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Effect of Tyrosine on Brain Catecholamine Turnover in Reserpine-Treated Rats

T. Oishi and R. J. Wurtman

Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.

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Summary

The injection of reserpine [2.5 mg/kg, intraperitoneally (i.p.)] increased brain concentrations of tyrosine and of the major catecholamine metabolites, 3-methoxy-4-hydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxy-4-hydroxy-phenylethylene-glycol-sulfate (MHPG-SO_4) in the rat. In reserpine-treated animals, tyrosine administration (200 mg/kg, i.p.) caused further increases in brain tyrosine, DOPAC, and HVA, but not in brain MHPG-SO_4. The increases in DOPAC and HVA levels were observed in a dopaminergic brain region (the corpus striatum) and a noradrenergic region (the cerebellum). These results support previous observations that catecholamine synthesis and release in both dopaminergic and noradrenergic neurons can depend in part upon brain tyrosine levels.

Introduction

Catecholamine biosynthesis is affected both by changes in the activity of tyrosine hydroxylase, the rate-limiting enzyme (*Nagatsu et al.*, 1964), and by changes in the enzyme's saturation with tyrosine, its dietary precursor (*Wurtman et al.*, 1974).

Tyrosine administration increases brain tyrosine concentrations (*Wurtman et al.*, 1974; *Gibson* and *Wurtman*, 1978), but has not been reported to cause subsequent changes in brain dopamine or norepinephrine levels. However, treatments that elevate brain tyrosine concentrations do increase brain levels of 3-methoxy-4-hydroxy-

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phenylethylene-glycol-sulfate (MHPG-SO_4), a norepinephrine metabolite, in rats injected with probenecid (*Gibson* and *Wurtman*, 1978), and in spontaneously hypertensive rats (*Sved et al.*, 1979 a), and also accelerate dopa accumulation in rats receiving a drug which inhibits brain dopa decarboxylase (*Wurtman et al.*, 1974). Tyrosine administration also increases brain levels of the dopamine metabolites 3-methoxy-4-hydroxy-phenylacetic acid (DOPAC) and homovanillic acid (HVA) in rats given drugs that block central dopaminergic receptors (*Scally et al.*, 1977), given reserpine chronically (*Sved et al.*, 1979 b), or previously subjected to lesions destroying 75—80% of the neurons of the nigrostriatal tract (*Melamed et al.*, 1980). On the other hand, tyrosine fails to affect the increase in brain dopamine among rats given a monoamine oxidase inhibitor (*Wurtman* and *Fernstrom*, 1976), or that of striatal HVA among animals treated with probenecid (*Scally et al.*, 1977). These observations suggest that tyrosine's ability to enhance catecholamine synthesis and release depends upon the extent to which the catecholamine neurons happen to be activated (*Wurtman et al.*, 1979).

Reserpine administration increases brain tyrosine (*Tagliamonte et al.*, 1971), DOPAC (*Andén et al.*, 1963), HVA (*Andén et al.*, 1964), and MHPG-SO_4 (*Bareggi et al.*, 1979) concentrations, concurrently depleting the brain of catecholamines. This report shows that tyrosine administration enhances brain catecholamine synthesis and the release of brain dopamine when catecholamine neurons are activated by a single dose of reserpine.

Materials and Methods

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 200—250 g were housed in groups of 6—8. The animals were exposed to light (Vita-Lite, 300 μW/cm^2; Duro-Test Corp., North Bergen, NJ) from 8 a.m. to 8 p.m. and the ambient temperature was maintained at 22 °C. Food (Charles River Rat-Mouse-Hamster Maintenance Formula) and water were provided *ad libitum*.

Reserpine (Sigma Chemical Co., St. Louis, MO), dissolved in 10% ascorbic acid, was administered intraperitoneally (i.p.). L-tyrosine (Sigma Chemical Co.), also injected i.p., was suspended in 0.9% saline.

