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Rapid recovery of vascular adrenergic nerves in the rat after chemical sympathectomy with 6-hydroxydopamine

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Summary

1. Twenty-four hours after the last of 4 intravenous doses of 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7) a complete impairment of adrenergic nerve function was observed in various organs of the rat.

2. A complete recovery of adrenergic nerve function in vascular smooth muscle was observed 7 days after the last dose of 6-hydroxydopamine whilst in non-vascular smooth muscle recovery took between 14 and 21 days.

3. On day 8, noradrenaline depletion produced by 6-hydroxydopamine was not as great in vascular tissues, such as the mesentery and renal artery, as in other tissues, such as the heart and salivary glands. Noradrenaline concentrations recovered much more rapidly in vascular than in other tissues.

4. Electron microscope studies of small mesenteric arteries showed a complete destruction of adrenergic nerve terminals 24 h after 6-hydroxydopamine (2×100 mg/kg). However, there was a reappearance of growing terminals within 7 days and after 28 days the regrowth of adrenergic nerve terminals seemed complete.

5. From the morphological and functional data it is concluded that 6-hydroxydopamine does produce complete destruction of vascular adrenergic nerve terminals. However, these terminals regenerate more rapidly than those in other tissues. This could explain the failure of intravenously administered 6-hydroxydopamine to prevent the development of experimental hypertension in the rat.

Introduction

6-Hydroxydopamine (6-OHDA) causes a long-lasting depletion of noradrenaline from various adrenergically innervated organs (Porter, Totaro & Stone, 1963; Laverty, Sharman & Vogt, 1965) and electron microscopic studies have shown that 6-OHDA produces a selective destruction of adrenergic nerve terminals in both the rat and cat (Thoenen & Tranzer, 1968). A short time after the administration of 6-OHDA this destruction is characterized by a virtually total impairment of adrenergic nerve function (Haeusler, Haefely & Thoenen, 1969; McGregor & Phelan, 1969; Finch & Leach, 1970a, b). This is followed by a period of increasing func-

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tional recovery of adrenergic nerves during the next few weeks (Haeusler *et al.*, 1969 ; De Champlain, 1971).

Several reports have shown that chemical sympathectomy with 6-OHDA fails to influence either the development or maintenance of several types of experimental hypertension in the rat, even after surgical removal of the adrenal medulla (Clarke, Smookler & Barry, 1970 ; Finch & Leach, 1970c, d ; Mueller & Thoenen, 1970 ; Yamori, Yamabe, De Jong, Lovenberg & Sjoerdsma, 1972). In contrast, it has been reported that immunosympathectomy or 6-OHDA given at birth (both methods produce a permanent sympathectomy) partially or completely prevent the development of several types of experimental hypertension in the rat (Ayitey-Smith & Varma, 1970 ; Clark, 1971 ; Clark, Laverty & Phelan, 1972). These conflicting observations could be explained by an incomplete sympathectomy of the vascular system by 6-OHDA in adult animals since it has recently been reported that blood vessels are more resistant to sympathectomy by 6-OHDA than other tissues (Haeusler, Haefely & Hürlimann, 1971 ; Berkowitz, Spector & Tarver, 1972). Alternatively, a rapid restoration of adrenergic nerve function may occur after 6-OHDA pretreatment since only the nerve terminals are destroyed by this method of sympathectomy (Tranzer & Thoenen, 1968).

The present studies show that 6-OHDA does in fact produce a complete destruction of vascular adrenergic nerve terminals but regeneration and thus recovery of adrenergic nerve function in blood vessels takes place within 7 days of the last dose of 6-OHDA.

Methods

Male Wistar rats (190–230 g) were used for all experiments.

Whole animal preparations

Anaesthetized and pithed preparations were set up as described previously (Finch & Leach, 1969). The pressor responses to physostigmine were studied in rats anaesthetized with urethane (1–1·5 g/kg i.p.) and those to tyramine in pithed rats. The pressor agents were injected intravenously in increasing doses at intervals of 15 minutes. Only one dose-response curve was obtained in each animal.

Pithed rat 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 intravenously before stimulation was started. In order to prevent the release of adrenal medullary catecholamines, bilateral adrenalectomy was performed under halothane anaesthesia 2 h before starting the experiment. These animals were given corticosterone (10 mg/kg i.m.) which has been shown to restore the impaired cardiovascular sensitivity after adrenalectomy (Drew & Leach, 1971).

In order to measure 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 central end of the cut cervical sympathetic trunk was 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 ±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 hypodermic needle) and then cut at the level of the kidney hilus. The isolated artery was floated on the surface of a 50 ml organ bath which was filled with Krebs–Henseleit solution (37° C) bubbled gently with 95% O_2 and 5% CO_2. The preparation was 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, the periarterial nerves were stimulated by means of a specially designed fluid electrode placed around the artery. Supramaximal stimulation at various frequencies with pulses of 1 ms duration for 20 s, was obtained with a Grass S7 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.

Estimation of tissue content of noradrenaline and adrenaline

Rats were stunned by a blow on the head. The organs to be assayed (heart, salivary gland, renal arteries and complete mesentery) were quickly removed and frozen at −75° C. After weighing, the tissues were homogenized in 0·4 N $HClO_4$. Catecholamines were adsorbed on alumina according to the method of Anton & Sayre (1962) and assayed fluorimetrically by the method of von Euler & Lishajko (1961).

