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Long-lasting peripheral and central effects of 6-hydroxydopamine in rats

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Summary

1. In newborn rats treated with 6-hydroxydopamine hydrobromide (6-OHDA) (50–150 mg/kg on 5–7 days) a widespread and long-lasting dose related sympathectomy was demonstrated.

2. When rats given 6-hydroxydopamine (100 mg/kg, seven treatments) in the neonatal period were killed at 10 weeks the concentration of noradrenaline (NA) in the heart, mesentery and vas deferens was significantly reduced. There was no alteration in the catecholamine content of the adrenal glands.

3. The amplitude of the responses of perfused mesenteric arteries from 6-hydroxydopamine treated rats to intra-arterial noradrenaline was not increased, compared with controls, but the duration of responses was increased.

4. 6-Hydroxydopamine given to newborn rats caused a long-lasting depletion of noradrenaline in three of the five regions of the central nervous system (cortex, cerebellum and spinal cord) studied. The concentration of noradrenaline in the pons-medulla was increased, but in the thalamic region was unchanged. The treated rats showed significantly lower exploratory activity.

5. Treatment of newborn rats with 6-hydroxydopamine thus has striking and long-lasting effects on peripheral and central adrenergic systems.

Introduction

As part of a study of the development of high blood pressure in rats of the New Zealand strain with genetic hypertension (Smirk & Hall, 1958 ; Phelan, 1968) we attempted to destroy the sympathetic nervous system by injecting newborn rats with antiserum to nerve growth factor (Levi-Montalcini, 1964): the sympathectomy thus produced was incomplete (Clark, 1971).

Immunosympathectomy of this type effectively reduces the sympathetic innervation of tissues having nerves which originate from paravertebral ganglia ; however, the adrenals and organs supplied by the prevertebral or pelvic ganglia are less affected (Vogt, 1964 ; Zaimis, Berk & Callingham, 1965 ; Iversen, Glowinski & Axelrod, 1966). When the denervation of large areas of the cardiovascular system is incom-

plete, only limited conclusions as to the role of the sympathetic nervous system in the pathogenesis of various types of experimental hypertension can be drawn from the study of immunosympathectomized animals (Ayitey-Smith & Varma, 1970; Finch & Leach, 1970; Clark, 1971).

Angeletti & Levi-Montalcini (1970a, 1970b) observed the complete absence of nerve cell bodies in a wide variety of sympathetic ganglia taken from mice which had been treated with 6-hydroxydopamine hydrobromide in the neonatal period. Angeletti (1971) found a reduction in the concentration of tissue noradrenaline in some peripheral organs and in the brain, and also showed that noradrenaline uptake was significantly less in rats which had been treated with 6-hydroxydopamine in the neonatal period than in untreated litter mates. Jaim-Etcheverry & Zieher (1971) reported that not all peripheral tissues were depleted uniformly of noradrenaline, and they could not detect any change in brain noradrenaline concentration. Thoenen (1971) has recently reported similar results. Jaim-Etcheverry & Zieher (1971) also found that the sympathetic ganglia were reduced in size, but there were remaining nerve cells which appeared normal on examination by light and electron microscopy. Such effects resulting from injections of 6-hydroxydopamine into newborn animals are in striking contrast to the effects of single or repeated doses in adult rats and cats, which result in an almost complete functional sympathectomy subsequent to degeneration of the adrenergic nerve terminals (Thoenen & Tranzer, 1968); however, the proximal parts of the sympathetic neurones are not affected, and when treatment with 6-hydroxydopamine ceases the nerve endings regenerate (de Champlain, 1971).

In the present paper we report the results of the administration of 6-hydroxydopamine in the neonatal period to normotensive and genetically hypertensive rats. The degree of sympathectomy varied, depending on the dose schedule used.

