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Importance of the sympathetic nervous system in the development of renal hypertension in the rat

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Summary

1. Chemical sympathectomy with 6-hydroxydopamine (6-OHDA) prevented the development of renal hypertension in 80% of weanling male rats of the C.F. strain.

2. Adult male rats treated with 6-OHDA developed hypertension on bilateral clamping of the renal arteries.

3. Demedullation of the adrenal glands followed by 6-OHDA treatment in adult rats prevented the development of hypertension in 75% of them.

4. Weanling rats treated with 6-OHDA did not have any measurable catecholamines in their hearts up to 72–78 days after treatment.

5. Unlike the weanling rats, the hearts of adult rats (demedullated or with intact medulla) showed significant refilling (40–50%) of catecholamine stores 60 days after 6-OHDA treatment.

6. It is concluded that the functional sympathetic nervous system is important in the development of renal hypertension in the rat.

Introduction

McCubbin & Page (1963) have suggested that chronic renal hypertension is neurogenic in origin. Studies by Koletsky & Pritchard (1963) and Pritchard, Ormond & Koletsky (1964) have indirectly implicated a chronic neurogenic mechanism as they could not detect vasoconstrictor material in the blood during the chronic phase of renal hypertension. The need for an intact sympathetic nervous system in the mediation of increased vascular resistance was shown by Laverty & Smirk (1961). They showed that acute sympathectomy reduces the increased resistance of perfused limb of a hypertensive rat to the same level as that seen in a sympathectomized normotensive limb. Spinalization lowers the blood pressure to the same level in renal hypertensive and normotensive rats (Taquini, 1963). Similarly, administration of general anaesthetics like pentobarbitone or chloralose lowers the blood pressure of hypertensive rats to normotensive level (Ogden, Collings & Saperstein, 1946; Grewal & Kaul, 1970).

The importance of an intact sympathetic nervous system in the maintenance of hypertension is evident from the fact that all drugs which interfere with the sympathetic nervous control of blood vessels can effectively bring down the pressure to normotensive levels in hypertensive animals.

In order to investigate this neurogenic component in hypertension, studies have been conducted by several groups of workers where the sympathetic nervous system has been destroyed by the use of nerve growth antiserum in rats (Dorr & Brody, 1966 ; Willard & Fuller, 1969 ; Ayitey-Smith & Varma, 1970). These studies have demonstrated that a functional sympathetic nervous system is important for the development of hypertension. However, recent studies by Finch & Leach (1970a) showed that pretreatment of adult rats with 6-hydroxydopamine (6-OHDA) did not prevent the development of hypertension.

6-OHDA destroys the sympathetic nerve endings (Thoenen & Tranzer, 1968). We have used it as a tool to investigate the functional significance of the sympathetic nervous system in the production of renal hypertension in weanling rats, adult rats and adult rats with demedullated adrenal glands.

Methods

In these studies weanling male rats, C.F. strain (2–3 weeks old) weighing 30–35 g and adult male rats (5–6 weeks old) weighing between 130–140 g were used. They were made hypertensive by bilateral clamping of the renal arteries with silver clips (Goldblatt, Lynch, Hanzal & Summerville, 1934). Chemical sympathectomy was performed before the induction of hypertension in both groups of rats using 6-OHDA. The doses used were 2×34 mg/kg intravenously on day 1, 2×68 mg/kg intravenously on day 7 and 1×15 mg/kg intravenously on day 14. The dose schedule of Thoenen & Tranzer (1968) was modified and an extra dose of 6-OHDA (1×15 mg/kg i.v.) was given on day 14. One week after the last injection the endogenous catecholamine content of the heart was determined in two animals and the silver clips were applied to the renal arteries of the rest of the rats. Control groups of both the weanling and adult rats had their renal arteries clamped at the same time. Two to three weeks later, blood pressure (1 mmHg$\equiv$1·333 mbar) was measured by a plethysmographic method from the tail under light ether anaesthesia (Byrom & Wilson, 1938). Blood pressure was measured thereafter every week for 7–9 weeks.

