Pharmacology Biochemistry and Behavior, Vol. 3, pp. 219–228. ANKHO International Inc., 1975. Printed in the U.S.A.

Effects of Chronic Intracranial Injection of Low and High Concentrations of Guanethidine in the Rat[1,2]

BARBARA K. EVANS

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

G. SINGER, S. ARMSTRONG AND P. E. SAUNDERS

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

AND

G. BURNSTOCK[3]

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

(Received 19 February 1975)

EVANS, B. K., G. SINGER, S. ARMSTRONG, P. E. SAUNDERS AND G. BURNSTOCK. *Effects of chronic intracranial injection of low and high concentrations of guanethidine in the rat.* PHARMAC. BIOCHEM. BEHAV. 3(2)219–228, 1975. – Low (64 μg in 2 μl) or high (320–1280 μg in 2 μl) doses of guanethidine sulphate were injected daily for up to 19 days into the lateral hypothalamus, substantia nigra, locus coeruleus, dorsal raphe nucleus, or amygdala region of the rat brain. Effects on monoamine-containing neurons were determined using fluorescence histochemistry. The noradrenergic terminals of the hypothalamus were depleted over a diameter of 7 mm by both low and high doses of guanethidine whereas, even with high doses, the dopaminergic terminals of the median eminence, amygdala and caudate nucleus were only partially depleted. Fluorescence levels of dopaminergic cell bodies of the substantia nigra and 5HT-containing cell bodies of the dorsal raphe nucleus were unaltered by low doses of guanethidine. Low doses of guanethidine did not affect the fluorescence of the noradrenergic cell bodies of the locus coeruleus, however high doses caused a substantial reduction in fluorescence levels. Normal levels of fluorescence were observed in all catecholamine-containing neurons within 14 days from cessation of injections. Thus, the axon retraction and eventual degeneration of peripheral sympathetic adrenergic neurons, which occurs as a result of chronic intraperitoneal injections of guanethidine, does not occur with the catecholamine-containing neurons in the central nervous system. The rapid recovery of central catecholamine-containing neurons is remarkable in view of the extensive areas of brain damage produced by chronic injection of such high concentrations of drug. Fluorescence in peripheral adrenergic nerves was unaffected by chronic injection of guanethidine into the lateral hypothalamus but adhesions of some internal organs were observed. Blood vessels in the vicinity of the cannula were heavily reinnervated by fluorescent fibres probably arising from intracranial catecholamine-containing neurons. Some of the advantages of intracranial injection of guanethidine compared to 6-hydroxydopamine for behavioral experiments are discussed.

Guanethidine Intracranial injection Catecholamine-depletion Lateral hypothalamus Amygdala Substantia nigra Dorsal raphe nucleus Locus coeruleus

---

[1] This work was supported by grants from the National Heart Foundation of Australia, the National Health and Medical Research Council and by Grant No. D65/15607 from the Australian Research Grant Committee.

[2] We are grateful to Louise Western and Linden Cooper for excellent technical assistance.

[3] Address from May 1974, Department of Anatomy, University College, London.

CHRONIC treatment with intraperitoneal injections of low doses of guanethidine depletes catecholamine stores, blocks nervous transmission and can produce damage, but not destruction, of adrenergic neurons in the peripheral nervous system which persists many months after cessation of treatment [11,12,14]. In contrast, chronic treatment with high doses of guanethidine produces retraction [15,16] and eventually destruction of whole sympathetic neurons [5,16]. However guanethidine does not cross the blood-brain barrier to any significant extent and thus noradrenaline levels of adrenergic neurons in the brain are unaltered or only temporarily reduced [3, 7, 8, 13, 21].

Intrahypothalamic injections of low concentrations of guanethidine deplete the terminals of central catecholamine-containing neurons, as shown by fluorescence histochemistry, and have pronounced effects on consumatory behavior and temperature regulation [1, 10]. However, these effects are short lasting and recovery of both catecholamine fluorescence and behavior is apparent within a week after cessation of treatment.

In the present study, these experiments have been extended to examine the effects of guanethidine injection on selected regions of the rat brain that contain different components of the monoaminergic systems, including the lateral hypothalamus (noradrenergic and dopaminergic terminals), the substantia nigra (dopaminergic cell bodies), the locus coeruleus (noradrenergic cell bodies), the dorsal raphe nucleus (5HT-containing cell bodies) and the amygdala (dopaminergic terminals) [22]. In addition, high concentrations have been injected to see if, as in the periphery, catecholamine-containing nerves are permanently damaged or destroyed.

METHOD

Animals

Naive, male Wister rats, weighing 250 g at the time of surgery, were housed in individual cages of wire mesh, in a continuously illuminated room, maintained at a temperature of 22° C.

Surgery

Animals were unilaterally or bilaterally prepared with indwelling cannulae aimed at one of the following 5 brain areas: the lateral hypothalamus, the dorsal raphe nucleus, the substantia nigra, the central nucleus of the amygdala and the locus coeruleus. The coordinates for these brain loci have been documented previously [11], except for the locus coeruleus and the dorsal raphe nucleus. Coordinates for the locus coeruleus were: AP −7.8mm, L ± 1.6mm and H −6.2mm, and the dorsal raphe: AP −5.6mm, L ± 0.8mm and H −5.5mm relative to bregma and in the plane of the Pellegrino and Cushman stereotaxic atlas [19].

Drugs and Solutions

Guanethidine sulphate (CIBA) was prepared in 4 concentrations. First a solution of 32 μg/μl in deionized distilled water was made isotonic to cerebrospinal fluid by addition of NaCl to 0.154 M. Injections of 0.154 M NaCl were used as placebo controls. Higher doses of 160 μg/μl, 320 μg/μl and 640 μg/μl were prepared, however, because of the limits of solubility, these doses appeared to be a mixture of solution and suspension of the drug. Due to the hypertonicity and low pH of these doses sodium sulphate was dissolved in H_2SO_4 adjusted to pH 5.5 and used as placebo control. Concentrations of 0.54M and 1.08M were prepared and stored at 4° C.