Methods

Newborn albino rats from the Otago stock colony and from the New Zealand strain with genetic hypertension (Phelan, 1968) were injected intraperitoneally on the first day of life, and thereafter as indicated in the section on results, with 6-hydroxydopamine hydrobromide freshly dissolved in 0·9% sodium chloride solution containing ascorbic acid (0·5 mg/ml). The volume injected varied between 0·1 and 0·2 ml, depending on the weight of the rat. As controls, litter mates were injected with similar volumes of the saline-ascorbic acid solution. Maximum litter size was restricted to nine rats. For some experiments also weight- and age-matched adult animals from the Otago stock colony were used as controls: these are hereafter called 'weight-matched controls'.

Systolic blood pressure was measured under light ether anaesthesia by a tail cuff method (Jones & Dowd, 1970), the reported pressures being the mean of three readings (1 mmHg≡1·333 mbar). Animals were killed by either decapitation or opening of the thorax under anaesthesia and removing the heart. Mesenteric arteries were isolated for perfusion with oxygenated Locke's solution at a constant rate (McGregor, 1965; Clark, 1971). The responses of the perfused arteries to perivascular nerve stimulation (75 V, 16 Hz, 5 ms, 100 pulses) or to intra-arterial injections of noradrenaline (Levophed, Winthrop) in doses (as base) of 0·05 μg, 0·1 μg, 0·3 μg and 0·5 μg were measured as changes in perfusion pressure detected by a Statham P23Gb transducer and displayed on a pen recorder.

Tissues required for assay of catecholamines were removed at death, immediately frozen over dry ice and stored at −15° C. The tissue concentrations of noradrenaline and adrenaline were measured fluorimetrically after homogenization in acetic acid and separation of catecholamines on Dowex 50 columns (Taylor & Laverty, 1969). Peripheral tissues assayed were heart, pancreas and adjoining mesentery (that is, the region bounded by stomach, spleen and duodenum), kidney, vas deferens and adrenals. In the central nervous system the spinal cord and either the whole brain or four brain regions, that is, cerebral cortex, thalamic area (including striate, hippocampus, hypothalamus and mid-brain regions), pons-medulla and cerebellum were assayed.

The exploratory behaviour of treated and control rats in response to a change in environment was measured over 3 min on 3 separate days in a Y-runway (Steinberg, Rushton & Tinson, 1961). The spontaneous nocturnal group activity of rats treated with 6-hydroxydopamine and rats treated with saline was measured in their normal cages by means of capacitance changes due to small displacements of a flexible false floor (Laverty & Meek, unpublished results). The number of rats was adjusted so that the total rat weight in each cage was approximately equal.

Results

Effects of different doses

Eighteen genetically hypertensive and eighteen normotensive female rats were injected daily from birth with 50 mg/kg 6-hydroxydopamine or with saline-ascorbic acid intraperitoneally for five treatments. The 6-hydroxydopamine treated animals appeared normal apart from exhibiting slight ptosis; they were killed when 3–4 months old. Superior cervical ganglia were removed from the normotensive rats and the number of nerve cells in longitudinal mid-sections counted (Clark, 1971).

Results from the genetically hypertensive animals are shown in Table 1: body weight was slightly lower in the 6-hydroxydopamine treated animals, blood pressure was significantly reduced ($P<0{\cdot}001$) and the noradrenaline concentration was significantly reduced ($P<0{\cdot}001$) in heart and spinal cord but not in pancreas and brain. Similar results were obtained in male genetically hypertensive rats ($n=6$). In normotensive female rats, 6-hydroxydopamine treatment did not affect blood pressure significantly (6-hydroxydopamine treated rats 113 ± 3 mmHg, $n=16$; saline treated rats 116 ± 3 mmHg, $n=8$). The number of nerve cells in mid-sections of ganglia from rats ($n=8$) treated with 6-hydroxydopamine was 150 ± 32; in saline treated rats ($n=7$) the comparable figure was 725 ± 66. The difference was significant ($P<0{\cdot}001$).