Adrenal demedullation was performed in adult rats. The adrenal gland was enucleated by making a small incision and squeezing out the contents of the gland. In order to check if all the medulla had been removed from the animals, histological as well as catecholamine estimation of the adrenal gland was carried out in some of the animals. These animals were then injected (3 days after demedullation) with 6-OHDA and the renal arteries were clamped bilaterally as described above.

Heart catecholamines were estimated by extracting the tissue with perchloric acid. The catecholamines were adsorbed on acid-washed alumina at pH 8·4 and eluted with 0·2 N acetic acid (Crout, Creveling & Udenfriend, 1961). Adrenal catecholamines were estimated as described by Bertler, Carlsson & Rösengren (1958).

Drugs

6-OHDA-HCl was dissolved in 0·001 N HCl previously bubbled with nitrogen. (−)-Noradrenaline-hydrogen tartrate and (−)-adrenaline hydrogen tartrate were also used. All the doses of these drugs refer to their bases.

Results

Effect of 6-OHDA on the development of renal hypertension in weanling rats

In the weanling rats, 6-OHDA treatment completely prevented the development of hypertension in sixteen out of twenty rats (Fig. 1). The hypertension which developed in the other four rats was slow compared with controls. The mean blood pressure reading in these four rats was 180 mmHg. The mean blood pressures of 6-OHDA treated rats from the third to the ninth week were significantly different from the control ($P<0{\cdot}001$) (Fig. 1). The initial blood pressure readings of the 6-OHDA treated rats were lower than controls.

In our laboratory the percentage of rats which develop hypertension after bilateral clamping of renal arteries is about 70%. The remaining 30% do not develop hypertension and/or die after surgery (mean of about 1,000 rats). In the control series described here, eleven out of seventeen rats had developed hypertension and the mean values for the controls plotted in Fig. 1 have been calculated from all the rats in this series. The values plotted for 6-OHDA treated rats are the mean of all twenty including the four rats which developed hypertension.

The catecholamine content of the hearts of two randomly selected rats from the 6-OHDA treated group was determined on the day on which the renal arteries were clamped. Neither of them showed detectable amounts of catecholamine. At the end of the experiment (approximately 72–78 days after the last dose of 6-OHDA) both the treated and control groups of rats were killed and the heart catecholamines were estimated. All the 6-OHDA treated rats seemed to be completely depleted except for two rats which showed some endogenous catecholamine content (12% of control) (Table 1). The four rats which developed hypertension had no detectable catecholamines in their hearts. The total adrenal catecholamines of the eight

