The contribution of the sympathetic nervous system to the development and maintenance of experimental hypertension in the rat

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Summary

1. Experimental hypertension in the rat, induced either by renal artery stenosis or by treatment with deoxycorticosterone acetate (DCA) developed maximally over a period of 8 weeks. In both types of hypertension the rate of development was unaffected by immunosympathectomy or by chemical sympathectomy following the administration of 6-hydroxydopamine.

2. The effect of 6-hydroxydopamine on chronic renal hypertensive rats was to produce a hypotensive action of longer duration than when similarly administered to DCA-induced hypertensive or normotensive rats. Reserpine (5–10 mg/kg intraperitoneally) produced a more marked hypotensive effect on both types of hypertensive rats although it was of much shorter duration.

3. It is concluded that experimental hypertension of renal origin or induced by DCA treatment can develop even though most of the sympathetic nervous system has been destroyed. The maintenance of chronic hypertension in these conditions may depend on the adrenal glands or a hormonal system as yet undetected.

Introduction

Experimental hypertension in the rat can be induced by various techniques such as renal artery stenosis (Wilson & Byrom, 1939), treatment with deoxycorticosterone and a high salt diet (Friedman, Friedman & Nakashima, 1951), radical denervation of the sino-aortic pressoreceptors (Krieger, 1964), and genetic inbreeding (Smirk & Hall, 1958 ; Okamoto & Aoki, 1963).

Studies on the induction and maintenance of experimental hypertension have suggested a neurogenic component. For instance salt hypertension using triiodothyronine (Dahl, Heine & Tassinari, 1962) was not produced in immunosympathectomized rats, suggesting a dependence on the sympathetic nervous system for the production of this type of hypertension (Willard & Fuller, 1969). Chronic renal hypertension is thought to be neurogenic in origin (McCubbin & Page, 1963) and this is supported by the fact that spinalization reduced the blood pressure to control levels in renal hypertensive rats (Taquini, 1963). Also Laverty & Smirk (1961) have shown that the increase in resistance to flow in the innervated hind limb preparation of renal hypertensive rats fell, after hexamethonium, to the same level as control preparations. Experiments using genetically hypertensive rats suggest that the

elevated blood pressure is also neurogenically maintained (Devine & Smirk, 1964 ; Phelan, 1968).

In order to investigate this neurogenic component in experimental hypertensive rats, 6-hydroxydopamine, which is thought to destroy nerve endings of the sympathetic nervous system (Thoenen & Tranzer, 1968), and nerve growth antiserum, which destroys sympathetic ganglia (Levi-Montalcini & Booker, 1960), were used in this study. Reserpine, which depletes endogenous catecholamines without significantly altering the uptake process (Carlsson, Rosengren, Bertler & Nilsson, 1957 ; Iversen, 1967), was also used to compare sympathectomy with short-term impairment of adrenergic nerve function.

Methods

Male CSE rats (Scientific Products) in groups of six were used throughout. Systolic blood pressure in the conscious animal was obtained by the tail cuff method, using a semi-conductor strain-gauge (Ether Ltd.) mounted on a tail clip for detection of the pulse which was visually displayed on an oscilloscope (Telequipment). The cuff was connected to a mercury manometer (1 mmHg≡1·33 mbar). Measurements were made with animals held in Bowman restraining cages and placed inside a warming cabinet (33° ± 1° C) for 15 min. Each determination was the mean of three readings.

Surgical procedures were carried out under halothane anaesthesia on rats weighing between 130 and 150 g. Renal hypertension was induced using silver clips, with an aperture of 0·254 mm, placed on the right renal artery, and the left kidney removed at the same operation. Hypertension was also induced using subcutaneous implants of deoxycorticosterone acetate (50 mg) and 0·9% w/v NaCl replacing the drinking water for the first 4 weeks. Unilateral nephrectomy was carried out at the start of this treatment.

Chemical sympathectomy, before the induction of hypertension, was carried out using rats weighing between 130 and 140 g. 6-Hydroxydopamine HBr was given 2 × 50 mg/kg intravenously on day 1, followed by 2 × 100 mg/kg on day 7 (Thoenen & Tranzer, 1968). Surgical procedures were carried out on days 8–10. Rats were immunosympathectomized using double strength cow nerve growth factor antiserum, injected subcutaneously on the day of birth and on the following 4 days with doses of 0·1 ml, 0·1 ml, 0·2 ml, 0·2 ml, 0·4 ml.