Procedure

Nine animals in the dorsal raphe nucleus group, 11 in the substantia nigra, 10 in the locus coeruleus, 10 in the lateral hypothalamus and 4 in the amygdala received treatment with the low dose of guanethidine (64 μg in 2 μl: bilaterally). Other animals from these groups received isotonic saline. Animals were put on the same food deprivation schedule and received the same injection procedure as has been reported previously [1]. A further 24 animals with hypothalamic implants received the higher concentrations. Four animals received 1280 μg in 2 μl of guanethidine daily for 12 days, 8 received 640 μg in 2 μl daily for 12 days, and 4 animals received 640 μg in 2 μl daily for 19 days. High doses of guanethidine were also given to rats cannulated in the locus coeruleus (640 μg in 2 μl, 4 rats) and amygdala (320 μg in 2 μl, 2 rats). Animals receiving low doses were bilaterally implanted and those receiving high doses were cannulated unilaterally. Preliminary work had shown that chronic bilateral high dose injections of guanethidine were lethal.

Low dose animals were sacrificed at 5 and 12 days during chronic treatment, and up to 12 days after the cessation of treatment. High dose animals were sacrificed on the last day of injection (12 or 19 days), and up to 23 days after the last injection.

Histochemistry

The Falck-Hillarp fluorescence histochemical method for localizing monoamines was used. Rats were guillotined and the brain quickly removed through the dorsal surface of the skull. After dissection, brain pieces of approximately 10 mm^3 were frozen in liquid propane cooled by liquid nitrogen and then freeze-dried at -38° C and 10^{-3} mm Hg, using P_2O_5 as a moisture trap, for 24–36 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 1½ 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 Otholux fluorescence microscope with an optical system as described elsewhere [5].

In this study no attempt was made to distinguish the specific fluorescences of adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine (5-HT fluorescence fades rapidly under control conditions). Anatomical references to neuron types and groups is that of Ungerstedt [22].

Various peripheral tissues from 8 rats injected with high doses of guanethidine (640 or 1280 μg in 2 μl) for 12 or 19 days were taken for fluorescence histochemical observation. The procedure was the same as that used for brain tissue excepting that incubation with paraformaldehyde was for 1 hr and sections were 10 μ thick. Tissues examined were right atrium, superior cervical ganglion, iris, pelvic ganglion and vas deferens.

The general appearance of the abdominal cavity was examined in all rats which received injections of high doses of guanethidine or the hypertonic placebo solutions.

RESULTS

Low Doses of Guanethidine – 64 μg in 2 μl

Placebo. Fluorescence of catecholamine containing neurons in placebo rats (injected with isotonic saline) was comparable to that of control, uncannulated rats. The density of the innervation was the same, as was the fluorescence intensity (Fig. 1A and C). There was a moderate degree of accumulation of fluorescence in the proximal portions of the severed axons caudal to the cannula due to the interruption of axonal flow [9]. The cannula track was approximately 1 mm in dia. and appeared as a region of orange autofluorescence due to necrosis and damage to the brain tissue [10].

Hypothalamus. There was a widespread depletion of catecholamines in the hypothalamus as shown by fluorescence histochemistry confirming the detailed results of earlier studies [1,10]. After 5 and 12 days chronic treatment there were no fluorescent fibres in the hypothalamus within a radius of at least 3 mm from the cannula. No fluorescent fibres were visible in the lateral hypothalamus, the arcuate nucleus, the paraventricular nucleus, the ventromedial nucleus or the anterior hypothalamus (Fig. 1 B and D). There was no accumulation of fluorescence adjacent to the cannula. The supraoptic nucleus, the ventral border of the median eminence and the A_{12} and A_{13} groups of dopaminergic cell bodies were resistent to the depleting action of guanethidine and still showed fluorescence following 12 days of treatment as reported in more detail elsewhere [12].

One day following cessation of treatment some fluorescence had returned to the perivascular adrenergic innervation of the pial arteries. Occasionally faintly fluorescent fibres were seen in the hypothalamus 2–3 mm from the cannula. By the second day of recovery a number of fluorescent fibres were seen throughout the hypothalamus and there was an accumulation of fluorescence caudal to the cannula. Twelve days after cessation of treatment the fluorescence of the hypothalamic region appeared normal.

Substantia nigra. After 5 days chronic treatment the dopaminergic neurons of the substantia nigra as close as 1 mm to the cannula still appeared normal. However the adrenergic innervation of the pial arteries on the ventral surface of the brain was totally devoid of fluorescence rostrally and caudally along the whole length of the tissue taken. Fluorescence levels in the hypothalamus were generally normal although some reduction in intensity was evident in the more caudal areas 2 mm and more from the cannula.

The A_9 dopaminergic neurons of the substantia nigra were still normally fluorescent after 12 days chronic treatment (Fig. 2D) and the ventral pial arteries totally depleted of fluorescence. There was now a considerable reduction in fluorescence levels of terminals in the hypothalamus to less than 50 percent of control levels including the paraventriculus, lateral hypothalamus, arcuate nuclei and the dorsal portion of the median eminence. As with the injections into the lateral hypothalamus, the supra-optic nucleus and ventral portion of the median eminence showed normal fluorescence.

One day following cessation of treatment the adrenergic innervation of the ventral pial arteries had recovered the majority of its fluorescence. By the fourth day of recovery the overall fluorescence of the substantia nigral region and the hypothalamus was normal. Fluorescent fibres were seen close to the cannula tracks and a small amount of accumulation of fluorescence caudal to the track was visible.

Locus coeruleus. Fluorescence levels in the A_6 group of noradrenergic cell bodies after 5 or 12 days chronic guanethidine and during recovery were comparable to those in placebo animals (Fig. 2A). The latero-ventral A_5 group also showed good fluorescence throughout treatment and recovery. The adrenergic innervation of the ventral pial arteries located 3–5 mm from the tip of the cannula was not depleted of catecholamines.

Dorsal raphe nucleus. The fluorescence of the 5HT-containing neurons of the dorsal raphe nucleus in placebo rats was considerably fainter than that of noradrenergic and dopaminergic neurons. There was no apparent change in fluorescence after 5 or 12 days of guanethidine treatment (Fig. 2C). The fluorescent innervation of the ventral pial arteries 3–5 mm from the cannulae was also unaffected by guanethidine.

Amygdala. The dopaminergic terminals of the caudate nucleus (Fig. 2E) and the central amygdaloid nucleus were also apparently unaffected after 13 days treatment. The adjacent hypothalamic areas and the pial artery ventral to them showed normal fluorescence levels.

Behavioral studies. Chronic guanethidine injections (64 μg in 2 μl) into the lateral hypothalamus resulted in a reduction of food and water intake and an increase in body temperature: after cessation of treatment these parameters returned to preinjection base levels [1].

