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STUDIES ON THE MECHANISM BY WHICH TYROSINE RAISES URINARY CATECHOLAMINES*

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Abstract—The source of urinary catecholamines and the mechanisms by which tyrosine administration raises these compounds were investigated in rats. Adrenalectomy blocked the tyrosine-induced rise in urinary epinephrine but not dopamine or norepinephrine. Following chemical sympathectomy with 6-hydroxydopamine (6-OHDA), the tyrosine-induced increase in urinary norepinephrine was diminished, but epinephrine and dopamine responses were unaffected. Cardiac norepinephrine, which is normally unchanged following tyrosine administration, became significantly elevated in 6-OHDA-pretreated animals. At the doses used in this study, tyrosine had no effect on the uptake or metabolic clearance of circulating catecholamines. We conclude that tyrosine augments the synthesis of the three catecholamines in sympathoadrenal cells by increasing the extent to which tyrosine hydroxylase is saturated with its amino acid substrate. Moreover, the effects of tyrosine on peripheral catecholamine synthesis may be enhanced under conditions of increased sympathetic activity.

Abundant evidence exists that the rates at which cells synthesize catecholamines are generally dependent on the conversion of tyrosine to DOPA by tyrosine hydroxylase [1]. Tyrosine hydroxylase activity is controlled by acute, short-term processes including, for example, kinetic changes caused by its phosphorylation [2] and by long-term variations in the amount of enzyme protein [3].

One additional factor acutely affecting the hydroxylation of tyrosine to DOPA is the availability of the amino acid precursor itself [4]. Studies with laboratory animals [5–7] and humans [8] have shown that administration of L-tyrosine can increase brain levels of catecholamine metabolites and urinary levels of the unchanged catecholamines. Theoretically, there are several mechanisms by which tyrosine could influence catecholamine output: it could act centrally to modulate sympathetic outflow; it could modify the peripheral metabolism of circulating catecholamines by inhibiting their enzymatic degradation or their renal clearance; or it could increase catecholamine synthesis within sympathoadrenal cells by enhancing the saturation of tyrosine hydroxylase. This study was designed to examine some of the various mechanisms by which tyrosine might enhance urinary catecholamine levels.

MATERIALS AND METHODS

Intact and adrenalectomized male Sprague–Dawley rats from Charles River Breeders (Wilmington, MA) were habituated in our laboratory for 1 week before use. During this period, animals had free access to food (Charles River Rat–Mouse–Hamster Maintenance Formula) and water; adrenalectomized rats drank 1% sodium chloride in place of tap water. On the evening before an experiment, animals were deprived of food but allowed to drink water; the next morning they were placed in groups and given the treatments described below. All injections were given intraperitoneally, unless otherwise indicated, and were followed immediately thereafter by 7 ml tap water given by stomach intubation in order to induce diuresis.

Adrenalectomized rats received 200 mg/kg tyrosine (as methylester hydrochloride) or 1% NaCl, and urine was collected for the next 3 hr. Control animals received tyrosine or saline. Chemical sympathectomy was produced by administration of 6-hydroxydopamine (6-OHDA) (four injections of 100 mg/kg each, at 48-hr intervals). Three days after the last injection, 24-hr urine samples were collected, and on day 4, the animals received tyrosine (200 mg/kg) or saline and urine was collected for the next 3 hr. At the end of the study period, animals were decapitated and bloods, tissues and urines were analyzed.

DL-[7-^{3}H]Norepinephrine ([^{3}H]NE, sp. act. 7.5 Ci/mmole; 0.27 mg per ml per μCi) was purchased from the New England Nuclear Corp. (Boston, MA). Rats received 25 μCi (Table 2) or 12.5 μCi (Table 3) subcutaneously with or 90 min before a tyrosine dose. After urines were collected, animals were killed and their hearts and adrenals were quickly removed for analysis.