Drugs. Reserpine phosphate (CIBA) was dissolved in 20% w/v ascorbic acid. 6-Hydroxydopamine HBr (generously donated by Drs. Thoenen & Hürlimann, Hoffman-La Roche, Basle) was dissolved in 0·001 N hydrochloric acid previously bubbled with nitrogen. Cow nerve growth factor anti-serum (Batch 4945/46/47) was generously donated by Dr. C. Edwards (Wellcome Research Laboratories, Beckenham, Kent).

Results

(a) Effects of 6-hydroxydopamine and immunosympathectomy on the development of experimental renal hypertension in the rat

In the majority of rats (80–90%), renal hypertension developed gradually over a period of 8 weeks, during which time the systolic blood pressure had reached a maximum of approximately 180 mmHg (Fig. 1). 6-Hydroxydopamine given before the

application of the renal clip did not prevent the development of the hypertension although a slight lag was seen in the rate of its development, presumably due to the initial lowering of the resting blood pressure caused by administration of 6-hydroxydopamine (Finch & Leach, 1970). Immunosympathectomized rats showed no difference in the rate of development of hypertension when compared with the control group. However, it was noted that 50% of the group died during the 8 week period.
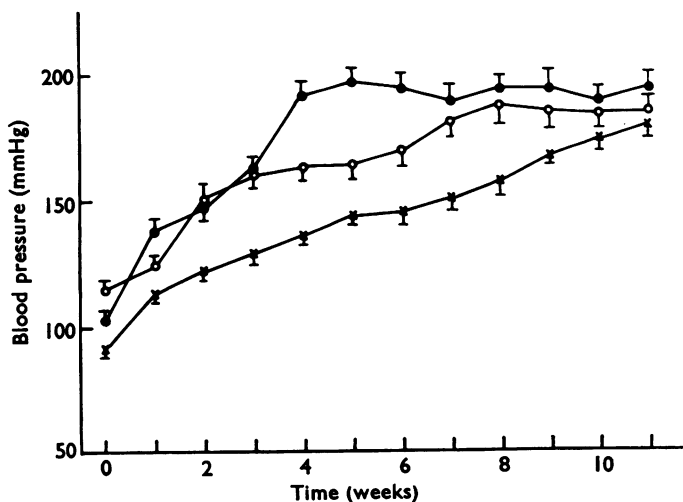

FIG. 1. Effect of immunosympathectomy and 6-hydroxydopamine on the development of hypertension in rats after partial occlusion of the left renal artery and right nephrectomy. Untreated rats (○──○); immunosympathectomized rats (●──●); 6-hydroxydopamine treated rats (×──×). Each point represents the mean systolic blood pressure ± standard error of a group of six conscious animals.
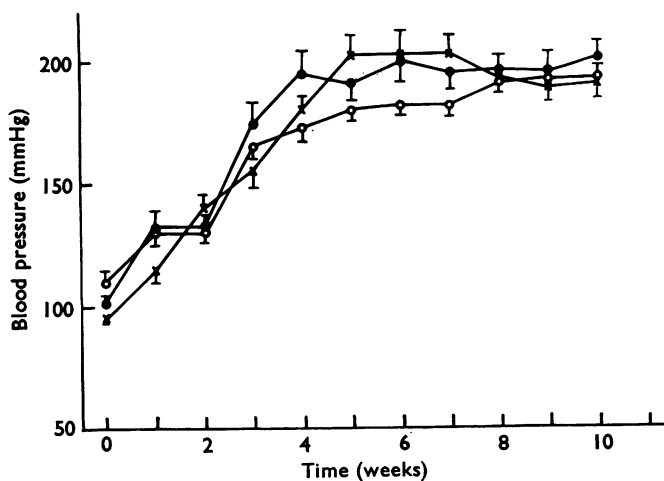

FIG. 2. Effect of immunosympathectomy and 6-hydroxydopamine on the development of DCA-induced hypertension in rats. Untreated rats (○──○); immunosympathectomized rats (●──●); 6-hydroxydopamine treated rats (×──×). Each point represents the mean systolic blood pressure ± standard error of a group of six conscious animals.

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(b) Effects of 6-hydroxydopamine and immunosympathectomy on the development of the hypertension induced by deoxycorticosterone in the rat

Development of hypertension was found to occur in all control animals after the treatment described earlier under **Methods**. It developed more rapidly than renal hypertension, the systolic blood pressure reaching a plateau of 190 mmHg after approximately 8 weeks (Fig. 2). 6-Hydroxydopamine given before treatment did not alter the pattern of development of the hypertension. Immunosympathecto- mized rats also showed no significant difference in the rate of development of hypertension when compared with the control group.