Rats were injected with reserpine (2.5 mg/kg) or its vehicle and, 30 minutes later, with tyrosine (200 mg/kg) or its vehicle. Animals were decapitated 90 minutes after the second injection, and their brains were quickly removed, bisected midsagitally and frozen. Half of each brain was used for assay of MHPG-SO_4 (*Meek* and *Neff*, 1972); an aliquot of each such homogenate in 0.15 M $ZnSO_4$ was also assayed for tyrosine (*Waalkes* and *Udenfriend*, 1957). Catecholamines, DOPAC and HVA were measured in the

other half brain using a high-performance liquid chromatograph with an electrochemical detector (HPLC-ED; Bioanalytical Systems, West Lafayette, Ind.). The tissue was homogenized in 4 ml of 0.1 M perchloric acid and centrifuged at 10,000 × *g* for 15 minutes. One hundred μl of the supernatant fluid was taken for analysis of dopamine and norepinephrine (*Felice et al.*, 1978; *Hefti et al.*, 1980), and 500 μl for assay of DOPAC and HVA (*Hefti*, 1979). In some experiments, the corpus striatum and cerebellum were dissected (*Glowinski* and *Iversen*, 1966) and assayed separately: corpus striatum was homogenized in 1.2 ml of 0.1 M perchloric acid, and cerebellum in 0.8 ml of 0.1 M perchloric acid. Aliquots (500 μl) of the supernatant fluid were taken for analysis of DOPAC and HVA as described above.

Data were analyzed by analysis of variance and Student's *t*-test.

Results

Tyrosine treatment increased brain tyrosine levels as reported previously (*Wurtman et al.*, 1974; *Gibson* and *Wurtman*, 1978), but did not cause significant changes in brain levels of dopamine or norepinephrine, or their metabolites DOPAC, HVA and MHPG-SO_4 (Table 1). Reserpine administration increased brain tyrosine, de-

Table 1. *Effect of tyrosine administration and reserpine pretreatment on concentrations of tyrosine, catecholamines and their metabolites in rat brain*

Tissue level		Treatment			
		Vehicle	TYR	Reserpine	Reserpine +TYR
Whole brain					
TYR	(μg/g)	14.1 ± 1.6	18.1 ± 1.0*	22.1 ± 2.2*	28.2 ± 2.2*†
DA	(ng/g)	728 ± 74	733 ± 27	65 ± 18*	56 ± 11*
DOPAC	(ng/g)	111 ± 8	102 ± 9	228 ± 17*	290 ± 21*††
HVA	(ng/g)	85 ± 6	85 ± 7	227 ± 26*	283 ± 15*†
NE	(ng/g)	320 ± 40	340 ± 16	72 ± 7*	73 ± 11*
MHPG-SO_4	(ng/g)	176 ± 12	181 ± 22	253 ± 37*	267 ± 24*
Corpus striatum					
DOPAC	(ng/g)	650 ± 59	603 ± 53	873 ± 92*	1173 ± 64*†
HVA	(ng/g)	186 ± 26	160 ± 24	504 ± 68*	695 ± 41*†
Cerebellum					
DOPAC	(ng/g)	23 ± 4	22 ± 5	29 ± 3**	49 ± 3*†
HVA	(ng/g)	6 ± 1	6 ± 1	54 ± 7*	58 ± 6*

Rats were killed 120 minutes after receiving reserpine (2.5 mg/kg, i.p.) or 90 minutes after tyrosine (200 mg/kg, i.p.).

TYR = tyrosine, DA = dopamine, NE = norepinephrine.

Values are expressed as means ± standard errors.

* $P < 0.01$, ** $P < 0.05$, compared to the group treated with vehicle.

