Pharmacology Biochemistry & Behavior, Vol. 5, pp. 207–213.

Intracranial Injections of 6-OHDA. Comparison of Catecholamine-Depleting Effects of Different Volumes and Concentrations

GREGORY L. WILLIS AND G. SINGER

Department of Psychology, La Trobe University, Bundoora, Victoria, Australia 3083

AND

BARBARA K. EVANS

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

(Received 1 June 1976)

WILLIS, G. L., G. SINGER AND B. K. EVANS. *Intracranial injections of 6-OHDA: comparison of catecholamine-depleting effects of different volumes and concentrations.* PHARMAC. BIOCHEM. BEHAV. 5(2) 207–213, 1976. – Fluorescence histochemistry was used to assess monoamine depletion after injections of 6-OHDA into selected brain areas. Two volumes (2 and 4 μl) and 4 concentrations (1, 2, 4 and 8 μg/μl) of 6-OHDA were injected into the olfactory tubercle, the posterior lateral hypothalamus and the lateral hyopthalamus. Selective destruction of catecholamine-containing neurons resulted from all injections of 6-OHDA with the exception of the 2 lowest doses (2 and 4 μl of 1 μg/μl) and the highest dose (4 μl of 8 μg/μl) which produced nonspecific damage of brain parenchyma. The results indicate that, in addition to the selection of an effective dose, it is also possible to choose a site of injection which will produce a maximal area of specific depletion. In cases where injections into terminal areas caused limited specific depletion the same dose injected into preterminal axons often caused a more widespread loss of fluorescence. With volume, concentration and anatomical location being important variables to consider, caution is needed in the interpretation of behavioural experiments. When using 6-OHDA it is necessary to show that specific depletion of catecholamines has been achieved.

6-OHDA Intracranial injections Olfactory tubercle Catecholamine depletion
Lateral hypothalamus Posterior lateral hypothalamus

THE effects of 6-hydroxydopamine (6-OHDA) on peripheral adrenergic neurons are well known. The terminal varicose regions of the nerve are first depleted of noradrenaline and then degenerate. However, the cell body is not seriously damaged and after several weeks the terminals regenerate [11]. Reports on the effects of intracranial injections of 6-OHDA on central nervous tissue are conflicting. These range from specific catecholamine depletion [12, 13, 14] to neurochemically nonspecific generalised damage [4, 6, 9, 10]. Recently it has been shown that high doses of 6-OHDA injected into various regions of the central nervous system cause generalised damage, but damage specific to catecholamine-containing neurons, as shown by fluorescence histochemistry, was not seen [6]. These different patterns of damage cannot easily be distinguised by biochemical assays and the specificity of 6-OHDA for central catecholamine-containing neurons has been questioned. However, it is still possible that the specificity of this drug is a function of the dose used.

In the present study the diffusion and damage patterns of different volumes and concentrations of 6-OHDA were examined using the fluorescent histochemical method. Two volumes (2 and 4 μl) and 4 concentrations (1, 2, 4 and 8 μg/μl) were selected since they, together with the doses used in the earlier study [6], cover the range of doses of 6-OHDA most commonly used in studies of the central nervous system.

METHOD

Animals

Forty seven naive male Wistar-derived rats weighing approximately 350 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 with temperature thermostatically controlled at 72 + 2°F. Rats had ad lib access to Mecon rat cubes and tap water, and were given at least 10 days to recover from surgery before injection of 6-OHDA.

Procedure

Surgery. Rats were anesthetised with a chloral hydrate/nembutal IP injection and stainless steel cannulae were implanted, with the aid of a stereotaxic instrument, in 1 of 3 brain areas: bilateral cannulation of the olfactory tubercle (N = 16, coordinates A + 2.5 mm, H − 8.5 mm and L ± 1.9 mm), and the posterior lateral hypothalamus (N = 20, coordinates A 0.8, H − 8.5 and L ± 1.9 mm) and unilateral cannulation of the lateral hypothalamus, (N = 11, coordinates A + 0.8 mm, H 8.1 mm and L ± 1.9 mm). All coordinates given are relative to bregma and in the plane of the Pellegrino and Cushman stereotaxic atlas [8].

