Increased Tyrosine Hydroxylase Activity after Drug-induced Alteration of Sympathetic Transmission

AFTER the destruction of sympathetic nerve endings by 6-hydroxydopamine[1,2], there is a compensatory increase in the catecholamine synthesis and tyrosine hydroxylase activity in the adrenal gland[3]. We have examined the actions of reserpine and phenoxybenzamine on adrenal tyrosine hydroxylase activity. These drugs, like 6-hydroxydopamine[2], interfere with postganglionic sympathetic transmission, but by different mechanisms[4-6]. We also studied the alterations of this enzyme in an embryologically related tissue, the superior cervical sympathetic ganglion. Reserpine and phenoxybenzamine increased the activity of adrenal tyrosine hydroxylase, and reserpine also increased the activity of tyrosine hydroxylase in the superior cervical ganglion. This increase in enzyme activity was prevented by interruption of nerve impulses by decentralization.

Sprague–Dawley male rats, 120–180 g, were obtained from Hormone Assay (Chicago, Illinois) and housed 72 h before use. The weights of control and treated animals were within a 20 g range at the start of each experiment. Animals were killed by a blow on the head and the adrenal glands were rapidly removed and placed in 2·0 ml. of 0·25 M sucrose at 4° C. For each assay of enzyme activity in the ganglion, two right or left superior cervical ganglia were pooled in 0·5 ml. of sucrose. Tissues were homogenized in all-glass homogenizers, centrifuged at 27,000*g* for 10 min, and the supernatant fluid was assayed for tyrosine hydroxylase[7]. Protein content was determined by the method of Lowry *et al.*[8]. Decentralization of the superior cervical ganglia was performed under ether anaesthesia 2 days before reserpine treatment. Adrenal catecholamines were isolated and determined by methods previously described[9,10].

Reserpine administered 24 and 48 h previously produced a three-fold increase in adrenal tyrosine hydroxylase activity. There was also a considerable reduction in the catecholamine content after reserpine. Similar results were obtained with phenoxybenzamine and 6-hydroxydopamine (Table 1).

Table 1. EFFECT OF DRUGS ON TYROSINE HYDROXYLASE ACTIVITY AND CATECHOLAMINE CONTENT OF THE RAT ADRENAL GLANDS

	Tyrosine hydroxylase ($\mu\mu$moles/pair of adrenals/h)	Catecholamines (% of controls)
Control	1·34 ± 0·23	
Reserpine	4·28 ± 0·34*	14 ± 2*
Control	1·54 ± 0·11	
Phenoxybenzamine	3·05 ± 0·21*	34 ± 7*
6-Hydroxydopamine	2·98 ± 0·14*	93 ± 6

The animals were injected 24 and 48 h before use with 5 mg/kg reserpine subcutaneously, 20 mg/kg phenoxybenzamine hydrochloride or 200 mg/kg 6-hydroxydopamine hydrobromide intravenously. Each value represents the mean ± *S.E.* of five or six determinations. Catecholamines were determined as adrenaline[8,9]. The mean value of five controls amounted to 18·6 ± 0·8 μg/pair of adrenals. The concentration of tyrosine in each assay was 15·5 μM, that of 6,7-dimethyl-5,6,7,8-tetrahydropteridine · HCl · $5H_2O$ 1·14 mM.
*$P < 0·01$.

The ganglia of the sympathetic nervous system and the adrenal medulla are both derived from the embryological neural crest. For this reason, we examined the effect of reserpine on the tyrosine hydroxylase activity in the superior cervical ganglion. Administration of reserpine resulted in a two-fold increase in the tyrosine hydroxylase activity (Table 2). The increase in tyrosine hydroxylase activity in the superior cervical ganglion could be dependent on neural impulses, a direct effect of the drug on the ganglion cell or a blood-borne factor. To eliminate the last two possibilities, the right superior cervical ganglion was decentralized by transection of the preganglionic trunk 2 days before the beginning of the reserpine treatment. Decentralization alone produced a negligible effect in the untreated rat. The interruption of nerve impulses by decentralization, however, abolished the rise of tyrosine hydroxylase produced by reserpine (Table 2).

