesized in peripheral sympahtoadrenal cells and in CNS neurons (Nielsen, 1976). Studies in our laboratory (Wurtman et al., 1974; Gibson and Wurtman, 1977) and elsewhere (Carlsson and Lindqvist, 1978) have shown that treatments that raise plasma tyrosine levels relative to those of other neutral amino acids (LNAA) (e.g., tyrosine injection; consumption of protein-containing meals) also cause parallel increases in brain tyrosine content and catechol synthesis. Moreover, such treatments accelerate the rates at which the dopamine metabolite, homovanillic acid (HVA), accumulates in corpora striata of rats given haloperidol (Scally et al., 1977) or reserpine (Sved et al., 1979a) and at which the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol sulfate (MHPG-SO₄) accumulates in brains of cold-stressed or probenecid-treated animals (Gibson and Wurtman, 1978) or spontaneously hypertensive rats (Sved et al., 1979b). Since tyrosine administration increases urinary catecholamine levels in rats (Alonso et al., 1980), catecholamine turnover in peripheral sympathoadrenal structures may similarly depend upon plasma amino acid levels.

Studies on human subjects have shown that oral tyrosine administration raises plasma tyrosine levels as well as the ratio of plasma tyrosine to the sum of the other LNAA which compete with tyrosine for brain uptake (Glaeser et al., 1979). If these increases are associated with enhanced brain and tissue tyrosine levels, and if tyrosine hydroxylase in humans, as in rats, is normally unsaturated with its amino acid substrate (Gibson and Wurtman, 1977; Carlsson and Lindqvist, 1978), then tyrosine administration might be expected similarly to enhance catecholamine synthesis in humans. Previously we found that giving tyrosine for a day along with protein-containing meals increased urinary catecholamine levels in humans, suggesting that both catecholamine synthesis in and release from peripheral cells was accelerated (Agharanya et al., 1981). In the present study we have examined the effect of a single oral dose of tyrosine, previously found to raise plasma tyrosine levels (Glaeser et al., 1979), on the excretion of catecholamines and their major metabolites (HVA; MHPG; and vanilmandelic acid (VMA)] among fasted subjects.

MATERIALS AND METHODS

Subjects

A group of 13 healthy male subjects, age 18-21 years, volunteered to participate in the study, in accordance with a protocol approved by the M.I.T. Committee on the Use of Humans as Experimental Subjects. All subjects were
admitted to the M.I.T. Clinical Research Center after an overnight fast. Baseline urinary samples (representing urine production between 6 AM and 8 AM) were collected, after which eight of the subjects (tyrosine-treated) ingested a single oral dose of L-tyrosine (two subjects, 100 mg/kg; six subjects, 150 mg/kg; Ajinomoto Co., Tokyo, Japan) mixed in water; the other five subjects (controls) were given only water. Subjects continued to fast for the next 8 hr, and urinary samples were obtained 2 hr (8 AM-10 AM), 4 hr (10 AM-12 noon), and 8 hr (12 noon-4 PM) after treatment. Urine was collected in plastic bottles containing 6 N HCl with 5% sodium metabisulphite. After measuring the volume and adjusting the urine to pH 3, aliquots of 20-50 ml were stored at -20°C until assayed.

**Assays**

**Urinary Catecholamines**

After thawing at room temperature, samples were mixed with 1/10 volume of 4 M HClO₄ and centrifuged at 30,000 × g for 15 min. The clear supernatant fluid was adjusted to pH 8.6 and passed over alumina columns as described by Anton and Sayre (1962). Catecholamines were eluted with 0.2 M acetic acid and the eluates were analyzed fluorimetrically. Dopamine (DA) was assayed by the method of Carlsson and Waldock (1958), and norepinephrine (NE) and epinephrine (E) were determined separately by the method of Peyrin and Cottet-Emard (1973).