Injections. Solutions containing 1, 2, 4 and 8 μg/μl of 2, 4, 5-trihydroxyphenlamine hydrochloride (6-OHDA, Astra) in distilled water containing 2 mg/ml ascorbic acid were prepared immediately prior to injection. Placebo injections were made with saline-ascorbic acid solutions isotonic with cerebrospinal fluid. The injection procedure has been described previously [2]. There were 8 treatment groups (Table 1) for each of the 3 anatomical locations. Thus, each treatment group received either 2 or 4 μl injections of one of the 4 concentrations.

TABLE 1

CONCENTRATIONS AND VOLUMES WHERE SPECIFIC DEPLETION OF CATECHOLAMINES OCCURRED

	2 μl	4 μl
1 μg/μl	None	None
2 μg/μl	PLH	PLH
	CLH	CLH
		LH(?)
4 μg/μl	TUB	TUB
	PLH	PLH
	CLH	CLH
	LH	
8 μg/μl	TUB	TUB (?)
	PLH	
	CLH	
	LH	

TUB—Olfactory tubercle injection and depletion.
PLH—Posterior lateral hypothalamus injection with lateral hypothalamus depletion.
CLH—Lateral hypothalamus injection with caudate nucleus depletion.
LH—Lateral hypothalamus injection and depletion.

Animals implanted in the olfactory tubercle (TUB) were injected unilaterally with 6-OHDA and contralaterally with either 2 or 4 μl of placebo. Animals implanted in the posterior lateral hypothalamus (PLH) received bilateral injections of 6-OHDA (N = 16). Control animals (N = 4) received bilateral injections of 2 or 4 μl of placebo. Rats unilaterally cannulated in the lateral hypothalamus (LH) received either 6-OHDA (N = 8) or 2 or 4 μl of placebo (N = 3).

Half of the animals in the TUB and PLH groups were killed 6 days postinjection while the other half were killed 10 days postinjection. All animals in the LH injected group were decapitated after 6 days.

Histochemistry. The Falck-Hillarp fluorescence histochemical method for localising monoamines was used [3]. 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 and cooled with liquid nitrogen and then freeze-dried at 38°C and 10^{-3} mm Hg, using P_2O_5 as a mositure trap, for 24–36 hr. The tissue was allowed to return to room temperature passively, then heated to 35°C before incubation in a sealed vessel at 80°C for 1.5 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 examined in a Leitz-Ortholux fluorescence microscope with an optical system as described elsewhere [3]. In this study no attempt was made to distinguish the specific fluorescence of adrenaline, noradrenaline, dopamine or 5-hydroxytryptamine.

RESULTS

In a previous study 3 areas of damage were distinguished on the basis of fluorescence histochemistry. There were also indentified in the present study: (a) cannulation damage resulting from chronic implantation of cannulae and placebo injection as indicated by nonspecific orange autofluorescence; (b) generalised damage attributable to injection of drug, also indicated by increased levels of autofluorescence, and (c) specific damage to catecholamine-containing neurons. In addition a fourth type of damage attributed to degeneration of distal portions of nerve processes following cannulation or generalised damage has been observed.

Within the range of doses of 6-OHDA used in this study little generalised damage was seen. However, there was a concentration-dependent variation in the extent of specific depletion of fluorescence from catecholamine-containing neurons by 6-OHDA. Injections near preterminal axons caused more extensive depletion of the terminals than similar injections directly into the terminal area. There was no apparent difference in fluorescence levels or extent of depletion in animals killed either six or ten days postinjection. The most effective dose for specific depletion of terminals was 2 μl of 8 μg/μl.

