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A comparison of the effects of chemical sympathectomy by 6-hydroxydopamine in newborn and adult rats

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Summary

1. The effects of chemical sympathectomy with 6-hydroxydopamine (6-OHDA) on the cardiovascular system of the rat were compared in, (a) 10-week-old rats treated during the first 14 days after birth with 150 μg/g subcutaneously, and (b) adult rats injected intravenously with 2×50 mg/kg on day 1 and 2×100 mg/kg on day 7 and the experiments performed on day 8.

2. Intravenous administration of 6-OHDA to adult rats almost completely abolished the pressor responses to stimulation of the entire sympathetic outflow in the pithed rat, the contractions of the lower eyelid to stimulation of the cervical sympathetic trunk and the vasconstrictor responses produced by periarterial nerve stimulation of the isolated renal artery preparation. Pressor responses to physostigmine and to tyramine were markedly reduced or abolished in anaesthetized and pithed rat preparations, respectively.

3. In corresponding experiments, 10-week-old rats treated as newborns with 6-OHDA showed a marked reduction in the stimulation-induced pressor responses and contractions of the lower eyelid, but completely normal vasoconstrictor responses to periarterial nerve stimulation of the isolated perfused renal artery were obtained. The pressor responses to physostigmine were slightly reduced but the tyramine responses were unchanged.

4. Treatment with 6-OHDA at birth caused an almost complete and long-lasting noradrenaline depletion in the heart, spleen, salivary glands and ileum but only a partial depletion in the mesentery from 10-week-old rats. These low noradrenaline levels showed no recovery in rats up to an age of 4 months. The tyrosine hydroxylase activity in both the cervical and stellate ganglia from 10-week-old rats was markedly reduced by treatment with 6-OHDA after birth.

5. Injections of 6-OHDA after birth produce an almost complete and permanent sympathectomy of various adrenergically innervated organs in the rat. The vascular system represents a major exception, exhibiting a surprisingly high resistance to this type of chemical adrenergic denervation.

Introduction

Morphological, biochemical and functional studies have demonstrated that 6-hydroxydopamine (6-OHDA) causes a selective destruction of adrenergic nerve

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terminals in adult animals (Thoenen & Tranzer, 1968; Haeusler, Haefely & Thoenen, 1969; Finch & Leach, 1970) and a destruction of the entire adrenergic neurone in newborn animals (Angeletti & Levi-Montalcini, 1970; Angeletti, 1971; Jaim-Etchevery & Zieher, 1971; Thoenen, 1971; Clark, Laverty & Phelan, 1972). Thus, 6-OHDA provides the unique possibility of achieving 'reversible sympathectomy' by treating adult animals and 'permanent sympathectomy' by administration to newborn animals.

After treatment of adult rats with 6-OHDA, the regeneration of adrenergic nerve terminals in blood vessels is much faster than in other organs with an adrenergic innervation and an almost complete functional recovery occurs within a few days after the injection of 6-OHDA (Finch, Haeusler, Kuhn & Thoenen, 1972). This rapid regeneration, after 6-OHDA, of vascular adrenergic nerve endings strongly questions the validity of long-term cardiovascular studies, e.g. studies on the role of the sympathetic nervous system in the development or maintenance of hypertension. In contrast, immunosympathectomy produces a permanent destruction of the peripheral adrenergic system (Levi-Montalcini & Angeletti, 1966; Zaimis, 1967). However, the sympathectomy is not complete (Finch & Leach, 1970), although several organs such as the heart and spleen show a marked depletion of noradrenaline (Iversen, Glowinski & Axelrod, 1966). Therefore, it was of interest to see whether administration of 6-OHDA to newborn rats would produce a complete as well as a permanent destruction of the adrenergic nerves supplying the vascular system.

Methods

Injection of 6-hydroxydopamine

Litters of rats (randomized colony of Wistar descent) were injected on the day after birth and for the following 13 days with 6-hydroxydopamine hydrobromide (150 μg/g body weight subcutaneously). The 6-OHDA was dissolved in 0·9% w/v NaCl solution containing 1% ascorbic acid and bubbled with nitrogen. The functional experiments were carried out 8 weeks after the last injection of 6-OHDA. Male rats (180–200 g) were injected intravenously with 6-hydroxydopamine hydrobromide (2 × 50 mg/kg on day 1 and 2 × 100 mg/kg on day 7) and functional experiments were carried out on day 8. Litters and adult rats for the control groups were injected with corresponding volumes of the vehicle solution.

