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Intracranial Injection of Drugs: Comparison of Diffusion of 6-OHDA and Guanethidine[1,2]

BARBARA K. EVANS

Department of Zoology, University of Melbourne, Parkville, Victoria 3052, Australia

S. ARMSTRONG, G. SINGER

Department of Psychology, LaTrobe University, Bundoora, Victoria 3083, Australia

R. D. COOK AND G. BURNSTOCK[3]

Department of Zoology, University of Melbourne, Parkville, Victoria 3053, Australia

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EVANS, B. K., S. ARMSTRONG, G. SINGER, R. D. COOK AND G. BURNSTOCK. *Intracranial injection of drugs: comparison of diffusion of 6-OHDA and guanethidine.* PHARMAC. BIOCHEM. BEHAV. 3(2) 205–217, 1975. – Marked differences in extent of diffusion have been shown with the fluorescence histochemical method between guanethidine and 6-OHDA (64 μg in 2 μl) when injected acutely or chronically into the lateral hypothalamus, the substantia nigra or the amygdala of the rat brain. Cannulation damage up to 1 mm in diameter and attributed to the implantation of cannulae and placebo injection was observed. A further area of generalized damage occurred following the injection of drugs and was far greater for 6-OHDA (2 mm) than for guanethidine (0.3 mm). Guanethidine, but not 6-OHDA, caused specific damage to catecholamine-containing neurons up to a distance of at least 3 mm and more from the cannula tip. These striking differences between the effects of intracranial injection of 6-OHDA and guanethidine are discussed in terms of the uptake and degradation of the two drugs and the anatomical features of the injection site; they are not explicable in terms of experimental conditions such as concentration, volume of injection, molecular weight or lipid solubility. The different patterns of damage would not easily be distinguished by biochemical analyses and the catecholamine specificity of 6-OHDA in studies of the central nervous system must be seriously questioned. Vascularization of chronically implanted cannula tracks and the presence of anatomical diffusion barriers are also discussed in relation to the diffusion of drugs injected intracranially.

6-OHDA Guanethidine Catecholamine-depletion Intracranial injection Drug diffusion

CONSIDERABLE attention has been given to problems associated with direct injection of drugs into the brain tissue of freely-moving animals [31, 35, 38]. In particular, areas of diffusion and concentrations within these areas, which may exert biochemical and subsequent behavioural changes, have been discussed in relation to cannula size, volume injected, drug concentration, pH, osmolarity etc. [1, 30, 31, 35, 36]. However, many experiments designed to investigate these problems have been limited in at least two ways. First, the drugs used have a short-term behavioural effect and thus studies of spread and diffusion have generally been limited to periods within one hour of injection. Second, the drugs injected have been mainly compounds exerting short-term effects because of their susceptibility to endogenous enzymatic processes so that histological or histochemical techniques used to assess the extent of diffusion cannot be extrapolated to drugs with long-term effects.

In the present study, the problem of diffusion is examined by comparing the effects of injection of two synthetic drugs, namely guanethidine and 6-hydroxydopamine (6-OHDA), into the lateral hypothalamus, the substantia nigra or the amygdala region of the rat brain. While no morphological studies have been carried out previously, biochemical analysis has shown that intraventricular injection of guanethidine lowers brain catecholamine levels [10]. Reports on the effects of intracranial injection of 6-OHDA are conflicting [34, 39, 44, 47]. Guanethidine has been shown to have a number of effects on peripheral sympathetic adrenergic neurons. It blocks nervous transmission, depletes neuronal noradrenaline stores, blocks uptake of noradrenaline into the neuron [3, 7, 16, 17, 41] and,

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[3] Address from May, 1975. Department of Anatomy, University College, London.

following chronic injection, causes long-lasting damage with low doses [13,14] and complete degeneration with high doses [6,19]. In peripheral systems 6-OHDA also specifically affects adrenergic neurons so that the terminal varicose regions of the nerve are depleted of noradrenaline and degenerate, although the cell body is not seriously damaged [26,42]. After several weeks, new nerve processes grow out to reinnervate the denervated tissue.

The advantage of selecting these particular drugs for intracranial injection is that they deplete neuronal catecholamine-containing stores and thus their actions can be sensitively monitored with the fluorescent histochemical method for localising monoamines. The pattern of tissue damage and subsequent vascularization produced by chronic implantation of cannulae and intracranial injections of drugs is also examined by light and electron microscopy.

METHOD

Animals

Thirty-five, naive, male Wistar-derived rats, 90–120 days old and weighing approximately 300 g at the time of surgery were used. After surgery, rats were housed individually in wire mesh cages (20 × 23 × 40 cm) in a room thermostatically controlled at 72 ± 2°F. Rats were fed ad lib Mecon rat cubes and supplied with tap water and given at least 10 days to recover from surgery.

Procedure

Surgery. Rats were starved 24 hr prior to surgery, and were anesthetized by a chloral hydrate/nembutal intraperitoneal injection. During surgery, stainless steel cannulae [8] were implanted with the aid of a stereotoxic instrument in one of three brain areas. First, bilateral cannulation of the lateral hypothalamus (N = 16), co-ordinates A + 0.8 mm, H − 8.5 mm and L ± 1.9 mm. Second, bilateral cannulation of the substantia nigra region (N = 9), just above the A9 dopamine cell bodies [45]; co-ordinates A − 3.2 mm, H − 7.5 mm, L ± 2.7 mm. Third, unilateral cannulation of the amygdaloid region (N = 10) just above and between the central and lateral amygdaloid nuclei and therefore at the ventral border of the caudate putamen; co-ordinates were A + 0.4 mm, H − 7.2 mm and L + 4.5 mm. All co-ordinates given are relative to bregma [33].

Drugs. A 32 μg/μl solution of 2,4,5-trihydroxyphenylamine hydrochloride (6-OHDA, Astra) in distilled water containing 2 mg/ml ascorbic acid was prepared daily immediately prior to injection. Guanethidine sulphate (Ciba) solution (32 μg/μl) in distilled water was prepared every fifth day and refrigerated during the intervening period.

Injections. The injection technique has been described previously [2]. Of the hypothalamic implants, 5 received guanethidine, 5 received 6-OHDA and 6 received isotonic saline. Rats received one, two or three 2 μl injections bilaterally, 24 hr apart at 11 a.m. each day. Rats from each treatment group were sacrificed for histochemical analysis 24 hr after the previous injection. Thus, the effective spread of 1, 2 or 3 drug injections after 24 hr was compared. Of the rats cannulated in the substantia nigra 3 received one bilateral injection of 6-OHDA (2 μl) and were killed 5 or 12 days later, 3 received 5 daily injections of guanethidine (2 μl) and were killed 24 hr later and the remainder received isotonic saline. The amygdaloid implants received 2 μl injections of guanethidine or isotonic saline daily for 13 days. Rats were killed 24 hr or 9 days later.

A volume of 2 μl was chosen in order to maximise differences in diffusion patterns of the two drugs. In fact no significant difference in catecholamine depletion patterns was seen when comparing 1 μl and 2 μl injections of guanethidine (Evans, unpublished observations). In experiments using 6-OHDA, large volumes of 4 and 5 μl have often been used [34,35].