In contrast, injections of guanethidine into the substantia nigra, locus coeruleus or dorsal raphe nucleus were shown in the present study to have no effects on food and water intake and temperature regulation; this is consistent with the lack of reduction of fluorescence levels.

High Doses of Guanethidine – 320, 640 or 1280 μg in 2 μl

Placebo. Placebo rats received chronic unilateral injections of either 0.54 or 1.08 M Na_2SO_4 solution. The fluorescence of catecholamine-containing nerves was comparable to that seen in control animals. The cannula track was brightly autofluorescent, indicating necrosis and damage to the surrounding brain tissue. This region of cannulation damage was approximately 1.3 mm in dia. and was generally brighter than the autofluorescent cannula tracks of the low dose placebo rats. There was an accumulation of specific fluorescence caudal to the cannula track (Fig. 3).

Hypothalamus. Chronic unilateral injection of guanethidine (640 or 1280 μg in 2 μl) for 12 or 19 days resulted in a large area of autofluorescent necrosis and generalized damage [12] approximately 4 mm across. This autofluorescent area occupied the majority of the ipsilateral hypothalamus and no fluorescent fibres were seen. Contralaterally only an occasional faintly fluorescent fibre was seen in the lateral hypothalamus, ventromedial hypothalamus and the median forebrain bundle. The contralateral supraoptic nucleus still showed bright fluorescence. Fluorescence was still evident in the A_{12} cell bodies of the arcuate nucleus, the ventral border of the median eminence (Fig. 5C) and the dopaminergic cell bodies of the ipsilateral substantia nigra. The dopaminergic terminals of the ipsilateral caudate nucleus showed a small reduction in fluorescence intensity. Fluorescent nerves were not seen in the ventral pial arteries.

Seven days following cessation of injections the auto-

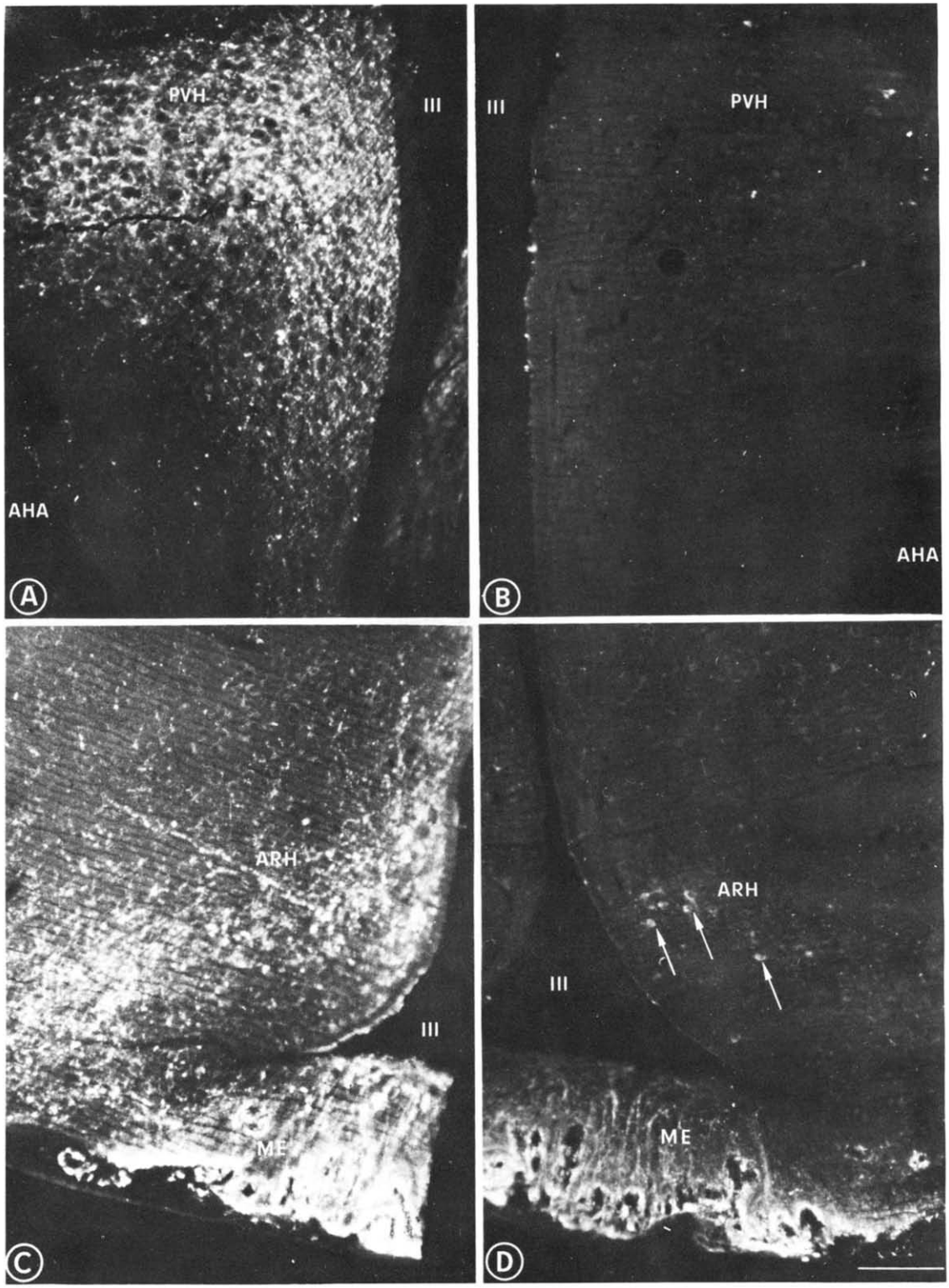

FIG. 1. Fluorescence histochemistry of the hypothalamic area of the rat brain. Rats had cannulae bilaterally implanted into the lateral hypothalamus and were killed after the following treatments. Calibration bar = 100 μ and applies to all figs. (A) Placebo daily for 12 days. Normal fluorescence levels are seen in the paraventricular nucleus. (B) Guanethidine (64 μg in 2 μl) daily for 5 days. The paraventricular nucleus is devoid of fluorescence. (C) Placebo daily for 12 days. Normal fluorescence levels are present in the arcuate nucleus and median eminence. (D) Guanethidine (64 μg in 2 μl) daily for 5 days. Fluorescent nerve terminals in the arcuate nucleus and dorsal region of the median eminence are depleted. Note that some fluorescence remains in the ventral border of the median eminence and in the A_{12} cell bodies (arrows). (PVH, paraventricular nucleus of the hypothalamus; AHA, anterior area of the hypothalamus; ARH, arcuate nucleus of the hypothalamus; ME, median eminence; 111, third ventricle.)