Whole animal preparations

Anaesthetized and pithed preparations were set up as described previously (Finch & Leach, 1969). Physostigmine and tyramine were injected intravenously, every 15 min, and only one response was obtained to each dose in each animal. The studies with physostigmine were carried out in rats anaesthetized with urethane (1–1·5 g/kg i.p.). Pithed preparations were used for stimulation of the entire sympathetic outflow (Gillespie & Muir, 1967); atropine (0·5 mg/kg) and tubocurarine (1 mg/kg) were given i.v. before stimulation was started. In order to prevent the release of adrenal medullary catecholamines, bilateral adrenalectomy was performed under halothane anaesthesia 2 h before the start of the experiment. These animals were supplemented with corticosterone (10 mg/kg i.m.) which has been shown to restore the cardiovascular sensitivity in this preparation (Drew & Leach, 1971).

Contractions of the lower eyelid

In order to record contractions of the lower eyelid, rats were anaesthetized with pentobarbitone (50 mg/kg i.p.). A tracheal cannula was inserted and the right cervical sympathetic trunk was carefully separated from the vagus with the aid of a dissecting microscope. The head of the rat was then secured in a holder and the cut cervical sympathetic trunk placed on a bipolar hook-electrode. The contractions of the lower eyelid in response to supramaximal stimulation, 1·0 ms pulse duration, were recorded isometrically by means of a thread tied through the eyelid and attached to a sensitive force displacement transducer (Shinkoh U-gage Type UL $\pm$2 g).

Isolated renal artery preparation

Rats were anaesthetized with ether and the left renal artery was cannulated from the aorta with a stainless steel cannula (No. 18 needle) and cut at the level of the

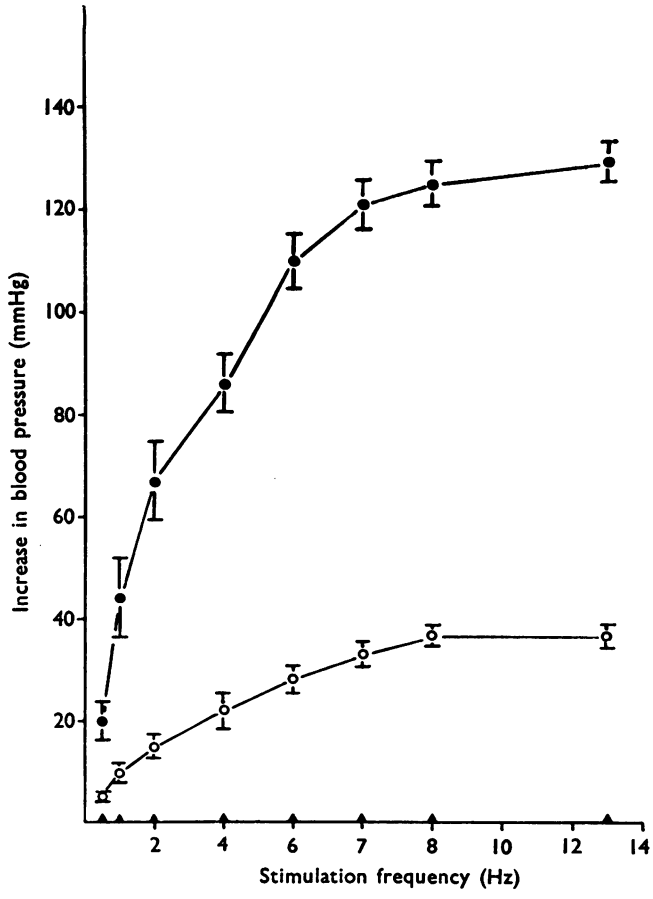

FIG. 1. Mean rise in blood pressure obtained in the Gillespie & Muir preparation (1967). Sympathetic outflow was stimulated with supramaximal voltage and increasing frequencies. (●——●) Controls; (○——○) rats treated at birth with 6-hydroxydopamine (14×150 μg/g subcutaneously) and experiments carried out 8 weeks after the last injection; (▲——▲) adult rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7 intravenously) and the experiments performed on day 8. Vertical bars show S.E.M. (n=8 for all groups).

kidney hilus. The isolated artery floated on the surface of a 50 ml organ bath which was filled with Krebs–Henseleit solution (37° C) and bubbled gently with 95% O_2 and 5% CO_2. The preparation was also perfused with oxygenated Krebs–Henseleit solution delivered from a Vario Perspex peristaltic pump. The flow rate was adjusted to 6 ml/min, which resulted in a basal perfusion pressure of 25–40 mmHg. After an equilibration period of 45 min, periarterial nerve stimulation was carried out with a specially designed fluid electrode placed around the artery. Supramaximal stimulation at varying frequencies, 1 ms pulse duration for 20 s, was obtained with a Grass S 7 stimulator. For dose-response curves, bolus injections (0·1 ml) of noradrenaline were given into the perfusion system 2 cm from the renal artery. Increases in perfusion pressure were recorded with a Statham P 23Dd pressure transducer.

