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Resistance of noradrenaline in blood vessels to depletion by 6-hydroxydopamine or immunosympathectomy

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Summary

1. The degree of the decrease in the noradrenaline concentrations caused by 6-hydroxydopamine or immunosympathectomy was different in different areas of the cardiovascular system.

2. In rats or guinea-pigs 6-hydroxydopamine depleted the noradrenaline content of the heart by 90%, of the mesenteric vein by 80% and of the mesenteric artery and aorta by 30–60%. Immunosympathectomy elicited a 70% reduction in the cardiac noradrenaline but only a 50% reduction in the noradrenaline of the blood vessels of the rat.

3. The tyrosine hydroxylase activity of the heart, blood vessels, or adrenal glands was not significantly altered 2 weeks after 6-hydroxydopamine. Nor was the monoamine oxidase activity in heart or blood vessels changed.

4. The inconsistent ability of both 6-hydroxydopamine and immunosympathectomy to abolish experimental hypertension may be due to the partial persistence of noradrenaline and functional sympathetic nervous system activity in the blood vessels.

Introduction

Many drugs which modify hypertension elicit their effects by interfering with the function of the sympathetic nervous system. Two drugs which drastically interfere with the activity of the sympathetic nervous system by means of a 'chemical sympathectomy' are 6-hydroxydopamine (6OH-D) (Thoenen & Tranzer, 1968) and nerve growth factor antiserum (Levi-Montalcini & Angeletti, 1966). Both these agents have only a partial or poor effect in antagonizing experimental hypertension (Varma, 1967 ; Finch & Leach, 1970a ; Müeller & Thoenen, 1970 ; Ayitey-Smith & Varma, 1970 ; Clark, 1971), although they were used in doses which were reported to cause an almost complete loss of the noradrenaline in the heart and spleen. This may be due to a resistance of the catecholamines in the adrenal gland to these drugs (Finch & Leach, 1970a). However, 6-hydroxydopamine fails to decrease the blood pressure even after adrenal demedullation (Finch & Leach, 1970b). Even more enigmatic was the observation that rats which were both immunosympathectomized and adrenalectomized had a normal or even increased urinary excretion of noradrenaline (Carpi & Oliverio, 1964). As these observations could be explained by a persistence of the vascular catecholamines, the relative sensitivity of the nor-

adrenaline in the heart and in the vascular smooth muscle to depletion by 6-hydroxydopamine or immunosympathectomy was studied in these experiments.

Methods

Male or female Sprague-Dawley rats and male Hartley Strain guinea-pigs were killed by cervical dislocation. The heart, aorta, superior mesenteric artery, abdominal vena cava and mesenteric vein (including the portal vein up to the diaphragm) were dissected out, freed from adhering tissue, immediately frozen on dry ice and stored frozen until analysis. Vessels were pooled for analysis as indicated in the tables. Rat blood pressures were measured in the unanaesthetized rat using the tail cuff method.

Noradrenaline in the heart and blood vessels was isolated by alumina adsorption, eluted and estimated fluorometrically after ferricyanide oxidation of 0·4 cc of the final acid eluate (Anton & Sayre, 1962). The catecholamines in the adrenal glands were determined by the method of Shore & Olin (1958). All values were corrected for recoveries ($70 \pm 10\%$) by using internal standards.

Tyrosine hydroxylase activity was measured in the 30,000 *g* supernatant fluid from a single pressed heart or in an aliquot of the homogenate of a pair of adrenal glands or two blood vessels by a modification (Tarver, Berkowitz & Spector, 1971) of the radioisotope method of Nagatsu, Levitt & Udenfriend (1964). Monoamine oxidase activity was measured in homogenized individual tissues by the radioisotope method of Wurtman & Axelrod (1963). Proteins were determined by the procedure of Lowry, Rosebraugh, Farr & Randall (1951).

Studies with 6-hydroxydopamine hydrobromide (6OH-D) were done 24 h after a single intraperitoneal dose or as described by Finch & Leach (1970a) using the schedule of Thoenen & Tranzer (1968). Male rats (130–140 g) were injected intravenously twice on day 1 of the experiment with 50 mg/kg, and twice on day 7 with 100 mg/kg ; rats were killed on day 15.