Urines were adjusted to the same volume with saline, and the radioactivity in aliquots was counted; the [^{3}H]NE in remaining portions was purified (by precipitation of proteins with 4 M perchloric acid, centrifugation, passage of the supernatant fluids over

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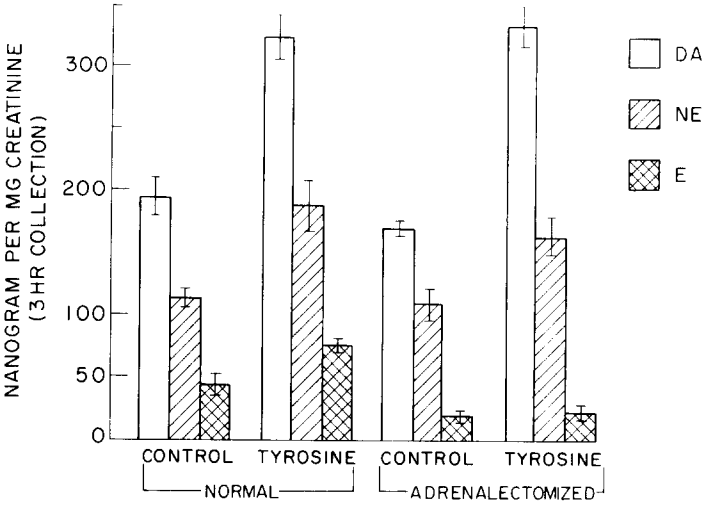

Fig. 1. Effect of tyrosine administration on urinary catecholamines in adrenalectomized rats. Groups of four normal and adrenalectomized rats received i.p. injections of tyrosine (200 mg/kg) or saline. Urines passed in the next 3 hr were collected and assayed for catecholamines. All values in the tyrosine-treated groups (normal animals) were significantly greater than those of the controls ($P < 0.001$). Dopamine and norepinephrine also differed between the two groups in adrenalectomized rats. Values are means ± S.E.M.

alumina at pH 8.6, and the elution of the [^{3}H]NE with 0.2 M acetic acid) and counted.

Tissue and urinary catecholamines were determined fluorimetrically using the trihydroxyindole method [9]. Serum and tissue tyrosine concentrations were also measured fluorimetrically [10].

RESULTS

Tyrosine administration to normal rats significantly raised all three catecholamines in urine (Fig. 1); it also elevated urinary norepinephrine (NE) and dopamine (DA) in adrenalectomized animals to the same levels as those obtained in normal rats. However, adrenalectomy caused a 60% fall in urinary epinephrine (E), and tyrosine loading had no further effect on the epinephrine level (Fig. 1). No treatment significantly affected urine volumes during the collection periods.

Chemical sympathectomy with 6-OHDA caused a significant decrease in urinary norepinephrine (Table 1)—24-hr excretions falling from 1.29 ± 0.08 to 0.83 ± 0.08 μg ($P < 0.005$)—but not in dopamine or epinephrine. Administration of tyrosine to such lesioned rats restored urinary norepinephrine, increasing these levels by 43% (($P < 0.025$) as compared with 15% ($P < 0.05$) in untreated rats (Table 1). 6-OHDA differentially affected tissue catecholamine levels (Fig. 2): whereas cardiac norepinephrine was depleted by 70%, adrenal catecholamines increased by 42%. Tyrosine administration to these chemically sympathectomized animals significantly raised cardiac norepinephrine ($P < 0.025$) but not adrenal epinephrine (Fig. 2); it failed to affect tissue catecholamine levels in intact rats.

Tyrosine administration concurrent with (Table 2) or 90 min after (Table 3) [^{3}H]NE failed to affect the urinary clearance or tissue uptake and retention of the tracer. Similarly, tyrosine had no effect on the specific activity of [^{3}H]NE in the heart and adrenals.

Table 1. Effect of 6-hydroxydopamine and tyrosine on urinary catecholamines*

	Urinary DA	Urinary NE	Urinary E
Control	284 ± 26	124 ± 7	84 ± 6
Tyrosine	426 ± 34†	141 ± 6‡	124 ± 8§
6-OHDA	309 ± 49	94 ± 6‡	82 ± 9
6-OHDA + tyrosine	446 ± 36	134 ± 12‖	88 ± 9

* Lesioned animals received 6-hydroxydopamine (6-OHDA) (100 mg/kg, i.p., four injections at 48-hr intervals). Four days after the last injection and after an overnight fast, the rats received tyrosine (200 mg/kg) or saline; urine was collected for the next 3 hr. Results are presented as means ± S.E.M. (ng/3 hr). There were six animals per group.

† $P < 0.005$ vs control.

‡ $P < 0.05$ vs control.

§ $P < 0.001$ vs control.

‖ $P < 0.025$ vs 6-OHDA.