Noradrenaline and tyrosine hydroxylase determinations

For the noradrenaline determinations the organs were quickly removed, frozen at −75° C, weighed and homogenized in 0·4 N $HClO_4$. Catecholamines were adsorbed

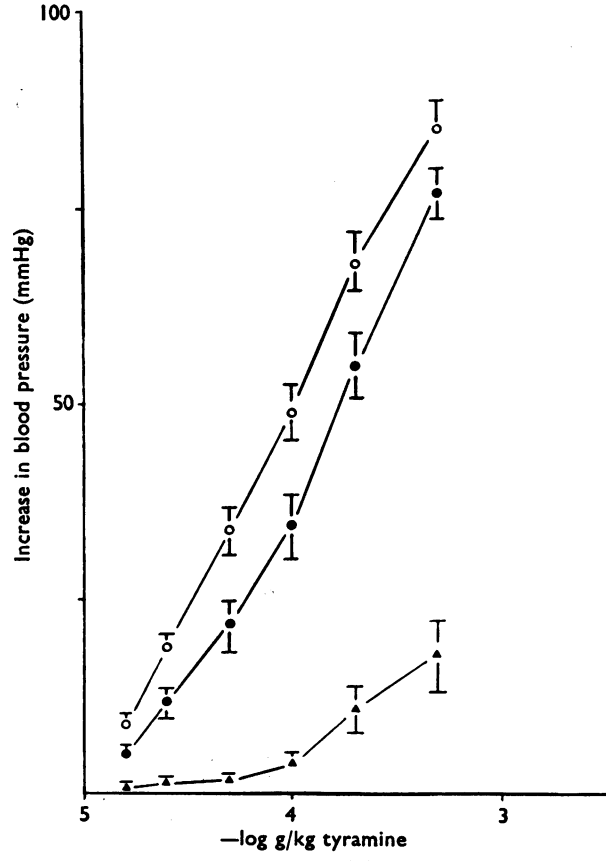

FIG. 2. Mean rise in blood pressure produced by intravenous doses of tyramine in pithed rat preparations. (●——●) Controls; (○——○) rats treated at birth with 6-hydroxydopamine (14×150 μg/g subcutaneously) and experiments were carried out 8 weeks after the last injection; (▲——▲) adult rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7, intravenously) and experiments performed on day 8. Vertical bars show S.EM. (n=8 for all groups).

on Alumina by the method of Anton & Sayre (1962). Noradrenaline was assayed fluorimetrically according to von Euler & Lishajko (1961). Tyrosine hydroxylase activity of the stellate and cervical ganglia was determined by the method of Levitt, Gibb, Daly, Lipton & Udenfriend (1967) with modifications described in detail by Mueller, Thoenen & Axelrod (1969).

Drugs

Atropine sulphate (C. H. Boehringer), corticosterone acetate (generously donated by Dr. R. Buckett, Organon Labs.), 6-hydroxydopamine hydrobromide (synthesized by Dr. A. Langemann, F. Hoffmann-La Roche & Co. Ltd.), (−)-noradrenaline (Fluka A. G.), physostigmine sulphate (Sandoz), tubocurarine chloride (Mann Labs., N.Y.), tyramine hydrochloride (Fluka A.G.). Doses are expressed in terms of salt except for noradrenaline which is expressed as base.