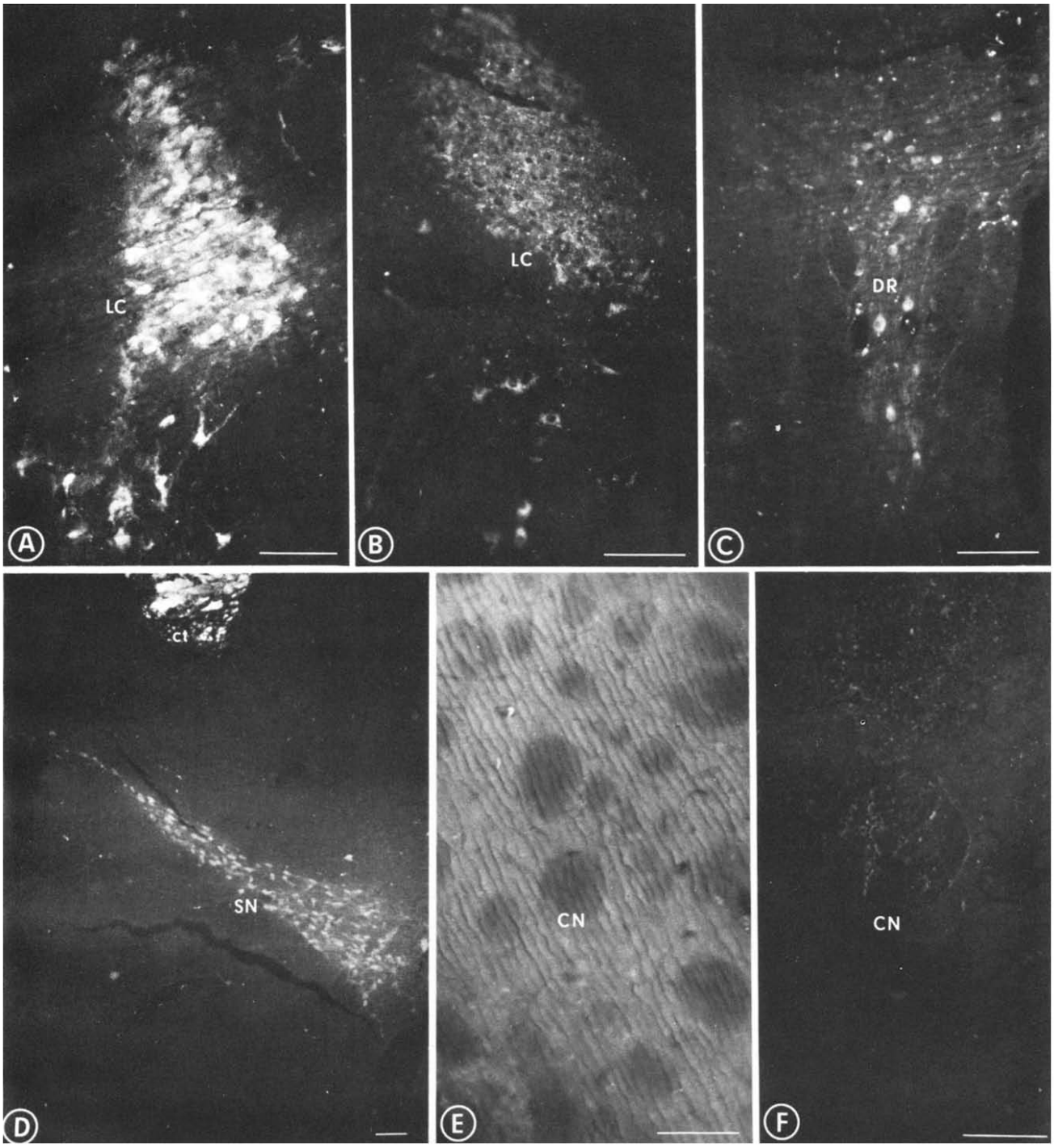

FIG. 2. Fluorescence histochemistry of the rat brain. Calibration bars = 100 μ. (A) and (B) Rats cannulated unilaterally into the locus coeruleus and killed after the following treatments. (A) Guanethidine (64 μg in 2 μl) daily for 12 days. Normal fluorescence is seen in the A_6 cell bodies of the locus coeruleus. (B) Guanethidine (320 μg in 2 μl) daily for 13 days. Fluorescence levels of the A_6 cell bodies of the locus coeruleus are reduced to about 50 percent. (C) Dorsal raphe nucleus. Rat cannulated into the dorsal raphe nucleus and killed after guanethidine injections (64 μg in 2 μl) daily for 12 days. Fluorescence levels are normal. (D) Substantia nigra. Rat cannulated bilaterally in the substantia nigra and killed after guanethidine injections (64 μg in 2 μl) daily for 12 days. Fluorescence levels in the A_9 cell bodies of the substantia nigra are normal. (E) and (F). Rats cannulated into the amygdala and killed after the following treatments. (E) Guanethidine (64 μg in 2 μl) daily for 13 days. The normal diffuse fluorescence of the caudate nucleus is evident. (F) Guanethidine (320 μg in 2 μl) daily for 13 days. Fluorescence in the caudate nucleus is absent. (LC, locus coeruleus; DR, dorsal raphe nucleus, SN, substantia nigra; CN, caudate nucleus; ct, cannula track.)