Histochemistry. The Falck-Hillarp fluorescence histochemical method for localizing monoamines was used [6]. Rats were guillotined and the brains were quickly removed through the dorsal surface of the skull. After dissection, brain pieces of approximately 10 mm^3 were frozen in liquid propane cooled with liquid nitrogen and then freeze-dried at −38°C and 10^{-3} mm Hg, using P_2O_5 as a moisture trap, for 36 hr. The tissue was then allowed to return to room temperature over 7 hr and heated to 35°C before incubation in a sealed vessel at 80°C for 1 1/2 hr with paraformaldehyde at optimal humidity. After vacuum embedding in paraffin wax, sections (15 μ) were cut, mounted with paraffin oil on heated glass slides, and then examined in a Leitz-Ortholux fluorescence microscope with an optical system as described elsewhere [6]. In this study no attempt was made to distinguish the specific fluorescences of adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine.

Light and electron microscopy. Tissue from the substantia nigra was fixed for ultrastructural study by injecting phosphate buffered 1% osmium tetroxide at pH 7.3 into the cannula for 10 min prior to the killing of the animal. This not only gave instant fixation of the tissue but also allowed precise localization of the drug treated site. Upon removal the tissue was diced, placed in buffered 1% OsO_4 for 1 hr and washed in buffer for 10 min. It was then post-fixed in buffered 5% glutaraldehyde for 1 hr, followed by another 10 min wash in buffer and then placed once again in buffered 1% osmic acid for 30 min. After a brief wash in distilled water the tissue was block stained in saturated aqueous uranyl acetate for 30 min, dehydrated in a graded series of alcohols, passed through various mixtures of propylene oxide and Araldite and finally embedded in Araldite. Sections 1μ thick were cut and stained with toluidine blue for light microscopy. Thin sections were cut from selected areas and examined in the electron microscope.

RESULTS

Fluorescence Histochemistry

Three areas of damage have been distinguished on the basis of fluorescence histochemistry (see Fig. 1). (1) Cannulation damage atributable to placement of cannula and placebo injection as indicated by non-specific orange autofluorescence. (2) Generalized damage attributable to injection of drugs. This additional damage is also indicated by autofluorescence and involves all cell types including catecholamine-containing neurons. The extent of this damage varies with different drugs. (3) Specific damage to catecholamine-containing nerves.

Cannulation Damage

In placebo animals (injected with isotonic saline) the general appearance of the catecholamine-containing nerve

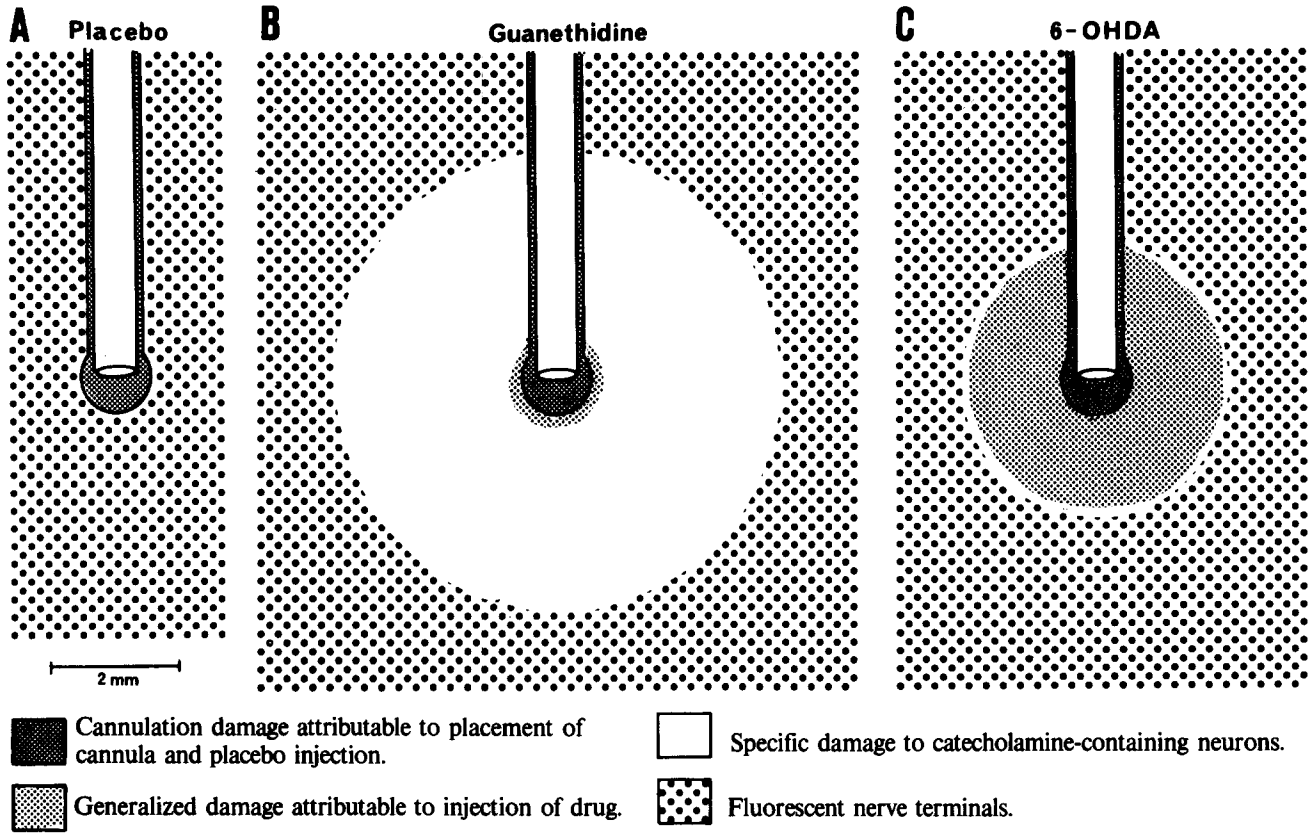

FIG. 1. Simplified diagrammatic representation of the extent of damage due to chronic injections (64 μg in 2 μl daily for 3 days) of (A) isotonic saline, (B) guanethidine and (C) 6-OHDA.

fibres was comparable to that seen in normal uncannulated animals. The levels of fluorescence were the same, as were the density and pattern of innervation.

The lesion due to cannulation was easily located since it was always highly autofluorescent (i.e. a non-specific usually orange-coloured fluorescence) and was surrounded by a narrow region which also exhibited an orange autofluorescence probably indicating a region of cell damage (Figs. 2 and 4B). The overall diameter of this autofluorescent region of damage was approximately 1 mm.

The implantation of a cannula caused severance of catecholamine-containing axons and subsequent interruption of axonal flow which resulted in a build-up of catecholamines in the proximal protions of the severed axons [11]. This was seen as a moderate degree of accumulation of fluorescence adjacent and caudal to the cannula track.

Generalized Damage

Other experimental details being the same, the extent of general damage adjacent to the cannula track was much greater with 6-OHDA than with guanethidine (Fig. 2). On the other hand the extent of drug-induced specific damage to catecholamine-containing nerves which occurred outside the region of general damage was extensive with guanethidine and barely detectable with 6-OHDA.