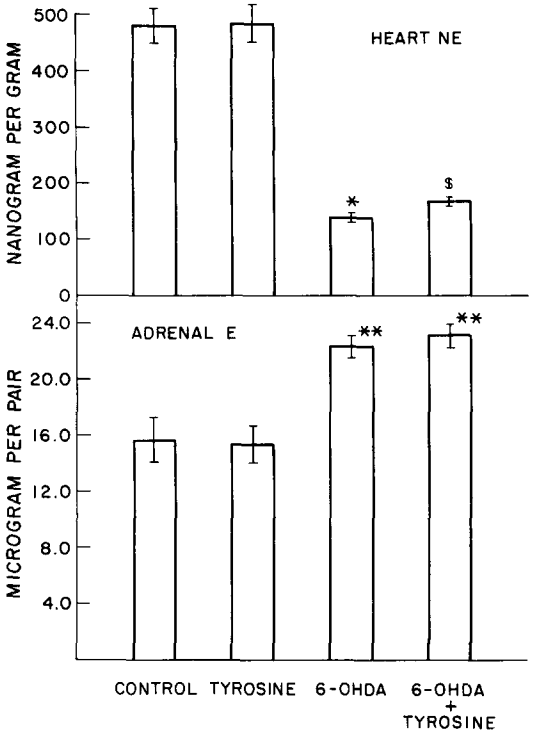

Fig. 2. Effect of 6-hydroxydopamine on tissue catecholamine levels. Animals were treated as described in Table 1. They were killed 3 hr after tyrosine injection and the tissues were removed and assayed for catecholamines. Values are means ± S.E.M. Key: (*) $P < 0.001$; (**) $P < 0.01$ compared to control; and ($) $P < 0.025$ compared to 6-OHDA alone.

Table 2. Cardiac uptake and urinary excretion of [^{3}H]NE following tyrosine loading*

	Control	Tyrosine-treated
	(dpm × 10^{-4})	
Cardiac uptake		
Total radioactivity	8.8 ± 1.0	9.6 ± 2.6
[^{3}H]NE extract	6.4 ± 0.9	7.7 ± 2.2
Levels in urine		
Total radioactivity		
3-hr Collection	653 ± 96	959 ± 137
7-hr Collection	1070 ± 78	1327 ± 145
Unchanged [^{3}H]NE		
3-hr Collection	70 ± 7	85 ± 16
7-hr collection	80 ± 9	93 ± 18

* Rats weighing approximately 200 g received [^{3}H]NE (25 μCi, s.c.) followed by 200 mg/kg tyrosine or saline i.p. Each rat also received 7 ml water by stomach tube to enhance urine flow. Urine was collected for the first 3 hr and then for an additional 4 hr after the animals again received 7 ml water. At the end of the 7-hr period animals were killed and cardiac [^{3}H]NE was assayed. Results are presented as means ± S.E.M. from four animals per group, per urine collection or per heart.

Table 3. Effect of tyrosine on the accumulation of [^{3}H]NE in the heart and adrenals*

	Control	Tyrosine-treated
	(dpm × 10^{-4})	
Cardiac uptake		
Total radioactivity	7.5 ± 0.6	8.4 ± 1.5
Unchanged [^{3}H]NE	6.7 ± 0.7	6.7 ± 1.1
Adrenal uptake		
Total radioactivity	0.15 ± 0.012	0.15 ± 0.025
Unchanged [^{3}H]NE	0.08 ± 0.006	0.08 ± 0.008

* Rats weighing approximately 185 g received [^{3}H]NE (12.5 μCi, s.c.) followed in 90 min by 200 mg/kg tyrosine or saline; they were killed 90 min later. Data are presented as means ± S.E.M. from four animals per group, per organ or pair.

DISCUSSION

Source of urinary catecholamines. Catecholamines are highly charged molecules and hence are unable to pass through the lipophilic blood–brain barrier without prior deamination and possibly *O*-methylation [11]. Hence, whatever effects exogenous tyrosine might exert on urinary catecholamine levels must reflect changes in the numbers or fates of catecholamine molecules released from peripheral tissues. Our data suggest that all of the increase in urinary epinephrine that follows the administration of tyrosine originates in secretion from the adrenal medulla: considerable amounts of epinephrine (40% of control; Fig. 1) continued to be present in urine after bilateral adrenalectomy, possibly coming from extra adrenal sites or from incompletely removed adrenal chromaffin tissue; however, urinary epinephrine levels failed to respond to tyrosine loading (Fig. 1). In contrast, adrenalectomy did not influence the outputs of dopamine or norepinephrine, suggesting that the adrenal contributes relatively little of these catecholamines to the urine.

