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Action of 6-hydroxydopamine on lamb sympathetic ganglia, vas deferens and adrenal medulla: a combined histochemical, ultrastructural and biochemical comparison with the effects of reserpine

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Summary

1. The effects of a single dose of 6-hydroxydopamine (6-OHDA) compared with those of chronic reserpine treatment were studied in lamb sympathetic neurones and adrenal medulla by a combination of fluorescence histochemistry, electron microscopy and radiochemical assay.

2. In sympathetic ganglia, 6-OHDA produced a rise in noradrenaline concentration within 24 h, and falls in tyrosine hydroxylase and monoamine oxidase activities, whereas reserpine caused a fall in noradrenaline, a rise in tyrosine hydroxylase activity and no change in monoamine oxidase activity. The fluorescence of intra- and postganglionic axons increased greatly within 24 h of 6-OHDA, and there was a corresponding accumulation of large dense-core vesicles within many axons whose neurotubules were disrupted. The changes were almost reversed after 3 weeks.

3. In the vas deferens, the concentration of noradrenaline and tyrosine hydroxylase and monoamine oxidase activities had all fallen 24 h after 6-OHDA treatment and had started to recover 3 weeks later. In the adrenal medulla, 6-OHDA did not alter NA concentrations but increased tyrosine hydroxylase activity whereas reserpine depleted noradrenaline and increased tyrosine hydroxylase activity.

4. The changes produced in sympathetic ganglia by 6-OHDA may be due both to a direct action on the axoplasmic transport of noradrenaline containing vesicles and indirectly to the reaction of the neurones to loss of the integrity of their axons.

Introduction

Reserpine and 6-hydroxydopamine (6-OHDA) both cause a profound and lasting depletion of noradrenaline from sympathetically innervated tissues (Porter, Totaro & Stone, 1963 ; Laverty, Sharman & Vogt, 1965) but the mechanism involved is different for the two drugs.

Reserpine inactivates the ATP-Mg^{++} dependent uptake of catecholamines into storage vesicles (Kirshner, 1962 ; Carlsson, Hillarp & Waldeck, 1963) and the doses usually employed cause few morphological changes in sympathetic neurones and adrenal medulla apart from loss of catecholamine specific fluorescence and depletion

of the electron-dense cores of the vesicles in both tissues (see Elfvin, 1968 ; Van Orden, Bensch & Giarman, 1967). In contrast, 6-OHDA causes considerable degenerative changes in sympathetic axon terminals but not in the adrenal medulla or sympathetic ganglia (see Thoenen, Tranzer & Haeusler, 1970).

Among the biochemical changes caused by reserpine, a 2–3 fold elevation of tyrosine hydroxylase activity has been observed within 48 h in both the rat adrenal gland and superior cervical ganglion (Mueller, Thoenen & Axelrod, 1969a). This was ascribed to trans-synaptic induction of the enzyme by the increased preganglionic sympathetic outflow which follows the loss of postganglionic sympathetic transmission. Although a similar induction of tyrosine hydroxylase was found in the adrenal medulla after 6-OHDA treatment (Mueller, Thoenen & Axelrod, 1969b), no biochemical changes in sympathetic ganglia have been reported with 6-OHDA.

The recovery of tissue noradrenaline after reserpine treatment begins within days and is complete within a few weeks. This has been correlated with the rapid transport to the axon terminals of new vesicles from their site of synthesis in the ganglia, a process which reserpine does not impair (see Dahlström, 1970). Recovery from the degenerative effects of 6-OHDA takes longer and is dependent on regenerative axon sprouting (see Thoenen *et al.*, 1970) but it is not known whether 6-OHDA interferes directly with the synthesis and transport of vesicles.

We have studied the time course of both morphological and biochemical changes in lamb sympathetic neurones after 6-OHDA, and compared them with those produced by reserpine in order to examine the reaction of the whole sympathetic neurone to these very different pharmacological attacks upon the integrity of its organelles and processes.

Methods

Twenty lambs of both sexes, 2–3 weeks old and weighing 3·5–5·3 kg were given either no treatment, a single intravenous injection of 100 mg/kg 6-OHDA HCl (equivalent to 82·5 mg/kg base) or daily intramuscular injections of 0·25 mg/kg reserpine (expressed as the base) for 1 week. Portions of the atria, vas deferens, adrenal medulla and sympathetic ganglia (superior cervical, stellate and paravertebral) were taken at intervals of 1, 7 and 21 days and processed for microscopy and biochemical assay as follows.

Fluorescence histochemical localization of catecholamines

The technique used was that of Falck & Hillarp (Falck, Hillarp, Thieme & Torp, 1962 ; Corrodi & Jonsson, 1967). Tissues were frozen in liquid nitrogen and freeze dried. They were then incubated at 80° C with formaldehyde gas, generated from paraformaldehyde in equilibrium with air of 70% relative humidity for 1 hour. The tissue was examined by fluorescence microscopy after vacuum embedding and sectioning and the specificity of the fluorescence tested by accepted procedures. In addition Nissl staining with cresyl-fast violet or neutral red was carried out on some sections which were examined by light microscopy.