When the number of daily injections was increased the extent of general damage increased accordingly. With guanethidine this increase was small and the difference between 3 and 12 injections was increased autofluorescence and a slightly greater (0.3 mm) spread of damage. However with 6-OHDA, injection for 3 days resulted in a region of generalized damage more than twice the size and with greatly increased autofluorescence than that after one injection. Such damage after guanethidine has only been seen when injections of 10 times the dose were administered over 12 days [15]. The extent of the specific depleting effect on catecholamine-containing nerves also increased as the number of injections of guanethidine were increased. Any similar increase in specific effects of 6-OHDA seemed to be masked by the great increase in general damage. Increasing the concentration of drug has also been found to increase the extent of generalized damage and specific drug-induced damage [15].

Specific Damage

Hypothalamus

Guanethidine. After a single injection of guanethidine (64 μg in 2 μl, one rat) there was clear depletion of catecholamine stores in the vicinity of the cannula tip (over a radius of about 1.5 mm) (Fig. 2A). The perivascular adrenergic fibres supplying the pial arteries ventral to the optic tract [32] showed no fluorescence over 2 mm or more rostral and caudal to the plane of cannulation. A small amount of the fluorescence build-up in the proximal portions of axons severed by the cannula was seen although

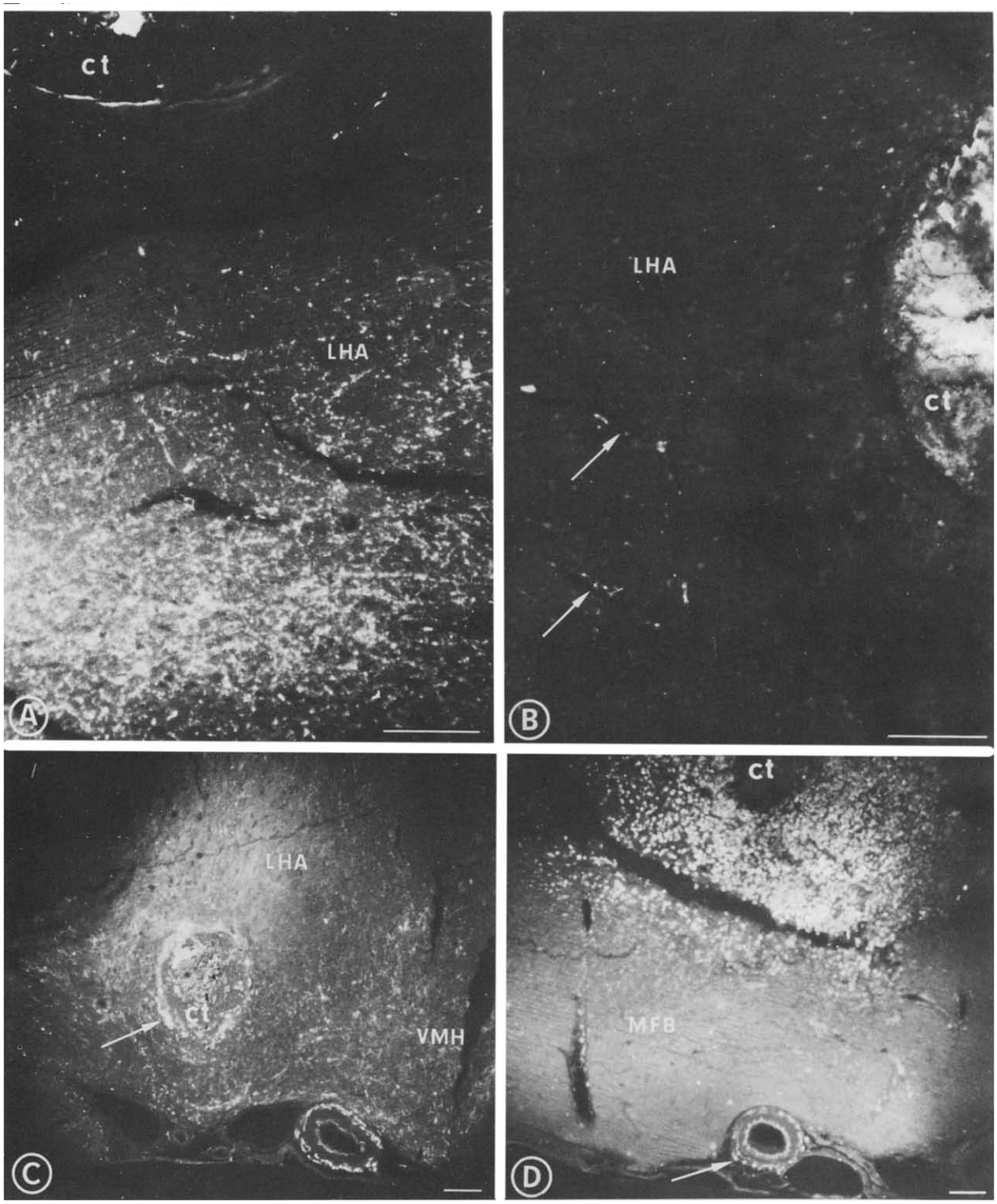

FIG. 2. Fluorescence histochemistry of the lateral hypothalamic area of the rat brain. Rats had cannulae bilaterally implanted into the lateral hypothalamus and were killed after the following treatments. Calibration bars = 100 μ. (A) Single injection of guanethidine (64 μg in 2 μl). Note the depletion of catecholamines from terminals within 0.5 mm of the region of cannulation damage. (B) Guanethidine (64 μg in 2 μl) daily for 3 days. No fluorescent nerve terminals are visible. Autofluorescent mast cells are clearly visible in the walls of a branching blood vessel (arrows). (C) Placebo injections daily for 3 days. Fluorescence levels are normal in the catecholamine-containing nerves of the hypothalamus and ventral pial artery. Some accumulation of fluorescence is evident adjacent to the cannulation region (arrow). (D) 6-OHDA (64 μg in 2 μl) daily for 3 days. Note the greatly increased region of autofluorescence associated with the cannula tract. The adrenergic nerves of the ventral pial artery are partially depleted (arrow). (LHA, lateral hypothalamic area; VMH, ventromedial nucleus of the hypothalamus; MFB, median forebrain bundle; ct, cannula track).