† $P < 0.01$, †† $P < 0.05$, compared to the group treated with reserpine.

creased dopamine and norepinephrine, and increased brain levels of all three catecholamine metabolites measured (DOPAC; HVA; MHPG-SO_4). Among rats pretreated with reserpine, tyrosine administration significantly enhanced the reserpine-induced increases in brain DOPAC and HVA levels, but not in brain MHPG-SO_4. The increases in dopamine metabolites after administration of reserpine plus tyrosine were also observed in the corpus striatum (HVA and DOPAC) and in the cerebellum (DOPAC) (Table 1).

Discussion

These observations show that tyrosine administration increases brain levels of the dopamine metabolites DOPAC and HVA among rats given a dose of reserpine which depletes brain dopamine and norepinephrine by 75—90%.

Tyrosine hydroxylase probably is unsaturated with tyrosine under physiological conditions: its K_m for tyrosine is on the same order as brain tyrosine levels (*Carlsson* and *Lindqvist,* 1978). The activity of tyrosine hydroxylase and its affinity for its cofactor are influenced by the physiological activity of the catecholaminergic neurons (*Thoenen,* 1972; *Roth et al.,* 1975) as well as by such factors as polyanions, phospholipids, salts, ions, and polysaccharides (*Lovenberg et al.,* 1978). Neural firing, possibly acting via calcium or cyclic AMP, causes the enzyme to be phosphorylated (*Joh et al.,* 1978), enhancing its activity and affinity for the tetrahydrobiopterin cofactor (*Goldstein et al.,* 1973; *Harris et al.,* 1974; *Costa et al.,* 1974). It has been estimated that, in unstimulated conditions, 80% of the tyrosine hydroxylase is in an "inactive" state with a low affinity ($K_m = 2$ mM) for tetrahydrobiopterin, while following exposure to phosphorylating conditions the enzyme exhibits high affinity ($K_m = 10\,\mu$M) for the cofactor, thus becoming dependent on available tyrosine levels (W. Lovenberg, R. Levine and L. Miller, submitted for publication). Reserpine, by depleting the catecholamines from brain neurons (*Holzbauer* and *Vogt,* 1956; *Carlsson et al.,* 1958), probably causes a compensatory acceleration in the firing frequency of these neurons (*Iggo* and *Vogt,* 1960; *Bunney et al.,* 1973) thereby increasing the affinity of their tyrosine hydroxylase for the pteridine cofactor (*Zivkovic* and *Guidotti,* 1974) and making the enzyme more dependent on tyrosine levels (*Wurtman et al.,* 1974).

In the present study, reserpine decreased brain dopamine and norepinephrine concentrations, concurrently increasing brain DOPAC and HVA (Table 1). The increase in these metabolites was

enhanced by tyrosine administration. However, tyrosine did not enhance the reserpine-induced increase in brain MHPG-SO_4. This finding may reflect reserpine's ability to suppress the uptake of newly synthesized cytoplasmic dopamine into synaptic vesicles (*Carlsson,* 1959; *Bertler,* 1961) which contain the enzyme (dopamine-β-hydroxylase) needed to convert dopamine to norepinephrine (*Potter* and *Axelrod,* 1963). This failure of uptake would make the newly synthesized dopamine vulnerable to mitochondrial monoamine oxidase, leading to enhanced production of the deaminated dopamine metabolites DOPAC and HVA, but no increase in the formation of MHPG-SO_4.

In reserpinized animals, we observed the enhanced increase in brain DOPAC caused by giving tyrosine in both the corpus striatum, a region rich in dopaminergic nerve terminals (*Carlsson,* 1959; *Bertler* and *Rosengren,* 1959; *Hillarp et al.,* 1966), and the cerebellum, where the predominant catecholamine neurotransmitter is norepinephrine (*Hillarp et al.,* 1966; *Vogt,* 1954). However, the increase in brain HVA in the corpus striatum was not seen in the cerebellum. Differences in the effects of reserpine on DOPAC and HVA levels within particular brain regions have been reported previously (*Andén,* 1964); their mechanism awaits investigation.

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Authors' address: Prof. Dr. *R.J. Wurtman*, Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.