**Urinary Catecholamine Metabolites**

Total MHPG (unconjugated plus conjugated forms), VMA, and HVA were determined by a high-pressure liquid chromatography with electrochemical detection (HPLC-ED) method developed in our laboratory (Alonso et al., 1981); this method is described briefly below. For MHPG, aliquots of 0.25 ml of urine were mixed with the same volume of 0.5 M sodium acetate buffer, pH 6.5, containing 4 mg/ml of EDTA, and 20 μl of glusulase (β-glucuronidase/aryl sulfatase from *Helix pomatia*; Calbiochem-Behring Corp., La Jolla, CA). After overnight hydrolysis, 100 mg NaCl was added to each sample and MHPG was extracted into 4 ml of HPLC-grade ethyl acetate (Burdick and Jackson, Muskegon, MO); this was centrifuged briefly, and then 3 ml of the organic phase was washed with 0.3 ml of 0.5 M KHCO₃, and passed through phase-separating filters (Brinkman Instruments Inc., Westbury, NY). The ethyl acetate extracts were evaporated to dryness under vacuum (Savant rotary vacuum, Savant Instruments, Hicksville, NY) and the dried residues dissolved in 50 μl of methanol and ap
plied to a thin-layer chromatographic plate (20 × 20 cm silica gel-coated Whatman LK-5D plate; Whatman, Clifton, NJ). The plates were developed to 10 cm using the organic layer of a chloroform:methanol:acetic acid:water mixture (120:20:30:25). A segment corresponding in location to that of authentic MHPG (chromatographed concurrently and identified by spraying with Folin-Ciocaltean phenol reagent; \( R_f = 0.60 \)) was eluted from each plate with 1 ml of HPLC-grade methanol. Aliquots of the methanol extract were evaporated to dryness and the dried residue was dissolved in 0.25 ml of 0.05 M sodium phosphate, monobasic, pH 5; 50-100 µl aliquots were then subjected to analysis by HPLC.

For VMA and HVA, 0.5 ml aliquots of urine were acidified with 50 µl of 0.6 M HClO\(_4\) containing 5 mg/ml of ascorbic acid. After saturation with NaCl, the acid metabolites were extracted into 4 ml of HPLC-grade ethyl acetate and 3 ml of the organic phase was passed through phase-separating filters. The ethyl acetate extracts were dried, and the residues then dissolved in 50 µl of methanol and separated by TLC as described for MHPG. The segments corresponding in location to authentic VMA and HVA (\( R_f = 0.22 \) and 0.82, respectively) were eluted together in 1 ml of HPLC-grade methanol. Aliquots of this methanol extract were evaporated to dryness, and the dried residues dissolved in 0.5 ml of the HPLC mobile phase; 50-100 µl aliquots were then injected into the HPLC system.

The liquid chromatographic system consisted of a µBondapak C\(_{18}\) reversed-phase column (30 × 3.9 mm, particle size 10 µm; Waters Assoc., Milford, MA), LC-4A amperometric controller with model TL-3 carbon paste electrode (Bioanalytical Systems, West Lafayette, IN), LC-22 Temperature Controller and LC-23 Column Heating Compartment (Bioanalytical Systems). Samples containing MHPG were assayed in separate chromatographic runs from those containing VMA plus HVA; they were injected into the HPLC using an automatic sample injector (WISP 710A; Waters Assoc.). The mobile phase was 0.05 M sodium phosphate, monobasic, pH 5, previously filtered (0.45 µm Millipore filters; Millipore Corp., Bedford, MA) and degassed. The detector potential was set at +0.7 V vs. a Ag/AgCl reference electrode (BAS). The flow rate was 1.5 ml/min. Typical retention times under these conditions (at 40 C) were 10.2 min for MHPG, 3.6 min for VMA, and 22.4 min for HVA. Chromatograms were recorded and analyzed using a 3385 Hewlett-Packard Automation System.

Concentrations of each compound were calculated from internal standard curves generated using pooled urine samples, and all values were corrected for internal recoveries. Recovery of MHPG was 40%, of HVA 70%, and of VMA 50%.