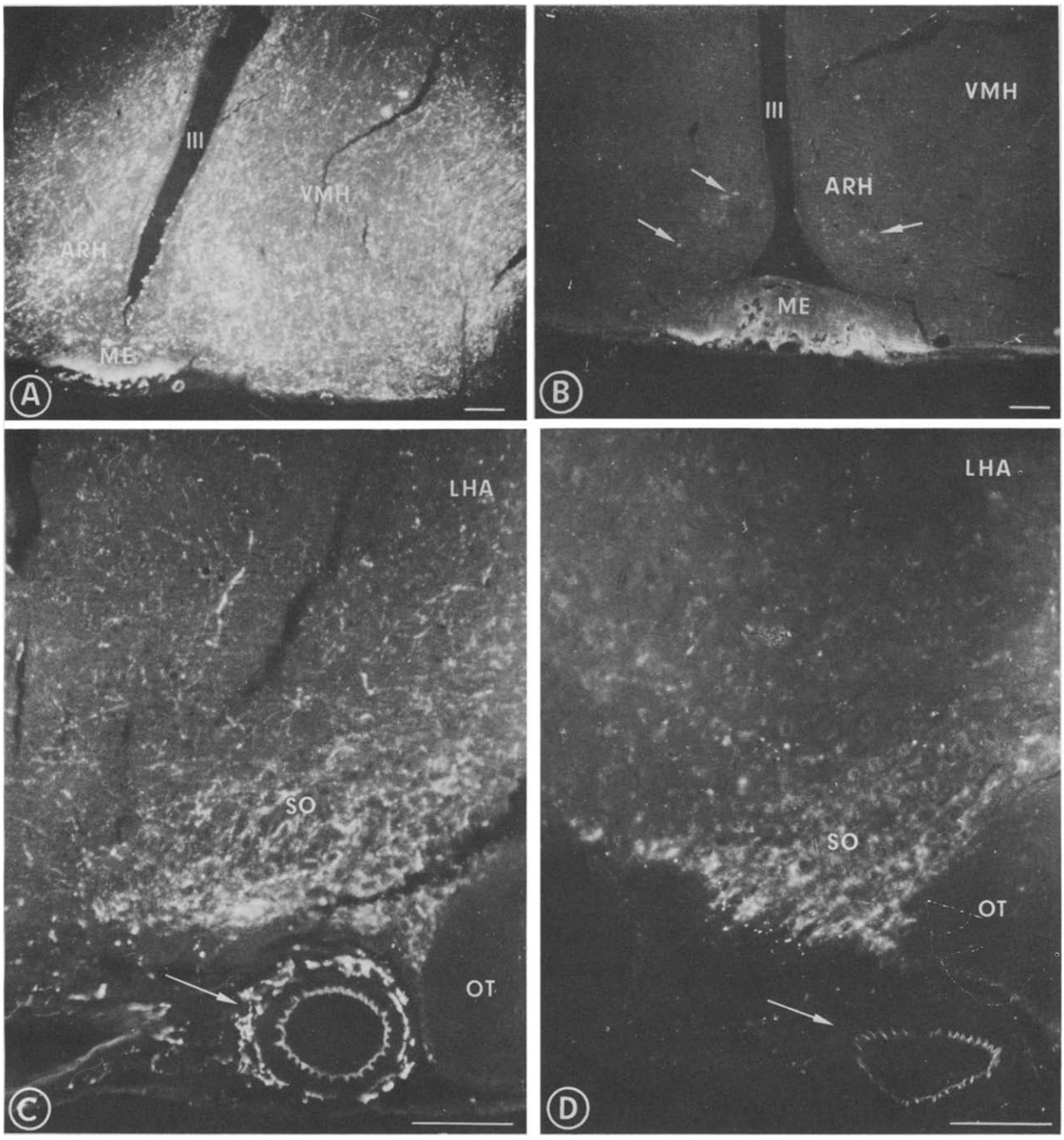

FIG. 3. Fluorescence histochemistry of the hypothalamic area of the rat brain. Rats had cannulae bilaterally implanted into the lateral hypothalamus and were killed after the following treatment. Calibration bars = 100 μ. (A) Placebo daily for 12 days. Normal fluorescence levels are present throughout the hypothalamus. (B) Guanethidine (64 μg in 2 μl) daily for 12 days. Fluorescent nerve terminals are generally absent throughout the hypothalamus. Note that some fluorescence remains in the ventral border of the median eminence and also in the A_{12} [45] cell bodies (arrows) of the arcuate nucleus. (C) Placebo injections daily for 3 days. Normal fluorescence levels are evident in the lateral hypothalamus, supraoptic nucleus and ventral pial artery (arrow). (D) Guanethidine (64 μg in 2 μl) daily for 3 days. The catecholamine-containing nerve terminals of the lateral hypothalamus and the ventral pial artery (arrow) are completely devoid of fluorescence. Some fluorescence clearly remains in the supraoptic nucleus. (ARH, arcuate nucleus of the hypothalamus; ME, median eminence; VMH, ventromedial nucleus of the hypothalamus; LHA, lateral hypothalamic area; SO, supraoptic nucleus, OT, optic tract; 111, third ventricle).

it was considerably less than that seen in placebo animals.

By the third injection of guanethidine (3 rats) there were virtually no fluorescent fibres visible in the various regions of the hypothalamus within a radius of at least 3 mm from the tip of the cannula (Fig. 2B and 3B). This involved the paraventricular and arcuate nuclei of the hypothalamus, the lateral and anterior hypothalamic areas and the ventromedial nucleus of the hypothalamus. The pial arteries were now totally devoid of fluorescence along the whole length of the piece of tissue taken. There was no longer any sign of accumulation of fluorescence adjacent to the cannula track.

Some regions, in confirmation of earlier results [2], specifically the ventral margin of the median eminence, the supra-optic nucleus, and the fluorescent cell bodies adjacent to the fornix and in the arcuate nucleus (Groups A13 and A12, [45]) were resistant to depletion by guanethidine and showed fluorescence after the third injection (Fig. 3B and 3D).

However, it is of interest that in a previous study [2] one rat, cannulated bilaterally in the lateral hypothalamus and given guanethidine (64 μg in 2 μl) for 12 days, was found, on sacrifice and examination, to have one cannula misplaced so that its tip lay within the optic tract. This was the only time in either that or the present study that the supra-optic nucleus was seen to be completely depleted of its fluorescence. Ipsilateral to this misplaced cannula the depletion of fluorescence in the hypothalamus was not complete and there remained a number of fluorescent fibres particularly in the paraventricular nucleus. The autofluorescence related to cannulation and injection was almost completely localized within the boundaries of the optic tract and was found over a distance of 2 mm rostral and caudal to the cannulation site. Contralaterally the cannula was correctly placed in the lateral hypothalamus and the hypothalamic area on this side was completely depleted of catecholamines with the exception of the supra-optic nucleus which still showed considerable fluorescence.

6-OHDA. After the first injection of 6-OHDA (64 μg in 2 μl, one rat) there was an increased accumulation of catecholamine fluorescence around the cannula track compared to that seen in placebo animals. This increased with further injections so that there was a massive accumulation of fluorescence evident following the third injection (3 rats). This was accompanied by an increased build-up of fluorescence along the axon pathways 3–4 mm caudal to the cannula.

No discrete area of catecholamine depletion was evident although there was a small decrease in general fluorescence of the lateral hypothalamus. The pial arteries lying directly below the cannula tract were only partially depleted (Fig. 2D). The supra-optic nucleus again appeared to be relatively unaffected, since, when the cannula was situated directly above the supra-optic nucleus it still showed considerable levels of specific fluorescence while the pial artery below it was depleted.

There was a great increase in the extent of generalized damage to the brain tissue in the region of the cannula. The autofluorescence around the cannula track was brighter than in either placebo or guanethidine treated animals and the area of damage after the third injection was 3–4 mm in diameter (Fig. 2D).

Substantia Nigra

Guanethidine. The dopaminergic cell bodies (A9, A10, [45]) of the substantia nigra were apparently unaffected by 5 daily injections of guanethidine (64 μg in 2 μl, three rats) (Fig. 4). They showed normal fluorescence even when within 1 mm of the region of generalized damage around the cannula track. However the fluorescent fibres innervating the pial arteries situated ventral to these neurons and up to 4 mm from the tip of the cannula were always depleted of their catecholamine stores. This depletion continued rostrally and caudally over the whole length of tissue taken. Fluorescent nerve fibres were seen throughout the hypothalamus although some depletion was evident in more caudal regions. The adrenergic nerves supplying the pial arteries ventral to the hypothalamus were depleted.