Ultramorphological studies

Small pieces of mesenteric arteries (0·5–1·5 cm from the small intestine) were examined 1, 3, 7, 14, 21 and 28 days after the first of two injections of 6-OHDA (2×100 mg/kg intravenously). Some preparations were incubated for 30 min at 37° C in Krebs–Henseleit solution containing 1 mg/ml 5-hydroxydopamine in order to test the ability of the adrenergic nerve terminals to take up this amine (Tranzer & Thoenen, 1967). Tissues were subsequently fixed in 3% glutaraldehyde in 0·1 M phosphate buffer for 2 h at pH 7·2 and 4° C, rinsed, and postfixed for one further hour in 2% osmium tetroxide in the same buffer. After dehydration and embedding in Epon, ultrathin sections were prepared, double-stained with uranyl acetate and lead citrate and examined with an EM 300 Philips electron microscope.

Administration of 6-hydroxydopamine

For the functional and biochemical experiments, rats were injected with 6-hydroxydopamine hydrobromide 2×50 mg/kg intravenously on day 1 and 2×100 mg/kg on day 7. 6-OHDA was dissolved immediately before injection in 0·001 N hydrochloric acid which had been gassed with nitrogen. Animals were then studied on days 8, 10, 14, 21 and 28. Rats used for electron microscopy were injected with 6-OHDA, 2×100 mg/kg intravenously within a 6 h period.

Statistical analysis

Student's t test was used for the evaluation of experimental data. P values $<0{\cdot}05$ were considered to be significantly different from controls. Throughout the paper, mean values are given together with the standard error of mean, and n is the number of experiments.

Drugs

Atropine sulphate (C. H. Boehringer A.G.), corticosterone acetate (generously donated by Dr. R. Buckett, Organon Labs.), guanethidine sulphate (Ciba-Geigy A.G.), 5-hydroxydopamine (3,4,5-trihydroxyphenethylamine hydrobromide), 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine hydrobromide) (both synthesized by Dr. A. Langemann, F. Hoffmann-La Roche), (−)-noradrenaline (Fluka A.G.), physostigmine sulphate (Sandoz), (+)-tubocurarine chloride (Mann Labs., N.Y.), tyramine hydrochloride (Fluka A.G.).

Results

Functional consequences of treatment with 6-hydroxydopamine

Anaesthetized and pithed preparations

In pithed rat preparations, stimulation of the entire sympathetic outflow through the pithing rod (Gillespie & Muir, 1967) was carried out in order to evaluate the

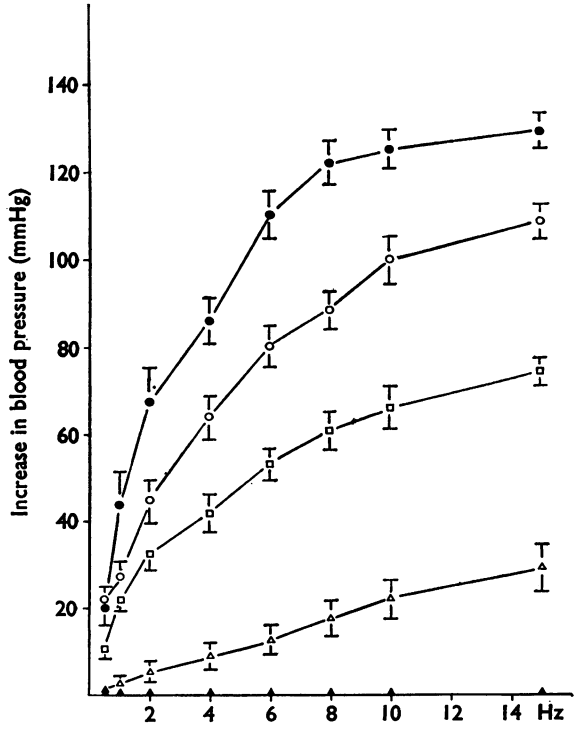

FIG. 1. Mean rise in blood pressure obtained in the Gillespie & Muir (1967) preparation. The entire sympathetic outflow was stimulated with supramaximal voltage and various frequencies. Controls (●——●); rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7), the experiments performed on day 8 (▲——▲); day 10 (△——△); day 14 (□——□); and day 28 (○——○). Vertical bars show S.E.M. (n=8 for all groups).

extent of the sympathectomy. The animals were also adrenalectomized since pretreatment with 6-OHDA does not deplete adrenal medullary amines (Thoenen & Tranzer, 1968). In control preparations, graded pressor responses were obtained with alterations in the frequency of stimulation (Figure 1). In animals pretreated with 6-OHDA (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7), the pressor responses to sympathetic nerve stimulation were completely abolished on day 8, whilst on days 10 and 14 a recovery of 50% of normal was observed. The accompanying positive chronotropic responses found in control preparations were not observed in those rats pretreated with 6-OHDA. By day 28 the cardiovascular responses to sympathetic nerve stimulation were similar in magnitude to those obtained in control preparations. Intravenous doses of tyramine (12·5–50 μg/kg) in normal pithed rat preparations produced a rise in blood pressure which was accompanied by a marked tachycardia with the higher doses (50–500 μg/kg). In rats pretreated with 6-OHDA (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7) and set up one day later, both the pressor and chronotropic responses to tyramine were almost completely abolished (Figure 2). However, between days 10 and 14, after treatment with 6-OHDA, the pressor responses were found to be comparable in magnitude to those observed in untreated preparations. The accompanying

