THE EFFECT OF 6-HYDROXYDOPAMINE ON NERVES IN THE RAT UPPER URINARY TRACT

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SUMMARY

The effect of a single large dose of 6-hydroxydopamine (6-OHDA) on adrenergic nerves in the rat upper urinary tract has been investigated by both light and electron microscopy. Reduction in the intensity of fluorescent catecholamine-containing nerves was seen 1–2 h after drug treatment. At 3–5 h, fluorescing nerves were absent from all preparations. Forty-eight hours after injection fine catecholamine-containing nerves could not be detected, although a few large nerves adjacent to vessels and in the adventitia of the upper urinary tract were associated with increased fluorescence. Using the electron microscope, axons related to smooth muscle were seen to be swollen and contained aggregations of small (50 nm diameter) granulated vesicles and electron-dense material 3–6 h after drug treatment. Other axons contained damaged mitochondria and were devoid of neurofilaments and neurotubules. Normal axons were also seen, some of which contained accumulations of small (50 nm diameter) agranular vesicles and occasional large (100 nm diameter) granulated vesicles. In the submucosa, damaged axons were observed lying close to, or occasionally running between, basal epithelial cells. Axons containing electron-dense clumps of synaptic vesicles were not observed in this situation. Normal axons containing accumulations of small agranular vesicles were identified, some of which were closely related to damaged axon profiles. Forty-eight hours after the drug treatment, some axons related to smooth muscle cells contained accumulations of electron-dense material. All other axons in the wall of the upper urinary tract appeared normal. These results are discussed in relation to the distribution of various types of autonomic nerve in the upper urinary tract.

INTRODUCTION

Following the original report by Porter, Totaro & Stone (1963), many studies have been made on the response of noradrenergic nerves to pre-treatment with 6-hydroxydopamine (6-OHDA). This substance has been shown to produce prolonged depletion of noradrenaline in sympathetically innervated tissue (Laverty, Sharman & Vogt, 1965), and Thoenen & Tranzer (1968) have described effective chemical sympathectomy following its administration. Evidence of fine-structural damage in adrenergic nerves following the use of this drug was first reported by Tranzer & Thoenen (1967). Similar studies by Bennett, Burnstock, Cobb & Malmfors (1970), Furness et al. (1970) and others on peripheral sympathetic nerves have confirmed and enlarged upon the original electron-microscopic observations. The use of 6-OHDA therefore provides an ideal technique for studying the distribution of noradrenergic nerves in sympathetically innervated tissue.

In a recent study on the innervation of the upper ureter (Dixon & Gosling, 1971), light microscopy demonstrated catecholamine-containing nerves in the
submucosa closely related to the basal layer of the epithelium. Electron microscopy of nerves in this region failed, however, to reveal intra-axonal accumulations of small dense-cored vesicles characteristic of adrenergic nerves. These conflicting observations suggested the need for further study using 6-OHDA, by means of which it might prove possible to obtain additional information pertaining to the frequency and distribution of submucosal noradrenergic nerves. In addition, such a study could help to reaffirm the distribution of catecholamine-containing nerves reported in previous studies on other parts of the upper urinary tract (Dixon & Gosling, 1970; Gosling & Dixon, 1970). Consequently, both light- and electron-microscopic techniques have been employed to examine the effect of 6-OHDA on nerves in the renal calix, pelvis and upper ureter of the rat.

MATERIALS AND METHODS

Male rats weighing 140-160 g were given 250 mg/kg 6-OHDA dissolved in 0.5 ml of saline solution containing 0.2 mg/ml ascorbic acid. This dose was administered intravenously into the tail vein 1-6, 24 and 48 h before death. The animals were killed by a blow on the head, and the kidney and proximal half of the ureter rapidly exposed. Portions of renal calix, pelvis and upper ureter were removed and processed either for light or electron microscopy.

The technique employed for the demonstration of tissue catecholamines was that described by Spriggs, Lever, Rees & Graham (1966). Material was frozen in propane pre-cooled in liquid nitrogen and cryostat sections 10-50 μm in thickness were prepared. These were dehydrated, heated at 80 °C in the presence of paraformaldehyde and then examined in a Zeiss photomicroscope fitted with a mercury-vapour light source (Wotan HBO 200-W), excitor filter BG 13/4 mm and barrier filter BG 9/1 mm.

For electron microscopy small portions of the renal calix, pelvis and upper ureter from drug-treated rats were fixed for 1 h in acetate-veronal buffered 1% osmium tetroxide at pH 7.3 (Palade, 1952). Dehydration was carried out in ascending concentrations of ethanol and the tissue embedded in TAAB epoxy resin. Thin sections were double stained with alcoholic uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) before examination in a Philips EM 300 electron microscope.

As control some animals were given 0.5 ml saline-ascorbic acid solution intravenously without 6-OHDA. Tissue from these animals was processed for light and electron microscopy as outlined above.