Female rats were immunosympathectomized as described by Finch & Leach (1970c) with double strength equine (instead of bovine) nerve growth factor antiserum. On the day of birth and for the next 4 days rats were injected subcutaneously with a dose of 0·1, 0·1, 0·2, 0·2 and 0·4 ml respectively. Rats were killed when they weighed 190–200 g. The controls were litter mates handled identically except they were injected with saline instead of nerve growth factor antisera.

Drugs

6-Hydroxydopamine HBr and (−)-6-hydroxydopa HBr were obtained from Hoffmann-La Roche (Nutley, N.J.). The dopa derivatives were dissolved in 0·9% sodium chloride which was acidified with a few drops of 1 N hydrochloric acid and injected immediately. Equine nerve growth factor antiserum was purchased from Burroughs Wellcome Co., North Carolina.

Results

Effect of 6-hydroxydopamine on the concentration of catecholamines in the rat and guinea-pig

The treatment of rats with two doses of 6OH-D caused a relatively smaller loss of noradrenaline from the blood vessels when compared with the heart or the spleen

(Table 1). The adrenals were not depleted by 6OH-D. Similar results were seen in the guinea-pig. Twenty-four hours after a single dose of 6OH-D 50 (mg/kg i.p.) the noradrenaline concentration in the aorta and the mesenteric artery was depleted by about 50%, the mesenteric vein 84% and the heart 96%.

Effect of (−)-6-hydroxydopa or a low dose of 6-hydroxydopamine on the concentration of noradrenaline in the rat heart and mesenteric artery

6-Hydroxydopa, which is probably decarboxylated *in vivo* to 6-hydroxydopamine, is also capable of depleting peripheral and central catecholamine stores (Ong, Crevling & Daly, 1969). The optical isomers of the compound have been synthesized and the laevorotory isomer is the most active (Berkowitz, Spector, Brossi, Focella & Teitel, 1970). When (−)-6-hydroxydopa was given in a dose of 100 mg/kg intraperitoneally once a day for 2 days the heart noradrenaline fell by 90% from a control level of 1·5 μg/g to 0·11 μg/g ($n=5$). The noradrenaline concentration in the mesenteric artery on the other hand was only decreased by 22% from 3·79 μg/g to 2·95 μg/g while that of the vena cava and mesenteric veins fell about 80%.

After a low dose of 6OH-D to rats, the noradrenaline in the mesenteric artery was also more resistant to depletion than that of the heart. Twenty-four hours after a dose of 6OH-D (3 mg/kg i.p.), the concentration of norepinephrine in the heart fell significantly by 27% but the mesenteric arterial concentration was unchanged.

Influence of immunosympathectomy on catecholamines in the heart and blood vessels of the rat

Results obtained in rats which were treated with nerve growth factor antisera (equine) are shown in Table 2. Like 6OH-D this treatment depleted noradrenaline to a greater extent in the heart (−70%) than in blood vessels (−42 to −52%).

TABLE 1. *Effect of 6-hydroxydopamine (6OH-D) on the concentration of catecholamines in the rat and guinea-pig*

Species and tissue	Noradrenaline (μg/g)† Control	6OH-D	% Decrease after 6OH-D
Rat			
Heart	1·13±0·13	0·15±0·02*	87
Aorta	0·41±0·23	0·16±0·02	61
Mesenteric artery	3·39±0·75	2·43±0·43	28
Mesenteric vein	2·30−2·50	0·50−0·70	75
Spleen	0·30±0·04	0·05±0·01*	83
Adrenal glands	22·60±0·74	24·90±0·79	0
Guinea-pig			
Heart	1·59±0·13	0·07±0·03*	96
Aorta	2·50±0·64	1·08±0·58	57
Mesenteric artery	2·27±0·09	1·12±0·15*	51
Mesenteric vein	0·79−1·00	0·13−0·33	84

† The results are the mean of five-six determinations ± S.E.M. for heart, spleen and adrenal catecholamines. Vessels were pooled: four aortas, three mesenteric arteries and five mesenteric veins for each determination and three determinations averaged except for the mesenteric vein where the range of two determinations is given. 6OH-D was administered to rats (140 g i.v.) twice within 24 h on day 1, 50 mg/kg twice on day 7, 100 mg/kg. The rats were killed on day 15. 6OH-D (50 mg/kg i.p.) was injected into guinea-pigs and sacrificed 24 h later. Adrenal noradrenaline is as μg/per pair of adrenal glands. * The difference between controls and treated animals statistically significant $P<0·05$.