Olfactory Tubercle

Injections of both volumes of the 2 lowered concentrations of 6-OHDA directly into the dopaminergic terminals of the olfactory tubercle produced no specific depletion of catecholamines, only nonspecific damage was seen (Table 1, Figure 1B cf. 1A). Higher doses of 6-OHDA (2 and 4 μl of 4 μg/μl) produced a partial decrease in fluorescence over a limited area (about 0.6 mm radius) from the cannula tip. After 2 μl of 8 μg/μl there was a decrease in fluorescence extending over a maximum distance of approximately 0.8 mm from the tip of the cannula (Figs. 1C, 1D). However, no specific depletion was seen after injection of 4 μl of 8 μg/μl. Very little accumulation of fluorescence adjacent to the area of damage was seen in any of the treatment groups.

Posterior Lateral Hypothalamus

Animals in these treatment groups were injected in the posterior lateral hypothalamus about 0.7 mm caudal to the area of observation where the majority of the fluorescent

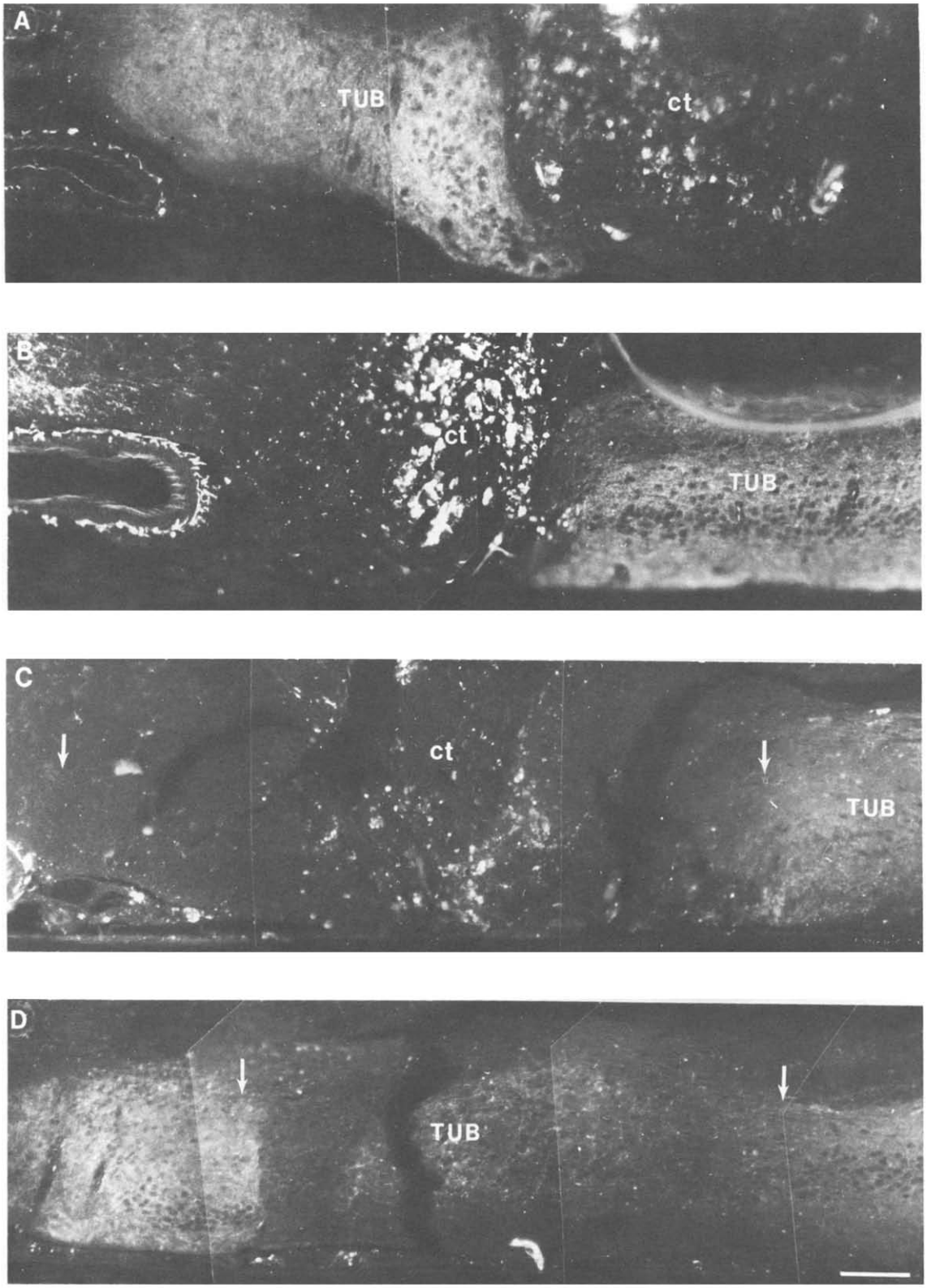