Electron microscopy

The superior cervical ganglia was fixed within 10 min of decapitation of the lamb in 3% gluteraldehyde buffered in 0·1 M sodium cacodylate (pH 7·25) at 4° C. After

initial fixation for 15–30 min, the tissues were trimmed into 1 mm^3 blocks and returned to the fixative for 4–24 hours. The tissues were washed thoroughly in the buffer and postfixed in 1% osmium tetroxide in 0·1 M sodium cacodylate buffer for 2 h at room temperature (22° C), after which the tissue was dehydrated rapidly through a series of acetone solutions, cleared in propylene oxide, embedded in Araldite which was then polymerized at 60° C. Thin (600–900 Å) sections were cut on a Cambridge ultramicrotome, picked up on 200-mesh carbon-coated copper grids, stained with uranyl acetate followed by lead citrate and viewed in a Siemens Elmiskop 1 electron microscope.

Biochemical estimations

Tissues were frozen in liquid nitrogen immediately on removal and stored at −25° C for a few days. They were weighed and then homogenized in 0·5 ml of 1·19% KCl in a Dual all-glass homogenizer (Size 20). Monoamine oxidase activity in a 25μl portion of the homogenate was determined by the method of Jarrott (1971) and noradrenaline content was assayed as described by Iversen & Jarrott (1970) using 10 μl of homogenate. The catecholamine content of the adrenal gland and atrial homogenates was determined by the tri-hydroxy indole procedure (Euler & Lishajko, 1961). Homogenates were then centrifuged at 27,000 *g* for 10 min and the tyrosine hydroxylase activity of a 50 μl portion of the supernatant was determined by the method of Mueller, Thoenen & Axelrod (1969a). Enzyme activities were calculated from the known specific activities of the substrates and expressed as (nmol or pmol/h)/mg wet wt.

Materials

6-Hydroxydopamine HCl (Axel Kistner AB, Göteborg) ; reserpine (Sepasil, Ciba) ; S-adenosyl-L-methionine ([^{3}H]-methyl, 4·7 Ci/mmol, The Radiochemical Centre, Amersham); tyramine ([^{3}H], generally labelled, 7·3 Ci/mmol, New England Nuclear Corp., Boston) ; L-tyrosine (3,5-[^{3}H], 8·9 Ci/mmol, The Radiochemical Centre). All other reagents were of analytical grade purity.

Results

Fluorescence histochemistry

In control lambs (Fig. 1a), the cell bodies of the sympathetic neurones in the superior cervical and stellate ganglia showed an evenly distributed green cytoplasmic fluorescence of variable intensity not involving the nucleus. Occasionally smaller intensely fluorescing cells were seen corresponding to those reported in other species (see Van Orden, Burke, Geyer & Lodoen, 1970), but they were comparatively sparse in the lamb. In addition a few bright varicose nerve terminals were seen within the ganglion, particularly in relation to blood vessels, but there was no obvious pericellular network of adrenergic terminals as described in some species (see Van Orden *et al.*, 1970). The nerve fibres entering and leaving the ganglia were only dimly fluorescent. The fluorescence of the adrenergic innervation of the atrium and vas deferens (Fig. 2a) was well developed, with bundles of dimly fluorescent preterminal axons giving off bright varicose terminals.

Within 24 h of 6-OHDA, the fluorescent nerve terminals in the atrium and vas deferens had disappeared, leaving bundles of preterminal axons that were more

fluorescent than normal (Fig. 2b). The fluorescence of the ganglion cell bodies was little altered but there was a striking increase in the fluorescence of axon bundles, both within the ganglia (Fig. 1c) and in nerve bundles such as the cardiac nerves. The smooth appearance of the axons was lost and many brightly fluorescing fibres became noticeably beaded and tortuous.

One week after 6-OHDA, the ganglion cell fluorescence was still unchanged but the increased fluorescence of the nerve fibres had begun to regress (Fig. 3c) and by 3 weeks only a small proportion of the axons remained bright and beaded. At this stage fluorescent varicose nerve terminals had begun to reappear in the vas deferens