RESULTS

Light microscopy

In all the regions examined, a marked reduction in the degree and number of fluorescing nerves (when compared with control preparations, Fig. 1) was obvious 2 h after 6-OHDA administration. This effect increased, so that catecholamine-containing nerves were undetected in tissue sections obtained 1-3 h later (Fig. 2). Twenty-four hours after treatment marked fluorescence was apparent in large nerves seen occasionally in the ureteric adventitia; nerves of similar size were only faintly fluorescent in control preparations. Finer nerves related to ureteric and renal caliceal and pelvic smooth muscle remained undetected 48 h after injection, although increased fluorescence persisted in some large adventitial and perivascular nerves.
Electron microscopy

The degree of damage sustained by adrenergic axons 1–2 h after drug administration was variable. At 3–6 h however, drug-induced changes were consistent and easily recognized. Consequently the results obtained during this time interval will be described in detail, since these are considered to provide a more accurate indication of the distribution of adrenergic nerves.

In the calix and pelvis numerous axon varicosities related to the outer layer of smooth muscle cells appeared damaged. These axons were swollen and each displayed a dense cytoplasmic matrix containing synaptic vesicles (Fig. 3). In some cases, masses of electron-dense material were observed in which individual profiles of synaptic vesicles could not be identified. Swollen axons containing similar abnormal inclusions were also identified on the external aspect of vascular smooth muscle and adjacent to ureteric muscle cells. When compared with the outer layer of smooth muscle in the renal calix and pelvis, ureteric muscle was associated with relatively few damaged terminal regions.

In the renal pelvis and ureter, other axon profiles included in both large and small axon bundles amongst the muscle cells were swollen and electron-translucent in appearance, but did not contain clumped masses of synaptic vesicles. These axons lacked a normal complement of neurofilaments and neurotubules and contained swollen and disrupted mitochondria. In addition, many such axons included occasional 100-nm diameter vesicles, each possessing a central electron-dense granule (Fig. 4). Small electron-dense particles reminiscent of glycogen granules were also observed in some of these swollen axons. Many nerve bundles also contained a few normal axons arranged either singly or in small groups (Fig. 5). Occasionally axons were observed adjacent to muscle cells which contained numerous 50-nm diameter agranular vesicles and normal mitochondria (Fig. 5).

In the submucosa close to the epithelial basal lamina many axons displayed signs of damage. These axons were swollen, devoid of neurofilaments and neurotubules and associated with an increase in the number of large granulated vesicles (Fig. 6). However, axons containing electron-dense aggregations of vesicles or clumps of osmiophilic material were not observed. In some subepithelial nerve bundles all the axons appeared damaged, whilst other bundles included damaged and undamaged axon profiles in varying proportions. Axon varicosities of normal appearance were also observed containing mitochondria, occasional large (100 nm diameter) granulated and numerous small (50 nm diameter) agranular vesicles (Fig. 6). These were often closely related (20 nm separation) to damaged axons and their frequency was similar to that observed in control preparations. Very occasionally, damaged axon profiles were observed beyond the basal lamina lying between adjacent basal epithelial cells (Fig. 7); epithelial nerves were not detected further towards the lumen.

Twenty-four hours after injection, axon profiles containing clumps of electron-dense material were observed among upper urinary tract smooth muscle. Such axons were similar in their distribution to those which contained clumped vesicles and other debris 3–6 h after 6-OHDA administration. The majority of the remainder appeared
normal although a few axons displayed a somewhat crenated axolemma and were devoid of neurofilaments and neurotubules. A similar proportion of axons containing electron-dense material was apparent in nerves adjacent to smooth muscle 48 h after drug treatment. All other axons in the wall of the upper urinary tract appeared undamaged and contained normal mitochondria, neurotubules and neurofilaments (Fig. 8).

DISCUSSION

The dose of 6-OHDA employed in the present investigation completely abolished fluorescence in fine nerves (presumably terminal sites) 3–5 h after intravenous administration. This effect persisted for 48 h and was associated with increased fluorescence of occasional large perivascular and periureteric nerves (presumably non-terminal sites) 24–48 h after drug treatment. These results are in agreement with the response obtained to high doses of 6-OHDA in other peripheral adrenergically innervated organs, as reported by Malmfors & Sachs (1968), Bennett et al. (1970) and Furness et al. (1970). Reduction in fluorescence intensity has been correlated with fine-structural damage to adrenergic nerve terminals by both Bennett et al. and Furness et al. These workers have described in detail the response of terminal regions to different doses of 6-OHDA at various time intervals. In the present electron-microscope study similar changes have been observed in numerous axons related to the outer muscle layer of the renal pelvis. These observations confirm those of an earlier study in which this muscle layer was reported to possess a rich supply of adrenergic nerve terminals (Gosling & Dixon, 1970). Similar changes have also been identified in perivascular axons and in nerves related to ureteric muscle. Absence of fluorescence 24 h after 6-OHDA treatment has been regarded as evidence for successful chemical sympathectomy by Furness et al. In this context, accumulations of small dense-cored vesicles typical of normal adrenergic terminals have not been observed in the present study in tissue obtained 24–48 h after injection. In these preparations, damaged axons containing dense bodies, clumped vesicles and other debris, have however, been observed, adding further support to the view that recovery of terminal regions has not been achieved.