TABLE 1. *Effects of treating genetically hypertensive female rats with 6-OHDA (50 mg/kg i.p.) for 5 days after birth*

	Treatment	
	6-OHDA (9)	Saline (9)
Body wt (g)	157± 2·3	169± 6·2
B.P. (mmHg)	134± 5·4*	166± 4·8
NA concentration (ng/g)		
Heart	231±15·1*	863±65·2
Pancreas-mesentery	309±39·5	397±32·6
Spinal cord	111±24·4*	262±19·0
Whole brain	315± 6·7	328±11·9

Rats aged 3-4 months when killed. * Significance of difference, $P<0{\cdot}001$.

The vasoconstrictor response to periarterial nerve stimulation was lower ($P<0{\cdot}001$) in 6-hydroxydopamine treated rats ($64 \pm 8{\cdot}4$ mmHg, $n=9$) than in saline treated litter mates ($161 \pm 10{\cdot}1$ mmHg, $n=8$).

In an attempt to obtain a more effective and widespread sympathectomy the daily dose of 6-hydroxydopamine given for the first five days of life was increased to either 100 mg/kg or 150 mg/kg, and in some cases a supplementary dose was given on day 12 of life (Fig. 1). The smallest responses to nerve stimulation and the lowest concentrations of noradrenaline occurred in rats given the extra dose of 6-hydroxydopamine on day 12. There was a significant ($P<0{\cdot}001$) positive correlation between vasoconstrictor response to periarterial nerve stimulation and the concentration of endogenous noradrenaline in the pancreas and adjacent mesentery.

Normotensive rats treated with 300 mg/kg of 6-hydroxydopamine for 5 days showed a high mortality, none surviving beyond the age of 10 days; rats treated with 150 mg/kg of 6-hydroxydopamine on days 1–5 and day 12, while appearing to thrive well up to the age of about 6 weeks, showed a proportion (about 20%) of sudden deaths at about this time. Rats treated with 100 mg/kg of 6-hydroxydopamine on days 1–5 and day 12 showed no excess mortality and appeared normal, apart from marked