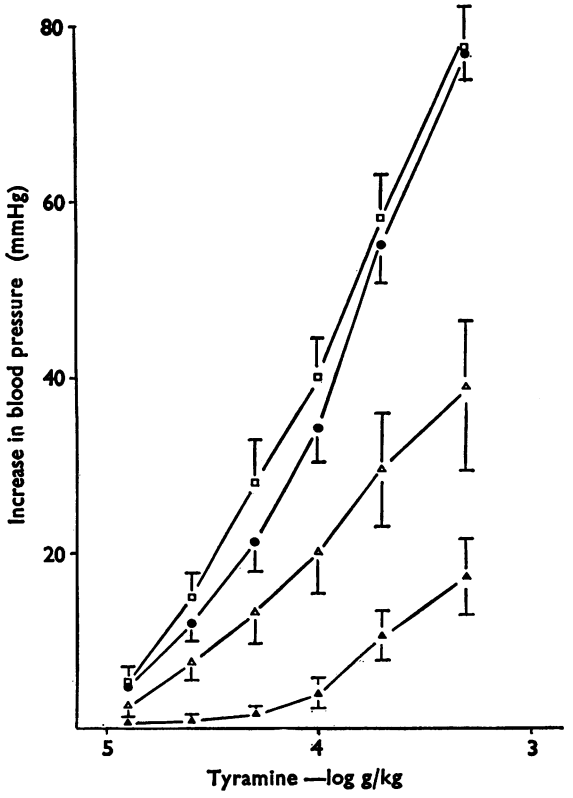

FIG. 2. Mean rise in blood pressure after intravenous doses of tyramine in pithed rat preparations. Controls (●——●); rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7), the experiments were performed on day 8 (▲——▲); day 10 (△——△); and day 14 (□——□). Vertical bars show S.E.M. (n=8 for all groups).

increases in heart rate were only seen on days 21 and 28 following treatment with 6-OHDA.

The centrally induced rise in blood pressure produced by intravenous administration of physostigmine was investigated in the rat anaesthetized with urethane (Figure 3). These pressor responses were greatly reduced on day 8 (1 day after the last dose of 6-OHDA), whilst almost normal responses were observed on day 14, only 7 days after the last dose of 6-OHDA.

In order to study the effects of sympathectomy by 6-OHDA on non-vascular smooth muscle, contractions of the lower eyelid were recorded after pre-ganglionic stimulation of the cervical sympathetic trunk. In untreated rats anaesthetized with pentobarbitone, frequency-dependent contractions were obtained even at low rates of stimulation (Figure 4). On day 8, one day after the last dose of 6-OHDA, no contractions were observed even at the higher rates of stimulation. Only a 50% recovery of the responses occurred between days 14 and 21 whilst, by day 28, the contractions to sympathetic nerve stimulation were almost identical to those observed in untreated rats.

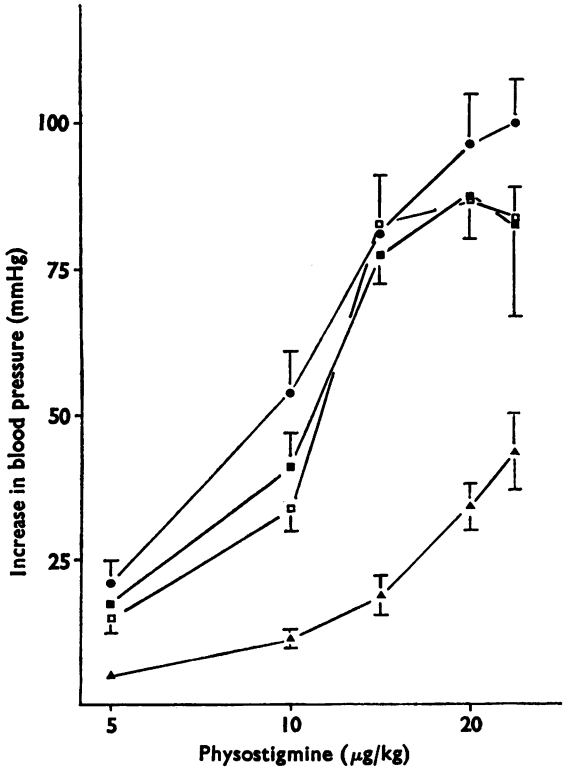

FIG. 3. Mean rise in blood pressure after intravenous doses of physostigmine in rats anaesthetized with urethane (1–1·5 g/kg i.p.). Controls (●——●); rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7), the experiments were performed on day 8 (▲——▲); day 14 (□——□); day 21 (■——■). Vertical bars show S.E.M. (n=8 for all groups).

