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A comparison of the effects of 6-hydroxydopamine immunosympathectomy and reserpine on the cardiovascular reactivity in the rat

L. FINCH AND G. D. H. LEACH

School of Studies in Pharmacology, University of Bradford, Bradford, 7, Yorkshire, U.K.

In the conscious rat, 6-hydroxydopamine, or reserpine (5 mg/kg) pretreatment produced a marked fall in the mean systolic blood pressure whilst immunosympathectomized rats had resting blood pressures just below that of control animals. In pithed preparations, 6-hydroxydopamine treatment or immunosympathectomy potentiated the pressor responses to injected noradrenaline; reserpine pretreatment did not potentiate the noradrenaline response to the same degree. Tyramine responses were abolished after 6-hydroxydopamine or reserpine pretreatment but were unaffected by immunosympathectomy. Stimulation of the sympathetic outflow by the Gillespie & Muir (1967) preparation was abolished after 6-hydroxydopamine and reserpine pretreatment, and reduced after immunosympathectomy. It is concluded that 6-hydroxydopamine produces a destruction of the sympathetic nerve endings, abolishing the physiological uptake process and, therefore, producing supersensitivity to injected noradrenaline. Immunosympathectomy, although showing a marked reduction in sympathetic nerve supply leaves a functional uptake process. Reserpine (5 mg/kg), given 6 h previously, depletes endogenous catecholamines without significantly altering the sensitivity to injected noradrenaline, the uptake process remaining functional.

6-Hydroxydopamine has been shown to deplete adrenergic nerves of their endogenous noradrenaline (Porter, Totaro & Stone, 1963; Laverty, Sharman & Vogt, 1965). Recently, electron microscope studies have shown that pretreatment with 6-hydroxydopamine leads to destruction of the adrenergic nerve endings and a consequent depletion of their amine stores (Thoenen & Tranzer, 1968).

Immunosympathectomy (IS) produced by nerve growth factor anti-serum has been shown to destroy sympathetic ganglia of the rat (Levi-Montalcini & Booker, 1960; Zaimis, 1967). Biochemical studies have further shown that the noradrenaline contents of some sympathetically innervated tissues are markedly reduced in IS animals (Zaimis, 1966; Iversen, Glowinski & Axelrod, 1966).

The administration of reserpine produces a long lasting depletion of catecholamines from the brain and peripheral tissues (Holzbauer & Vogt, 1956; Carlsson, Rosengren & others, 1957; Shore, 1962), although it is not thought to interfere with the uptake of catecholamines into the sympathetic nerves (Iversen, 1967). Supersensitivity develops slowly in reserpinized tissues and does not resemble that seen after cocaine (Trendelenburg, 1963). After chronic post-ganglionic denervation, supersensitivity to noradrenaline has been demonstrated on the nictitating membrane (Trendelenburg & Weiner,

1962). Zaimis (1967) showed that in the IS rat the pressor and inotropic responses to noradrenaline and adrenaline were prolonged when compared with control animals. More recently workers have shown that supersensitivity to noradrenaline occurs in the nictitating membrane after pretreatment with 6-hydroxydopamine (Häusler, Thoenen & Haefely, 1968). We have investigated the effects of 6-hydroxydopamine reserpine and IS on the cardiovascular system of the rat.

EXPERIMENTAL

Methods

All experiments were made using male C.S.E. rats (Scientific Products), of 180–200 g.

Mean systolic blood pressure in conscious animals was measured, using the tail cuff method, and a semi-conductor strain gauge (Ether Ltd.), mounted on a tail clip for detection of the pulse and visually displayed on an oscilloscope (Telequipment). Measurements were made with animals held in Bowman restraining cages and placed inside a warming cabinet (33° ± 1°) for 15 min. Each determination was the mean of three readings.