Effect of 6-hydroxydopamine on tyrosine hydroxylase and monoamine oxidase activity in rat blood vessels and adrenals

Table 3 shows that there was no significant change in the activities of monoamine oxidase or tyrosine hydroxylase in the heart of rats treated with 6OH-D (50 mg/kg twice on day one and 100 mg/kg twice on day 7, killed on day 15) although cardiac tyrosine hydroxylase did decline 30%. The mesenteric artery and the aorta in the same rats also did not show any change in the activity of these enzymes.

Discussion

These studies show a different sensitivity of the noradrenaline contained in the heart and the noradrenaline contained in blood vessels of rats or guinea-pigs towards 6OH-D. In agreement with the studies of Thoenen & Tranzer (1968) 85–95% of heart noradrenaline may be depleted by 6OH-D. However, quantitatively different effects occur in the blood vessels of the same animals. The noradrenaline in the mesenteric artery and aorta is depleted only 30–60%. The ability of the drug to deplete mesenteric vein noradrenaline (75–85%) is intermediate between the heart and arteries. A quantitatively greater effect of 6OH-D on heart than on blood vessels is also suggested by the studies of Fleisch, Saul & Mailing (1970); Goldman & Jacobowitz (1971) and Häusler, Haefely & Huerlimann (1971). Moreover, Stone,

TABLE 2. *Effect of immunosympathectomy on catecholamines in the heart and blood vessels of the rat*

Tissue	Noradrenaline (μg/g)† Control	Immunosympathectomy	% Change
Heart	1·00±0·06	0·30±0·13*	70
Aorta	0·64±0·10	0·33±0·03	48
Mesenteric artery	4·20±0·24	2·00±0·05*	52
Mesenteric vein	(1·94–2·58)	(1·28–1·35)	42

† Double strength horse nerve growth factor antiserum was injected subcutaneously on five consecutive days beginning on the day of birth. Rats were killed when they weighed 200 g. Five hearts, three groups of aortas or mesenteric arteries and two groups of mesenteric veins were assayed and the results expressed as mean ± S.E. of the mean. Tissues pooled as in Table 1. * Statistically significant as compared to control animals ($P<0·05$).

TABLE 3. *Effect of 6-hydroxydopamine on tyrosine hydroxylase and monoamine oxidase activity in the heart, brain, adrenal and cardiovascular systems of the rat*

Tissue*	Tyrosine hydroxylase† Control	6OH-D	Monoamine oxidase† Control	6OH-D
Heart	0·12±0·04	0·08±0·04	8·80±2·6	7·2 ±2·1
Mesenteric artery	1·52±0·28	1·64±0·44	6·16±1·0	8·07±2·4
Aorta	—	—	5·5 ±0·5	4·6 ±0·8
Brainstem	—	—	19·1 ±2·0	16·6 ±1·8
Adrenals	4·72±0·32	5·24±0·32	—	—

* Heart, adrenal gland pairs, and brainstem were analysed individually. The results of three-four tissues were averaged ± the standard deviation. For tyrosine hydroxylase two blood vessels were pooled and the results of three-four groups averaged. For monoamine oxidase, vessels were analysed individually. 6OH-D was administered intravenously twice within 24 h to 130 g male rats on day 1 of the experiment (50 mg/kg) and on day 7 (100 mg/kg) and rats were killed on day 15. † Tyrosine hydroxylase and monoamine oxidase were assayed as described in **Methods** and **Results** are expressed as (nmol dopa/h)/mg protein and (nmol indole acetic acid/20 min)/mg protein respectively.

Stavorski, Ludden, Wenger, Ross, Totaro & Porter (1963) found that the 6OH-D congener, 6-amino dopamine, depleted the heart and spleen noradrenaline in the dog to a much greater extent than that of the blood vessels.