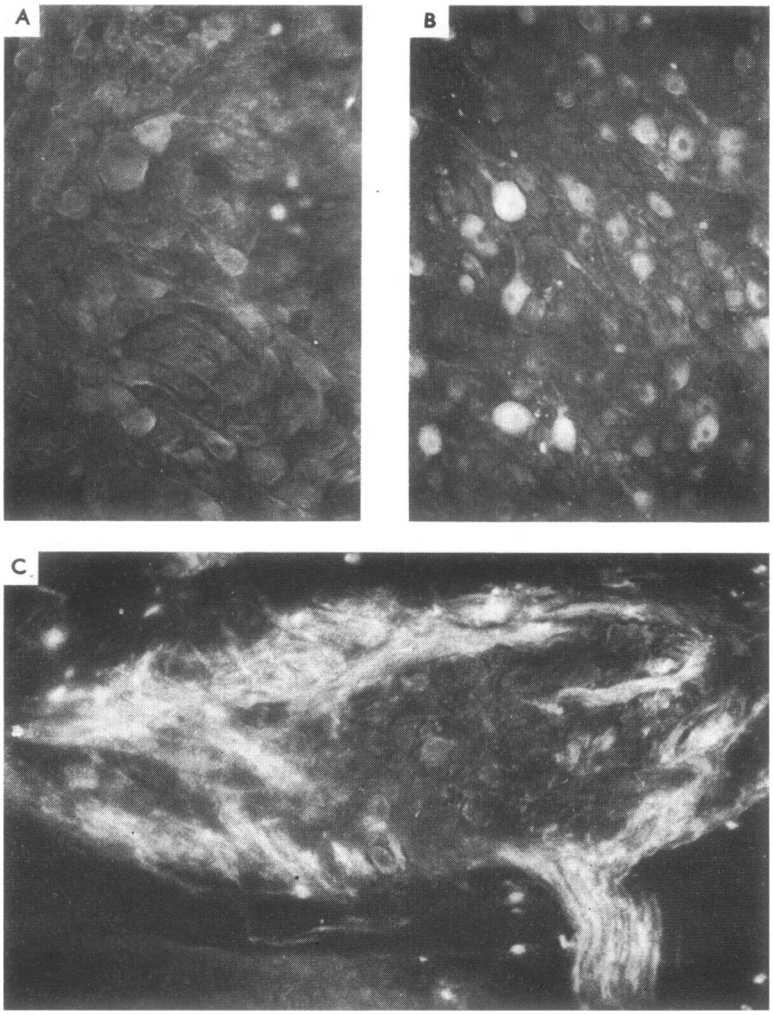

FIG. 1. Fluorescence histochemistry of the superior cervical ganglion. Magnification × 68. (A), Normal ganglion. Note variation in fluorescence intensity of cell bodies and the weak fluorescence of the nerve fibres. (B), Ganglion 1 day after chronic reserpine treatment ((0·25 mg/kg)/day for 7 days). There is already recovery of fluorescence in the cytoplasm of the cell bodies, some of which show supranormal fluorescence but the fluorescence of the nerve fibres is weak. (C), Ganglion 1 day after 6-OHDA (100 mg/kg). The intra- and post-ganglionic axon bundles show a considerable increase in fluorescence whereas the fluorescence of the cell bodies is hardly altered.

(Fig. 2d) but not in the atrium. At no stage did the fluorescence of the adrenal medulla (Fig. 2f) or of the small cells of the ganglia alter significantly.

In contrast to these results, 1 day after chronic reserpine treatment, the fluorescence of the ganglion cells was very variable with some cells showing supranormal fluorescence, particularly concentrated in a halo around the nucleus, while others were very dim. The intense fluorescence of the occasional small ganglion cell and the dim fluorescence of the nerve fibres within the ganglion were both slightly