Anaesthetized and pithed preparations were set up as described previously (Finch & Leach, 1969). In some experiments the pithed preparation was used for stimulation of the entire sympathetic outflow (Gillespie & Muir, 1967); submaximal stimulation from a Multitone stimulator at strengths of 15–30 V, 0·03 ms duration and a frequency of 3 pulses/s was applied for periods of 18 s. Stimulations were repeated at not less than 10 min intervals. Atropine (0·5 mg/kg) and tubocurarine (1 mg/kg) were given before beginning stimulation.

Slices of atria and ventricles were prepared for histochemical fluorescence microscopy according to the method of Spriggs, Lever & others (1966), with additional details described by Clarke, Jones & Linley (1969).

Pretreatment

Chemical sympathectomy was carried out in rats of 160–175 g. 6-Hydroxydopamine hydrobromide was given 2 × 50 mg/kg intravenously on day 1, followed by 2 × 100 mg/kg on day 7 and experiments were made on day 8–10 (Thoenen & Tranzer, 1968). Reserpine 5 mg/kg was given intraperitoneally 6 h before recordings were taken. Immunosympathectomized rats were obtained using double strength cow nerve growth anti-serum, injected subcutaneoulsy on the day of birth and on the following four days with doses of 0·1, 0·1, 0·2, 0·2, 0·4 ml.

Drugs and solutions

All stock solutions of drugs were diluted in 0·9% w/v NaCl solution before use. Noradrenaline acid tartrate (Hoechst), calculated as base, was stored in 0·01N HCl. The following drugs were calculated as salt; atropine sulphate (Northern Pharmaceuticals); desipramine (Geigy); dimethylphenylpiperazinium iodide (Aldrich); mecamylamine hydrochloride (Merck, Sharp & Dohme); reserpine phosphate (CIBA) dissolved in 20% ascorbic acid; tubocurarine chloride (Burroughs Wellcome); and tyramine hydrochloride (BDH). 6-Hydroxydopamine HBr, generously donated by Dr's Thoenen and Hürlimann (Hoffmann-La Roche, Basle) was dissolved in 0·001 N

hydrochloric acid previously bubbled with nitrogen. Cow nerve growth anti-serum (Batch Ex 4945/46/47) was generously donated by Dr. C. Edwards (Wellcome Research Laboratories, Beckenham, Kent).

RESULTS

Effect of 6-hydroxydopamine reserpine and IS on the mean resting blood pressure of rats

In the conscious untreated rat the mean resting systolic blood pressure was found to be 115 mm of Hg whilst 6-hydroxydopamine treated rats or rats pretreated with reserpine 6 h previously exhibited much lower pressures of 94 and 84 mm of Hg respectively (Table 1). IS rats had slightly lower blood pressures (103 mm of Hg) than the control group. After anaesthesia (sodium pentobarbitone, 60 mg/kg), the mean resting blood pressures of 6-hydroxydopamine- and reserpine-treated rats were markedly lower than those of the corresponding control animals (Table 1). After pretreatment with mecamylamine (10 mg/kg), or pithing, the blood pressures of IS and 6-hydroxydopamine rats did not significantly differ from their respective control groups. However, rats pretreated with reserpine showed significantly elevated pressures in both cases when compared with control preparations.

Effect of 6-hydroxydopamine, reserpine and IS on noradrenaline, tyramine and dimethylphenylpiperazinium (DMPP) sensitivity in pithed rat preparations

In the pithed rat preparation the mean pressor response to a range of noradrenaline doses (0·125–1 μg/kg) was found to be potentiated in both magnitude and duration after pretreatment with 6-hydroxydopamine (Fig. 1). IS rats also showed potentiated