FIG. 1. Fluorescence histochemistry of the olfactory tubercle from rats killed after various treatments. Calibration bar = 100 μ. (A) 2 μl of placebo. Only cannulation damage is evident. (B) 2 μl of 1 μg/μl of 6-OHDA. No increase in area of damage when compared to (A). (C) 2 μl of 8 μg/μl of 6-OHDA. An area of specific depletion of catecholamines is seen (↓↓) surrounding the area of cannulation damage. (D) Section approximately 0.7 mm anterior to the section seen in (C). Note the area of specific depletion of catecholamines (↓↓). (TUB, olfactory tubercle; ct, cannula track).

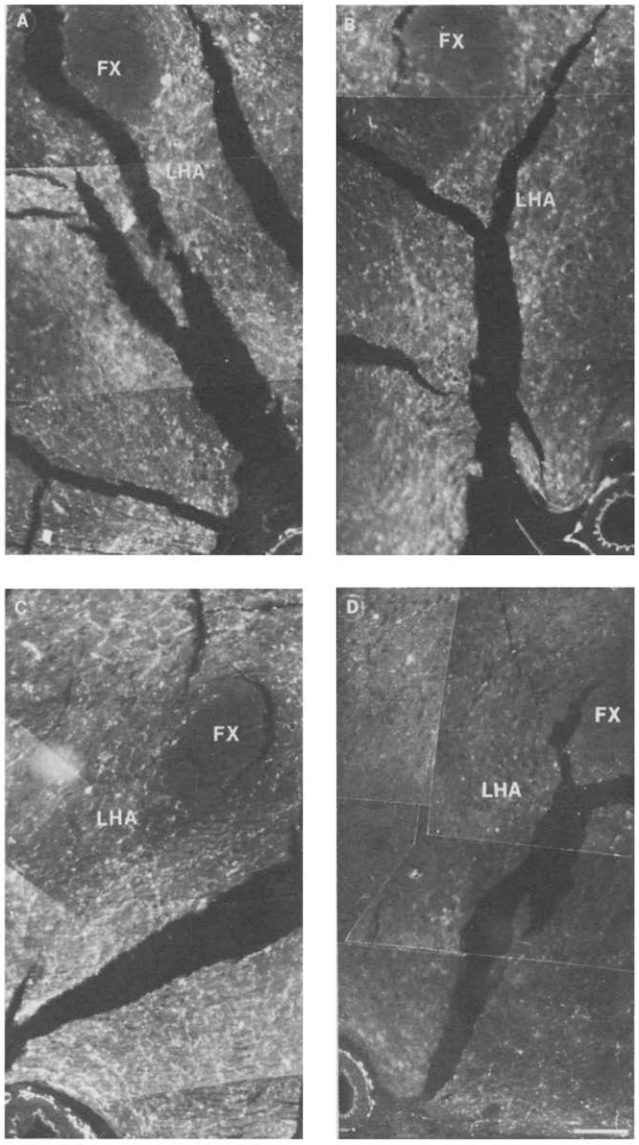

FIG. 2. Fluorescence micrographs of the lateral hypothalamus approximately 0.8 mm anterior to the site of injection in the posterior lateral hypothalamus. Calibration bar = 100 μ. (A) 2 μl of 1 μg/μl of 6-OHDA. (B) 4 μl of 1 μg/μl of 6-OHDA. (C) 2 μl of placebo. Fluorescence levels in (A), (B) and (C) are normal. (D) 2 μl of 8 μg/μl of 6-OHDA. Fluorescence levels in the LHA are considerably decreased. (FX, Fornix; LHA, Lateral Hypothalamus).