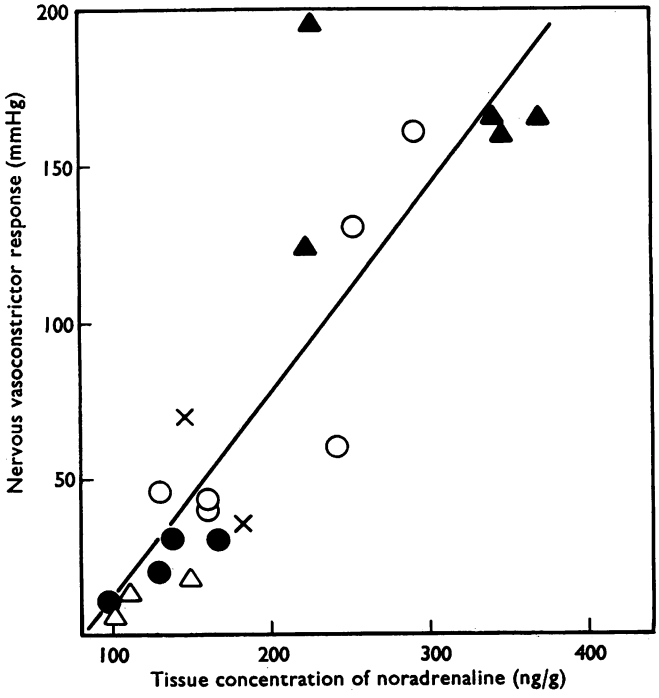

FIG. 1. Effect of 6-OHDA in the neonatal period on the vasoconstrictor response of saline-perfused mesenteric arteries of 3–4 month old rats to stimulation of the periarterial nerves (ordinate) and on the concentration of endogenous NA in the pancreas and adjacent mesentery (abscissa). The linear relation between the two variables is significant ($r=0{\cdot}88$, $P<0{\cdot}001$, $d.f.=18$). Drugs were administered intraperitoneally daily to newborn normotensive rats as follows: ▲, Controls; injected with 0·1–0·2 ml of a saline-ascorbic acid solution for 5 days. Concentration of endogenous NA in heart 718 ng/g (range 897–660 ng/g; $n=5$). ○, Treated; 6-OHDA, 100 mg/kg for 5 days. Heart NA, 224 ng/g (407–117 ng/g; $n=6$). ×, Treated; 6-OHDA, 150 mg/kg for 5 days. Heart NA, 127 ng/g (143–110 ng/g; $n=2$). ●, Treated; 6-OHDA, 100 mg/kg for 5 days with a further dose on day 12. Heart NA 169 ng/g (211–106 ng/g; $n=4$). △, Treated; 6-OHDA, 150 mg/kg for 5 days with a further dose on day 12. Heart NA 170 ng/g (180–142 ng/g; $n=3$).

ptosis and some weight loss compared with their saline treated litter mates. Slight diarrhoea, which accompanied the sympathectomy, was easily corrected by adding small amounts of calcium carbonate mixed with dried milk powder to the diet.

Sympathetic nerve function and catecholamines in peripheral tissues

A treatment which appeared to combine effective sympathectomy with minimum toxicity was chosen. Newborn male rats were injected intraperitoneally with 6-hydroxydopamine (100 mg/kg) on 7 alternate days from days 1 to 13. Litter mate controls were injected concurrently with drug solvent. Rats were killed 10 weeks after birth and tissues taken for mesenteric artery perfusion and for noradrenaline estimations. In addition to litter mate controls, untreated rats matched for weight and age were also studied. This latter group was selected from relatively light for age, but otherwise healthy, rats present normally in the Otago stock colony.

Periarterial nerve stimulation caused the perfusion pressure of mesenteric arteries isolated from 6-hydroxydopamine treated rats to rise by only $4 \pm 0{\cdot}7$ mmHg; the comparable responses of arteries from saline treated controls and weight-matched controls were $137 \pm 18{\cdot}2$ mmHg and $127 \pm 10{\cdot}5$ mmHg, respectively.

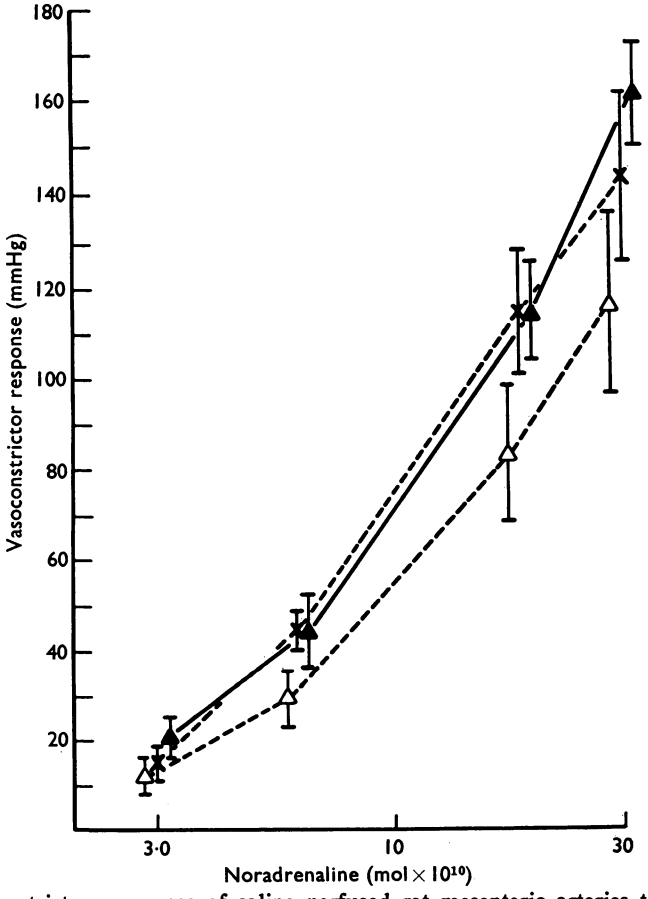

FIG. 2. Vasoconstrictor responses of saline perfused rat mesenteric arteries to injected NA: six rats treated in the neonatal period with 6-OHDA 100 mg/kg i.p., alternate days from birth, seven treatments (△); six saline injected litter mates (▲); and six rats matched for weight as well as age (×). Means ± S.E. are shown.