6-OHDA is known to cause selective destruction of adrenergic neurons [12, 13] and such denervation is accompanied by the supersensitivity of surviving neurons [14] and by an increase in the activity of the preganglionic nerves to the adrenals [15]. Destruction of peripheral sympathetic nerves with 6-OHDA resulted in a significant depletion of urinary norepinephrine; by contrast, levels of dopamine and epinephrine remained unaltered (Table 1). Tyrosine administration to lesioned rats raised urinary norepinephrine by 43%, but only by 15% in normal animals. This may reflect a relationship between sympathetic neuronal firing rates (presumably enhanced in the surviving noradrenergic neurons) and the extent to which they respond to additional precursor, since rate dependence has been noted previously for noradrenergic [16] and dopaminergic [17] brain neurons. Similarly, as illustrated in Fig. 2, tyrosine had no effect on cardiac norepinephrine in normal rats; however, in chemically sympathectomized animals, it raised this level by over 30% ($P < 0.025$), suggesting that the increased firing rate of the surviving cardiac nerves in the latter group exaggerated the susceptibility of these neurons to changes in substrate supply.

The relative resistance of urinary dopamine to 6-OHDA (Table 1) suggests that it and urinary norepinephrine are not derived from the same tissue source. Recent studies support the speculation that dopamine may function in its own right in some peripheral tissues [18]. Specific dopamine receptors, together with significant quantities of the amine, have been identified in various peripheral tissues including the renal vascular bed [19, 20]. In laboratory animals, we have found that urinary dopamine levels are greater than those of norepinephrine and epinephrine combined [5]. Urinary dopamine in humans is almost 10-fold greater than norepinephrine [21], and yet plasma dopamine levels reportedly are less than 25% those of norepinephrine [22]. This suggests that much of the urinary dopamine may originate from the kidney. In rats, a significant amount of the amine may be derived from dopamine or DOPA present in cereal-containing chow routinely consumed by the animals [23]; dietary cereals may also contribute to the dopamine in human urine.

Since the conversion of tyrosine to dopamine requires the participation of tyrosine hydroxylase, it seems likely that the increased urinary dopamine found in tyrosine-treated rats derives from cells known to contain this synthetic enzyme, e.g. the vasomotor nerves of the kidney [20, 24]; post-ganglionic sympathetic perikarya in ganglia; sympathetic terminals resistant to 6-OHDA; and small intensely fluorescent (SIF) cells in ganglia [25]. The cells responsible for urinary dopamine respond to treatments other than 6-OHDA differently from the cells that contribute norepinephrine and epinephrine to the urine. For instance, when animals are fasted for 5 days, or fed diets lacking in proteins but rich in carbohydrates, urinary dopamine falls dramatically, whereas norepinephrine and epinephrine are elevated. On the other hand, in animals fed protein-rich diets, urinary dopamine concentrations rise while those of norepinephrine and epinephrine are depressed (J. C. Agharanya and R. J. Wurtman, manuscript submitted for publication). In other studies, we have found that, when animals are treated with sufficient nicotine (12.5 mg/kg, s.c.), a potent adrenergic stimulating agent, to increase urinary norepinephrine 2-fold and epinephrine 5-fold, urinary dopamine remains unchanged. These observations support the notion that most urinary dopamine in rats derives from dopamine-containing neuronal elements distinct from those releasing norepinephrine and epinephrine.

Effect of tyrosine on the metabolism of catecholamines. To investigate possible influences of tyrosine on the metabolism of endogenous catecholamines, we administered the amino acid concurrently with or 90 min after a tracer dose of [^{3}H]NE. We hypothesized that this molecule is metabolized similarly to endogenous, circulating norepinephrine and hence its uptake and metabolism can be taken as indices of those of the endogenous catecholamine. As shown in Tables 2 and 3, tyrosine had no discernible effect on the uptake of the ^{3}H-amine into adrenals or cardiac sympathetic nerves, nor did tyrosine influence [^{3}H]NE excretion into urine. These data indicate that the amino acid does not increase urinary norepinephrine by, for example, increasing the proportion of circulating catecholamine that passes into the urine.

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