terminals of the lateral hypothalamus are located. No specific depletion of fluorescence was seen with both volumes of the lowest concentration (Figs. 2A, 2B cf 2C), or the 4 μl volume of 8 μg/μl. All the remaining doses caused considerable specific depletion of the hypothalamic terminals (Fig. 2D). The 2 μl volume of 8 μg/μl also caused partial depletion of terminals in the median eminence, the arcuate nucleus and the ventromedial hypothalamus. Accumulation of fluorescence in the proximal portions of axons severed by cannula insertion and injection of drug occurred in most animals. Greater accumulation occurred at those concentrations and volumes which were most effective in decreasing fluorescence.

In 4 animals (2 μl of 1 μg/μl, 2 × 2 μl of 4 μg/μl and 2 μl of 8 μg/μl), the area of generalised damage extended caudally into the dopaminergic cell bodies of the substantia nigra. There was no alteration in the fluorescence levels or morphology of these cell bodies, even those situated immediately adjacent to the area of nonspecific damage (Fig. 3A).

Lateral Hypothalamus

Injections into this area produced variable results. Partial depletion of adrenergic terminals was caused by the doses of 4 μl of 2 μg/μl, 2 μl of 4 μg/μl and 2 μl of 8 μg/μl. Depletion caused by the 2 μl volume of 8 μg/μl was limited to an area with a radius of approximately 0.8 mm from the cannula tip. The dopaminergic terminals of the caudate nucleus were also examined. The 2 μl injection of 8 μg/μl caused a total depletion of fluorescence of this structure (Fig. 3C cf. 3B), with smaller decreases in fluorescence levels seen with 2 and 4 μl volumes of the 2 and 4 μg/μl concentrations. The other doses did not affect the fluroescence levels of the caudate nucleus.

DISCUSSION

When using 6-OHDA injections into the CNS to achieve specific depletion of catecholamine-containing neurons, volume and concentration of the drug as well as anatomical locus of injection are important variables to be considered.

Of the 4 concentrations and 2 volumes used in this study, both volumes of the lowest concentration caused no loss of fluorescence outside the area of nonspecific damage. With increasing concentrations of 6-OHDA the extent of nonspecific damage remained approximately the same, while the area of specific depletion gradually increased to reach a maximum at 8 μg/μl. In an earlier study [6] it was shown that concentrations of 32 μg/μl produced a larger area of generalised damage but virtually no specific depletion of catecholamines. These findings indicate that too large or too small a dose of 6-OHDA causes only nonspecific damage while the medium range of doses is optimal for selective depletion of catecholamines.

Of equal importance, with regard to extent of the specific depletion caused by 6-OHDA, was the anatomical placement of the injection. In these cases where injections into terminal areas caused limited specific depletion, injection of the same dose near the preterminal axons often caused a more widespread loss of fluorescence. A schematic interpretation of the different effects of 6-OHDA injected into areas containing catecholaminergic axons and terminals is shown in Fig. 4.

Injections into the lateral hypothalamus had little effect upon noradrenergic terminals close to the site of injection while causing a considerable reduction in fluorescence in the olfactory tubercle and caudate nucleus. This could have been due to the close proximity of the nigro-striatal bundle passing through the lateral hypothalamus and terminating in the olfactory tubercle and caudate nucleus. Similarly, the decrease in fluorescence of the lateral hypothalamus following injections into the posterior lateral hypothalamus can be attributed to the drug acting on the ascending fibres of the dorsal and/or ventral noradrenergic bundles which terminate in the lateral hypothalamus.