In 6-hydroxydopamine treated rats the vasoconstrictor response of the perfused mesenteric arteries to injections of intra-arterial noradrenaline was slightly reduced at all dosage levels when compared with both types of controls (Fig. 2). However, the duration of the responses to noradrenaline, that is the interval from the time of injection to the return of the perfusion pressure to 25% above the initial baseline, was significantly prolonged in 6-hydroxydopamine treated rats (Fig. 3): the mean duration of response to 0·3 μg noradrenaline, for example, was $47 \pm 6{\cdot}8$ s in 6-hydroxydopamine treated rats, $16{\cdot}5 \pm 2{\cdot}1$ s in age-matched controls and $17{\cdot}0 \pm 1{\cdot}3$ s in weight-matched controls ($n=6$ in each case).

The baseline perfusion pressures in the perfused arteries of 6-hydroxydopamine treated rats and in the two groups of controls did not differ significantly.

The body weights, blood pressures and concentrations of noradrenaline in various peripheral tissues of the rats used in the perfusion experiments are set out in Table 2. The body weights ($P<0{\cdot}05$) and systolic blood pressures ($P<0{\cdot}01$) of the 6-

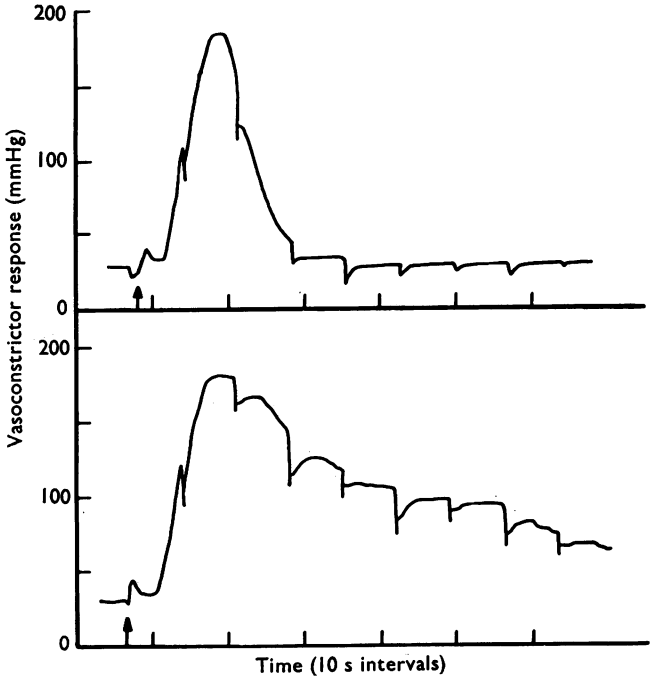

FIG. 3. Responses to 0·3 μg NA of perfused mesenteric arteries obtained from a saline injected control rat (upper trace) and from a rat treated in the neonatal period with 6-OHDA (100 mg/kg i.p. alternate days from birth, seven treatments; lower trace). The arrows show the point of injection of NA; the regular transient falls in pressure are a pump artefact.