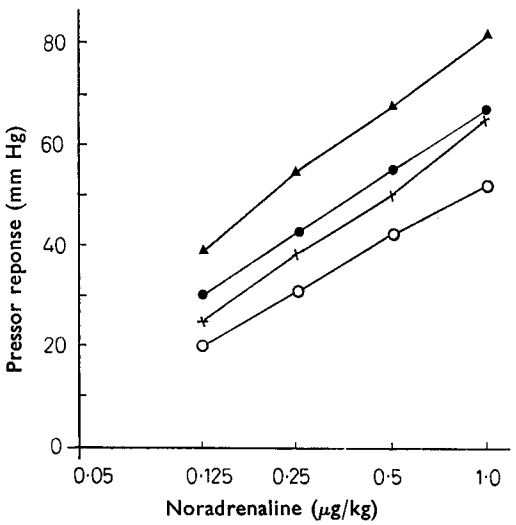

FIG. 1. Noradrenaline log dose-response curves obtained using pithed rat preparations. Each line represents the mean of four experiments. (○) control; (×) reserpine 5 mg/kg, 6 h previously; (●) Immunosympathectomized rats; and (▲) 6-hydroxydopamine pretreatment.

responses but the effect was not as marked as after 6-hydroxydopamine pretreatment. Reserpinized preparations showed only a slight increase in sensitivity to noradrenaline when compared with control preparations. However, reserpine pretreatment (5 mg/kg, 6 h previously) caused a marked slowing of the heart. Desipramine (0·5 mg/kg),

Table 1. *Effect of 6-hydroxydopamine, reserpine and immunosympathectomy on the resting blood pressure of rats.* The treatments significantly reduced the blood pressure in conscious and pentobarbitone treated animals.

Pretreatment	Conscious†	Pentobarbitone (60 mg/kg, i.p.)‡	Pentobarbitone and mecamylamine (10 mg/kg, i.v.)	Pithed preparations‡
Untreated	..115±3 (12)	119±2 (17)	73±2 (17)	50±1·5 (18)
6-Hydroxydopamine ..	.. 94±1·9 (13)*	89±3·3 (5)*	65±3·5 (5)	52±5·8 (6)
Reserpine (5 mg/kg, 6 h previously)	.. 84±3 (7)*	80±4·5 (7)*	80±2·3 (8)*	64±3 (9)*
Immunosympathectomy	103±3 (7)*	101±2·7 (8)*	69±2·8 (9)	51±2·2 (6)

* $P < 0{\cdot}05$ compared with untreated groups.
† Indirect measurement (mean systolic blood pressure in mm of Hg ± standard error of mean).
‡ Direct measurement (mean blood pressure in mm of Hg ± standard error of mean).
( ) indicates number of experiments.

which is known to block the uptake of noradrenaline into adrenergic nerve terminals, was seen to potentiate the noradrenaline pressor responses in both magnitude and duration, in rats pretreated with reserpine, IS and the untreated control preparations (Fig. 2). In 6-hydroxydopamine rats, desipramine produced only a slight potentiation of noradrenaline responses, suggesting that 6-hydroxydopamine almost completely destroys the physiological uptake mechanism. It can also be seen that noradrenaline sensitivity after desipramine is approximately equal for the control and all the pretreated preparations.

6-Hydroxydopamine and reserpine pretreatment abolished the response to tyramine in pithed rat preparations (Table 2), whereas, IS rats gave similar responses to those

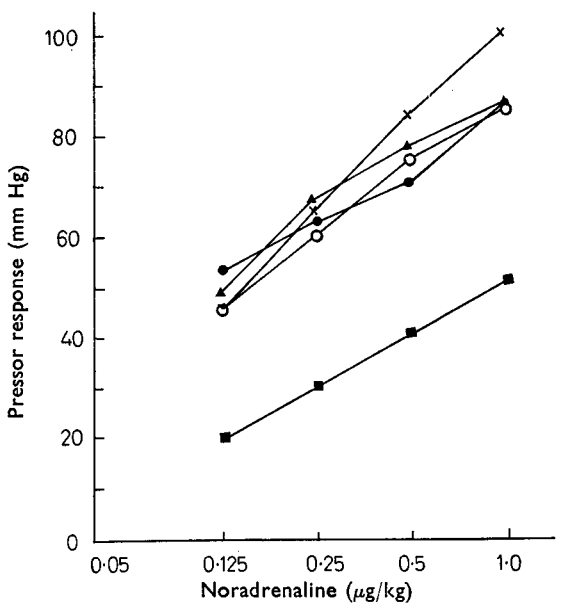

FIG. 2. Noradrenaline log dose-response curves before and after desipramine (0·5 mg/kg, i.v.) obtained using pithed rat preparations. Each line represents the mean of four experiments. (■) Untreated controls. After desipramine (○) control; (×) reserpine 5 mg/kg, 6 h previously: (●) immunosympathectomized rats; and (▲) 6-hydroxydopamine pretreatment.