(c) Comparison of the effects of 6-hydroxydopamine on resting systolic blood pressures of chronic hypertensive and normotensive rats

Chronic hypertensive rats were considered to be those with a systolic blood pressure in excess of 160 mmHg and of 8 weeks duration. The effect of 6-hydroxy- dopamine (2 × 50 mg/kg on day 1 and 2 × 100 mg/kg on day 7) on the resting blood pressure was studied in these and normotensive rats. This dose of 6-hydroxy- dopamine is thought to produce almost complete chemical sympathectomy (Thoenen & Tranzer, 1968 ; Finch & Leach, 1970). The effect on rats made hypertensive by deoxycorticosterone treatment was to produce a lowering of the blood pressure to 140 mmHg between days 8–10, returning to original levels of 200 mmHg by day 14 (Fig. 3). 6-Hydroxydopamine lowered the blood pressure to 145 mmHg between days 9–10 in chronic renal hypertensive rats and it gradually returned to its original level after a period of 5 weeks. In normotensive rats with systolic blood pressures of 120 mmHg, 6-hydroxydopamine lowered the blood pressure to 94 mmHg on day 8, after which the pressures returned to normotensive levels by day 14 (Fig. 3).
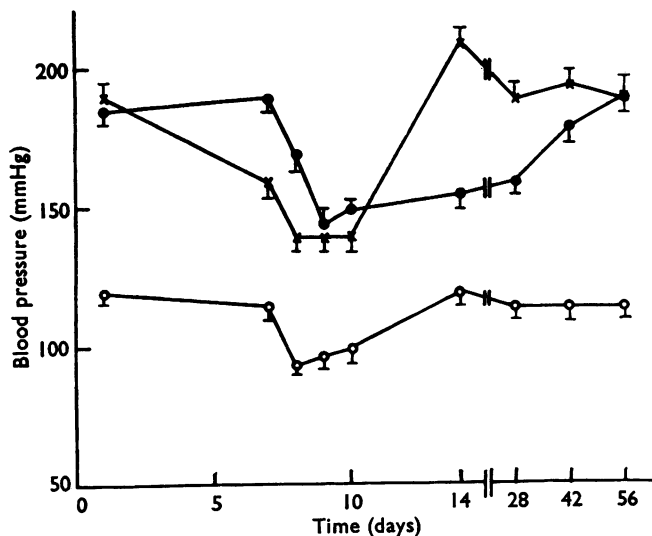

FIG. 3. Effect of 6-hydroxydopamine on the systolic blood pressure of conscious chronic hypertensive and normotensive rats. 6-Hydroxydopamine 2×50 mg/kg on day 1 and 2×100 mg/kg on day 7 was given intravenously to normotensive rats (O——O) ; chronic renal hypertensive rats (●——●) ; DCA-induced hypertensive rats (×——×). Each point repre- sents the mean systolic blood pressure ± standard error of a group of six animals.

(d) Effect of reserpine on the resting systolic blood pressure of chronic hypertensive and normotensive rats

The acute effect of reserpine (0·5 mg–10 mg/kg) given intraperitoneally was studied on the resting systolic blood pressure of conscious rats. In normotensive rats, reserpine (5–10 mg/kg) produced a maximal lowering of blood pressure to 95 mmHg within 6 h (Fig. 4). This effect persisted for 24 h after the injection and returned to pre-administration levels after 60 hours. Reserpine (5–10 mg/kg) produced a maximal hypotensive effect in chronic renal hypertensive and DCA-induced hypertensive rats within 6–9 h after injection, the systolic blood pressure being lowered from 185 mmHg to 125 mmHg. The hypotensive effects of reserpine (5–10 mg/kg) followed the same time course in normotensive and hypertensive rats. These results are in agreement with those of Henning (1969a) for renal hypertensive rats in which blood pressure recordings were made by direct cannulation techniques.