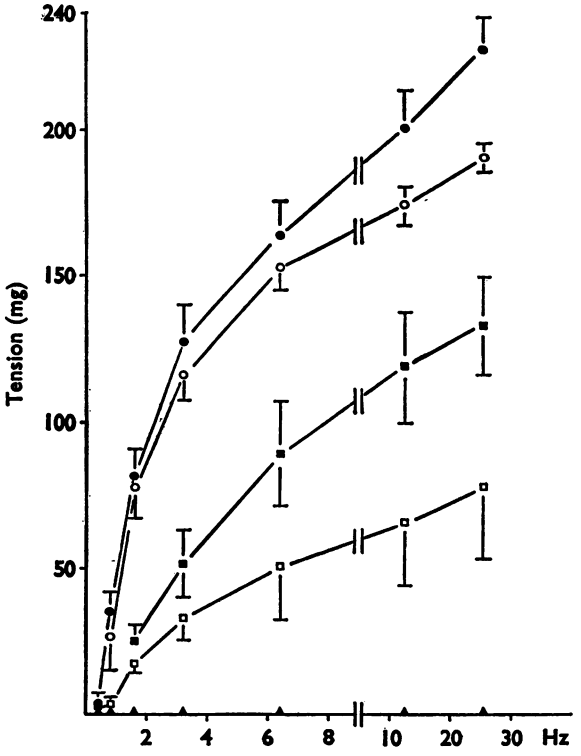

FIG. 4. Contraction of the right lower eyelid to electrical stimulation of the cervical sympathetic trunk. Controls (●——●); rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7), experiments were performed on day 8 (▲——▲); day 14 (□——□); day 21 (■——■); and day 28 (○——○). Vertical bars show S.E.M. (n=8 for all groups).

Isolated renal artery preparation

Periarterial nerve stimulation of the isolated renal artery preparation induces a vasoconstrictor response which is measured as an increase in the perfusion pressure (Figure 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 vasoconstrictor response was due to a direct stimulation of vascular smooth muscle cells. Arteries from rats treated with 6-OHDA showed on day 8 responses similar in magnitude to those perfused with guanethidine, thus indicating a marked sympathectomy by 6-OHDA in this tissue. By day 10, however, there was some recovery in the responses to nerve stimulation and by day 14, the responses were identical to those from untreated rats. None of the preparations obtained from animals treated with 6-OHDA exhibited supersensitivity to noradrenaline when it was added to the perfusion fluid.

Recovery of noradrenaline levels after 6-hydroxydopamine

The functional studies indicate that a more rapid recovery of adrenergic nerve function occurred in vascular smooth muscle (7 days after the last dose of 6-OHDA) than in non-vascular or cardiac muscle (14–28 days after the last dose of 6-OHDA). It was of interest, therefore, to study whether the levels of catecholamines showed a

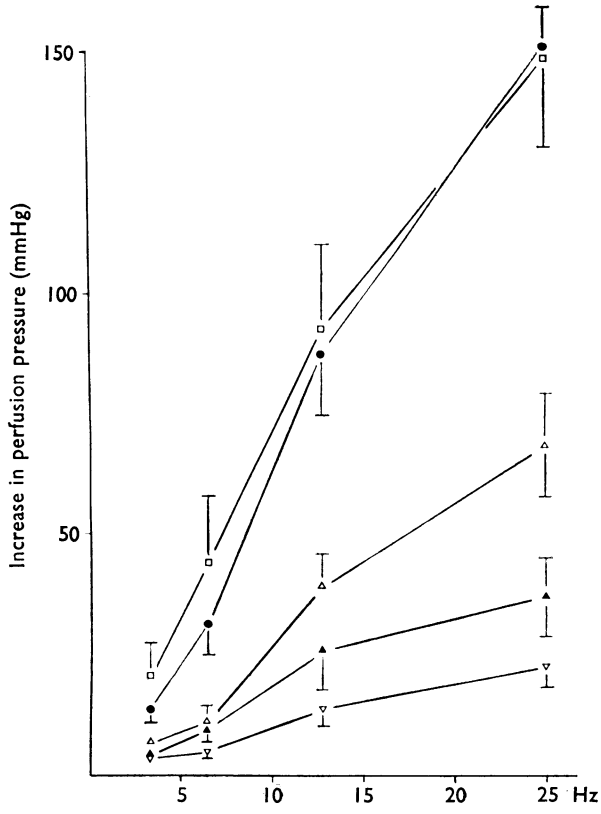

FIG. 5. Vasoconstrictor responses to periarterial nerve stimulation of the isolated renal artery preparation. Stimulation was carried out for 20 s periods at various frequencies with supramaximal voltage. Controls (●——●); 1×10^{-5}M guanethidine added to the perfusion fluid (▽——▽); rats treated with 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7), the experiments were performed on day 8 (▲——▲); day 10 (△——△); and day 14 (□——□). Vertical bars show S.E.M. ($n=10$ for all groups).

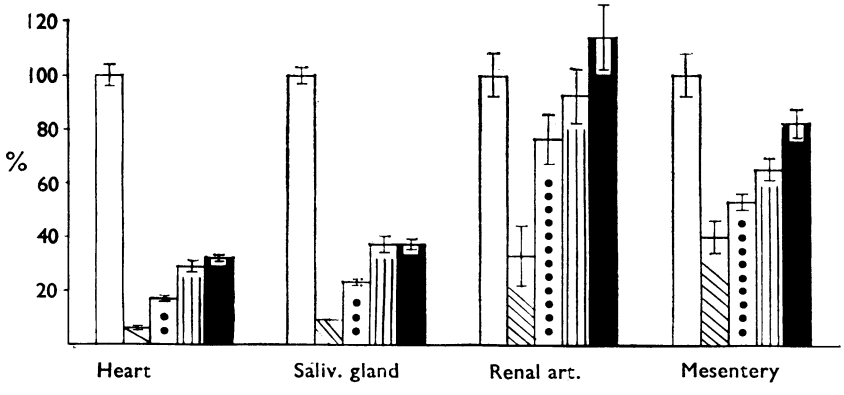

FIG. 6. Effect of 6-hydroxydopamine (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7) on the noradrenaline content of various organs. Untreated controls (100%) □; day 8 ▨; day 14 ▤; day 21 ▥; day 28 ■. Vertical bars show S.E.M. Each column represents the mean of 5 determinations from 3–6 pooled organs.