Reserpine, phenoxybenzamine and 6-hydroxydopamine all produce a marked increase in adrenal tyrosine hydroxylase activity. These three agents interfere with sympathetic function by different mechanisms. 6-Hydroxydopamine causes a selective destruction of sympathetic nerve terminals[1,2], reserpine depletes the sympathetic nerve transmitter[4] and phenoxybenzamine produces an α-adrenergic blockade[5]. The impairment of sympathetic function by these mechanisms probably produces a reflex increase in sympathetic nerve activity[6] which may elevate the activity of tyrosine hydroxylase. Evidence for such a mechanism is provided by our observations on tyrosine hydroxylase activity in sympathetic ganglia. Reserpine increased tyrosine hydroxylase activity in sympathetic ganglia as well as the adrenal gland, and the effect on the ganglion could be prevented by interruption of nerve impulses by decentralization.

Table 2. EFFECT OF RESERPINE ON TYROSINE HYDROXYLASE ACTIVITY OF NORMAL AND DECENTRALIZED RAT SUPERIOR CERVICAL GANGLION

	Tyrosine hydroxylase activity ($\mu\mu$moles/mg protein/h)	
	Normal	Decentralized
Control	35·8 ± 2·8	28·8 ± 1·9
Reserpine	75·9 ± 4·1*	32·8 ± 3·8

Each value represents the mean ± *S.E.* of six determinations. The animals were pretreated with 5 mg/kg reserpine subcutaneously 24 and 48 h before use. The concentration of tyrosine in each assay was 0·65 μM, that of 6,7-dimethyl-5,6,7,8-tetrahydropteridine · HCl · $5H_2O$ 0·72 mM.
* $P < 0·01$.

It has previously been shown that increased nervous activity resulting from nerve stimulation[11] or phenoxybenzamine[12] produces an increase in the synthesis of the neurotransmitter, noradrenaline, without an increase in tyrosine hydroxylase. This effect has been attributed to an increase in tyrosine transport, an increase in co-factor concentration or a decrease in end product inhibition[11,12]. Our results indicate that tyrosine hydroxylase activity can be increased by prolonged and/or intense sympathetic nerve activity induced by drugs. This conclusion is supported by preliminary observations that several hours are required before an increase in enzyme activity can be detected.

Two mechanisms seem to be concerned with the control of tyrosine hydroxylase activity, one involving rapid regulation by end product inhibition, tyrosine transport or cofactor concentration and another by increasing the enzyme activity. Preliminary observations indicate that this increased activity is due to an increased amount of enzyme protein.

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1 Tranzer, J. P., and Thoenen, H., *Experientia*, **24**, 155 (1968).
2 Thoenen, H., and Tranzer, J. P., *Naunyn-Schmiedebergs Arch. Pharmak. Exp. Path.*, **261**, 271 (1968).
3 Mueller, R. A., Thoenen, H., and Axelrod, J., *Science*, **163**, 468 (1969).
4 Carlsson, A., Rosengren, E., Bertler, A., and Nilsson, J., in *Psychotropic Drugs*, **6**, 363 (Elsevier, Amsterdam, 1957).
5 Nickerson, M., and Gump, W. S., *J. Pharmacol. Exp. Therap.*, **97**, 25 (1949).
6 Iggo, A., and Vogt, M., *J. Physiol.*, **150**, 114 (1960).
7 Levitt, M., Gibb, J. W., Daly, J. W., Lipton, M., and Udenfriend, S., *Biochem. Pharmacol.*, **16**, 1313 (1967).
8 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
9 Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exp. Therap.*, **138**, 360 (1962).
10 Euler, U. S. von, and Lishajko, F., *Acta Physiol. Scand.*, **51**, 348 (1961).
11 Sedvall, G. C., and Kopin, I. J., *Biochem. Pharmacol.*, **16**, 39 (1967).
12 Dairman, W., Gordon, R., Spector, S., Sjoerdsma, A., and Udenfriend, S., *Mol. Pharmacol.*, **4**, 457 (1968).

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