The variation in extent of specific depletion in relation to the anatomical location of injection could be due to a

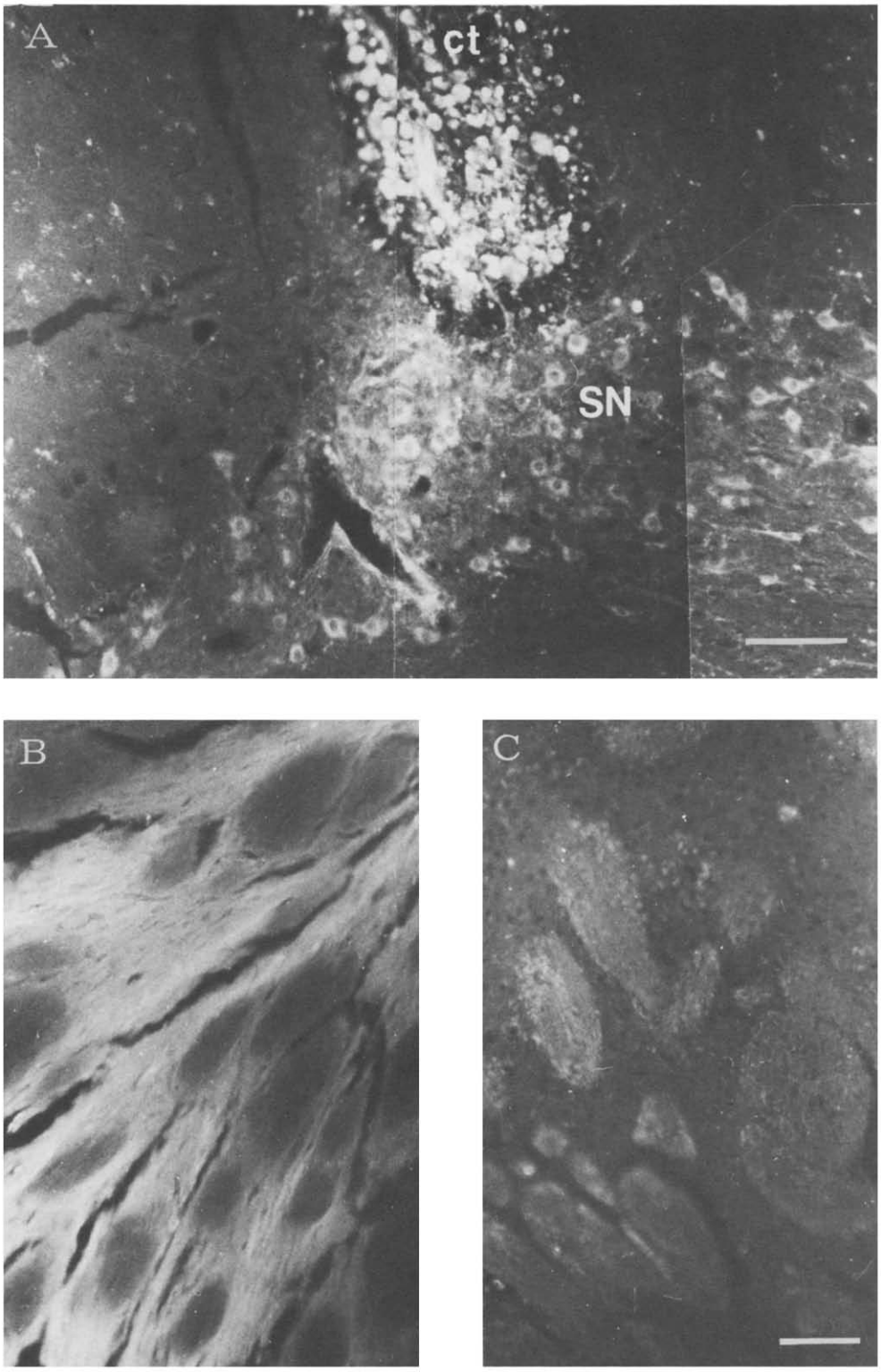

FIG. 3. Fluorescence micrographs of rat brain. Calibration bars = 100 μ. (A) 2 μl of 4 μg/μl of 6-OHDA. Non-specific damage in the zona compacta. Adjacent dopaminergic cell bodies appear normal. (B) Uninjected control. Normal fluorescence of the caudate nucleus. (C) 2 μl of 8 μg/μl of 6-OHDA. Specific depletion of fluorescence in the caudate nucleus after injection of 6-OHDA into the lateral hypothalamus. (SN, substantia nigra; ct, cannula track).