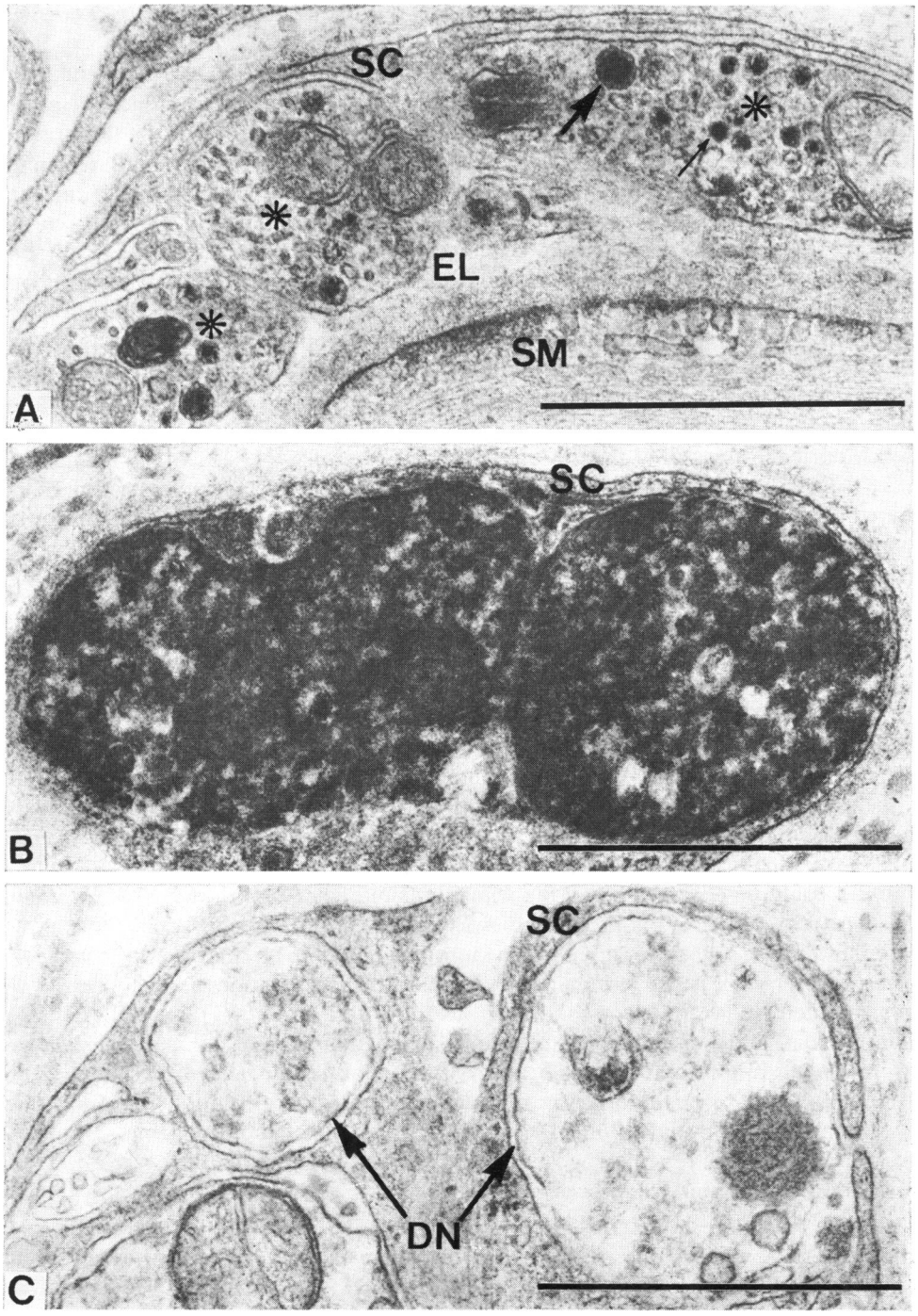

FIG. 7. Adrenergic axon profiles in transverse sections of rat mesenteric arteries, incubated with 5-hydroxydopamine followed by glutaraldehyde/osmium fixation. Calibration bars 1 μm × 56,000. (A) Control. A group of adrenergic axons (*) are partially enclosed by a Schwann cell (SC). They are separated from the smooth muscle cell (SM) by a thin external elastic lamella (EL). A typical nerve terminal with large ——▶ and small ——→ dense core vesicles is indicated. (B) One day after 6-hydroxydopamine. Degenerating adrenergic nerve terminals with dense osmiophilic cytoplasm. SC, Schwann cell. (C) Three days after 6-hydroxydopamine. The Schwann cell (SC) appears to have engulfed the degenerating nerves (DN), in which osmiophilia is now reduced. The remaining osmiophilic inclusions may represent lysed mitochondria and vesicles.

comparably rapid recovery after the administration of 6-OHDA. Treatment with 6-OHDA (2×50 mg/kg on day 1 and 2×100 mg/kg on day 7) produced a great depletion of noradrenaline in the heart to 6% of control values on day 8 (Figure 6). During the next 20 days there was only a gradual recovery, the values reaching 30% of control at the end of this period. Similar results were obtained for the depletion and recovery of noradrenaline in the salivary gland, which was taken as another example of non-vascular tissue. In the renal artery, the depletion by 6-OHDA was not as large (concentration of noradrenaline was 35% of controls on day 8) as in the heart and salivary glands (Figure 6). Furthermore, the recovery was much more rapid and a normal noradrenaline content was reached again by day 14. In the whole mesentery (without the gut) which contains both large and small blood vessels, the depletion and recovery of noradrenaline was similar to that observed in the renal artery. However, the noradrenaline content did not reach normal values until day 28 after the last dose of 6-OHDA. It is interesting that the renal artery has a very high noradrenaline content (6–8 μg/g) when compared with the heart (1–1·5 μg/g), the salivary glands (1·5–2 μg/g) and the mesentery (0·5–0·8 μg/g).