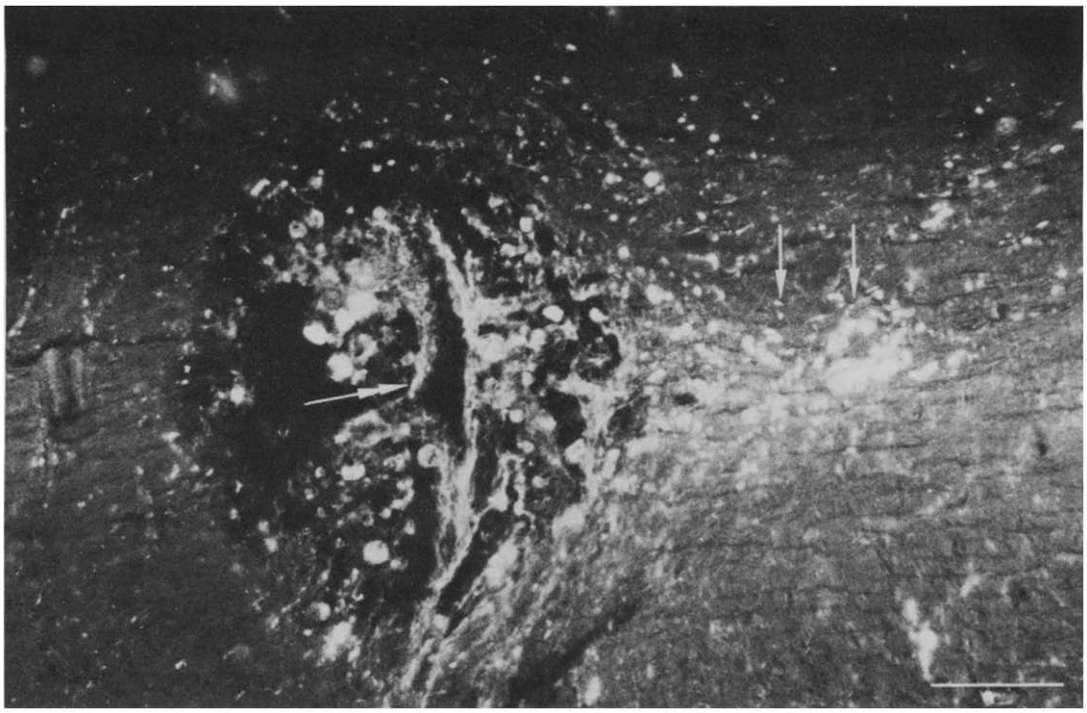

FIG. 3. Fluorescence histochemistry of cannula track located in the lateral hypothalamus. Rat was killed following hypertonic placebo injections (1.08 M Na_2SO_4) daily for 12 days. Note the accumulation of fluorescence in proximal portions of severed axons (single arrows). Note also the large blood vessel with plexus of fluorescent nerve terminals invading the region of cannulation damage (double headed arrow). Calibration bar = 100 μ.

fluorescent area of nonspecific damage had reduced considerably (Fig. 4). Fluorescence was absent throughout most of the ipsilateral hypothalamus, however an occasional fluorescent fibre was seen in the paraventriculus and along the base of the brain (Figs. 4 and 5A). Contralaterally there was a greater recovery of fluorescence levels. The ventral pial arteries showed normal brightly fluorescent adrenergic innervation as did the supraoptic nucleus and the median eminence (Fig. 4). There was a considerable accumulation of fluorescence caudal to the cannula track in the proximal portions of severed axons.

After 14 days of recovery, the generalized damage around the cannula track had reduced to an area more comparable to that seen after placebo injections. Most regions of the ipsilateral and contralateral hypothalamus showed substantial fluorescent adrenergic innervation although still somewhat less than in placebo animals (Figs. 5B and D). This situation persisted 24 days following cessation of injections.

Locus coeruleus. Unilateral injection of guanethidine (320 or 640 μg in 2 μl) chronically for 10 or 13 days caused a partial bilateral reduction in the intensity of fluorescence, to about 50 percent, particularly on the ipsilateral side, of the A_6 group of noradrenergic cell bodies of the locus coeruleus (Fig. 2B). The latero-ventral A_5 group was apparently unaffected by the treatment and anterior to the track the substantia nigral dopaminergic neurons (A_9 and A_{10}) showed normal fluorescence. Adrenergic terminals in the more caudal regions on the hypothalamus showed some reduction in fluorescence whereas the anterior hypothalamus and nucleus striae terminalis showed normal fluorescence. Ventral pial arteries were totally depleted in all sections. There was no accumulation of fluorescence in the vicinity of the cannula track which was seen as an autofluorescent region of generalized damage [12] approximately 2 mm in dia.

Nine days after the last injection, the appearance of catecholamine-containing cell bodies and terminals throughout the brain was comparable to that seen in placebo animals. There was no evidence of fluorescence accumulation in the vicinity of the cannula track. The autofluorescent region of generalized damage associated with the cannula was now approximately 1.5 mm in dia.

Amygdala. Thirteen days of chronic unilateral guanethidine injections (320 μg in 2 μl) into the amygdala depleted the dopaminergic terminals of the caudate nucleus (Fig. 2F) and reduced fluorescence levels in the central amygdaloid nucleus. The fluorescence of the ipsilateral anterior amygdaloid area and caudate nucleus 2–3 mm anterior to the cannula, and the contralateral catecholamine-containing terminals was comparable to the placebo situation. There was a small decrease in fluorescence levels in the ipsilateral hypothalamus. The ventral pial arteries showed a normal fluorescent innervation in all sections.

Nine days following the last injection, fluorescence had returned to the central amygdaloid nucleus and the majority of the caudate nucleus except in the immediate vicinity of the cannula.

Examination of peripheral organs. Normal fluorescence levels were seen in the adrenergic nerves of the atrium,