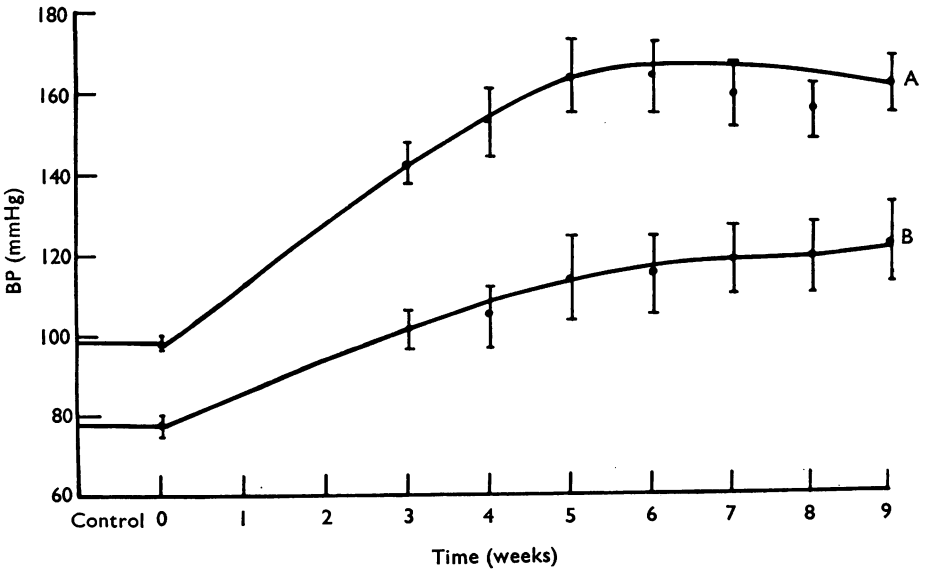

FIG. 1. Effect of 6-OHDA on the development and maintenance of hypertension in weanling rats. A, Control; B, 6-OHDA treated (for doses see text). Note that all the blood pressure readings of 6-OHDA treated rats are significantly different from controls ($P<0{\cdot}001$) and that 80% of 6-OHDA treated rats did not develop hypertension.

TABLE 1. *Effect of 6-hydroxydopamine on the catecholamine content of weanling and adult rat hearts and adrenals*

Series	Treatment	Weanling rats			Adult rats		
		Catecholamine content (μg/g)					
		Heart	Adrenals		Heart	Adrenals	
			Adrenaline	Noradrenaline		Adrenaline	Noradrenaline
1	Control	0·52±0·01 (12)	948 ±112 (8)	93 ±23 (8)	—	—	—
	6-OHDA	0 (14) 0·063 (2)	607*± 71 (9)	187*±30 (8)	—	—	—
	Controls	—	—	—	0·47±0·05 (6)	695±48 (6)	181±23 (5)
2	Demedullated controls	—	—	—	0·40±0·05 (5)	—	—
	6-OHDA	—	—	—	0·15±0·02 (6)	875±75 (6)	152±25 (5)
	Demedullated 6-OHDA	—	—	—	0·23±0·01 (9)	—	—

The doses of 6-OHDA used were as described in the text. Weanling rats killed 72–78 days after the last dose of 6-OHDA and adult rats killed 60 days after the last dose of 6-OHDA. In the adult rats there was no significant difference in the endogenous catecholamine content of the control and demedullated control hearts. Similarly 6-OHDA treated rats and demedullated rats treated with 6-OHDA showed no significant difference in the catecholamine content of their hearts. All values are the mean±S.E.M. Figures in parentheses indicate the number of observations. *$P<0·05$.

rats examined did not show any significant difference between the control and the 6-OHDA treated rats but the adrenaline content was slightly and significantly reduced, whilst the noradrenaline content was significantly increased (Table 1).

Effect of adrenal demedullation on the development of hypertension in the control and 6-OHDA treated rats

In the second series of experiments using adult rats, the development of hypertension in the following four sets of experiments was studied. There were six to nine animals in each set of experiments. A: Normal rats with bilaterally clamped renal arteries; B: demedullated rats with bilaterally clamped renal arteries; C: normal rats treated with 6-OHDA with bilaterally clamped renal arteries; D: demedullated rats treated with 6-OHDA with bilaterally clamped renal arteries.

Groups A and B developed hypertension normally and demedullation had no effect on its development (Fig. 2—lower graph). In group C hypertension developed