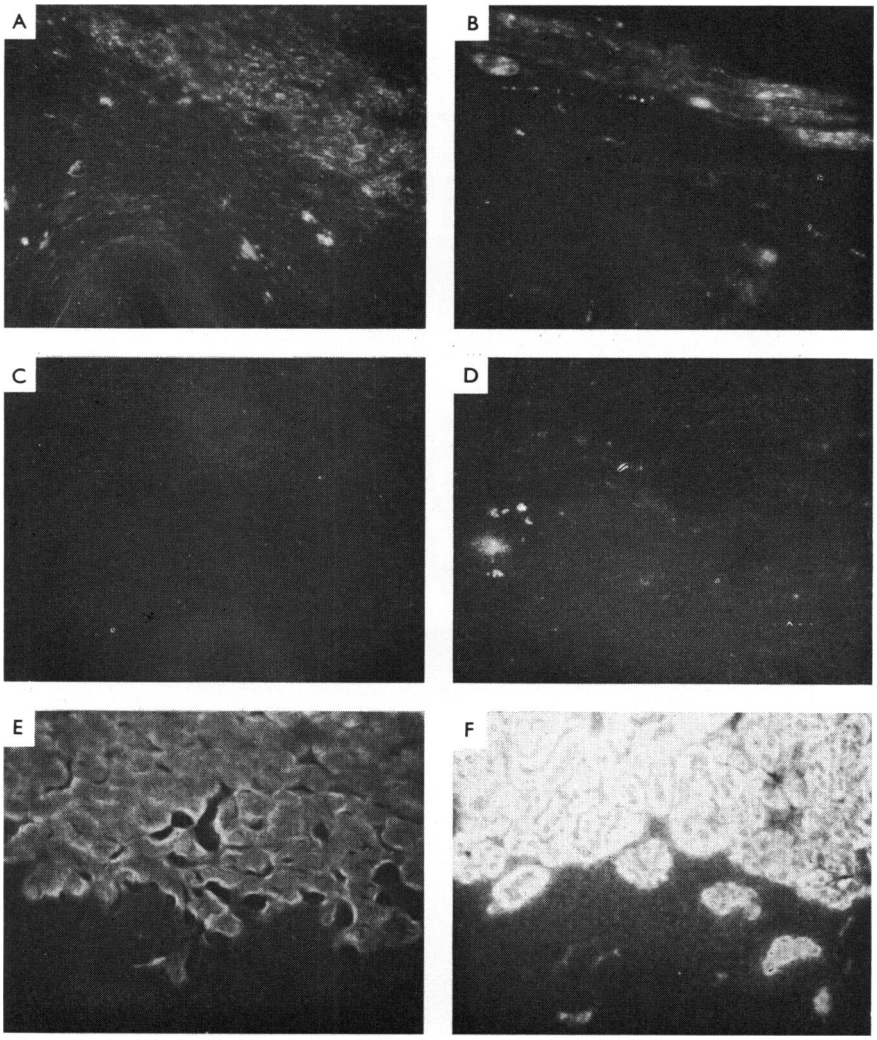

FIG. 2. Fluorescence histochemistry of the vas deferens and the adrenal gland. (A), Normal vas deferens with varicose fluorescent nerve terminals in the inner circular smooth muscle layer and in cross section in the outer longitudinal layer. The lumen is situated below; ×68. (B), Vas deferens 1 week after 6-OHDA (100 mg/kg). The fluorescent axon terminals have disappeared, leaving preterminal axon bundles which are more fluorescent than normal; ×170. (C), Vas deferens after chronic reserpine treatment ((0·25 mg/kg)/day for 7 days). All specific fluorescence has been depleted; ×68. (D), Vas deferens 3 weeks after 6-OHDA (100 mg/kg). Some varicose axon terminals have reappeared; ×170. (E), Adrenal medulla at its junction with the cortex (below) showing reduced fluorescence 1 day after chronic reserpine treatment; ×68. (F), Adrenal gland 1 day after 6-OHDA (100 mg/kg). No obvious change in the fluorescence intensity of the medulla can be seen; ×170.

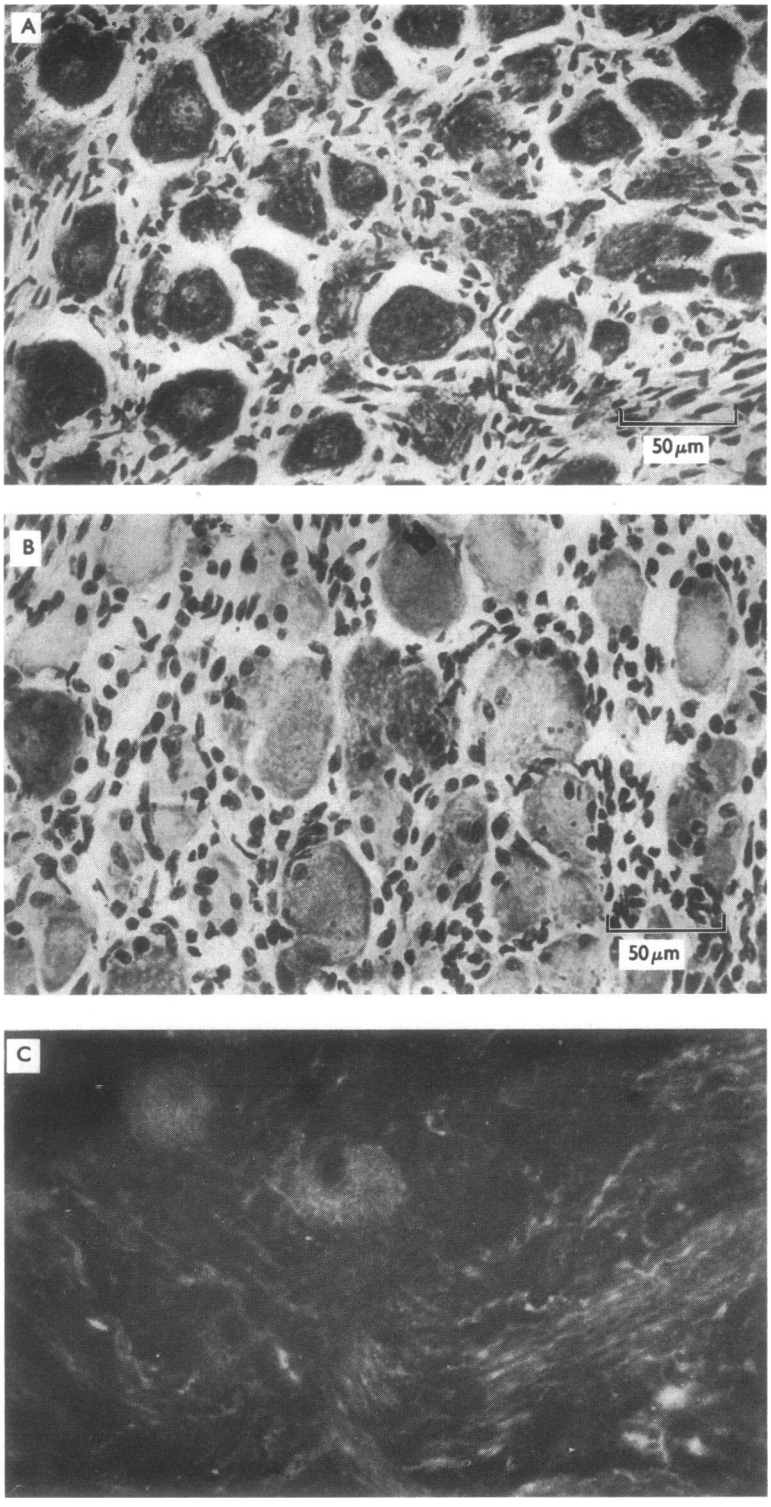

FIG. 3. Chromatolytic changes in the superior cervical ganglion after 6-OHDA. Magnification ×185. (A), Normal ganglion stained with cresyl-fast violet. The granular basophilic Nissl substance is dispersed throughout the cytoplasm and the nuclei are centrally located. (B), One week after 6-OHDA (100 mg/kg), most cells show chromatolytic changes which include eccentric nuclei, dispersion of the Nissl substance and swelling of the cell body. Some proliferation of interstitial cells has occurred. (C), Fluorescence histochemistry 1 week after 6-OHDA showing the swollen neurones with eccentric nuclei. The intensity of fluorescence of the cell bodies is unchanged but it is less evenly distributed. The nerve fibres still show some increased fluorescence and tortuosity compared to their normal dim and smooth appearance but these changes are less marked than at 1 day.

reduced (Fig. 1b). Reserpine treatment virtually abolished the specific fluorescence of the adrenergic innervation of the atrium and vas deferens (Fig. 2c) and the adrenal medulla fluorescence was also diminished (Fig.2e).