Electron microscope studies

The adrenergic nerve terminals of the mesenteric arteries (diameter 150–200 μm) were usually located within the adventitia just outside the external elastic lamella. The typical features of these nerves were revealed in control arteries incubated with 5-hydroxydopamine (Figure 7A). Vesiculated axon profiles (i.e. terminal and pre-terminal) contained both large and small dense core vesicles in which the electron density of the cores was noticeably enhanced compared with unincubated tissues. The vesiculated and non-vesiculated (i.e. non-terminal) profiles were usually associated with Schwann cell processes. As many as 300 axon profiles could be observed along the whole circumference of an artery. In addition to these adventitial axon profiles, there were mostly large non-vesiculated axon bundles outside the adventitia, which accompanied the blood vessels. One day after 6-OHDA, all nerve terminals showed signs of degeneration characterized by a general increase in the electron density of the whole profile (Figure 7B). Intact non-terminal and pre-terminal axon profiles could only rarely be observed. However, while it appeared that most adventitial non-terminal axon profiles were degenerated, those in the large axon bundles outside the adventitia remained unchanged. Three days after 6-OHDA many but not all of the detectable axon profiles appeared to have undergone lysis and electron lucent structures were observed engulfed by Schwann cell processes (Figure 7C).

Seven days after 6-OHDA some nerve terminals, probably in the process of regeneration, could be observed. In most of them the vesicles looked empty even after incubation with 5-hydroxydopamine (Figure 8A). Fourteen and 21 days after 6-OHDA the ultrastructure of the axon profiles was similar to that of control animals and after incubation in 5-hydroxydopamine the electron density of the cores of the large and small vesicles was enhanced. One week later the adrenergic nerve terminals were indistinguishable from those of controls (Fig. 8B) both with regard to ultrastructure and their ability to accumulate and store 5-hydroxydopamine.

In a further series of experiments, 6-OHDA was administered to spontaneously hypertensive rats of the Japanese strain (Okamoto & Aoki, 1964). The pattern of

degeneration and regrowth of adrenergic nerves in the mesenteric arteries of these animals was similar to that of normotensive rats after 6-OHDA treatment.

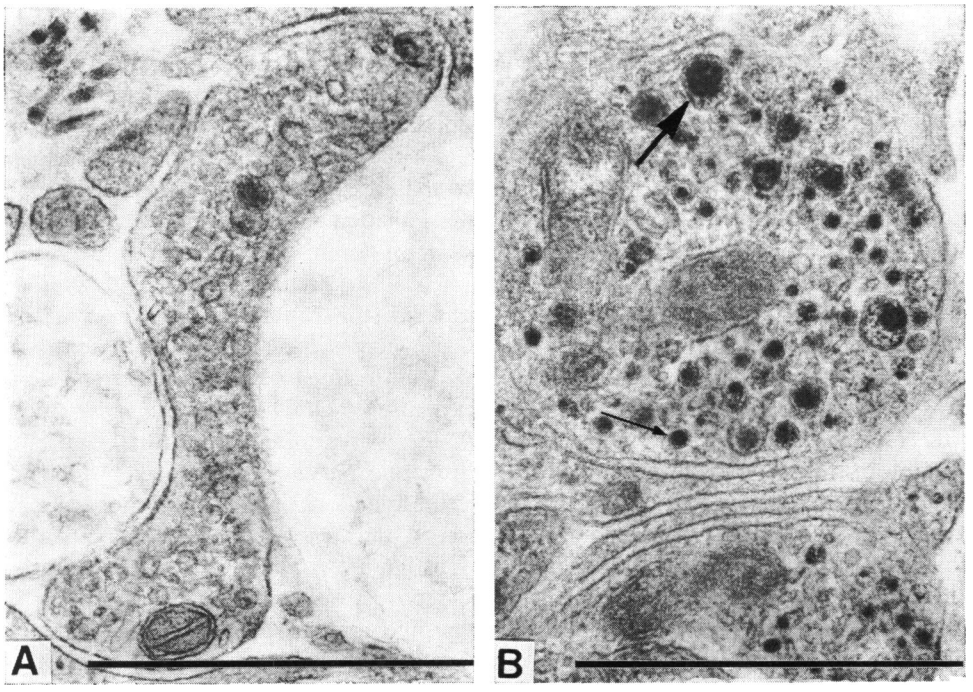

FIG. 8. As in Fig. 7. (A) Seven days after 6-hydroxydopamine. A probably regenerating nerve terminal. In spite of incubation with 5-hydroxydopamine the vesicles look empty. The electron lucent structures on either side may be lysed terminals (compare Fig. 7C). (B) Twenty-eight days after 6-hydroxydopamine. The adrenergic nerve terminals again resemble those from control animals. The small ⟶ and large ⟶▶ vesicles contain electron dense cores, which were particularly prominent after incubation with 5-hydroxydopamine.