In the muscle layer of the ureter 3–6 h after 6-OHDA administration, a number of non-terminal axons (i.e. axons without accumulations of synaptic vesicles) appear normal, while others are swollen and contain damaged mitochondria, occasional large granulated vesicles, and lack neurotubules and neurofilaments. The latter axons do not include changes associated with damaged terminal regions, such as, for example, aggregations of synaptic vesicles. These observations suggest a number of possible explanations. Firstly, the fine-structural changes induced by 6-OHDA may vary along the length of an adrenergic fibre. Hence a section through one part of an axon could appear normal and give no indication of the damage sustained by another segment of the same axon. The proportion of such axons could be assessed by studying serial sections. Secondly, some adrenergic nerves may escape damage, perhaps as a result of low drug concentration in the vicinity of their terminal sites. In the present
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study this seems unlikely, since all adrenergic terminal regions examined show evidence of damage following treatment with 6-OHDA. Thirdly, a proportion of axons may be non-adrenergic; the presence of undamaged axons containing accumulations of agranular vesicles confirms this possibility.

After 48 h all non-terminal axons are normal in appearance and only those features associated with damaged terminal regions remain. This observation indicates that those pre-terminal axons which sustained damage between 1 and 6 h after drug treatment are capable of undergoing reversible fine-structural changes.

Of particular interest is the behaviour of submucosal nerves after 6-OHDA administration. As reported elsewhere (Dixon & Gosling, 1971), adrenergic nerve terminal regions have not been identified close to urinary tract epithelium, although fluorescent catecholamine-containing nerves have been detected in this situation. In the present study these observations have been resolved by the use of 6-OHDA. Thus a number of submucosal nerves have been identified as pre-terminal adrenergic axons; degenerative changes characteristic of nerve terminals have not been observed. These observations indicate that adrenergic axons run through the submucosa close to the epithelium before reaching their terminal sites. The latter may well be urinary tract or vascular smooth muscle since these are the only situations in which adrenergic nerve terminals have been observed. Nevertheless, some sub- or intra-epithelial fibres must be relatively close to their terminal sites, since the formaldehyde-fluorescence technique has demonstrated catecholamine-containing nerves in similar situations.

The presence of normal axons containing accumulations of agranular vesicles confirms the presence of non-adrenergic nerves in the submucosa. The absence of obvious effector target sites in this situation suggests that these non-adrenergic terminal regions may be sensory in function. While the vesicle content of these axons is similar to that described in cholinergic terminals (Dixon, 1966), it is noteworthy that acetylcholine has been implicated in sensory nerve conduction by a number of workers (for review see Nadol, Brzin & De Lorenzo, 1970). However, if these axons subserved a purely sensory role the significance of closely related pre-terminal adrenergic axons remains obscure. One explanation for this close association is suggested by the work of Ehinger, Falck & Sporrong (1970) and Nilsson & Sporrong (1970) who proposed that interaction between adjacent axons can occur. Thus the present results suggest the hypothesis that sensory non-adrenergic terminal regions may influence adjacent pre-terminal adrenergic nerves.

REFERENCES


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Fig. 1. A photomicrograph of a portion of normal ureter showing numerous fluorescent nerves related to a small artery (a). Fine nerves are also observed in the ureteric muscle coat (m) and in the submucosa. Some of the submucosal nerves are closely related to the epithelium (e). x 250.

Fig. 2. A section through the ureter 4 h after an intravenous injection of 250 mg/kg 6-OHDA. Note complete absence of fluorescing nerves related to small vessels (a) and the lack of nerves in both the muscle coat (m) and the submucosa of the ureter. Autofluorescence is apparent in vessel walls, submucosa and epithelium (e). x 250.
Fig. 3. An electron micrograph of the terminal portion of two axons lying close to a smooth muscle cell (sm) in the renal calix. One axon is swollen with a disrupted axolemma; the other contains electron-dense material and clumped synaptic vesicles. × 45000.

Fig. 4. A bundle of axons in the ureteric muscle coat 4 h after 250 mg/kg 6-OHDA intravenously. Several axons contain clumps of electron-dense material, while others are swollen and appear electron-translucent. × 17100.
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Fig. 5. An axon bundle among ureteric muscle 4 h after injection of 6-OHDA. Some axons are swollen and lack filaments and tubules (s) although others appear normal (n) in cross-section. A few of the latter contain accumulations of agranular vesicles (v). × 14,000.

Fig. 6. A small nerve bundle lying close to ureteric epithelium (e) 6 h after 6-OHDA treatment. Several damaged axon profiles containing swollen mitochondria and large granulated vesicles (gv) are observed in company with an undamaged axonal varicosity packed with small agranular vesicles (av) and a few large granulated vesicles (gv). × 35,000.
Fig. 7. Three swollen axon profiles (ax) occur in the intercellular spaces between basal ureteric epithelial cells 6 h after 6-OHDA treatment. A small nerve bundle (n) containing both damaged and undamaged axon profiles lies in the submucosa. × 17100.

Fig. 8. A large nerve bundle in the ureteric muscle coat 48 h after 6-OHDA administration. Some axon profiles contain accumulations of electron-dense material (arrows), while the majority are normal in cross-section and display the usual axonal organelles. × 11200.
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