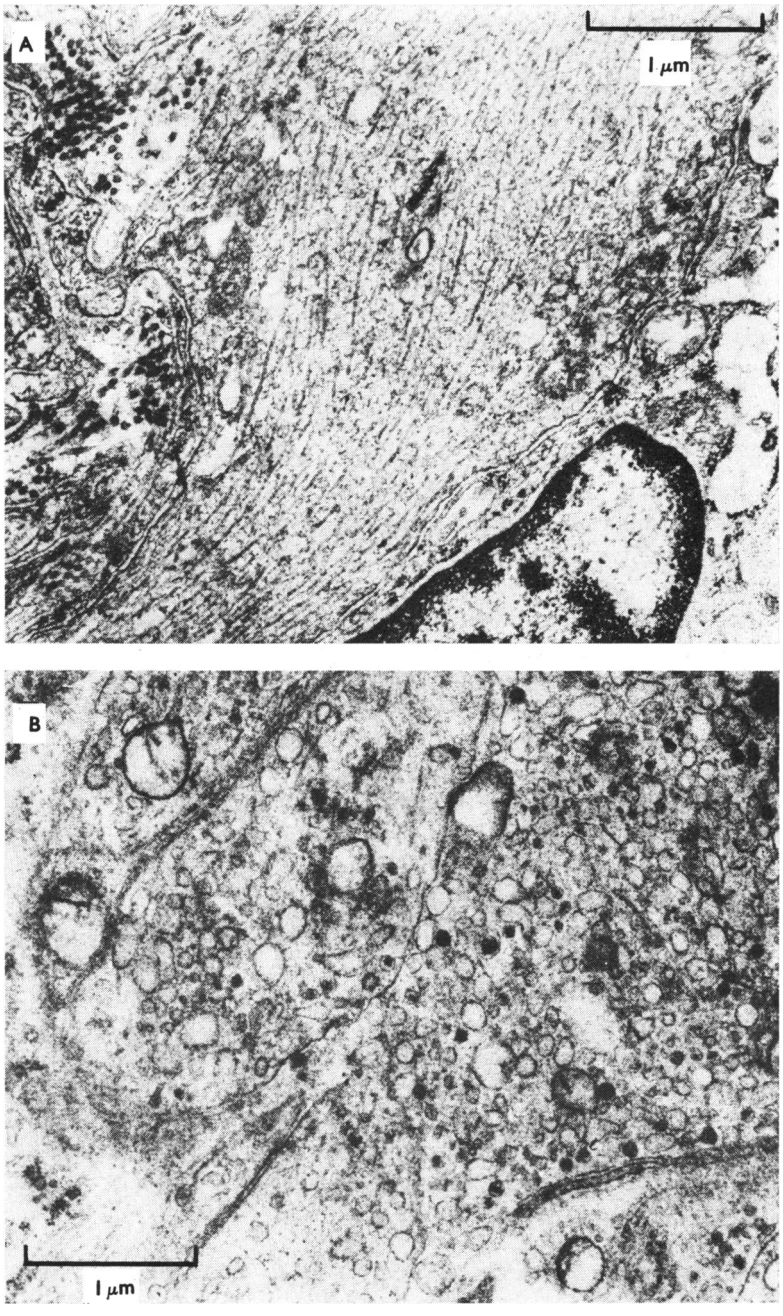

FIG. 4. (A), Electron micrograph showing normal axon profiles within the neuropil of the ganglion containing longitudinally arranged neurotubules and surrounded by Schwann cell. (B), Axon profile after 6-OHDA (100 mg/kg) showing the disruption of the neurotubules and accumulation of electron-lucid vesicles and large granular vesicles.

Light microscopy

The superior cervical ganglia were composed mainly of typical neurone cell bodies 20–45 μm in diameter with basophilic Nissl substance distributed in clusters throughout the cytoplasm (Fig. 3a). After 6-OHDA they showed chromatolytic changes that were most obvious after 1 week (Fig. 3b). These consisted of swelling with dispersion of the Nissl substance in the pale cytoplasm and displacement of the nucleus to one side. There was proliferation of small interstitial cells 4–7 μm in diameter with dark elongated nuclei that was most noticeable after 3 weeks. No such changes were seen after reserpine treatment.

Electron microscopy

A systematic study of the time course of the changes in ganglia produced by 6-OHDA is still in progress and therefore the results reported here will be confined to the acute changes seen in the neuronal processes within the superior cervical ganglion and in the cardiac nerves.

In normal ganglia (Fig. 4a), the axons and other nerve processes in the neuropil contained longitudinal arrays of neuro-tubules, a few mitochondria, irregular elements of endoplasmic reticulum and randomly dispersed granular vesicles. Several unmyelinated axons were enclosed by the one Schwann cell and were connected to the extracellular space by mesaxons. It was not possible to distinguish between pre- and postganglionic unmyelinated axons on morphological grounds, although basal portions of ganglion cell processes often contained organelles found in the perikaryon.