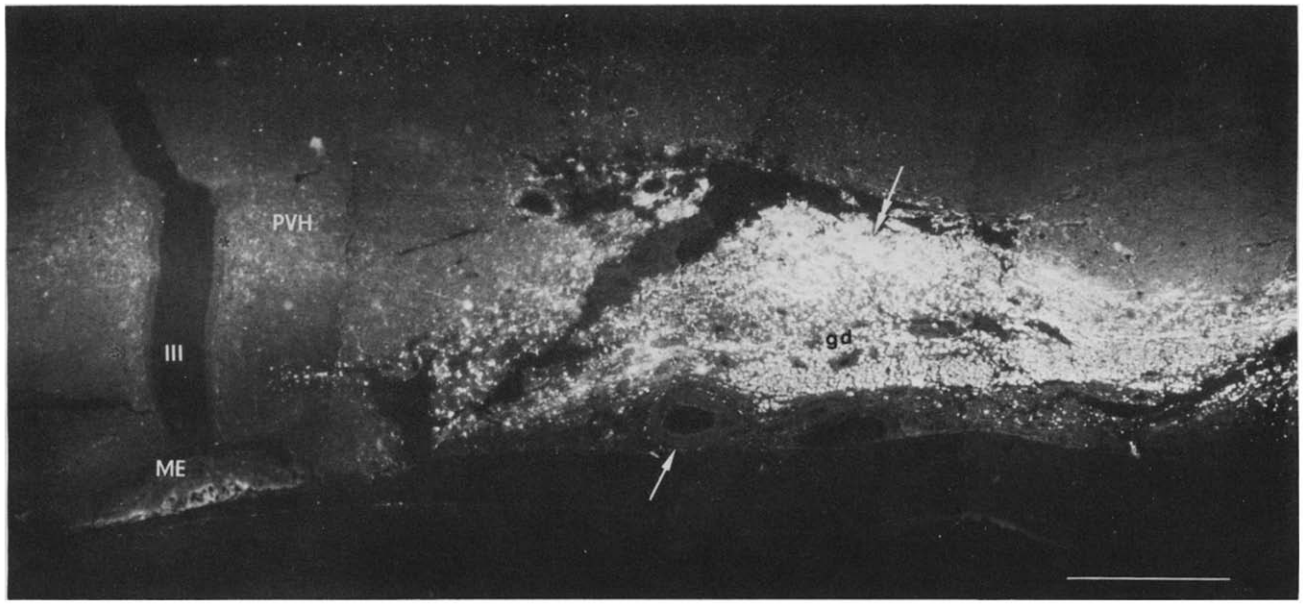

FIG. 4. Fluorescence micrograph showing the extensive generalized damage following unilateral injection of high doses of guanethidine (1280 μg in 2 μl) into the lateral hypothalamus daily for 12 days. Rat was killed 7 days after the last injection. Levels of specific fluorescence throughout the hypothalamus are low. Note the large extent of autofluorescent generalized damage [10]. Fluorescent terminals are evident in the ventral border of the median eminence and some fluorescence has returned to the nerve terminals of the paraventricular nuclei and the adrenergic plexus of the ventral pial artery (single arrow). Accumulation of fluorescence (double headed arrow) is evident in the proximal portions of severed axons within and adjacent to the area of generalized damage. Calibration bar = 500 μ. (PVH, paraventricular nucleus of the hypothalamus; ME, median eminence; gd, generalized damge, lll, third ventricle.)

superior cervical ganglion, iris, pelvic ganglion and vas deferens from rats injected into the lateral hypothalamus with guanethidine (640 or 1280 μg in 2 μl) daily for 12 or 19 days.

Post mortem examinations of the peritoneal body cavity showed that many of the guanethidine injected rats had large adhesions between lobes of the liver, mesentery, epididymal fat and other organs. Such adhesions were only rarely seen in placebo animals.

DISCUSSION

Chronic intracranial injection of guanethidine in the rat produced greater effects on noradrenaline-containing neurons compared to those containing dopamine. At low doses (64 μg in 2 μl), noradrenaline terminals of the hypothalamic region were depleted quickly and over a large area. However the dopamine stores of the caudate nucleus, amygdala and median eminence were apparently unaffected by these doses. In fact, even high doses of guanethidine (320–1280 μg in 2 μl) did not deplete the dopaminergic neurons of the median eminence and substantia nigra, although they caused a marked reduction of fluorescence in the caudate nucleus and amygdala regions. A similar situation has been reported following intracranial 6-OHDA injection where noradrenergic neurons were affected at lower doses while higher doses [22] and pargyline (to reduce 6-OHDA deamination) [4] were required to cause similar effects to dopaminergic neurons. 6-Hydroxydopa also affected noradrenergic and dopaminergic neurons differentially [20]; the dose sufficient to affect dopaminergic neurons was 4 times that required to similarly affect noradrenergic neurons. Differences between the properties of the amine uptake pump of noradrenergic and dopaminergic neurons in the central nervous system have been reported [6]. Since both guanethidine and 6-OHDA are concentrated within the neuron by this pump, the differing sensitivities of dopaminergic and noradrenergic neurons may reflect a different degree of uptake of these neurotoxic drugs.

Monoamine-containing cell bodies in the hypothalamus and the substantia nigra (dopamine), the locus coeruleus (noradrenaline) and the dorsal raphe nucleus (5-HT) were not depleted by low doses of guanethidine, nor was there any change in food and water intake and temperature regulation. At high doses, noradrenergic cell bodies showed a partial reduction of fluorescence but there was no apparent change in fluorescence levels of dopaminergic cell bodies.

A striking difference has been shown between the effects of guanethidine on noradrenergic neurons of the central and peripheral nervous systems (see Fig. 6). Chronic intraperitoneal injections of low doses of guanethidine (5 mg/kg/day) caused long-lasting damage to the short adrenergic neurons of the pelvic plexus [11, 12, 14]. Even one year after cessation of injections, noradrenaline levels in these cells were only about 10 percent of normal. At higher doses (30–60 mg/kg/day) this depletion was followed by retraction of nerve fibres and eventually the degeneration of more than 95 percent of all peripheral noradrenergic neurons [5, 15, 17]. In contrast, even with the high doses used, guanethidine only partially depleted the noradrenergic cell bodies in the locus coeruleus. Fluorescence returned to these cell bodies and also to the terminals in the hypo-