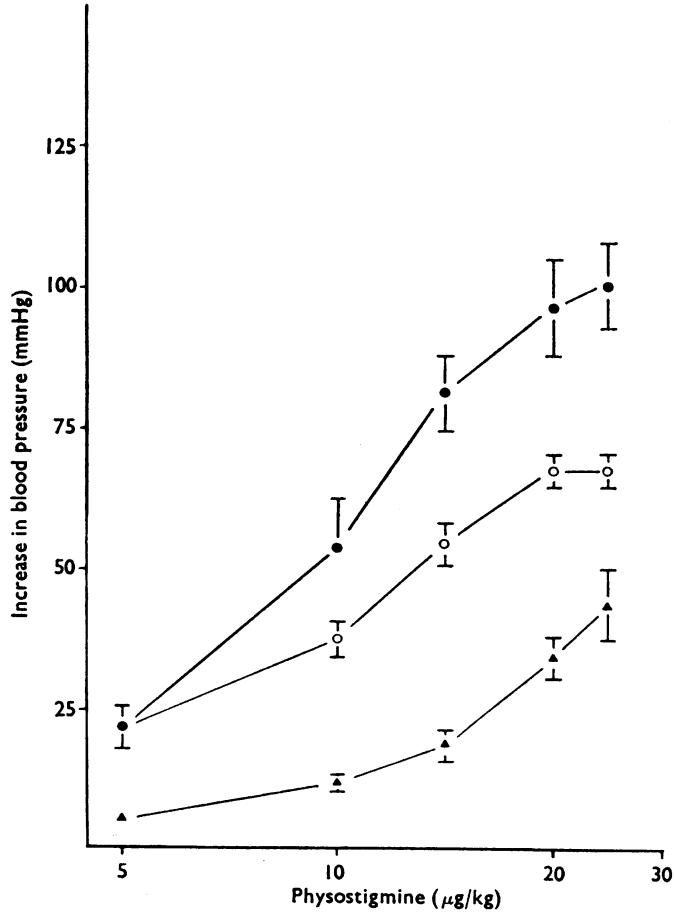

FIG. 3. Mean rise in blood pressure produced by intravenous doses of physostigmine in rats anaesthetized with urethane (1–1·5 g/kg intraperitoneally). (●——●) Controls; (○——○) rats treated at birth with 6-hydroxydopamine (14×150 μg/kg subcutaneously) and experiments carried out 8 weeks after the last injection; (▲——▲) adult rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7 intravenously) and experiments performed on day 8. Vertical bars show S.E.M. (n=8 for all groups).

Results

Functional consequencies of 6-hydroxydopamine treatment in newborn and adult rats

In pithed rat preparations, stimulation of the entire sympathetic outflow was carried out in order to evaluate the extent of sympathectomy by the different treatments with 6-OHDA. The animals were adrenalectomized since pretreatment with 6-OHDA in either newborn or adult rats does not deplete adrenal medullary amines (Table 1; Thoenen & Tranzer, 1968). In untreated preparations, graded pressor responses were obtained with alterations in the frequency of stimulation within the range of 0·5–10 Hz (Fig. 1). Animals previously treated with 6-OHDA at birth showed a marked reduction in the pressor responses, especially at the lower rates of stimulation. However, in preparations treated with 6-OHDA intravenously (2×50 mg/kg and 2×100 mg/kg), the pressor responses to sympathetic nerve stimulation were completely abolished.

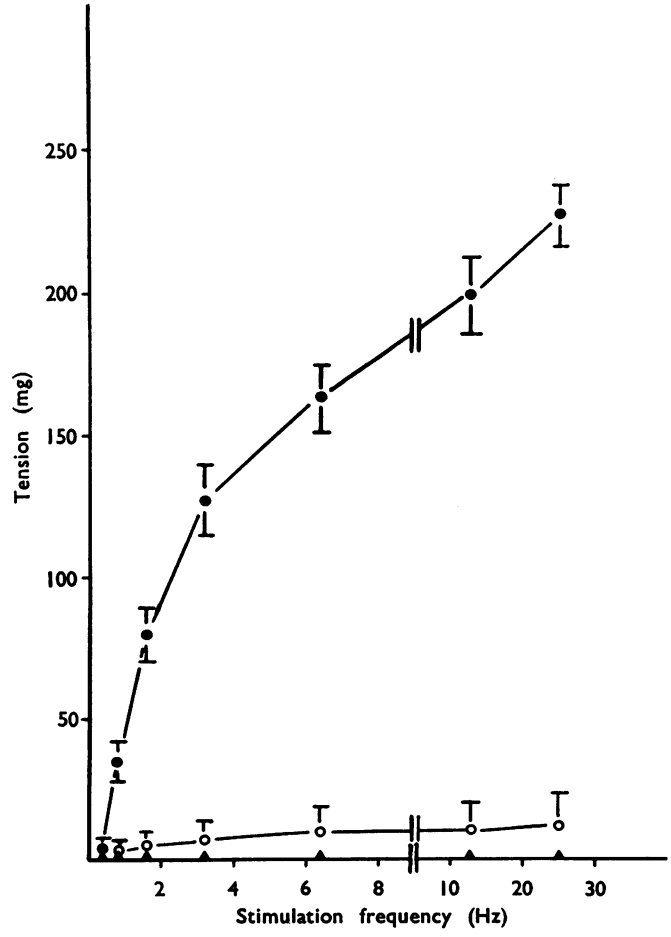

FIG. 4. Contraction of the right lower eyelid of the rat to stimulation of the cervical sympathetic trunk. (●——●) Controls; (○——○) rats treated at birth with 6-hydroxydopamine (14×150 μg/g subcutaneously) and experiments carried out 8 weeks after the last injection; (▲——▲) adult rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7 intravenously) and experiments performed on day 8. Vertical bars show S.E.M. ($n=8$ for all groups).

In normal pithed rat preparations, tyramine (12·5–500 μg/kg i.v.) produced a dose-dependent rise in blood pressure which was accompanied by a marked tachycardia at the higher doses (50–500 μg/kg) (Fig. 2). Preparations from animals which had been treated after birth with 6-OHDA showed a slightly enhanced pressor response to tyramine. However, the chronotropic responses to tyramine were markedly reduced and in most cases completely abolished. Intravenous administration of 6-OHDA to adult rats abolished both the pressor and chronotropic responses to low doses of tyramine and markedly reduced the responses to the higher doses (100–500 μg/kg). In the urethane-anaesthetized rat, intravenous administration of physostigmine (5–25 μg/kg) produced a short-lasting rise in blood pressure (Fig. 3). These pressor responses were only slightly reduced after treatment with 6-OHDA at birth, but greatly diminished after intravenous administration to the adult animals.