The mechanism for the relative resistance of some vascular beds to the action of 6OH-D is not clear. There is evidence (Goldman & Jacobowitz, 1971 ; McGregor & Phelan, 1969) that the sympathetic nerves in the blood vessels regenerate faster than those in the heart. If this is the reason for the differential effects of 6OH-D, the differences should be less apparent shortly after administration of the drug than 1 or 2 weeks later. However, our results on the acute effects of (−)-6-hydroxydopa in the rat and 6OH-D in the rat and guinea-pig show that even 24 or 48 h after drug administration the noradrenaline content of the aorta and mesenteric arteries was depleted to a lesser extent than that of the heart. Thus, a faster regeneration of vascular sympathetic nerves cannot entirely explain the relative resistance of the aorta and mesenteric artery.

An alternate explanation for this differential effect could be that less 6OH-D reaches the sympathetic nerves in the aorta and mesenteric artery than the nerves in the heart. This view is supported by the observation that noradrenaline, which has a structure similar to 6OH-D, does not readily reach the sympathetic nerves in arteries if it is applied into the lumen of the vessel (De La Lande, Frewin & Waterson, 1967) and that the *in vivo* uptake of noradrenaline by blood vessels is much less that that of the heart (Berkowitz, Tarver & Spector, 1971).

However, whatever the mechanism, the tissue differences noted could possibly be overcome if enough 6OH-D were used. The direct application of the drug in the immediate vicinity of arteries clearly destroys periarteriolar sympathetic nerves (Siggins & Bloom, 1970). The resistance of vascular noradrenaline to these drugs particularly in the mesenteric artery, does not reflect a general insensitivity of this tissue to catecholamine depleting drugs, since reserpine depletes the noradrenaline in the mesenteric vessels or very low concentrations (Berkowitz, *et al.*, 1971).

An increased activity of tyrosine hydroxylase in the adrenal glands was observed 2 days after a large dose of 6OH-D (Thoenen, Müeller & Axelrod, 1969a, 1969b). In contrast, we did not find any significant change in adrenal tyrosine hydroxylase activity 15 days after the first of two doses of 6OH-D (Table 3) despite the negligible concentrations of noradrenaline in heart and spleen. Thus the increase in adrenal tyrosine hydroxylase after 6OH-D may not be sustained. The unchanged tyrosine hydroxylase activity in the mesenteric artery after 6OH-D is further evidence that the sympathetic nerves in this vascular bed have not been completely destroyed.

Our studies show that 6OH-D also fails to alter monoamine oxidase activity in the blood vessels or in the heart and thus confirms recent studies of Lowe & Horita (1970) and Jarrot (1971). The data could be explained by the observation that a large proportion of the MAO-activity in the blood vessels is located extraneuronally (De La Lande, Hill, Jellett & McNeil, 1970).

Immunosympathectomy with antisera to the nerve growth factor depletes the noradrenaline content of the heart to a larger extent than that of the aorta, mesenteric artery or mesenteric vein. The only tissues in which the vasculature has been reported to be depleted of noradrenaline by immunosympathectomy have been in skeletal muscles and iris while adrenergic terminals in the intestines were resistant to immunosympathectomy (Hamberger, Levi-Montalcini, Norberg &

Sjöqvist, 1965). Finch & Leach (1970c), have reported that catecholamine containing nerve fibres were still present in the coronary vessels after rats had been immunosympathectomized. Moreover, mesenteric arteries are still able to respond to sympathetic nerve stimulation (Clark, 1971) and rats are not hypotensive (Finch & Leach, 1970c) after immunosympathectomy.

The persistence of urinary catecholamine metabolites (Ceasar, Ruthven & Sandler, 1969) in immunosympathectomized rats and the ability to increase the amounts of noradrenaline in the urine of rats which have been immunosympathectomized and adrenalectomized (Carpi & Oliverio, 1964) may also be explained by the present data. It is probable that the major source of excreted norepinephrine in the urine derives from blood vessels (Spector, Tarver & Berkowitz, 1971 ; Bigelow, Dairman, Weil-Malherbe & Udenfriend, 1969). Thus, the continued presence of noradrenaline and metabolites in the urine of these rats may result from only a partial depletion of vascular noradrenaline.

The reason for the resistance of experimental hypertension to either 6OH-D or antiserum to nerve growth factor (Varma, 1967 ; Finch & Leach, 1970a, b) could be explained by the presence of sufficient noradrenaline in critical sites in the blood vessels to maintain an elevated total peripheral resistance.

These studies also indicate that when studying the disposition of noradrenaline *in vivo* consideration should be given to the handling of the biogenic amine by the vasculature.

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