6-OHDA. 6-OHDA induced damage appeared to be confined to the area of generalized damage around the cannula track over a radius of about 1 mm (64 μg in 2 μl, three rats). Dopaminergic neurons situated approximately 2 mm from the tip of the cannula showed completely normal fluorescence and the pial artery ventral to these neurons also showed normal fluorescence levels.

There was very little accumulation of fluorescence adjacent to the cannula tracks in both placebo and 6-OHDA treated animals indicating that the cannulation and 6-OHDA diffusion had not affected any major bundles of catecholamine-containing nerves. The fluorescence of the hypothalamic regions was normal.

Amygdala

Guanethidine. There was little increase in the autofluorescence or in the total area of damage associated with the cannula track as a result of the 13 days of guanethidine treatment (64 μg in 2 μl/day, six rats) as compared to that seen after 3, or 5 days of guanethidine injection in other regions of the brain. There was no evidence of fluorescence accumulation beside the cannula track.

Guanethidine injections for 13 days caused no apparent depletion of the dopaminergic terminals of the nucleus caudatus putamen, nor of the nucleus amygdaloideus centralis. The adjacent hypothalamus was similarly unaffected and had normal fluorescence levels. The pial arteries on the ventral surface of the hypothalamus within 2–3 mm of the cannula track also showed normal fluorescent adrenergic innervation throughout. This was in marked contrast to injection into the lateral hypothalamus or substantia nigra where after the same treatment blood vessels 5 mm and more away from the cannula were totally depleted of noradrenaline stores.

Vascularization of Cannula Tracks

In studies involving chronically implanted cannulae, some times over a period of weeks, the situation is considerably different from those where both the cannulation and injection are acute. In a previous study [2] where rats were cannulated for over 4 weeks there was a greatly increased number of large and small blood vessels supplying the damaged region around the cannula track. Arteries were seen branching from the ventral pial arteries and passing up to ramify amongst the damaged tissue (Fig. 5). A similar pattern of vascularization has never been

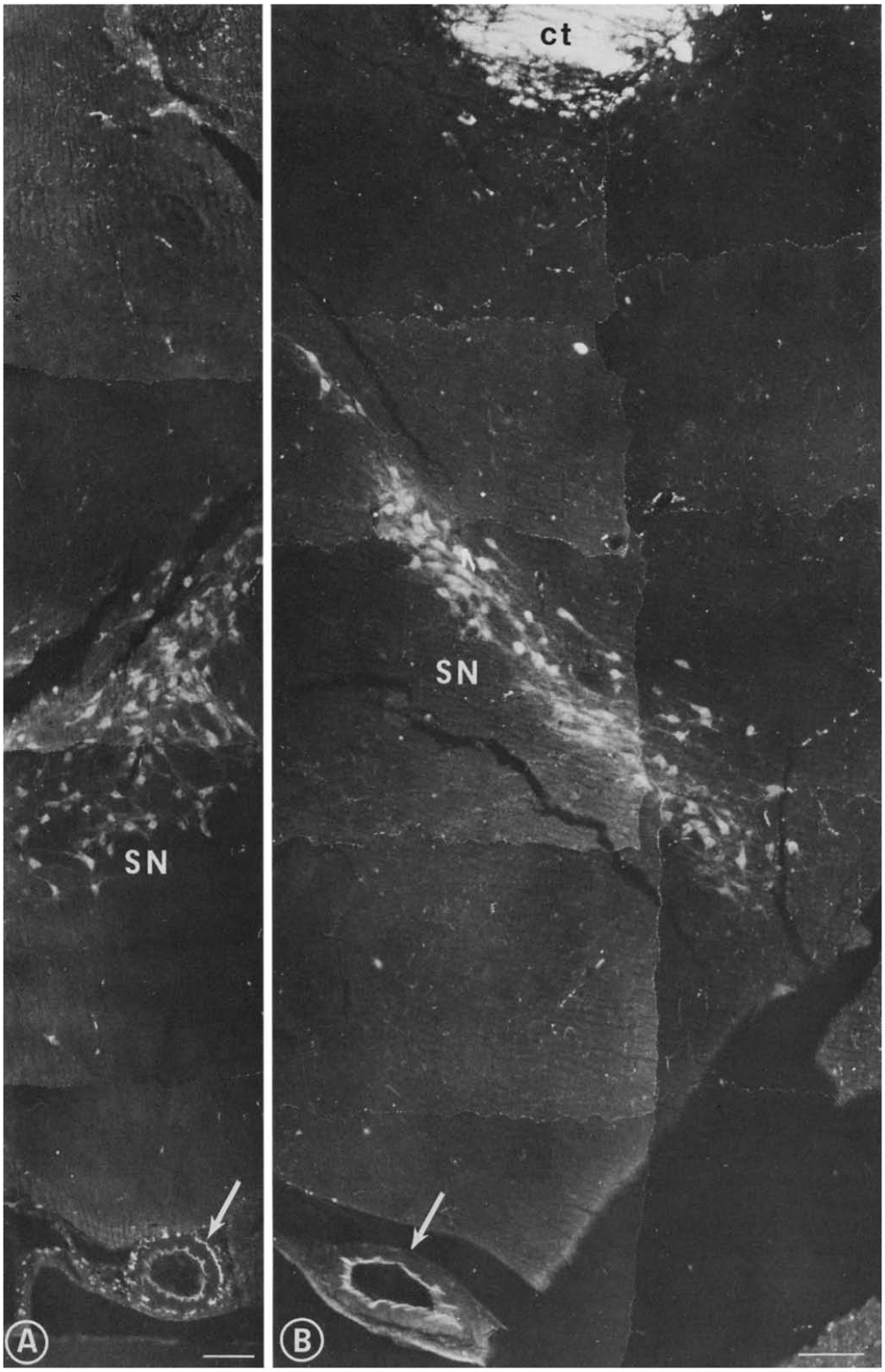

FIG. 4. Fluorescence histochemistry of the substantia nigral region. Rats had cannulae bilaterally implanted into the substantia nigra and were killed after the following treatment. Calibration bar = 100 μ. (A) Placebo injections daily for 12 days. Fluorescence of the A9 [45] dopaminergic neurons and of the ventral pial artery (arrow) is normal. (B) Guanethidine (64 μg in 2 μl) daily for 12 days. The ventral pial artery (arrow) is completely devoid of fluorescence. However the A9 [45] cell bodies of the substantia nigra show normal fluorescence levels. (SN, substantia nigra; ct, cannula track).