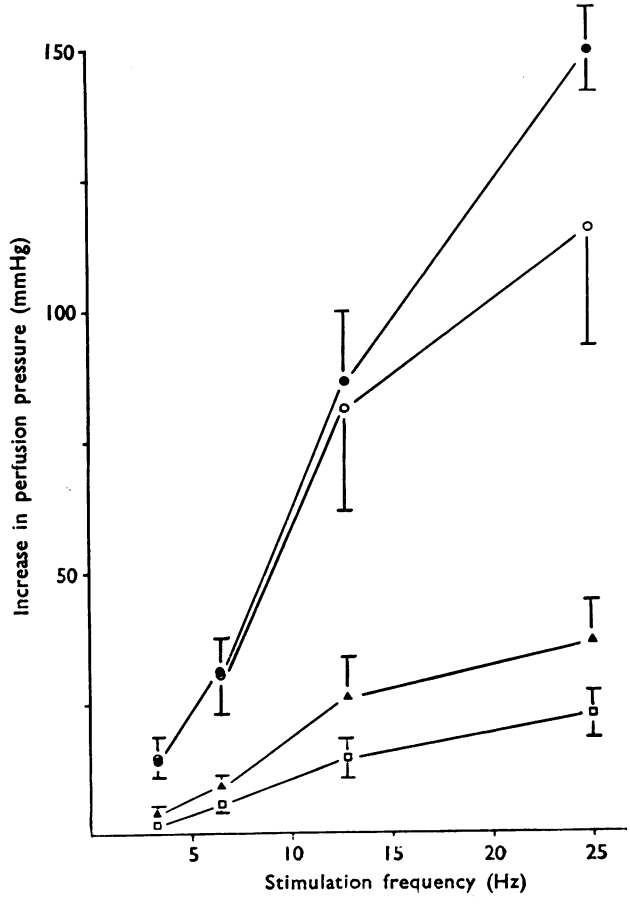

FIG. 5. Vasoconstrictor responses to periarterial nerve stimulation of the isolated renal artery preparation. The preparation was stimulated for periods of 20 s at varying frequencies, with supramaximal voltage. (●——●) Control; (○——○) rats treated at birth with 6-hydroxydopamine (14×150 μg/g subcutaneously) and experiments carried out 8 weeks after the last injection; (▲——▲) adult rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7 intravenously) and the experiments performed on day 8; (□——□) 1×10^{-5}M guanethidine added to the perfusion fluid. Vertical bars show S.E.M. ($n=8$ for all groups).

Contractions of the lower eyelid were obtained by stimulation of the cervical sympathetic nerve and frequency-dependent responses were obtained in untreated animals (Fig. 4). 6-OHDA given at birth markedly reduced these contractions. However, after treatment of adult rats with 6-OHDA, they were abolished (Fig. 4).

Periarterial nerve stimulation of the isolated renal artery preparation produced vasoconstriction which was measured as an increase in the perfusion pressure (Fig. 5). Addition of guanethidine (1×10^{-5}M) to the perfusion fluid almost completely abolished the responses to periarterial nerve stimulation, suggesting that only a small portion of the vasoconstriction was due to a direct stimulation of vascular smooth muscle cells. Treatment with 6-OHDA at birth did not influence these vasoconstrictor responses produced by periarterial nerve stimulation. In contrast, preparations from rats treated with 6-OHDA intravenously and set up on day 8 showed responses similar to those perfused with guanethidine (Fig. 5). The magnitude of the vasoconstrictor responses to exogenous noradrenaline was not affected by either type of treatment with 6-OHDA.

Effect of 6-hydroxydopamine at birth on catecholamine levels and tyrosine hydroxylase activity

Table 1 shows the long-lasting noradrenaline depletion in rats treated at birth with 6-OHDA (14×150 μg/kg). However, there were marked differences in the degree of depletion as shown by the mesentery (50–60%), which was taken as an example of vascular smooth muscle, whilst non-vascular tissue and cardiac muscle were depleted to less than 5% of control levels (Table 1). Since no recovery in the levels of noradrenaline was observed up to an age of 16 weeks, it would seem that this treatment with 6-OHDA leads to a permanent sympathectomy. There was also a slight decrease in brain catecholamines in the 10-week-old rats which suggests

TABLE 1A. *Effect of* 6-*hydroxydopamine* (14×150 μg/g s.c.) *after birth on the catecholamine content of various organs in adult rats* (10 *weeks old*)