One day after 6-OHDA administration the most striking change both within the ganglion and postganglionic nerves was that the neurotubular system of some axon profiles was greatly disrupted and there was an accumulation of a variety of vesicles and large electron-lucid tubules (Figs. 4b & 5). Many of the vesicles contained an electron dense core and were of a more regular shape and size (circ. 900 Å) than the other elements. They resembled the granular vesicles found more sparsely distributed in the normal ganglion and accumulated in ligated nerves (Geffen & Ostberg, 1969). The electron-lucid vesicles were very much larger and more irregularly shaped. They had a unit membrane and could have been derived from swollen elements of the endoplasmic reticulum. In some longitudinal profiles, distorted neurotubules were seen which were possibly connected with the electron-lucid structures but not with the electron-dense vesicles. The axons containing the large accumulations of vesicles were also swollen in comparison with the normal profiles, which are possibly cholinergic fibres, within the ganglion. The affected axons were often partly exposed from their Schwann cell sheath and their axon membrane was possibly disrupted in places (Figs. 4b & 5).

Other changes seen in the neurones after 6-OHDA treatment, such as swelling of the rough endoplasmic reticulum, were not confined to the ganglion cells alone. The rough endoplasmic reticulum of the Schwann cells surrounding the affected neurones and axons was similarly affected, and the endothelial cells of some capillaries were so swollen that the lumen was nearly occluded.

Radiochemical assays

The absolute values of the biochemical changes following treatment with 6-OHDA and reserpine are shown in Table 1 for the sympathetic ganglia and Table 2 for the adrenal medulla and vas deferens.

Administration of a single dose of 6-OHDA produced the following changes in tissue catecholamines. The noradrenaline concentrations in the superior cervical, stellate and remaining paravertebral ganglia were elevated approximately 2-fold after one day but had returned to control levels by 7 days where it remained at 21 days. In contrast, the noradrenaline concentrations in the vas deferens and atria were greatly reduced at both 1 and 7 days. The noradrenaline concentration in the vas deferens but not the atrium had partly recovered at 21 days. In the adrenal gland, the catecholamine concentration was not significantly altered.

Treatment with 6-OHDA also affected enzyme activities. The tyrosine hydroxylase activity of the sympathetic ganglia fell by approximately 30% after 1 day and 60% after 7 days but at 21 days, it had returned to control levels. The tyrosine hydroxylase activity of the vas deferens was reduced by 70% at 1 day and was recovered by 21 days. In contrast to the ganglia and vas deferens, the tyrosine hydroxylase activity of the adrenal gland was elevated by 25% after 7 days and

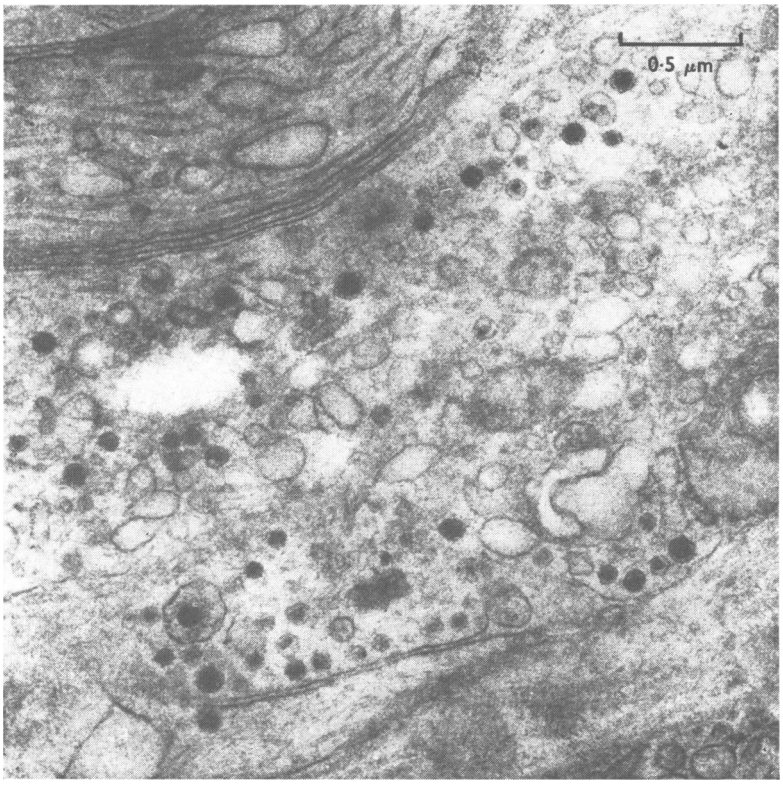

FIG. 5. Electron micrograph showing axon profiles after 6-OHDA (100 mg/kg). The upper axon contains both electron-lucid tubules and neurotubules. In the lower axon, which contains both electron lucid vesicles and large granular vesicles, the neurotubules have been entirely disrupted.