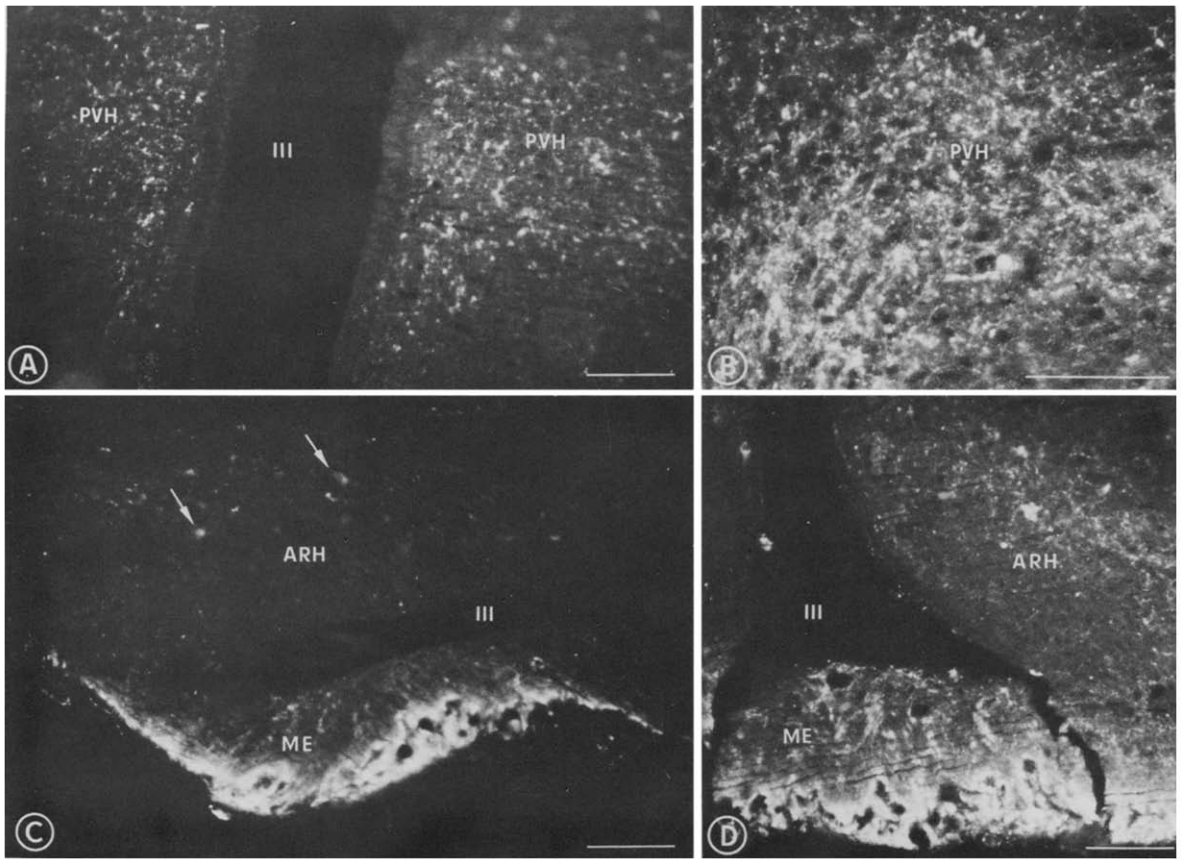

FIG. 5. Fluroescence histochemistry of the hypothalamic area of the rat brain. Rats were unilaterally cannulated into the lateral hypothalamus and killed after the following treatments. Calibration bars = 100 μ. (A) Rat killed 7 days after guanethidine (1280 μg in 2 μl) daily for 12 days. Note that there is some return of fluorescence to the terminals of the paraventricular nucleus. (B) Rat killed 14 days after guanethidine (640 μg in 2 μl) daily for 12 days. Fluorescence of the paraventricular nucleus is now almost normal. (C) Guanethidine (640 μg in 2 μl) daily for 19 days. Fluorescence levels are generally depleted excepting the ventral border of the median eminence and the A_{12} cell bodies of the arcuate nucleus (arrows). (This section is oblique instead of transverse.) (D) Rat killed 14 days after guanethidine (640 μg in 2 μl) daily for 12 days. Fluorescence of the arcuate nucleus and median eminence is now almost normal. (PVH; paraventricular nucleus of the hypothalamus; ARH, arcuate nucleus of the hypothalamus; ME, median eminence; 111, third ventricle.)

thalamus within days and fluorescence levels were comparable to normal after only 14 days. There is no evidence, therefore, for a degenerative effect of guanethidine on these central neurons. Retraction of nerve terminals apparently does not take place since almost immediately upon cessation of injections there was an accumulation of fluorescence due to axonal flow [9] in the proximal portions of severed axons adjacent to the cannula track. Also the return of fluorescence is seen as a slow increase in intensity of fluorescence over an apparently normal density of innervation. These results suggest that there are some basic differences in the amine uptake pump, noradrenaline storage and/or general metabolism of the central noradrenergic neurons as compared to the noradrenergic neurons of the peripheral sympathetic nervous system. Marked differences in the sensitivity of central and peripheral noradrenergic neurons to agents which block the amine uptake pump have been shown [6]. The different properties are perhaps to be expected since central and peripheral noradrenergic neurons almost certainly have a different developmental origin.

The unusually bright and numerous fluorescent fibres observed in the present study innervating blood vessels adjacent to the cannula may represent a collateral sprouting of central monoamine neurons to innervate the proliferating blood vessels often seen in the vicinity of the cannula [10]. It has been suggested that regenerating central catecholamine neurons may be able to innervate the walls of intracranial vessels [2]. The adrenergic supply to pial vessels arises in the superior cervical ganglion and in normal circumstances some intracranial vessels also show adrenergic innervation from this source [18].

The use of intracranial guanethidine injection as a tool in the study of the central nervous system and in particular behavior relationships has certain advantages. For example, in low doses it appears to be more selective in the depletion of catecholamine neurons than does 6-OHDA, it causes only minimal generalized damage [12] and does not appear to affect dopamine neurons. Thus, low doses seem to have possibilities where a specific and comparatively widespread depletion of noradrenergic nerve terminals is required. One