Organ	Control	6-OHDA	6-OHDA as % of controls
Heart	1,139± 83	61±13	5
Spleen	876±119	17± 7	1·9
Salivary glands	1,552±136	32±21	2
Brain	479± 24	395±14	83
Uterus	653± 85	52± 8	8
Mesentery	672±108	432±60	64
Ileum	274± 24	0	0
Adrenal glands	19·9±0·5	29·8± 4	150

TABLE 1B. *Effect of* 6-*hydroxydopamine* (14×150 μg/g s.c.) *after birth on the catecholamine content of various organs in adult rats* (16 *weeks old*)

Organ	Control	6-OHDA	6-OHDA as % of controls
Heart	1,140± 74	97± 5	8·5
Spleen	1,522±194	44±14	3
Salivary glands	1,857±119	44±26	2·3
Brain	444± 13	413±37	93
Mesentery	407±100	219±25	54
Ileum	379± 40	0	0
Adrenal glands	40·3±1·7	31±3·8	77

Values indicate estimations of noradrenaline (mean±S.E.) as ng/g of tissue except for the adrenal glands where values refer to the sum of noradrenaline and adrenaline as μg/pair of organs ($n=5$).

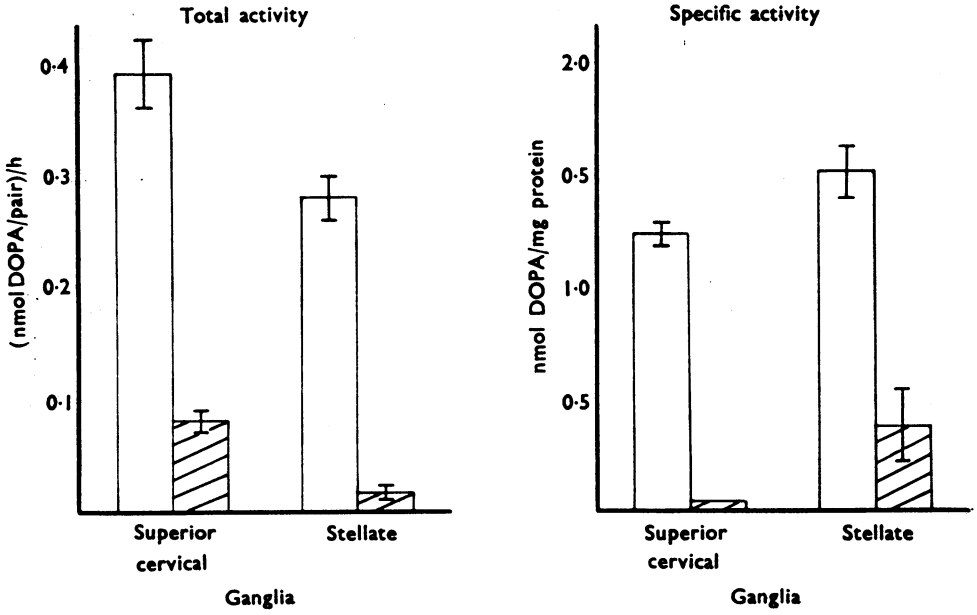

FIG. 6. Tyrosine hydroxylase activity of the superior cervical and stellate ganglia from 10-week-old rats. □ Control; ■ rats treated at birth with 6-hydroxydopamine (14×150 μg/g subcutaneously). Vertical bars represent S.E.M. (n=8 for both groups).

that 6-OHDA is capable of penetrating into the brain of newborn rats. In 8-week-old rats treated at birth with 6-OHDA, the size of the stellate and superior cervical ganglia was markedly reduced as compared with untreated animals. Furthermore, the tyrosine hydroxylase activity was markedly diminished in both these ganglia (Fig. 6).

Discussion

The biochemical and functional results obtained in rats treated with 6-OHDA at birth demonstrate that a virtually complete sympathectomy of some adrenergically innervated organs can be achieved. This finding is in general agreement with other reports in which lower doses of 6-OHDA were used and in which the sympathectomy was not as complete (Angeletti, 1971; Jaim-Etcheverry & Zieher, 1971; Thoenen, 1971; Clark, *et al.*, 1972). The sympathectomy by 6-OHDA, when administered for the first 14 days after birth, is permanent since no recovery in catecholamine levels was observed up to an age of 4 months. The apparent inability of the adrenergic nerves to regenerate is explained by a destruction of the cell bodies in their respective sympathetic ganglia. Evidence for such a destruction is derived from the marked reduction of the tyrosine hydroxylase activity in the stellate and superior cervical ganglia found in the present experiments. This confirms the earlier morphological studies in both rats and mice (Angeletti & Levi-Montalcini, 1970). In addition, there was a significant reduction in the total brain catecholamines in 10-week-old rats, suggesting that 6-OHDA is capable of penetrating into the brain of newborn rats. Recently it has been demonstrated that 6-OHDA given to newborn rats, causes a marked and permanent depletion of noradrenaline

in the cortex, cerebellum and spinal cord region, whilst, in the pons-medulla, the levels are increased (Clark, *et al.*, 1972). This property of 6-OHDA is different from nerve growth factor antiserum which does not destroy neurones within the central nervous system (Levi-Montalcini & Angeletti, 1966).