TABLE 1. *Noradrenaline (NA), tyrosine hydroxylase (TH) and monoamine oxidase (MAO) activities of lamb sympathetic ganglia at various times after 6-OHDA and reserpine*

	Superior cervical ganglion			Stellate ganglion			Sympathetic chain		
	NA (ng/mg)	TH ((pmol/h)/mg)	MAO ((nmol/h)/mg)	NA (ng/mg)	TH ((pmol/h)/mg)	MAO ((nmol/h)/mg)	NA (ng/mg)	TH ((pmol/h)/mg)	MAO ((nmol/h)/mg)
Controls ($n=5$)	2·56± 0·615	14·77± 2·154	8·17± 0·847	1·56± 0·141	7·61± 1·008	4·51± 0·160	1·13± 0·210	4·11± 0·894	5·28± 0·925
1 day after 6-OHDA ($n=3$)	5·27± 0·788†	9·13± 0·506†	8·37± 1·346NS	3·22± 0·689*	6·17± 0·449NS	5·41± 0·251†	2·58± 0·732*	3·55± 0·523NS	4·17± 0·213NS
7 days after 6-OHDA ($n=4$)	3·03± 1·198NS	3·92± 0·854†	3·63± 0·921†	2·44± 1·054NS	3·00± 0·916†	3·45± 0·368†	2·45± 1·569NS	2·73± 1·573NS	3·08± 0·731*
21 days after 6-OHDA ($n=3$)	2·69± 0·552NS	14·40± 1·550NS	3·47± 0·227†	2·40± 0·276*	12·15± 2·087NS	2·20± 0·447†	2·13± 0·696NS	5·28± 2·585NS	2·76± 0·307†
1 day after chronic reserpine treatment ($n=5$)	0·69± 0·172†	24·50± 5·942*	7·55± 0·451NS	0·33± 0·029†	10·72± 0·861†	4·57± 0·347NS	0·28± 0·090†	7·70± 0·818†	4·76± 1·094NS

Each value is the mean±S.E. Differences between control and experimental means were analysed by a t test. * $P<0·1$; † $P<0·05$; NS=not significant.

TABLE 2. *Noradrenaline (NA), tyrosine hydroxylase (TH) and monoamine oxidase (MAO) activities of adrenal gland and vas deferens and NA of atrium at various times after 6-OHDA and reserpine*

	Adrenal gland			Vas deferens			Atrium
	NA (μg/mg)	TH ((pmol/h)/mg)	MAO ((nmol/h)/mg)	NA (ng/mg)	TH ((pmol/h)/mg)	MAO ((nmol/h)/mg)	NA (ng/mg)
Controls ($n=5$)	1·57± 0·253	28·32± 3·366	1·90± 0·225	1·79± 0·075	1·36± 0·376	1·44± 0·252	1·68± 0·241
1 day after 6-OHDA ($n=3$)	1·40± 0·044NS	29·56± 2·903NS	1·84± 0·113NS	0·32± 0·026†	0·41± 0·202†	1·27± 0·063NS	0·13± 0·058†
7 days after 6-OHDA ($n=4$)	1·50± 0·117NS	36·85± 2·940*	2·20± 0·089NS	0·62 n=2	0·34	0·88	0·09± 0·037
21 days after 6-OHDA ($n=3$)	1·16± 0·244NS	43·10± 7·055*	1·77± 0·237NS	1·30 n=2	1·04	0·62	0·19± 0·010
1 day after chronic reserpine treatment ($n=5$)	0·069± 0·0020†	40·92± 8·83*	2·42± 0·173NS	0·24 n=2	1·41	1·92	0·34± 0·159

* $P<0·1$, † $P<0·05$, NS=not significant when compared with control values.

40% after 21 days. The monoamine oxidase activity of the sympathetic ganglia was depressed by approximately 50% after 7 days and remained at this level for 21 days. In the vas deferens, it fell by 40% and had not recovered by 21 days. The monoamine oxidase activity of the adrenal gland was not significantly altered at any time.

After chronic reserpine pretreatment, a considerable depletion of endogenous noradrenaline in sympathetic ganglia, vas deferens, atria and adrenal gland was found. The monoamine oxidase activity in all tissues was not significantly changed whereas the tyrosine hydroxylase activity of sympathetic ganglia and adrenal gland was elevated.

Discussion

The principal finding to emerge from this study is that the deleterious effects on sympathetic neurones of a single large dose of 6-OHDA were not confined to the axon terminals but involved the entire neurone.

We have confirmed, in the lamb, the findings in the rat that 6-OHDA rapidly depletes the noradrenaline content of the heart and vas deferens but not the adrenal medulla and that recovery is more rapid in the vas deferens (Thoenen *et al.*, 1970). The reappearance of fluorescent nerve terminals in the vas but not the atrium after 3 weeks is likely to be due to quicker regeneration of shorter postganglionic axons. The parallel fall and recovery in tyrosine hydroxylase activity in the vas deferens supports this interpretation and confirms the fall reported after 6-OHDA treatment in the rat heart (Mueller *et al.*, 1969b). The decrease in monoamine oxidase activity of the lamb vas deferens is greater than that reported in the rat spleen and submaxillary gland after 6-OHDA (Jarrott, 1971) but was similar to that found after surgical denervation of the rat vas deferens (Jarrott & Iversen, 1971), suggesting that the fraction of monoamine oxidase lost resided in the dense sympathetic innervation of the vas deferens. The slower recovery of monoamine oxidase activity than noradrenaline may be due to the slower axoplasmic transport of mitochondria compared to noradrenaline storage vesicles (Banks, Mangnall & Mayor, 1969) during nerve regeneration.