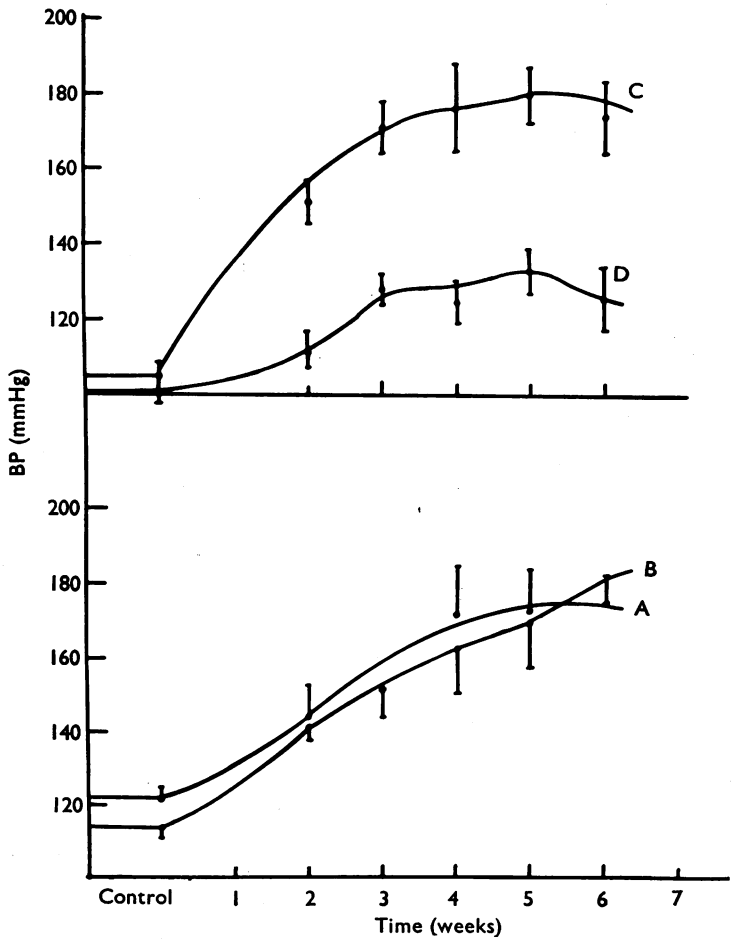

FIG. 2. Effect of 6-OHDA on the development and maintenance of hypertension in control and demedullated renal hypertensive rats. Upper graph: C, control 6-OHDA treated rats; D, demedullated rats treated with 6-OHDA. Lower graph: A, control renal hypertensive rats; B, demedullated renal rats. Note that demedullation prevented the development of hypertension in 6-OHDA rats (75%) but did not affect the development of renal hypertension in control rats. All values are the mean of six–nine rats.

as in the controls, but six out of eight demedullated rats, treated with 6-OHDA (group D) did not develop hypertension. The two rats which developed hypertension had a mean blood pressure reading of 162 mmHg. The values for these two rats have been included in Fig. 2 (upper graph).

At the end of the experiments all the animals were killed (about 60 days after the last dose of 6-OHDA) and the catecholamine content of the hearts and adrenals were estimated. Unlike the weanling rats, these adult rats had a significant amount of catecholamine in their hearts at this time (Table 1). There was no significant difference between the values for catecholamines in the 6-OHDA treated rats and the rats which were demedullated and given 6-OHDA (Table 1). The amounts of adrenaline and noradrenaline were also insignificantly different from controls in the 6-OHDA treated animals.

Discussion

Our experiments show that most of the weanling rats (80%) treated with 6-OHDA failed to develop hypertension while matched control rats with bilateral renal clamps did. The main difference in two groups of rats was the absence of a functional sympathetic nervous system, as 6-OHDA destroys adrenergic nerve endings (Thoenen & Tranzer, 1968). In the 6-OHDA treated weanling rats there was a marked destruction of the sympathetic nervous system as judged from cardiac catecholamine concentrations. In fourteen out of twenty rats there was no measurable amount of catecholamine present even 72–78 days after 6-OHDA treatment. In two rats only was there a detectable amount of catecholamine in the heart (12% of control) (Table 1). The remaining four rats were killed during the course of the experiments from the sixth week onwards and none of these rats had any detectable amount of catecholamines in their hearts. It is well known that an intact sympathetic nervous system is necessary for the binding of endogenous or exogenous catecholamines, and the absence of catecholamine would indicate marked destruction of sympathetic fibres, at least to the heart.

Adult rats with 6-OHDA and with bilateral renal clamping developed hypertension. This is in conformity with the results obtained by Finch & Leach (1970a), who, however, have shown that there is a considerable time lag in the development of hypertension, whereas in our experiments it was less pronounced.