Urinary creatinine was measured colorimetrically by the Jaffe method (Bonsnes and Taussky, 1945).

Statistics

Data were processed by 2 × 4 factorial analysis of variance for repeated measurements (Winer, 1970). To control for variations in the excretion of any
compound that might have arisen from sample-to-sample differences in urine volume, we also performed analysis of covariance, linear correlation, and regression analysis (Armitage, 1974). There was no statistically significant difference between urinary catecholamine or metabolite values from the two subjects receiving 100 mg/kg of tyrosine and the six receiving 150 mg/kg; hence data for all eight of the tyrosine-treated subjects were pooled.

RESULTS

For the 2 hr after tyrosine ingestion, the excretions of the catecholamines and their metabolites were elevated; in contrast, no significant changes were observed among control subjects given water (Figs. 1 and 2). These increases were significant for all compounds studied, assessed by $2 \times 4$ factorial analysis of variance with repeated measurements for the same subject. The percentage

![Graph showing urinary levels of catecholamines](image)

Fig. 1. Urinary levels of catecholamines in normal humans after tyrosine administration. Thirteen subjects fasted overnight; the next morning eight received a single oral dose (100 or 150 mg/kg) of L-tyrosine mixed in water; while five controls received only water. Urinary samples were obtained 0, 2, 4, and 8 hr after tyrosine or water ingestion. Data are given as $\mu g$ excreted/hr (mean ± SEM); • $p < 0.01$ by ANOVA for repeated measurements; •• $p < 0.005$. 
Fig. 2. Urinary levels of catecholamine metabolites in normal humans after tyrosine administration. Experimental conditions were as described in Fig. 1. Data are given as μg excreted/hr (mean ± SEM); • $p < 0.001$ by ANOVA for repeated measurements; **$p < 0.005$.

Mean increases at 2 hr for each compound were: NE, 82% ($p < 0.01$); E, 275% ($p < 0.005$); DA, 178% ($p < 0.005$); MHPG, 225% ($p < 0.001$); VMA, 210% ($p < 0.005$); HVA, 254% ($p < 0.001$). No significant changes in blood pressure, pulse rate, or other side effects were noted during the study.

Since the urine volume and creatinine excretion also were elevated during the 2 hr after tyrosine ingestion (Table I), we performed covariance and regression analyses to determine whether the tyrosine-induced variations in catecholamine metabolite excretion might have been due to concurrent diuresis. No relationships were found to exist between urine volumes or creatinine contents and the tyrosine-induced variations in the other compounds studied: individual correlation coefficients were very low, and in some cases even negative.

The mean values of each catecholamine or metabolite in the base-line urine samples (6 AM-8 AM) did not differ among the control and tyrosine-treated groups. Among samples representing urines produced during the 2 hr after treat-
Table I. Effects of a Single Oral Dose of Tyrosine on Urinary Volume and Creatinine Excretion in Fasted Human Subjects

<table>
<thead>
<tr>
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<th>Hours after tyrosine or water ingestion (mean ± SEM)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Controls (n = 5)</td>
<td></td>
</tr>
<tr>
<td>Urine volume (ml/hr)</td>
<td>39.8 ± 10.7</td>
</tr>
<tr>
<td>Creatinine (mg/hr)</td>
<td>73.5 ± 4.2</td>
</tr>
<tr>
<td>Tyrosine-treated (n = 8)</td>
<td></td>
</tr>
<tr>
<td>Urine volume (ml/hr)</td>
<td>23.9 ± 4.0</td>
</tr>
<tr>
<td>Creatinine (mg/hr)</td>
<td>63.4 ± 4.6</td>
</tr>
</tbody>
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a sub p < 0.01 differs from zero time group by ANOVA for repeated measurements.