Discussion

Although in renal hypertension it is thought that renin is released during the acute phase with the subsequent formation of angiotensin (Gollan, Richardson & Goldblatt, 1948 ; Koletsky & Pritchard, 1963), no substantial increase in angiotensin levels have been detected during the chronic stages. It has therefore been suggested that a neurogenic factor is responsible for the elevated blood pressure (McCubbin & Page, 1963 ; Page & McCubbin, 1965). In the experiments described in this paper 6-hydroxydopamine, known to destroy adrenergic nerve endings (Thoenen & Tranzer, 1968 ; Finch & Leach, 1970), failed to prevent the development of renal hypertension in rats. It has also been shown that 6-hydroxydopamine does not affect the development of spontaneous hypertension in rats (Sjoerdsma & Yamori, unpublished observations). Immunosympathectomy also failed to alter the development of renal hypertension, but this procedure has been shown to produce
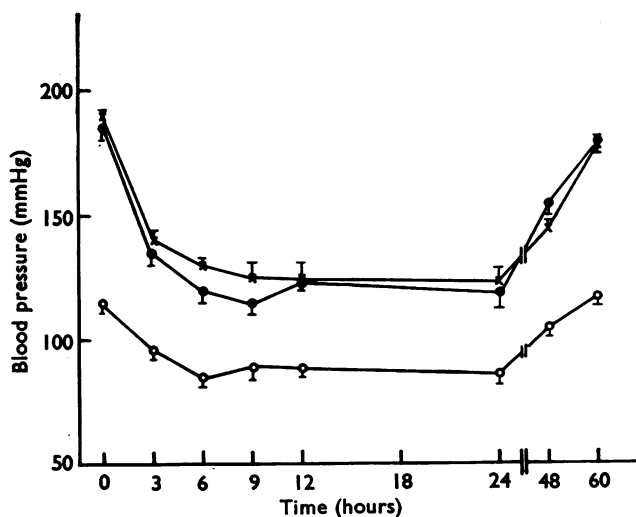

FIG. 4. Effect of reserpine on the systolic blood pressure of conscious chronic hypertensive and normotensive rats. Reserpine (10 mg/kg intraperitoneally) was given to normotensive rats (O——O) ; chronic renal hypertensive rats (●——●) ; DCA-induced hypertensive rats (×——×). Each point represents the mean systolic blood pressure ± standard error of a group of six animals.

incomplete sympathectomy and to leave a partially functional sympathetic nervous system (Iversen, Glowinski & Axelrod, 1966 ; Finch & Leach, 1970). These results are in part agreement with Dorr & Brody (1966) and Wilson (1966) who found that immunosympathectomized rats developed renal hypertension, but did not maintain the elevated blood pressure for a long period. In order to investigate the chronic phase of renal hypertension, 6-hydroxydopamine and reserpine were given separately to rats with hypertension of 8 weeks duration. It was seen that reserpine lowered the blood pressure more than 6-hydroxydopamine but chemical sympathectomy exerted a more prolonged action.

Hypertension induced by deoxycorticosterone treatment is characterized by cardiac enlargement and is thought to involve a nervous pathway (Green, Saunders, Wahlgren & Craig, 1952). Also an increased cardiovascular reactivity to various pressor agents has been demonstrated (Sturtevant, 1956). The experiments reported in this paper show that the development of hypertension in response to deoxycorticosterone was not altered by 6-hydroxydopamine pretreatment or by using immunosympathectomized rats, thus confirming the observations of Varma (1967). 6-Hydroxydopamine given to DCA-induced rats maintained in their maximally hypertensive state for a further 8 weeks showed a similar lowering in blood pressure to that of renal hypertensive rats, but returned to hypertensive levels after 7 days. Reserpine produced similar effects to those seen in chronic renal hypertensive rats.

Although chemical sympathectomy is thought to destroy completely the functional sympathetic supply to the cardiovascular system (Finch & Leach, 1970), it does not deplete adrenal catecholamines (Thoenen & Tranzer, 1968). Also, 6-hydroxydopamine pretreatment causes a compensatory increase in tyrosine hydroxylase activity in the adrenal glands of the rat (Mueller, Thoenen & Axelrod, 1969) whilst in immunosympathectomized mice there is a two-fold increase in turnover of adrenal catecholamines (Iversen *et al.,* 1966). It is therefore possible that in both immunosympathectomized or 6-hydroxydopamine pretreated rats, the development of hypertension depends on the catecholamines still present in the adrenal medulla. However, experimental hypertension in the rat is thought to be independent of the adrenals when an adequate salt intake is maintained (Floyer, 1951 ; Fregly, 1957 ; Nolla-Panades & Smirk, 1964).

Evidence for implicating the sympathetic nervous system in the maintenance of experimental hypertension has been investigated by several workers. Rats made hypertensive by DCA treatment have been shown to have a defect in the storage and retention characteristics of the sympathetic granular fraction (Krakoff, de Champlain & Axelrod, 1967). More recently an increased turnover of noradrenaline has been demonstrated in this type of hypertension (de Champlain, Mueller & Axelrod, 1969). These workers also showed an increase in the rate of synthesis of noradrenaline in the adrenals of rats with DCA-induced hypertension. In renal hypertensive rats Henning (1969b) has demonstrated an increase in sympathetic nerve activity using a tyrosine hydroxylase inhibitor.

In conclusion, our experiments show that both renal and DCA-induced hypertension can develop and be maintained even though the majority of the sympathetic nervous system has been destroyed. If there is no functional nervous system present then perhaps a hormonal system as yet undetected may exist and be responsible for the maintenance of the experimental hypertension.

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