The pressor responses produced by stimulation of the entire sympathetic outflow were reduced after treatment with 6-OHDA at birth, but not completely abolished as in the adult rats which had been treated with 6-OHDA intravenously. The rises in blood pressure produced by physostigmine were only slightly affected and those in response to tyramine were not affected by the treatment with 6-OHDA in newborn animals. Also, in isolated perfused renal artery preparations, the vasoconstrictor responses to periarterial nerve stimulation were not impaired. This poor effect on vascular adrenergic innervation of 6-OHDA, when given at birth, contrasts with the virtually complete denervation of non-vascular tissues as indicated by the loss of the contractor responses of the lower eyelid to sympathetic nerve stimulation and the pronounced reduction in the noradrenaline content of heart, spleen, salivary glands and ileum. The reason for this preferential destruction of some ganglia is unknown and a matter of pure speculation. One possible explanation is that ganglia at birth are in different stages of development and the ones supplying the blood vessels may be more mature and therefore more resistant to destruction by 6-OHDA. It is interesting that peripheral adrenergic neurones also exhibit this difference in susceptibility to nerve growth factor antiserum (Levi-Montalcini & Angeletti, 1966).

In some preliminary experiments it was found that 6-OHDA in a dose of 150 μg/rat for 14 days was the highest tolerated by the litters and a better survival rate was achieved if the litters were first injected on the day after birth (Finch, unpublished observations). Therefore, the dose levels used in the present experiments would seem to produce the most effective sympathectomy possible with this method of administration of 6-OHDA. Even so, the functional results with adult rats previously treated at birth with 6-OHDA compare very favourably with the previous findings with immunosympathectomized rats (Finch & Leach, 1970).

The intravenous administration of 6-OHDA (2×50 and 2×100 mg/kg) to adult rats produced almost a complete impairment of adrenergic nerve function 24 h after the final dose of 6-OHDA and confirms the previous reports in which similar doses of 6-OHDA were used (Finch & Leach, 1970 ; DeChamplain, 1971). As far as the vascular system is concerned, it is not quite clear whether the destruction of adrenergic nerve endings is complete one day after the last administration of 6-OHDA. Although the pressor responses to stimulation of the entire sympathetic outflow were abolished in pithed rat preparations, the injections of tyramine or physostigmine were still followed by a small rise in blood pressure. There is some parallel in rats treated at birth with 6-OHDA in which, on a percentage scale, the pressor responses to stimulation of the entire sympathetic outflow were more affected than those to physostigmine. These differences could be explained by the assumption that electrical stimulation of preganglionic autonomic nerves via a steel rod inserted into the vertebral canal does not excite all sympathetic nerves. In this case, the survival of a small portion of vascular adrenergic nerve endings after 6-OHDA may have remained undetected. However, morphological studies have indicated that at least in the mesenteric vascular bed the destruction of the adrenergic nerve endings by 6-OHDA is complete (Finch, *et al.*, 1972). For this reason we favour

the explanation that both tyramine and physostigmine release catecholamines from some chromaffin tissue and that intravenous administration of 6-OHDA to adult rats produces a virtually complete adrenergic denervation of arterial blood vessels as it does in most of the other adrenergically innervated peripheral tissues. The high residual noradrenaline content (50% of controls) found in the mesentery after treatment of adult rats with 6-OHDA (Haeusler & Haefely, 1970 ; Haeusler, Haefely & Huerlimann, 1971) probably represents noradrenaline located in the long non-terminal sympathetic nerve fibres which accompany the mesenteric blood vessels and which are not destroyed by 6-OHDA. The absence of a supersensitivity to exogenous noradrenaline observed in the present experiments in the isolated renal artery preparation after administration of 6-OHDA to adult rats does not necessarily militate against the completeness of the adrenergic denervation. Other large conductance vessels such as the rabbit aorta also do not show a prejunctional type of supersensitivity after the elimination of adrenergic nerve terminals (Bevan & Verity, 1967).

It seems reasonable to conclude that 6-OHDA, administered to adult rats, produces a virtually complete adrenergic denervation, not only in non-vascular tissues but also in blood vessels. In contrast to non-vascular tissues, however, regeneration of vascular adrenergic nerve endings is particularly rapid (Finch, *et al.*, 1972) and, therefore, severely restricts the usefulness of this type of chemical adrenergic denervation for long-term studies in such important fields as hypertension. 6-OHDA given at birth produces a permanent sympathectomy but, unfortunately, it is complete only in non-vascular tissues and not in blood vessels.