TABLE 2. *Effects of treating normotensive male rats with 6-OHDA (100 mg/kg i.p. on alternate days from birth, seven treatments)*

	Treatment		Untreated normotensive wt-matched controls (6)
	6-OHDA (6)	Saline (6)	
Body wt (g)	145± 4·2*	209±22·7	142± 7·8
B.P. (mmHg)	112± 6·7†	137± 2·5	—
NA concentration (ng/g)			
Heart	152±15·1‡	929±25·2	941±38·4
Pancreas-mesentery	94±13·8‡	311±18·6	335±32·4
Kidney	376±30·8	476±34·4	—
Vas deferens	5000±800 ‡	9500±530	12800±1260

Rats aged 10 weeks when killed. Significances of difference: * $P<0{\cdot}05$; † $P<0{\cdot}01$; ‡ $P<0{\cdot}001$.

hydroxydopamine treated rats were significantly lower than the respective values for age-matched litter mate controls. The concentrations of endogenous noradrenaline in heart, pancreas-mesentery and vas deferens were significantly reduced ($P<0.001$) compared with age-matched or weight-matched controls. The concentration of noradrenaline in the kidneys was also reduced but the difference for age-matched controls was not significant. The total amount of noradrenaline and adrenaline in the adrenals of 6-hydroxydopamine treated rats (noradrenaline, 7.3 ± 0.59 μg/pair of adrenals; adrenaline, 15.5 ± 2.47 μg/pair) did not differ from that in the adrenals of saline treated litter mates (noradrenaline, 7.8 ± 0.61 μg/pair; adrenaline, 16.5 ± 1.20 μg/pair).

Changes in the central nervous system

It was noted earlier that rats treated with 50 mg/kg of 6-hydroxydopamine for 5 days after birth had considerable depletion of spinal cord noradrenaline (Table 1), whilst the concentration of noradrenaline in the whole brain was unchanged. These rats, and all rats treated with higher doses of 6-hydroxytryptamine, failed to show clonic convulsions after decapitation (Laverty, 1971).

Rats treated with 6-hydroxydopamine (100 mg/kg, alternate days from birth, seven treatments) had concentrations of endogenous noradrenaline significantly lower than normal in three of the five regions (cortex, cerebellum and spinal cord) of the central nervous system studied (Table 3); in the thalamus the concentration of noradrenaline appeared unaltered and in the pons-medulla there was a significantly higher concentration of noradrenaline (Table 3).

On casual observation 6-hydroxydopamine treated rats did not appear to behave differently from litter mate controls. Treated animals showed obvious ptosis and were lighter in weight. However, when first placed in a Y-runway the 6-hydroxydopamine treated animals showed a significantly lower activity than controls (Table 4). When studied on 2 subsequent days the saline treated animals showed the

TABLE 3. *Effects on the concentration of NA in brain regions of rats treated with 6-OHDA (100 mg/kg, i.p., alternate days from birth, seven treatments)*

	Treatment	
	6-OHDA	Saline
Cortex (ng/g)	21 ± 6·9†	177 ± 12·7
Thalamus	413 ± 32·3	400 ± 24·5
Pons-medulla	975 ± 71·4†	467 ± 25·3
Cerebellum	52 ± 8·4*	189 ± 16·3
Spinal cord	68 ± 18·0*	203 ± 16·3

Rats aged 10 weeks when killed. Means are of six–twelve observations (±S.E.M.). Significances of difference: * $P<0.05$; † $P<0.001$.

TABLE 4. *Effects of 6-OHDA (100 mg/kg i.p. on alternate days from birth, seven treatments) on exploratory behaviour of normotensive male rats in a Y-runway*

	6-OHDA treated (7)		Saline treated (litter mates) (6)	
	Entries	Rearings	Entries	Rearings
1st exposure to runway	8·9 ± 0·55*	9·1 ± 0·63†	12·2 ± 0·60	18·3 ± 1·59
2nd exposure	8·4 ± 0·97	9·4 ± 1·49	8·2 ± 0·65	10·3 ± 1·65
3rd exposure	8·4 ± 0·72	7·0 ± 0·58	8·8 ± 0·48	7·8 ± 1·67

Means ± S.E.M. are shown. Significances of difference from controls: * $P<0.005$; † $P<0.001$.

expected drop in activity; however, the activity of the 6-hydroxydopamine treated rats remained at its initial level and the significant difference between the two groups was abolished.