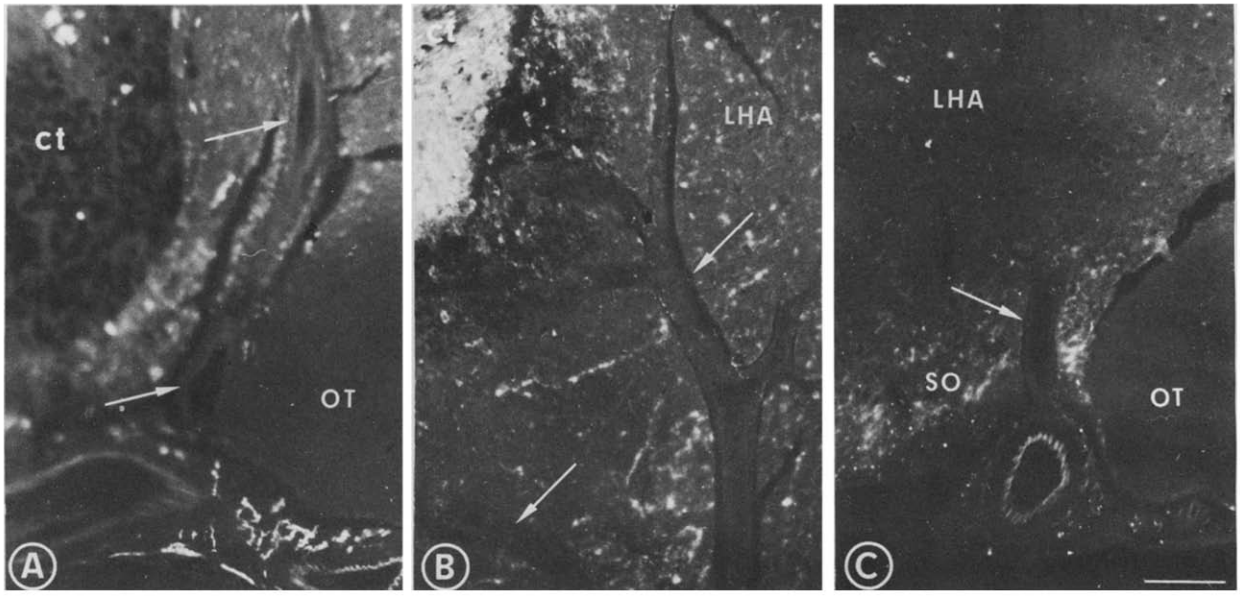

FIG. 5. Fluorescence micrographs showing increased vascularization to the cannulation area arising from the ventral pial artery. Rats had cannulae bilaterally implanted into the lateral hypothalamus and were killed after the following treatment. Calibartion = 100 μ and applies to A, B, C. (A) Placebo injections daily for 3 days. Note the relatively large artery (arrows), devoid of adrenergic innervation, which arises from the ventral pial artery. (B) The same rat as in A. but several sections away showing branches of the artery (arrows) which arose from the ventral pial artery ramifying around the area of cannulation damage. These are likely to become innervated at a later stage [15]. (C) Guanethidine (64 μg in 2 μl) daily for 3 days. A large branch (arrow) from the ventral pial artery (which has been depleted of its fluorescence) passes through the supraoptic nucleus. This vessel in later sections was seen to ramify in the areas of cannulation and generalized damage. OT, optic tract; LHA, lateral hypothalamic area; SO, supraoptic nucleus; ct, cannula track).

observed in the comparable regions of the brain from uncannulated rats but has been seen often in both placebo and drug-treated animals.

There appeared to be a correlation between the time elapsed since implantation cannulae and the amount of vascularization seen. Increased vascularization was usually seen when the cannula had been implanted for at least four weeks.

Light and Electron Microscopy

Microscopical examination of the placebo and guanethidine treated tissue revealed that the autofluorescence in the region of cannulation damage was due to the presence of numerous macrophages containing debris and lipid droplets (Fig. 6). Accompanying these cells were many other haematogeneous cells, especially plasma cells. Neuropil was not observed in this area.

Numerous clear spaces, indicative of extensive degeneration within the neuropil were seen in the region of generalized damage of guanethidine treated tissue. Myelinated axons showed varying degrees of degeneration; the organelles, especially tubules and filaments, of some were only slightly disrupted (Fig. 7A) whilst other axons were shrunken and occasionally electron-dense. Frequently the inner tongue of oligodendroglial cytoplasm was enlarged (Fig. 7A). Few astrocytes and their processes were observed and these were often disrupted. Intact neurons were not seen. Occasional macrophages, mostly with lipid inclusions, were scattered throughout this area which accounted for the autofluorescence. In the 6-OHDA treated tissue, degeneration of neuropil within the area of generalized damage was virtually complete except for occasional tracts of myelinated axons. There was usually a distinct demarcation between the area of generalized damage and the surrounding neuropil. Despite the broad similarity between the appearance of the regions of generalized damage resulting from injections of guanethidine and 6-OHDA, it seems likely that more extensive ultrastructural studies may reveal some differences as well as similarities in morphology.

In the region of specific damage of guanethidine-treated tissue, the neuropil remained relatively compact although many terminal axons had degenerated in some areas (Fig. 7B). Some degenerating myelinated axons were also observed.

Fenestrated capillaries were not observed in any of the regions.

DISCUSSION

Marked differences in extent of diffusion have been shown between intracranially injected guanethidine and 6-OHDA (see Fig. 1). These differences reflect the problems encountered in endeavouring to lay down any general rules governing the diffusion of intracranially-injected substances. Studies of the comparatively passive diffusion of physiologically inert substances (such as dyes and large protein molecules) of different molecular weights, concentrations, volumes, etc. have been carried out [30]. However extrapolation from these situations to those involving physiologically active drugs and putative transmitter sub-