REFERENCES

ANGELETTI, P. U. (1971). Chemical sympathectomy in newborn animals. *Neuropharmac.*, **10**, 55–59.

ANGELETTI, P. U. & LEVI-MONTALCINI, R. (1970). Sympathetic nerve cell destruction in newborn mammals by 6-hydroxydopamine. *Proc. natn. Acad. Sci., U.S.A.*, **65**, 114–121.

ANTON, A. H. & SAYRE, D. F. (1962). A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

BEVAN, J. A. & VERITY, M. A. (1967). Sympathetic nerve-free vascular muscle. *J. Pharmac. exp. Ther.*, **157**, 117–124.

CLARK, D. W. J., LAVERTY, R. & PHELAN, E. L. (1972). Long-lasting peripheral and central effects of 6-hydroxydopamine in rats. *Br. J. Pharmac.*, **44**, 233–243.

DECHAMPLAIN, J. (1971). Degeneration and regrowth of adrenergic nerve fibers in the rat peripheral tissues after 6-hydroxydopamine. *Canad. J. Physiol. Pharmac.*, **49**, 345–355.

DREW, G. M. & LEACH, G. D. H. (1971). Corticosteroids and their effects on cardiovascular sensitivity in the pithed adrenalectomized rat. *Arch. Int. Pharmacodyn. Ther.*, **191**, 255–260.

EULER, U. S. VON & LISHAJKO, K. (1961). Improved technique for the fluorimetric estimation of catecholamines. *Acta physiol. scand.*, **51**, 348–355.

FINCH, L., HAEUSLER, G., KUHN, H. & THOENEN, H. (1972). The recovery of vascular adrenergic nerve function in the rat after chemical sympathectomy with 6-hydroxydopamine. *Br. J. Pharmac.*, **44**, 357–358P.

FINCH, L. & LEACH, G. D. H. (1969). The role of the sympathetic nervous system in the cardiovascular responses to angiotensin in the pithed rat. *Br. J. Pharmac.*, **36**, 481–488.

FINCH, L. & LEACH, G. D. H. (1970). A comparison of the effects of 6-hydroxydopamine, immunosympathectomy and reserpine on the cardiovascular reactivity in the rat. *J. Pharm. Pharmac.*, **22**, 354–360.

GILLESPIE, J. S. & MUIR, T. C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. *Br. J. Pharmac. Chemother.*, **30**, 78–87.

HAEUSLER, G. & HAEFELY, W. (1970). Pre- and post-junctional supersensitivity of the mesenteric artery preparation from normotensive and hypertensive rats. *Naunyn-Schmiedebergs Arch. Pharmak.*, **266**, 18–33.

HAEUSLER, G., HAEFELY, W. & HUERLIMANN, A. (1971). Effect of surgical and chemical adrenergic denervation on vascular responses. In: *Vascular Neuro-Effector Systems*, ed. Bevan, J. A., Furchgott, R. F., Maxwell, R. A. and Somlyo, A. P., pp. 141–159. Basel: Karger.

HAEUSLER, G., HAEFELY, W. & THOENEN, H. (1969). Chemical sympathectomy of the cat with 6-hydroxydopamine. *J. Pharmac. exp. Ther.*, **170**, 50–61.

IVERSEN, L. L., GLOWINSKI, J. & AXELROD, J. (1966). The physiological disposition and metabolism of norepinephrine in immunosympathectomized animals. *J. Pharmac. exp. Ther.*, **151**, 273–284.

JAIM-ETCHEVERRY, G. & ZIEHER, L. M. (1971). Permanent depletion of peripheral norepinephrine in rats treated at birth with 6-hydroxydopamine. *Eur. J. Pharmac.*, **13**, 272–276.

LEVI-MONTALCINI, R. & ANGELETTI, P. U. (1966). Immunosympathectomy. *Pharmac. Rev.*, **18**, 619–628.

LEVITT, M., GIBB, J. W., DALY, J. W., LIPTON, M. & UDENFRIEND, S. (1967). A new class of tyrosine hydroxylase inhibitors and a simple assay of inhibition *in vivo*. *Biochem. Pharmac.*, **16**, 1313–1321.

MUELLER, R. A., THOENEN, H. & AXELROD, J. (1969). Increase in tyrosine hydroxylase activity after reserpine administration. *J. Pharmac. exp. Ther.*, **169**, 74–79.

THOENEN, H. (1971). Biochemical alterations induced by 6-hydroxydopamine in peripheral adrenergic neurones. In: *6-Hydroxydopamine and Catecholamine Neurones*, ed. Malmfors, T. and Thoenen, H., pp. 75–85. Amsterdam: North Holland.

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

ZAIMIS, E. (1967). In: *The Scientific Basis of Medicine*, Annual Reviews, pp. 59–73. London: Athlone Press.

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