The nocturnal activity of the 6-hydroxydopamine treated rats and saline-treated controls was monitored for 4 nights. There was a consistently lower level of activity in the rats treated with 6-hydroxydopamine.

Discussion

Treatment of newborn rats with 6-hydroxydopamine hydrobromide produces a long-lasting sympathectomy which is dose-dependent and affects many adrenergically innervated peripheral organs. It does not, however, affect the content of catecholamines in the adrenal medulla. There are significant changes in the concentration of catecholamines in several regions of the central nervous system.

The sympathectomy achieved by treatment of newborn rats with 50 mg/kg of 6-hydroxydopamine for 5 consecutive days, though extensive, was not complete. That the treatment affected tissues innervated by paravertebral ganglia was evident from the low concentration of noradrenaline in the heart and the reduced number of nerve cells in the superior cervical ganglia. The lower than normal response of perfused mesenteric arteries to nerve stimulation indicates that there is some effect on organs innervated via prevertebral ganglia, though the endogenous noradrenaline concentration in the mesentery was not markedly affected.

The effects of increasing (a) the dose of 6-hydroxydopamine and (b) the length of the course of treatment were investigated. Ptosis could be seen in all treated rats, but was most marked in those rats treated with the largest doses of 6-hydroxydopamine. The effects of 6-hydroxydopamine treatment were assessed in the rat mesentery, one of the regions in which the endogenous noradrenaline concentration is least affected by immunosympathectomy (Iversen *et al.*, 1966) or low doses of 6-hydroxydopamine (Thoenen, 1971), and in which sympathetic function can be assessed in the perfused mesenteric arteries. With different dose schedules it was possible to obtain graded reductions in the concentrations of endogenous noradrenaline and in the responses to nervous stimulation in this region. It was decided that 100 mg/kg intraperitoneally of 6-hydroxydopamine given on alternate days from birth was the most suitable dosage schedule, even though considerable weight loss and some diarrhoea, both probably consequences of effective sympathectomy, occurred in rats treated in this way. The concentration of endogenous noradrenaline in the heart and pancreas-mesentery was reduced to 15% and 33%, respectively, of the control values. In the perfused mesenteric arteries the response to periarterial nerve stimulation was virtually non-existent. In the vas deferens from the treated rats the noradrenaline concentration had fallen by only 50%. The noradrenergic innervation of this organ is supplied by pelvic ganglia which lie peripherally, are highly resistant to immunosympathectomy, and have certain properties reminiscent more of parasympathetic than of typical orthosympathetic ganglia (Blackman, Crowcroft, Devine, Holman & Yonemura, 1969).

It is possible that the failure of 6-hydroxydopamine treatment to reduce the catecholamine content of the adrenal medulla may mask the effects of a widespread peripheral sympathectomy. Angeletti (1971) and Thoenen (1971) have both reported minimal changes in the adrenals of newborn rats treated with 6-hydroxydopamine. The secretion of catecholamines from the adrenals may play an import-

ant role in cardiovascular and metabolic adaptations of 6-hydroxydopamine treated rats to environmental changes. Our preliminary results indicate that 6-hydroxydopamine treatment will be useful in studying the pathogenesis of hypertension. The lower than usual blood pressures recorded in 6-hydroxydopamine treated genetically hypertensive rats and in normotensive rats are consistent with our earlier results using immunosympathectomized rats (Clark, 1971).

Angeletti (1971) found that four treatments of newborn rats with 50 mg/kg of 6-hydroxydopamine produced animals with an extensive peripheral sympathectomy, a lower concentration of brain noradrenaline compared with controls, and the uptake of noradrenaline in heart, spleen, pancreas and brain was reduced.