obtained in control preparations. The pressor responses to DMPP, a ganglion stimulating agent, were markedly reduced after reserpine pretreatment whilst responses with 6-hydroxydopamine rats were potentiated. The responses to DMPP in IS rats were unaltered from those seen in the control pithed preparations.

Table 2. *The effect of 6-hydroxydopamine, reserpine and immunosympathectomy on tyramine and dimethylphenylpiperazinium responses in the pithed rat.* 6-Hydroxydopamine and reserpine abolished the response to tyramine. The response to DMPP was reduced after reserpine but potentiated after 6-hydroxydopamine.

Pretreatment	Mean blood pressure rise (Expressed in mm of Hg ± standard error of mean)			
	Tyramine		Dimethylphenylpiperazinium	
	125 μg/kg	250 μg/kg	50 μg/kg	100 μg/kg
Untreated	28±2·9 (8)	49±4·4 (8)	12±2·1 (6)	31±3·1 (6)
6-Hydroxydopamine	6±0·9 (8)*	8±1·2 (8)*	21±2·2 (7)*	41±2·6 (8)*
Reserpine (5 mg/kg, 6 h previously)	3±0·8 (8)*	6·5±1·5 (8)*	4±1·0 (6)*	11±1·2 (6)*
Immunosympathectomy	29±3·8 (8)	39±4·2 (8)	11·5±2 (6)	33·5±2·2 (6)

* $P < 0{\cdot}05$ compared with untreated groups.
( ) indicates number of experiments.

Effect of 6-hydroxydopamine, reserpine, IS on the Gillespie and Muir preparation

Using the stimulation parameters described under methods, reproducible submaximal pressor responses were obtained in all preparations. Pretreatment with reserpine or 6-hydroxydopamine produced a marked reduction in the magnitude of the pressor responses, together with a latency of 8–12 s in onset of the effect (Fig. 3). IS rats showed an overall reduction in the size of the pressor response, which was not as marked as after reserpine or 6-hydroxydopamine pretreatments; the onset of the response was normal in comparison with control preparations.

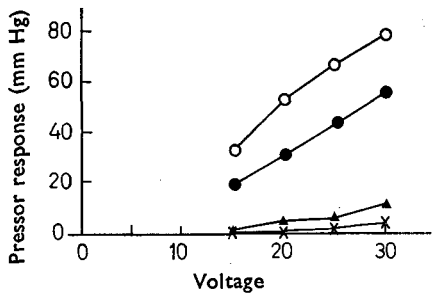

FIG. 3. The effect of catecholamine depletion on the pressor responses obtained from Gillespie and Muir preparations. Sympathetic outflow stimulated for 18 s at a pulse duration of 0·03 ms, a frequency of 3/s and repeated every 10 min at varying voltage. Each line represents the mean of four experiments. (○) control; (×) reserpine 5 mg/kg, 6 h previously; (●) immunosympathectomized rats and (▲) 6-hydroxydopamine pretreatment.

Histochemical fluorescence studies

Pretreatment with 6-hydroxydopamine or reserpine completely abolished the specific fluorescence attributable to adrenergic nerves in both atria and ventricle slices.

This is in agreement with catecholamine analysis of these pretreatments (Thoenen & Tranzer, 1968; Clarke & Jones, 1969; Carlsson & others, 1957).

IS rats showed a marked reduction in fluorescence intensity attributable to adrenergic nerves. However, in two preparations, isolated coronary vessels with adrenergic nerve attachments were observed.