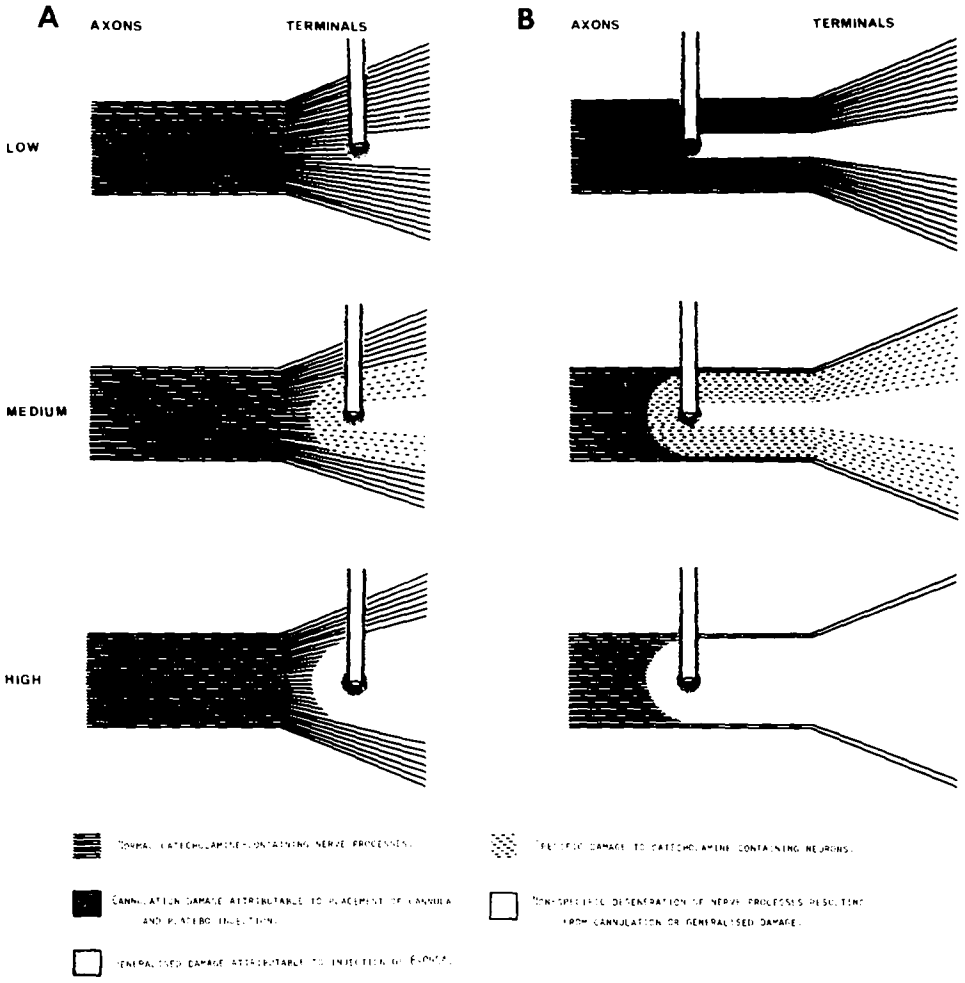

FIG. 4. Diagrammatic interpretation of damage resulting from injection of low, medium and high doses of 6-OHDA into areas containing catecholaminergic nerve terminals (A) and axons (B) in the CNS. This is based on the results of this study and an earlier study [6].

combination of the limited extent to which 6-OHDA diffuses [6] and the differences in its uptake and action on axons and terminals. When the drug is injected into an area where diffusion is the only means by which it is distributed to individual terminals then widespread depletion is unlikely to occur. However, when 6-OHDA is injected near axons, in addition to a comparable area of damage at the site of injection, there is also depletion of the larger terminal region supplied by these axons. This may be a result of axonal uptake and transport of 6-OHDA into nerve terminals which has been shown to occur in peripheral adrenergic nerves [5].