Our results extend and confirm the conclusion of Jaim-Etcheverry (1971) and Thoenen (1971) that the sympathectomy produced by treating newborn rats with 6-hydroxydopamine is long-lasting, but does not affect all adrenergically innervated organs to the same extent. Our impression is that the effect of 50 mg/kg of 6-hydroxydopamine daily is very similar to that obtained by treating rats with antiserum to nerve growth factor for the first five days of life (Clark, 1971). We were able to demonstrate that increasing the dose and duration of treatment with 6-hydroxydopamine produced a more widespread and complete sympathectomy in our rats. The variation in the effects of 50 mg/kg of 6-hydroxydopamine, as reported by different groups of workers, may arise from the differences in dose schedules, differences in strains of rats or differences in the methods of assessing the extent of the changes in the nervous system.

Whether acute or moderately long-term sympathectomy sensitizes blood vessels to the vasoconstrictor effects of noradrenaline is controversial (Haeusler, Haefely & Huerlimann, 1971). In perfused mesenteric blood vessels from rats sympathectomized from birth with higher doses of 6-hydroxydopamine the magnitude of the vasoconstrictor response to injected noradrenaline was less than or equal to the response of normal vessels over a range of doses. This result is similar to that obtained by Haeusler *et al.* (1971) in mesenteric arteries from adult rats treated with 6-hydroxydopamine for at least 10 days before the experiment. They found no evidence for the development of postjunctional supersensitivity in chronically denervated mesenteric blood vessels. However, the increased duration of the response to noradrenaline in our work indicates that the properties of the smooth muscle of these blood vessels, which have never been normally innervated, may differ from those of vessels which are acutely denervated.

In rats treated at birth with a low dose of 6-hydroxydopamine there were indications that the treatment was affecting the central nervous system. These rats, when killed by decapitation, did not show the usual clonic convulsions ; this absence of kicking activity was also found in rats treated systemically with drugs which penetrated the blood-brain barrier and depleted the central nervous system of catecholamines. Rats treated with antiserum to nerve growth factor, or those treated with a sedative (pentobarbitone) (Laverty, 1971) showed normal convulsive behaviour when decapitated. The concentration of noradrenaline in the spinal cord of rats treated with 6-hydroxydopamine was reduced to approximately 40% of normal. In rats treated with antiserum to nerve growth factor the concentration of noradrenaline in the spinal cord (Phelan, unpublished observations) and in the brain (Iversen *et al.*, 1966) remained normal.

A lower than normal concentration of noradrenaline in the cortex, cerebellum and spinal cord, and a higher than normal concentration of noradrenaline in the pons-medulla were found in rats given 100 mg/kg of 6-hydroxydopamine during the neonatal period. The absence of significant changes in the noradrenaline concentration in the thalamic region may indicate only that further subdivision of this region is required. The regional variations may reflect differences in the susceptibilities of catecholamine neurone tracts or in the relative permeability of the blood-brain barrier. The increased concentration of noradrenaline in the pons-medulla may suggest that only the central nervous system nerve axons are destroyed and that the cell bodies remain intact.

In spite of these gross changes in the concentration of catecholamines in several brain regions, 6-hydroxydopamine treated rats behaved on superficial inspection in a manner similar to untreated litter mates. Nocturnal activity and exploratory behaviour were decreased less than might have been expected from the magnitude of the biochemical changes.

We have established that a long-lasting sympathectomy of the rat is possible by treatment of the newborn with 6-hydroxydopamine, and that the degree of the sympathectomy is to some extent controllable by varying the size of the dose and the schedule by which it is given. The regimen we have suggested is not necessarily the optimum for all purposes or for all strains of rats but has proved in our hands to produce a widespread sympathectomy.

The permanent dose dependent sympathectomy and the depletion of catecholamines in the central nervous system produced by treatment of neonatal animals with 6-hydroxydopamine is potentially a very powerful tool for investigating the role of the nervous system in a variety of experimental situations.

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