In demedullated rats treated with 6-OHDA the application of silver clips to both the renal arteries did not lead to hypertension for 7 weeks. These results are not completely in agreement with those described by Finch & Leach (1970b). In their experiments the demedullated and 6-OHDA treated rats did not develop hypertension for the first 5 weeks while the untreated control rats and demedullated rats had developed hypertension by the third week. In their earlier experiments also (Finch & Leach, 1970a), there seems to be a significant difference between the blood pressure readings of the control and 6-OHDA rats from the third to the eighth week. However, their demedullated and 6-OHDA treated rats became hypertensive after the ninth week. There are certain methodological differences between their experiments and ours. They demedullated their animals after treatment with 6-OHDA while we removed the medulla a week before the first injection of 6-OHDA. They gave saline to their rats for 2 weeks after demedullation whereas we did not. They unilaterally nephrectomized their rats but ours had both kidneys intact. These

methodological differences may have something to do with the differences observed in our experiments.

Out of the four 6-OHDA treated rats which developed stable hypertension, two developed hypertension at the end of the sixth week and the other two at the end of the eighth week. None of the other sixteen weanling rats developed hypertension during the total observation period of 9 weeks. It is difficult to understand why these four rats became hypertensive as the catecholamine content of their hearts was the same as in the other rats and the two rats which showed slight amount of catecholamines did not develop hypertension.

In all the 6-OHDA treated adult rats the catecholamine content of their hearts was 40% of the controls when measured after approximately 8 weeks. The hearts of a few rats from this group examined 1 week after 6-OHDA injection contained no detectable catecholamine. The demedullated rats gave similar catecholamine values. The demedullation was done on the assumption that the adrenal glands may be releasing enough circulating catecholamine to replenish the catecholamine stores in adrenergic nerve endings, as 6-OHDA treatment does not deplete adrenal catecholamines (Thoenen & Tranzer, 1968), and it increases catecholamine synthesis by causing a compensatory increase in tyrosine hydroxylase activity (Mueller, Thoenen & Axelrod, 1969). Our experiments would seem to suggest that there is a certain amount of regeneration of sympathetic nervous system in the adult rats given 6-OHDA. These results are in agreement with those of Thoenen & Tranzer (1968). It also seems that the presence or absence of adrenal medulla does not make any difference to the rate of repletion of cardiac stores.

Our results suggest that in weanling rats one can get a greater degree of destruction of sympathetic nerve endings with 6-OHDA and that regeneration of the sympathetic nervous system is minimal. This has some similarity to immunosympathectomy where the neurones are most vulnerable during the first 2 weeks after birth (Hamburger, Levi, Montalcini, Norberg & Sjoquist, 1965).

The importance of the sympathetic nervous system in the maintenance of increased vascular resistance has been amply demonstrated (Laverty & Smirk, 1961 ; Taquini, 1963 ; Grewal & Kaul, 1970). Evidence for an increase in sympathetic activity in renal hypertensive rats has been adduced from studies using a tyrosine hydroxylase inhibitor (Henning, 1969). Recent observations of De Champlain, Krakoff & Axelrod, 1966, 1967, 1968, and Louis, Spector, Tabei & Sjoerdsma, 1969, have shown an alteration in the sympathetic function during experimental hypertension. All the drugs that interfere with sympathetic nervous control (ganglion blocking agents, adrenergic nerve transmission blocking agents and depletors of catecholamine) of cardiovascular system lower the blood pressure to normotensive levels in renal hypertensive rats. Our experiments seem to indicate that a functional sympathetic system is important for the development and maintenance of hypertension.

REFERENCES

AYITEY-SMITH, E. & VARMA, D. R. (1970). An assessment of the role of the sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats. *Br. J. Pharmac.*, **40**, 175–185.

BERTLER, A., CARLSSON, A. & RÖSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.*, **44**, 273–292.

BYROM, F. & WILSON, C. (1938). Plethysmographic method for measuring systolic blood pressure in the intact rat. *J. Physiol., Lond.*, **93**, 301–304.