In four instances injections into the posterior lateral hypothalamus extended caudally into the dopaminergic cell bodies of the zona compacta but no changes in normal fluorescence or morphology were observed. This is in conflict with previous reports of the destruction of dopaminergic cell bodies of the substantia nigra by 6-OHDA [1, 7, 10, 12].

Considering the concentrations and volumes used in this study as well as the location of injection, the most widespread specific depletion of nerve terminals occurred after injection of the 8 μg/μl concentration of 6-OHDA into their preterminal axon bundles. Previous studies show that very high doses of 6-OHDA produce only generalised damage [6]. Low doses also cause generalised damage but there is a limited range of doses where 6-OHDA produces specific depletion of catecholamines. When using 6-OHDA as a tool in behavioural studies, it is necessary to show that specific damage to catecholamine-containing neurons has been achieved.

REFERENCES

1. Agid, Y., F. Javoy, J. Glowinski, D. Bouvet and C. Sotelo. Injection of 6-hydroxydopamine into the substantia nigra of the rat. II. Diffusion and specificity. *Brain Res.* 58: 291–301, 1973.

2. Armstrong, S., G. Burnstock, B. Evans and G. Singer. The effects of intrahypothalamic injections of guanethidine on catecholamine fluorescence, food intake and temperature regulation in the rat. *Pharmac. Biochem. Behav.* 1: 307–312, 1973.

3. Burnstock, G., B. Evans, B. J. Gannon, J. W. Heath and V. James. A new method of destroying adrenergic nerves in adult animals using guanethidine. *Br. J. Pharmac.* **43**: 295–301, 1971.
4. Butcher, L., S. Eastgate and G. Hodge. Evidence that punctate intracerebral administration of 6-hydroxydopamine fails to produce selective neuronal degeneration. *Arch. Pharmac.* **285**: 31–70, 1974.
5. Cobb, J. and T. Bennett. An electron nicroscopic examination of the short term effects of 6-hydroxydopamine on the peripheral adrenergic nervous system. In: *6-Hydroxydopamine and Catecholamine Neurons,* edited by T. Malmfors and H. Thoenen. Amsterdam: North-Holland Publishing Co., 1971.
6. Evans, B., S. Armstrong, G. Singer, R. Cook and G. Burnstock. Intracranial injection of drugs: comparison of diffusion of 6-OHDA and guanethidine. *Pharmac. Biochem. Behav.* **3**: 205–217, 1975.
7. Hökfelt, T. and U. Ungerstedt. Specificity of 6-hydroxydopamine induced degeneration of central monoamine neurons: an electron and fluorescence microscopic study with special reference to intracerebral injection of the nigra-striatal dopamine system. *Brain Res.* **60**: 269–297, 1973.
8. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
9. Poirier, L. J., P. Langelier, A. Roberge, R. Boucher and A. Kitsikis. Non-specific histopathological changes induced by the intracerebral injection of 6-hydroxydopamine (6-OH-DA). *J. Neurol. Sci.* **16**: 401–416, 1972.
10. Sotelo, C., F. Javoy, Y. Agid and J. Glowinski. Injection of 6-hydroxydopamine in the substantia nigra of the rat. I. A morphological study. *Brain Res.* **58**: 269–290, 1973.
11. Thoenen, H. and J. P. Tranzer. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **261**: 271–288, 1968.
12. Ungerstedt, U. 6-Hydroxydopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmac.* **5**: 107–110, 1968.
13. Ungerstedt, U. and G. W. Arbuthnott. Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigro striatal dopamine system. *Brain Res.* **24**: 485–493, 1970.
14. Uretsky, N. J. and L. L. Iversen. Effect of 6-hydroxydopamine on catecholamine-containing neurons in the rat brain. *J. Neurochem.* **17**: 269–278, 1970.