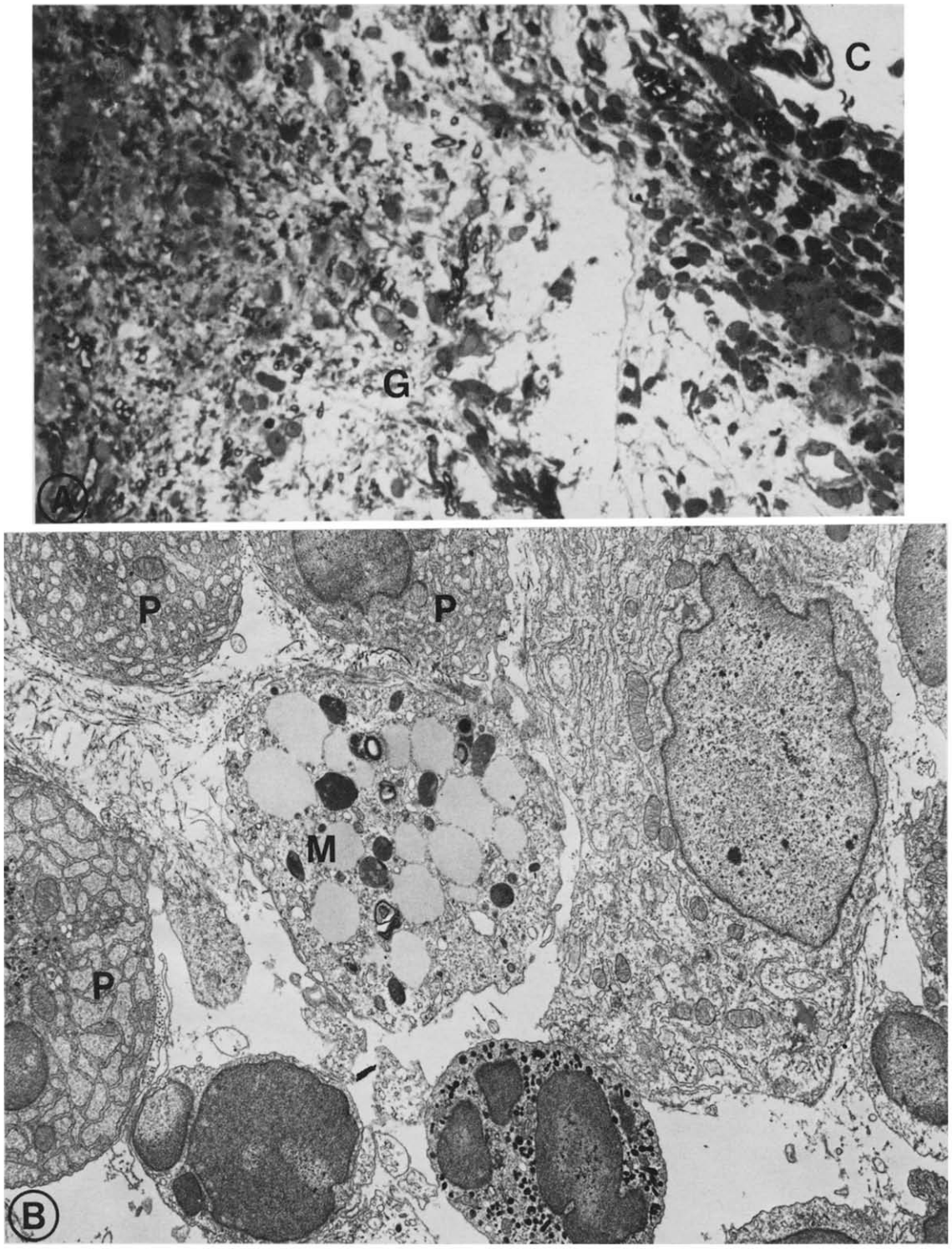

FIG. 6. Micrographs of regions of the substantia nigra. Rats had cannulae bilaterally implanted into the substantia nigra and were killed after the following treatment. (A) Guanethidine (64 μg in 2 μl) daily for 12 days. Portion of a thick toluidine-blue stained section showing the site of cannulation (c) and associated haematogenous cells, the region of generalized damage (G) and the edge of the area of specific damage (S), ×430. (B) Placebo daily for 12 days. Electron micrograph showing some of the haematogenous cells present close to the site of cannulation. M, macrophase containing debris and lipid droplets; P, plasma cells. ×6,520.

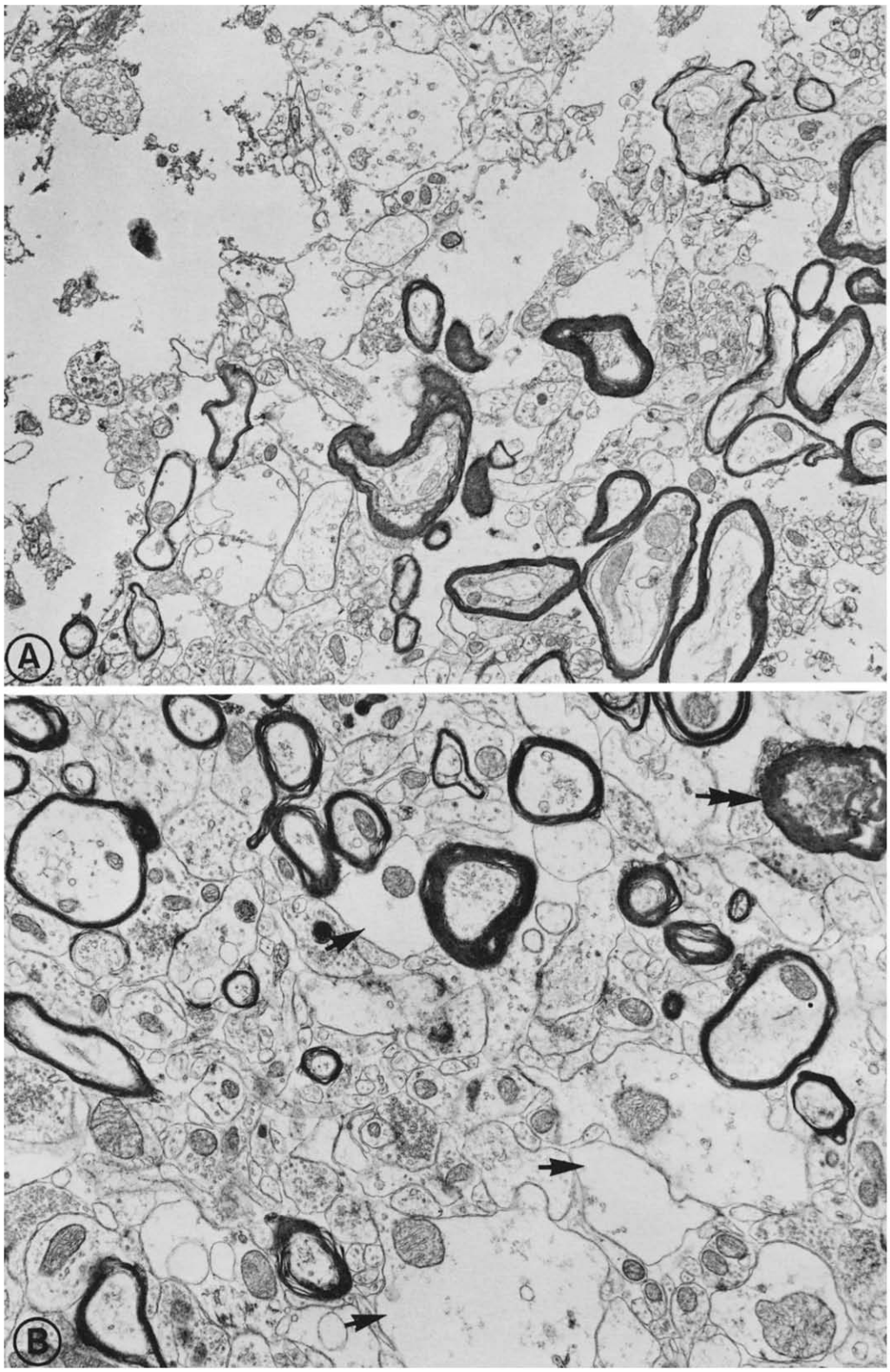

FIG. 7. Electron micrographs of the substantia nigra. Rats, cannulated bilaterally into the substantia nigra, received 12 daily injections of guanethidine (64 μg in 2 μl) prior to killing. (A) This micrograph illustrates the disruption of the neuropil seen in the area of generalized damage ×7,690. (B) Region of specific damage showing disruption of a myelinated nerve fibre (double arrow) and some terminal axons (arrows). ×13,195.

stances is somewhat tenuous. Despite comparable experimental conditions such as concentrations, volume of injection, molecular weights and lipid solubility, and despite the fact that both guanethidine and 6-OHDA show specificity for catecholamine-containing neurons, the diffusion patterns of these drugs were markedly different. Clearly consideration of these factors alone can be misleading. The differences may be explained by their unique pharmacological properties. In peripheral tissue guanethidine is taken up specifically into adrenergic neurons by the amine uptake pump [29,37] where it causes a blockade of nervous transmission, depletion of noradrenaline [3, 7, 17] and, at higher doses, degeneration of the neuron itself [6,19]. However, in addition, guanethidine blocks the membrane uptake pump [12, 14, 16, 41] thus limiting its own further accumulation. This situation may also occur in the brain whereby guanethidine, although taken up specifically into the adrenergic terminals of the hypothalamus may then inhibit further uptake thus allowing the drug to diffuse over greater distances.