CROUT, J. R., CREVELING, C. R. & UDENFRIEND, S. (1961). Norepinephrine metabolism in rat brain and heart. *J. Pharmac. exp. Ther.*, **132**, 269–277.

DE CHAMPLAIN, J., KRAKOFF, L. R. & AXELROD, J. (1966). A reduction in the accumulation of H^3-norepinephrine in experimental hypertension. *Life Sci.*, **5**, 2283–2291.

DE CHAMPLAIN, J., KRAKOFF, L. R. & AXELROD, J. (1967). Catecholamine metabolism in experimental hypertension in rat. *Circulation Res.*, **20**, 136–145.

DE CHAMPLAIN, J., KRAKOFF, L. R. & AXELROD, J. (1968). Relationship between sodium intake and noradrenaline storage during development of experimental hypertension. *Circulation Res.*, **23**, 479–490.

DORR, L. D. & BRODY, M. J. (1966). Preliminary observations on the role of sympathetic nervous system in development and maintenance of experimental renal hypertension. *Proc. Soc. exp. Biol. Med.*, **123**, 155–158.

FINCH, L. & LEACH, G. D. H. (1970a). The contribution of the sympathetic nervous system to the development and maintenance of experimental hypertension in the rat. *Br. J. Pharmac.*, **39**, 317–324.

FINCH, L. & LEACH, G. D. H. (1970b). Does the adrenal medulla contribute to the maintenance of experimental hypertension? *Eur. J. Pharmac.*, **11**, 388–391.

GOLDBLATT, H., LYNCH, J., HANZAL, R. F. & SUMMERVILLE, W. W. (1934). Studies on experimental hypertension. *J. exp. Med.*, **59**, 347–379.

GREWAL, R. S. & KAUL, C. L. (1970). Mechanism of the antagonism of the hypotensive effect of guanethidine by propranolol. *Br. J. Pharmac.*, **38**, 771–775.

HAMBERGER, B., LEVI, MONTALCINI, R., NORBERG, K. A. & SJOQUIST, F. (1965). Monoamines in immunosympathectomized rats. *Int. J. Neuropharmac.*, **4**, 91–95.

HENNING, M. (1969). Noradrenaline turnover in renal hypertensive rats. *J. Pharm. Pharmac.*, **21**, 61–62.

KOLETSKY, S. & PRITCHARD, W. H. (1963). Vasopressor material in experimental renal hypertension. *Circulation Res.*, **13**, 552–556.

LAVERTY, R. & SMIRK, F. H. (1961). Observations on pathogenesis of spontaneous inherited hypertension and constricted renal artery hypertension in rats. *Circulation Res.*, **9**, 455–464.

LOUIS, J., SPECTOR, S., TABEI, R. & SJOERDSMA, A. (1969). Synthesis and turnover of noradrenaline in the heart of the spontaneously hypertensive rat. *Circulation Res.*, **24**, 85–91.

MCCUBBIN, J. W. & PAGE, I. H. (1963). Renal pressor system and neurogenic control of arterial pressure. *Circulation Res.*, **12**, 553–559.

MUELLER, R. A., THOENEN, H. & AXELROD, J. P. (1969). Adrenal tyrosine hydroxylase. Compensatory increase in activity after chemical sympathectomy. *Science, N.Y.*, **163**, 468–469.

PRITCHARD, W. H., ORMOND, A. P. jun. & KOLETSKY, S. (1964). Differences in levels of circulating vasopressor material in dogs with acute and chronic renal hypertension. *Proc. Soc. exp. Biol. Med.*, **117**, 127–131.

TAQUINI, A. C. (1963). Neurogenic component of peripheral resistance in renal hypertension. *Circulation Res.*, **12**, 562–567.

THOENEN, H. & TRANZER, J. P. (1968). Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Arch exp. Path. Pharmak.*, **261**, 271–288.

WILLARD, P. W. & FULLER, R. W. (1969). Functional significance of the sympathetic nervous system in the production of hypertension. *Nature, Lond.*, **233**, 417–418.

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