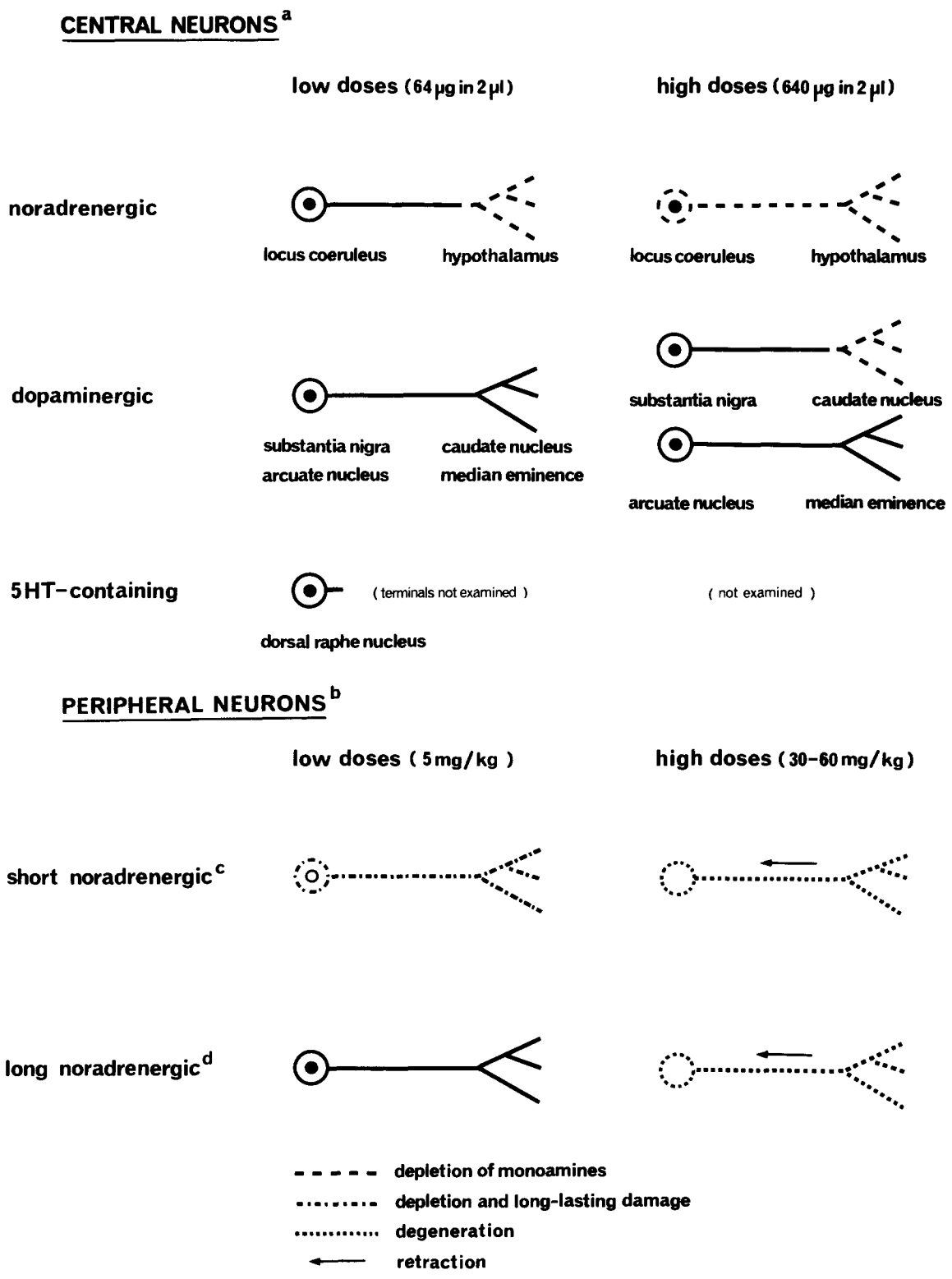

FIG. 6. Sensitivity of monoamine-containing neurons to chronic guanethidine injections. (a) intracranial injections, 12–19 days. (b) intraperitoneal injections, 4–12 weeks. (c) short noradrenergic neurons located in the pelvic plexuses. (d) long noradrenergic neurons comprising the remainder of the sympathetic system.

further advantage is that the return of normal fluorescence upon cessation of injections allows a further experimental control. However, chronic injection of high doses is of little value due to the massive damage and incapacitating effects on the animals behavior.

REFERENCES

1. 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.
2. Björklund, A. and U. Stenevi. Growth of central catecholamine neurons into smooth muscle grafts in the rat mesencephalon. *Brain Res.* **31**: 1–20, 1971.
3. Boura, A. L. and A. F. Green. Adrenergic neuron blocking agents. *Rev. Pharmac.* **5**: 183–212, 1965.
4. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **174**: 413–420, 1970.
5. 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.
6. Carlsson, A. Effects of drugs on amine uptake mechanisms in the brain. In: *New Aspects of Storage and Release Mechanisms of Catecholamines,* edited by H. J. Schümann and G. Kroneberg. Berlin: Springer Verlag, 1970, pp. 223–233.
7. Cass, R. and B. A. Callingham. Some effects of drugs which influence sympathetic transmission on tissue catecholamine levels in the rat. *Biochem. Pharmac.* **13**: 1619–1625, 1964.
8. Dagirmajian, R. The effects of guanethidine on the noradrenaline content of the hypothalamus in the rat and cat. *J. Pharm. Pharmac.* **15**: 516–521, 1963.
9. Dahlström, A. and K. Füxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta physiol. scand.* **62**: suppl. 232: 4–55, 1964.
10. Evans, B. K., S. Armstrong, G. Singer, R. D. Cook and G. Burnstock. Intracranial injections of drugs: comparison of diffusion of 6-OHDA and guanethidine. *Pharmac. Biochem. Behav.* **3**: 205–217, 1975.
11. Evans, B., B. J. Gannon, J. W. Heath and G. Burnstock. Long-lasting damage to the internal male genital organs and their adrenergic innervation in rats following chronic treatment with the antihypertensive drug guanethidine. *Fert. Steril.* **23**: 657–667, 1972.
12. Evans, B., T. Iwayama and G. Burnstock. Long-lasting supersensitivity of the rat vas deferens to norepinephrine after chronic guanethidine administration. *J. Pharmac. exp. Ther.* **185**: 60–69, 1973.
13. Furst, C. I. The biochemistry of guanethidine. *Adv. Drug Res.* **4**: 133–161, 1967.
14. Gannon, B. J., T. Iwayama, G. Burnstock, J. Gerkens and M. L. Mashford. Prolonged effects of chronic guanethidine treatment on the sympathetic innervation of the genitalia of male rats. *Med. J. Aust.* **2**: 207–208, 1971.
15. Heath, J. W., B. K. Evans and G. Burnstock. Axon retraction following guanethidine treatment. Studies of sympathetic neurons *in vivo*. *Z. Zellforsch.* **146**: 439–451, 1973.
16. Heath, J. W., B. K. Evans, B. J. Gannon, G. Burnstock and V. B. James. Degeneration of adrenergic neurons following guanethidine treatment: An ultrastructural study. *Virchows Arch path. Anat. Physiol.* **II**: 182–197, 1972.
17. Heath, J. W., C. E. Hill and G. Burnstock. Axon retraction following guanethidine treatment: studies of sympathetic neurons in tissue culture. *J. Neurocytol.* **3**: 263–276, 1974.
18. Owman, Ch., L. Edvinsson, B. Falck and K. C. Nielsen. Amine mechanisms in brain vessels, with particular regard to autonomic innervation and blood-brain barrier. In: *Pathology of Cerebral Microcirculation,* edited by J. Cervós–Navarro. Berlin: Walter de Gruyter and Co., 1974, pp. 184–199.
19. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
20. Sachs, C. and G. Jonsson. Changes in central noradrenaline neurons after systemic 6-hydroxydopamine administration. *J. Neurochem.* **21**: 1517–1524, 1973.
21. Sanan, S. and M. Vogt. Effect of drugs on the noradrenaline content of brain and peripheral tissues and its significance. *Br. J. Pharmac Chemother.* **18**: 109–127, 1962.
22. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand. Suppl.* **367**: 1–48, 1971.