DISCUSSION

Pretreatment with reserpine or 6-hydroxydopamine produced a significant fall in the resting systolic blood pressure in the conscious rat, although IS rats showed only a slight fall when compared with control animals. These results with IS rats confirm the findings of Zaimis (1967). The difference in resting blood pressures may be attributable to several factors. Firstly reserpine or 6-hydroxydopamine pretreatments, at the dose levels used, are more efficient at depleting endogenous catecholamines (Shore, 1962; Thoenen & Tranzer, 1968; Clarke & Jones, 1969) compared with IS procedures Iversen & others, 1966; Zaimis, 1967). Also the increased turnover of adrenal catecholamines in IS animals may be the reason for their near normal resting blood pressures (Iversen & others, 1966). Recently Thoenen, Mueller & Axelrod (1969) have demonstrated an increased adrenal tyrosine hydroxylase activity in rats pretreated with reserpine or 6-hydroxydopamine. However, in our experiments compensation of the blood pressure may not have taken place in the time interval of the pretreatment procedures.

Most sensitivity experiments concerned with denervation and blockade of sympathetic function have been made on cats and, therefore, results described in this paper cannot be fully compared with those described by Trendelenburg (1963). However, 6-hydroxydopamine pretreatment potentiated the pressor responses to noradrenaline in the pithed rat preparation suggesting that this pretreatment produces supersensitivity similar to denervation. These conclusions are in accord with the findings of Thoenen & Tranzer (1968) in which 6-hydroxydopamine produced a depletion of noradrenaline in sympathetically innervated nerve endings to an extent comparable with that seen after surgical denervation or IS procedures. Recently these workers also showed potentiation of the noradrenaline response on the nictitating membrane in cats pretreated with 6-hydroxydopamine (Häusler & others, 1968). The tyramine responses were almost completely abolished and desipramine failed to further potentiate injected noradrenaline to any marked degree in the pithed rat preparation pretreated with 6-hydroxydopamine. These results suggest that destruction of the sympathetic nerves supplying the cardiovascular system was virtually complete together with the elimination of their physiological uptake mechanism. Further evidence in support of these findings is the fact that sympathetic stimulation (Gillespie & Muir preparation) produces a pressor response which in terms of latency and magnitude is most likely attributable to stimulation of the adrenal medulla. Also Malmfors & Sachs (1968) showed pretreatment with 6-hydroxydopamine prevented the retention of endogenous noradrenaline and α-methyldopa in the mouse iris.

IS rats showed increased responsiveness to noradrenaline in pithed preparations but this was less marked than that seen in 6-hydroxydopamine-pretreated animals. Pressor responses to tyramine and DMPP were found to be normal yet responses after sympathetic stimulation (Gillespie & Muir preparation) were markedly reduced compared with normal preparations. These results suggest that although IS rats have a

severely reduced sympathetic supply to the cardiovascular system, near normal physiological function is capable of being maintained. It is also supported by the results of histochemical fluorescence showing that some sympathetic nerves were still present in the heart. Although the uptake process may be reduced after IS, the results suggest that a sufficient proportion still remains functional as shown by the fact that injected noradrenaline responses were capable of substantial potentiation after desipramine in IS pithed preparations. These conclusions are in accordance with the findings of Iversen & others (1966) who found that noradrenaline uptake was reduced in various sympathetically innervated tissues of IS rats, but in none of these experiments did they find the uptake process completely abolished.

Reserpine has been used throughout these experiments as a known depletor of adrenergic catecholamines. The fact that tyramine responses and sympathetic nerve stimulation were abolished suggests a complete depletion of sympathetic neuronal stores of catecholamines supplying the cardiovascular system. Desipramine markedly potentiated the noradrenaline responses and strongly suggests that the uptake processes still remain functional, although the responses to injected noradrenaline showed a slight increase when compared with control groups. This confirms the work of Zaimis (1966) who suggested that potentiated responses to noradrenaline in reserpinized rats may be due to increased cardiac output and not due to catecholamine depletion.

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