Discussion

The starting point for the present investigation was the unexpected but repeatedly confirmed observation that the intravenous administration of 6-OHDA, in doses sufficient to produce chemical sympathectomy, was followed by a reduction in blood pressure which was only short-lasting. After 6-OHDA, the blood pressure returned to normal within 3–5 days both in normotensive and hypertensive rats (Clarke *et al.*, 1970 ; Finch & Leach, 1970c, d ; Mueller & Thoenen, 1970 ; Yamori *et al.*, 1972). In contrast, the recovery of the noradrenaline content in several non-vascular organs of the rat was rather slow, reaching 10, 30 and 60% of controls in the spleen, heart and the vas deferens, respectively, after 4 weeks (Thoenen & Tranzer, 1968). The question, therefore, arose as to whether the time-course of the functional recovery of the adrenergic nerves after 6-OHDA differs in vascular and in non-vascular tissues.

One day after the last injection of 6-OHDA the vasoconstrictor responses to periarterial nerve stimulation in the isolated perfused renal artery preparation were nearly abolished. However, there was a very rapid recovery of the adrenergic nerve function and 6 days later, the responses were indistinguishable from those of untreated controls. Particular emphasis is laid on these experiments since they are

the only ones in which vascular adrenergic nerve function could be studied without any interference with the adrenals or with the adrenergic innervation of the heart.

A very similar time-course of functional recovery was observed when pressor responses to tyramine were studied in pithed rat preparations or pressor responses to physostigmine in rats anaesthetized with urethane. Here again, the pressor responses had virtually returned to normal 7 days after the last injection of 6-OHDA. However, when compared with the isolated perfused renal artery preparation, the situation is less clear since catecholamines released from the adrenal medulla, which is not affected by 6-OHDA, will contribute to the pressor responses to both tyramine and physostigmine. This is indicated by the fact that even one day after the last injection of 6-OHDA the pressor responses to tyramine and physostigmine were not completely abolished. The contribution of the adrenal medulla will tend to cause an overestimation of the rate with which the functional recovery of the vascular adrenergic nerve endings takes place, whereas the recovery of the cardiac adrenergic nerves may be rather slow. This slow recovery is suggested by a slow rise of the noradrenaline content of the heart after 6-OHDA and the observation that 7 days after 6-OHDA the tachycardia due to tyramine was virtually absent. Therefore, the slow recovery of cardiac adrenergic nerve function after 6-OHDA will lead to an underestimation of the rate of the functional recovery of the vascular adrenergic nerves, since adrenergic activation of the heart certainly contributes to the pressor responses to tyramine and physostigmine. These two opposing processes, which obscure the assessment of the recovery of vascular adrenergic nerve function based on pressor responses to tyramine and to physostigmine seem to balance each other. In any case, the recovery rate of the tyramine- and physostigmine-induced pressor responses in the whole animal is strikingly similar to that of the adrenergic nerve function in the isolated perfused renal artery preparation.

In the pithed rat, stimulation of the entire sympathetic outflow according to the method of Gillespie & Muir (1967) causes pressor responses, which are the result of an activation of vascular and cardiac adrenergic nerves and of the adrenal medulla. The contribution of the adrenal medulla was eliminated by adrenalectomy in our experiments. Under these conditions the pressor responses to stimulation of the entire sympathetic outflow were completely inhibited one day after the administration of 6-OHDA. Then, a rapid initial recovery set in, reaching 20% and 50% of the pressor responses of the untreated controls after 3 and 7 days, respectively. This rapid initial phase was followed by a rather slower phase of further functional recovery. We are inclined to assume that the initial phase is due to the rapid restoration of vascular adrenergic nerve function and the following delay due to the slow recovery of cardiac adrenergic nerves.

The lower eyelid of the rat was taken as an example to study the functional recovery after 6-OHDA in a non-vascular smooth muscle. The initial recovery in this organ was rather slow. In the renal artery preparation a supersensitivity to noradrenaline could not be detected after 6-OHDA and, therefore, the restoration of the pressor responses to periarterial nerve stimulation is a good indication of the recovery of the vascular adrenergic nerves. It cannot be decided whether the smooth muscles of the orbit also failed to develop supersensitivity, since noradrenaline given intravenously did not produce reproducible contractions of the lower eyelid. If there was supersensitivity, the functional recovery of the lower eyelid preparation would have been slower than indicated by the curves of Figure 5.

The isolated renal artery and the lower eyelid of the rat may be considered as the two preparations of vascular and non-vascular smooth muscle in which the functional recovery could be studied without interference by other organs such as the adrenals or the heart. Therefore, they would most reliably show differences in the functional recovery of vascular and non-vascular adrenergic nerve terminals. A comparison of the recovery curves of both preparations (Figs. 4 and 5) leads to the conclusion that the functional recovery of adrenergic nerve terminals in vascular smooth muscle is more rapid than that in other smooth muscle. This conclusion is supported by the time-course of the restoration of the noradrenaline content in various organs. The renal artery and the mesentery, which were taken as examples for vascular adrenergic nerves, regained their normal noradrenaline content within 3 weeks after the last injection of 6-OHDA. During the same period the noradrenaline content in the heart and salivary glands rose to only 40% of that of the controls.