DISCUSSION

These data show that a single oral dose of tyrosine can increase urinary levels of the catecholamines and their chief metabolites MHPG, VMA, and HVA. Maximal increases are observed during the initial 2 hr after tyrosine ingestion; thereafter catecholamine levels return to base line but metabolite levels remain somewhat elevated (Figs. 1 and 2). The time course for tyrosine’s effect on urinary catecholamines tends to parallel the increases in plasma tyrosine levels and in the plasma tyrosine ratio (i.e., plasma tyrosine/other serum LNAA) described previously (Glaeser et al., 1979), with maximal values also occurring during the 2 hr after tyrosine ingestion. In experimental animals, the flux of tyrosine into the brain depends on the plasma tyrosine ratio (Wurtman et al., 1974; Gibson and Wurtman, 1977; Fernstrom and Faller, 1978); furthermore, tyrosine doses which elevated this ratio in the rat also proportionately elevate brain and adrenal tyrosine levels, and can accelerate catecholamine synthesis in and release from central and peripheral tissues under appropriate conditions, i.e., when neurons are
depolarizing frequently or when catecholamine turnover is increased (Scally et al., 1977; Gibson and Wurtman, 1978; Melamed et al., 1980; Alonso et al., 1980).

The present findings suggest that a similar sequence occurs in humans consuming tyrosine and that the rapid rise in urinary levels of the catecholamines and their metabolites reflects accelerations in catecholamine synthesis and release. We are not yet able to draw conclusions about the anatomic loci of the observed effect. Most of the catecholamine molecules in the body and in the urine (except some originating directly in the kidney or in tuberoinfundibular dopamine neurons) derive from the peripheral tissues (Axelrod, 1965); hence, the increase in urinary catecholamines probably reflects an effect on sympathetic-adrenal cells and on peripheral cells producing dopamine.

The origins of the catecholamine metabolites in human urine are less well established. Their levels have been proposed as an index of central catecholamine turnover (DeMet and Halaris, 1979), and several particular norepinephrine metabolites (urinary MHPG and VMA) have been proposed as the basis for a biochemical classification of depressive disorders (Maas et al., 1968; Schildkraut et al., 1978). Using a direct method to calculate venous-arterial differences in humans, Maas et al. (1979) estimated that 63% of urinary MHPG derived from CNS neurons. Some of the MHPG formed in brain may be converted to other products in the periphery, such as VMA, and appear in the urine as those compounds (Blomberg et al., 1980). Destruction of peripheral sympathetic terminals in animals normally lowers urinary MHPG (Hoeldtke et al., 1974). It seems likely that a portion of the large increase in urinary MHPG (225%) and VMA (210%) which follows tyrosine administration reflects CNS norepinephrine synthesis and release, but the exact portion cannot now be estimated. [MHPG can, of course, also be formed from epinephrine, and tyrosine administration does elevate urinary epinephrine levels (Fig. 1).]

Urinary excretion of HVA (Rinne et al., 1966; Lenman et al., 1977) is altered in Parkinson’s disease and other movement disorders presumably related to deficiencies in dopaminergic neurons. Even though the kidney contains dopamine (Sandler and Ruthven, 1969) as well as enzymes to convert dopamine to HVA, it has been shown in monkeys that urinary HVA normally originates from HVA in the blood (Elchisak et al., 1978) which, in turn, represents the contribution of brain and peripheral sources. Recent studies in this laboratory have shown that oral L-tyrosine elevates the concentration of HVA in CSF of parkinsonian patients (Growdon and Melamed, 1980). Thus, the elevated urinary levels of HVA found in this study might partly reflect an acceleration of dopamine synthesis in brain neurons.

Our findings indicate that tyrosine administration to humans may, as it does in rats, accelerate catecholamine synthesis and release, probably in both central and peripheral catecholaminergic cells. These findings support the possibility that tyrosine alone, or more sophisticated amino acid mixtures, may
have clinical application in diseases resulting from insufficient catecholamine release. In one such disease, depression, tyrosine has already been found to be useful in two small studies (Gelenberg et al., 1980; Goldberg, 1980) involving three patients, one of whom (Gelenberg et al., 1980) was studied using a placebo-controlled, crossover design.

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REFERENCES