6-OHDA has quite different properties. It is an extremely powerful reducing agent and once injected reacts very quickly with surrounding tissue forming considerable amounts of H_2O_2 [34]. Thus because of this great reactivity, it may not diffuse in an intact state far from the site of injection. It is this property of 6-OHDA which may explain the large degree of generalized damage to brain tissue observed in the present study when compared to that seen after saline or guanethidine injection. Peripheral intravenous injections of 6-OHDA have shown that it is taken up specifically into adrenergic terminals which then degenerate [42]. However because of the density of the brain parenchyma and the lack of rapid removal via the circulation due to ischaemic trauma, rapid dispersal of 6-OHDA following intracranial injection is limited and thus its specificity for adrenergic neurons is less evident. Poirier *et al.* [34] also found very little damage specific to catecholamine-containing neurons after injection of 6-OHDA whereas other workers have reported specific degeneration of catecholaminergic neurons [44, 45, 46, 47].

In experiments using cultured newborn rat sympathetic ganglia, Hill *et al.* [20] found that while low doses of 6-OHDA specifically affected the adrenergic neurons, high doses caused non-specific damage resulting in the death of all cell types present in the culture. In contrast, at low or high doses, the damage resulting from guanethidine was confined to adrenergic neurons. These findings may explain the differences between the results reported elsewhere [34, 39, 45] regarding the specificity of action of 6-OHDA on adrenergic neurons. Injection of very small amounts of 6-OHDA results in specific damage to adrenergic neurons whereas injection of larger doses causes a generalized damage to the surrounding brain tissue. Also considering the limited diffusion of 6-OHDA, the total dose injected may be more relevant than the concentration of the solution.

Following injection into the substantia nigra, 6-OHDA damage was localized around the cannula tract and the dopaminergic cell bodies even as close as 1 mm away maintained normal fluorescence levels. With guanethidine the dopaminergic neurons were also apparently unaffected but the adrenergic nerves innervating pial artery ventral to these neurons were depleted. This could be interpreted in terms of guanethidine reaching the artery via the vasculature. However this seems unlikely because guanethidine has been shown, by its depleting action, to have diffused throughout the hypothalamus over a radius of at least 3 mm while the dopaminergic cell bodies of Groups A12 and A13 [45] located amongst these depleted fibres showed normal fluorescence. In another study [15] it has been that dopaminergic neurons are considerably more resistant to guanethidine than noradrenergic neurons. Thus it is not unreasonable to assume that the guanethidine reached and depleted the adrenergic innervation of the ventral pial artery by diffusion through the brain tissue.

Light and electron microscopic examination of the area of cannulation damage showed that the orange autofluorescence was related to the presence of haematogenous cells, especially macrophages containing debris. The presence of a necrotic region following traumatic damage to the central nervous system has previously been described [9, 21, 24, 25, 40]. While the surrounding area of generalized damage was broadly comparable after guanethidine and 6-OHDA treatment, differences are likely to be revealed following more extensive study. For example, 6-OHDA caused a more widespread, rapid and complete degeneration of the neuropil than that produced by guanethidine. this difference is probably related largely to the reducing nature of 6-OHDA. The occurrence of degenerating myelinated axons as well as some degenerating terminal axons in the neuropil of the regions of specific damage may indicate Wallerian or retrograde degeneration as a result of damage to nerve fibres in the regions of cannulation and generalized damage.

In addition to the individual properties of substances injected into the brain other factors appear to be involved in the subsequent patterns of diffusion, particularly the anatomical features of the region in the vicinity of the cannula. Routtenberg [35] suggested that heavily myelinated fibre tracts may limit the diffusion of substances through the brain. This seems a reasonable explanation for the lack of depletion of catecholamines in the hypothalamus following guanethidine injections into the amygdala. There was no change in the fluorescence of hypothalamic fibres as close as 2 mm to the amygdaloid cannula, a distance over which complete depletion occurred when the cannula was placed in the lateral hypothalamus. The heavily myelinated fibres of the internal capsule may have limited the diffusion of guanethidine, which is largely ionized under physiological conditions and thus relatively insoluble in lipids. Similar reasoning may explain the unusual resistance to depletion by guanethidine of the supra-optic nucleus which lies in close proximity to the heavily myelinated fibres of the optic tract. The only time the supra-optic nucleus was depleted was when the cannula tip lay within the optic tract. In this case the myelinated fibres limited the spread of guanethidine so that the damage was chiefly located within the optic tract itself.

It may be almost impossible to detect with biochemical analysis differences in catecholamine levels due to generalised damage as compared to specific damage to catecholamine-containing neurons in brain regions treated with 6-OHDA since both will result in a decrease in catecholamine levels. Therefore the generalized damage caused by the application of 6-OHDA as shown in the present study could be interpreted as being specific destruction of catecholamine-containing nerves [48]. Furthermore other biochemical changes such as reduction in acetylcholine acetylase levels [23] may be interpreted as adrenergic cholinergic interaction when in fact both changes in

enzyme levels are the result of generalized neuronal damage caused by 6-OHDA. The present findings throw some doubt on the noradrenergic specificity of 6-OHDA used in studies of the central nervous system even in cases where behavioural data is supported by data from biochemical analysis. Thus injections of 6-OHDA may be little more selective than electrolytic lesions. For example there are recent reports that 6-OHDA lesions are similar to electrolytic lesions in the classical hypothalamic syndrome and are therefore the result of catecholamine depletion [48].

The role of the vasculature in the diffusion of intracranially injected substances is not yet understood. Certainly the blood-brain barrier appears to function in either direction thus preventing not only substances passing from the blood to the brain but also in the opposite direction [27]. However it is clear that guanethidine, which normally does not cross the blood-brain barrier following peripheral injection [17] is able to diffuse into and completely deplete the adrenergic innervation along the whole length of the pial arteries ventral to the hypothalamus. This depletion occurred even when the cannulation was in the substantia nigra, and the anterior regions of the hypothalamus showed normal fluorescence levels.

The observations made in the present study of an increased vascular supply in the vicinity of the cannula tract introduces further complexity. It is usual for vascular invasion to take place at sites of damage or pathology of the central nervous system [28]. Poirier [34] reported a proliferation of sub-ependymal blood vessels following intraventricular injections of 6-OHDA. These newly formed blood vessels often have greatly differing properties to the normal vascular supply of the region in particular, the blood-brain barrier regions of the brain [5, 27, 43]. Although fenestrated capillaries were not observed in the present study, fenestrations were observed in capillaries within neurilemomas and were suggested to have arisen as a result of focal haemorrhages [22]. Thus, particularly in experiments where cannulae are chronically implanted, the probability of diffusion of injected substances through a vasculature which may have altered blood-brain properties must be considered.

The present study indicates that in experiments using intracranial injections of drugs, the unique properties of the drug used, the neuroanatomical structure of the injection site and particularly if using chronically implanted cannulae, the possibility of an altered vascular supply to the area are important factors in the extent of diffusion and should be considered in addition to the volume, concentration and osmolarity of the injected solution.

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