The striking changes within 24 h of a single large dose of 6-OHDA on the fluorescence, ultrastructure and biochemistry of the ganglia were surprising in view of previous negative reports (Laverty *et al.*, 1965 ; Malmfors & Sachs, 1968 ; Thoenen & Tranzer, 1968 ; Jonsson & Sachs, 1970). Nevertheless the various changes observed were consistent with and complementary to each other. The considerable increase in fluorescence of some axons within the ganglion and postganglionic nerves correlated with the increased numbers of dense-core vesicles accumulated within them and with the 2-fold rise in noradrenaline content of the ganglia. Since the fluorescence intensity of the ganglion cells was not increased and that of the few intraganglionic nerve terminals around blood vessels was abolished, the rise in ganglion noradrenaline could only have occurred within the non-terminal adrenergic axons which emerge from or pass through the ganglia.

Increased fluorescence of preterminal axons has been noted previously (see Malmfors & Sachs, 1968) and ascribed to an effect similar to that produced by a ligation, where noradrenaline-containing vesicles accumulate immediately proximal to the constriction as a result of axoplasmic transport (see Dahlström, 1970). Such an

explanation does not suffice for our observations that the increase in axon fluorescence is not confined to the preterminal axons (Malmfors & Sachs, 1968) but occurs within intra- and postganglionic nerve trunks. Since some axons were found to be packed, before and after emerging from the ganglia, with a variety of vesicles, including many with electron-dense cores similar to those described just proximal to a ligation (Mayor & Kapeller, 1967; Geffen & Ostberg, 1969), it is more likely that a diffuse chemical rather than a focal mechanical interuption of the axoplasmic transport had occurred.

A possible explanation for the axonal changes observed may reside in an effect of 6-OHDA on the neurotubular system, which has been implicated in the mechanism of rapid axoplasmic transport (see Schmitt, 1968). Dahlström (1970) has shown that local application of colchicine and vinblastine, two alkaloids which disrupt neurotubules, causes a considerable increase in the fluorescence of intra- and postganglionic axons similar to the increase in axon fluorescence seen in our experiments. One of the most striking ultrastructural changes seen in axons affected by 6-OHDA was the disruption of the regular longitudinal arrangements of neurotubules and their replacement by swollen electron-lucid tubules and vesicles, some of which appeared connected to the vestiges of neurotubules.

The chromatolytic changes in the neurone cell bodies of the ganglia within 1 week of 6-OHDA provides an explanation for the sharp fall in ganglion tyrosine hydroxylase activity when a rise might have been anticipated as a result of the inducing stimulus of increased sympathetic drive to the ganglia. Reserpine, which caused such an induction of ganglion tyrosine hydroxylase in the lamb in our experiment and in the rat (Mueller *et al.*, 1969a), did not produce chromatolysis. A similar induction of tyrosine hydroxylase activity was found in the lamb adrenal medulla after both 6-OHDA and reserpine, confirming that reported in the adrenal medulla of other species (Mueller *et al.*, 1969a, b).

The chromatolytic reaction in the ganglia after 6-OHDA could also account for the pronounced and prolonged fall in monoamine oxidase activity after 6-OHDA but not reserpine treatment. This fall in monoamine oxidase activity would have contributed to the rise in ganglion noradrenaline by permitting extravesicular accumulation of noradrenaline in the axons. Inhibition of the enzyme produces a 3-fold increase in the amount of noradrenaline accumulated in axons above a ligature (Dahlström, Jonason & Norberg, 1969). There is evidence that extravesicular noradrenaline fluoresces more intensely than an equivalent amount of the vesicular bound form (Van Orden, Schaefer, Burke & Lodoen, 1970), and this too may have contributed to the increased fluorescence of the axons after 6-OHDA.

No morphological or biochemical changes in sympathetic ganglia after 6-OHDA have been reported hitherto, except in new-born mice and rats where much smaller doses of 6-OHDA for 1 week led to destruction of the sympathetic ganglion cells (Angeletti & Levi-Montalcini, 1970). This was interpreted as revealing an early vulnerability of the sympathetic ganglia to 6-OHDA, which is subsequently lost. An alternative explanation is that in the adult, preferential uptake into the axon terminals sequesters a considerable proportion of circulating 6-OHDA thereby protecting the ganglia, whereas this axonal uptake is poorly developed in new born rats (Iversen, de Champlain, Glowinski & Axelrod, 1967). The concept that the site of action of the drug is determined by its accumulation through uptake rather than a differential susceptibility of parts of the neurone is supported by the failure

of 6-OHDA to deplete the adrenal medulla, which lacks an effective catecholamine uptake mechanism (Kirpekar & Cervoni, 1963).

The most unified interpretation we have for the changes caused by 6-OHDA in the more proximal parts of the sympathetic neurone is that they are the consequences both of a direct action of the drug as well as an indirect response of the cell body to loss of its axon terminals.

A direct action of the drug may explain the rapid and extensive disruption of the fine structure of the axons particularly the neurotubules, thereby interrupting axoplasmic transport of adrenergic vesicles (Geffen & Livett, 1971). Indirectly, the readjustment of the protein synthesis of the neurone during the period of chromatolysis toward repair of the integrity of its axon could account for the fall in tyrosine hydroxylase in the face of the inducing stimulus of increased preganglionic activity. This reaction to axon injury could also explain the fall in monoamine oxidase, which in turn would have contributed to the increase in noradrenaline content of the ganglion and the bright fluorescence of the non-terminal axons.

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