The results discussed so far can be interpreted in two ways: (1) Vascular and non-vascular adrenergic nerve terminals are equally destroyed by 6-OHDA, however, the former regenerate faster. (2) 6-OHDA causes a rather short-lasting noradrenaline depletion and functional impairment of vascular adrenergic terminals without destroying them. In order to distinguish between these two possibilities electron microscope studies were carried out on small mesenteric arteries and these showed that virtually all adventitial axon profiles degenerated within the first 24 h after 6-OHDA treatment, while the large axon bundles outside the adventitia apparently were left unaffected. Seven days after 6-OHDA, vesiculated axon profiles reappeared indicating regeneration. Their ability to take up and store amines (5-hydroxydopamine), however, seems to be developed only two weeks later when most vesiculated axon profiles contained dense core vesicles. The undamaged large axon bundles outside the adventitia may be the morphological correlate of the high residual noradrenaline content determined biochemically in the arteries shortly after 6-OHDA. Fluorescence histochemical studies (Lorez, to be published) seem to confirm this view. The ratio of adrenergic nerve terminals to non-terminal axons is obviously lower in vascular tissue than in the heart or the salivary glands (for comparison see noradrenaline content in the various organs one day after 6-OHDA in Figure 6). This different ratio may lead to the erroneous conclusion that some vascular beds are resistant to the action of 6-OHDA, particular when mainly biochemical determinations of the noradrenaline content are considered.

No explanation can be given for the particularly rapid regeneration of the adrenergic nerve terminals in the mesenteric arteries after 6-OHDA. Whether this fast regeneration is also valid for adrenergic nerve terminals of other vascular beds cannot be answered with certainty at the moment, though it seems very likely. Furthermore, our results have shown that the time-course of regeneration of the vascular adrenergic nerve terminals is similar in normotensive and genetically hypertensive rats. Therefore, it seems reasonable to conclude that the failure of 6-OHDA to influence hypertension in rats over a reasonably long time is due to the rapid regeneration of the vascular adrenergic nerve terminals.

REFERENCES

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.

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.

BERKOWITZ, B. A., SPECTOR, S. & TARVER, J. H. (1972). Resistance of noradrenaline in blood vessels to depletion by 6-hydroxydopamine or immunosympathectomy. *Br. J. Pharmac.*, **44**, 10–16.

CLARK, D. W. J. (1971). Effects of immunosympathectomy on development of high blood pressure in genetically hypertensive rats. *Circulation Res.*, **28**, 330–336.

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.

CLARKE, D. E., SMOOKLER, H. H. & BARRY, H. (1970). Sympathetic nerve function and DOCA-NaCl induced hypertension. *Life Sci.*, **9**, 1097–1108.

DE CHAMPLAIN, J. (1971). Degeneration and regrowth of adrenergic nerve fibers in the rat peripheral tissues after 6-hydroxydopamine. *Can. 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. *Archs 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. & 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. (1970a). A comparison of the effects of 6-hydroxydopamine, immunosympathectomy and reserpine on the cardiovascular reactivity in the rat. *J. Pharm. Pharmac.*, **22**, 354–360.

FINCH, L. & LEACH, G. D. H. (1970b). Effects of 6-hydroxydopamine on the perfused rat mesentery preparation. *J. Pharm. Pharmac.*, **22**, 543–544.

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

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.*, **30**, 78–87.

HAEULSER, G., HAEFELY, W. & HÜRLIMANN, A. (1971). Effect of surgical and chemical adrenergic denervation on vascular responses. In: *Physiology and Pharmacology of Vascular Neuroeffector System*, ed. by Bevan, J. A., Furchgott, R. F., Maxwell, R. A. & Somlyo, A. P., pp. 139–159. Basle: Karger.

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

LAVERTY, R., SHARMAN, D. F. & VOGT, M. (1965). Action of 2,4,5-trihydroxyphenylethylamine on the storage and release of noradrenaline. *Br. J. Pharmac. Chemother.*, **24**, 549–560.

MCGREGOR, D. D. & PHELAN, E. L. (1969). Vasoconstrictor responses to sympathetic nerve stimulation in perfused mesenteric arteries of rats following administration of 6-hydroxydopamine. *Proc. Univ. Otago Med. Sch.*, **47**, 19–21.

MUELLER, R. A. & THOENEN, H. (1970). Effect of 6-hydroxydopamine hydrobromide and adrenalectomy on the development of deoxycorticosterone-trimethylacetate-NaCl hypertension in rats. *Fedn Proc.*, **29**, 546.

OKAMOTO, K. & AOKI, K. (1963). Development of a strain of spontaneously hypertensive rats. *Jap. Circul. J.*, **27**, 282–293.

PORTER, C. C., TOTARO, J. A. & STONE, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *J. Pharmac. exp. Ther.*, **140**, 308–316.

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

TRANZER, J. P. & THOENEN, H. (1967). Electronmicroscopic localization of 5-hydroxydopamine (3,4,5-trihydroxyphenylethylamine), a new 'false' sympathetic transmitter. *Experientia (Basle)*, **23**, 743–745.

TRANZER, J. P. & THOENEN, H. (1968). An electronmicroscopic study of selective, acute degeneration of sympathetic nerve terminals after administration of 6-hydroxydopamine. *Experientia (Basle)*, **24**, 155–156.

YAMORI, Y., YAMABE, H., DE JONG, W., LOVENBERG, W. & SJOERDSMA, A. (1972). Effect of tissue norepinephrine depletion by 6-hydroxydopamine on blood pressor in spontaneously hypertensive rats. *Eur. J. Pharmac.*, **17**, 135–140.

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