

INTRANEURAL DISTRIBUTION OF EXOGENOUS NOREPINEPHRINE IN THE CENTRAL NERVOUS SYSTEM OF THE RAT

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ABSTRACT

Catecholaminergic neurons, which take up and retain exogenous norepinephrine labeled with tritium, were studied by means of high resolution radioautography, in the substantia nigra, the substantia grisea periventricularis, and the locus coeruleus of the rat. Under the conditions required for the radioautographic detection of exogenous norepinephrine- ^3H , it was established that (1) glutaraldehyde was the most suitable fixative for preserving the labeled amine *in situ*; (2) norepinephrine- ^3H itself, rather than metabolites, accounted for most of the reactions detected in catecholaminergic neurons. At various time intervals after an intraventricular injection of norepinephrine- ^3H , the tracer reached a concentration 15–100 times higher, and disappeared at a slower rate, in presynaptic axons ($t_{1/2}$: 4 hr) than in nerve cell bodies ($t_{1/2}$: 0.8–1.3 hr). After pretreatment with a monoamine oxidase inhibitor, the radioautographic reactions increased and persisted longer, especially in the preterminal axons. Within neurons, the labeled amine was ubiquitously distributed in the nerve cell body and concentrated in presynaptic axons and synaptic terminals of various morphological types. Although large granular vesicles were usually present in the labeled axonal bulbs, no structural characteristic could be specifically ascribed to catecholaminergic neurons. It is suggested that exogenous norepinephrine bound to macromolecular complexes is present in all parts of catecholaminergic neurons and mainly concentrated within presynaptic axons.

INTRODUCTION

Catecholamines, synthesized in neurons, may act as neurotransmitters in the central nervous system (31). Nerve cells which normally contain endogenous catecholamines have the ability to take up and retain exogenous norepinephrine, labeled and injected in the lateral ventricle of the rat brain (18, 16, 29). Thus, reaping profit from the neuronal uptake of norepinephrine- ^3H , high-resolution radioautography (1, 2, 9) may provide new information which cannot be derived from neurochemical and histofluorescence methods alone: the ultrastructural distribution and turn-

over of exogenous norepinephrine within neurons maintained intact in their natural environment.

MATERIALS AND METHODS

Nine adult male Wistar rats (body weight 215 ± 15 g), pretreated or not with a monoamine oxidase (MAO)¹ inhibitor (β -phenylisopropyl hydrazine,

¹ The following abbreviations were used in this paper: SN, substantia nigra; SGP, substantia grisea periventricularis; LC, locus coeruleus; MAO, monoamine oxidase.

Catron, 5 mg/kg i.p., 18 and 2 hr before), were lightly anesthetized with sodium pentobarbital and given 500 μ Ci of DL-norepinephrine- 3 H in the left lateral ventricle of the brain² (Table I, Exp. 1-9). One of the pretreated rats received a single dose of reserpine (Serpasil^R, Ciba, Fairlawn, N.J., 10 mg/kg i.p.) 30 min after norepinephrine- 3 H (Exp. 7). In a control experiment, 500 μ Ci of DL-normetanephrine- 3 H, instead of norepinephrine- 3 H, were administered to another animal pretreated with MAO inhibitor (Exp. 10). In each case, the tracer was diluted in 70 μ l of saline and injected for 4 min under stereotaxic control (26, 4).

At various time intervals after label administration, awake rats were reanesthetized with chloral hydrate and the central nervous system of all animals was fixed (see Table I). In Exp. 1-7 and 10, this was done in vivo by intraaortic high-flow rate perfusion of 3.64% glutaraldehyde in 0.05 M phosphate buffer (11) for 30 min, followed by a 30-min immersion of the whole brain in the same solution. Coronal brain slices were then postfixed with 2% OsO₄ in 0.1 M phosphate buffer for 2-3 hr. In one rat given norepinephrine-8- 3 H (Exp. 8), the perfusate was 1% glutaraldehyde + 1% formaldehyde in 0.3 M phosphate buffer with CaCl₂ added, and the slices were either postfixed directly with 2% OsO₄ in 0.3 M phosphate buffer containing 0.4 M sucrose, or immersed for 4 hr in 5% K₂Cr₂O₇ buffered to pH 4.1 with 0.2 M sodium lactate prior to osmium tetroxide postfixation (36, 35). In another animal injected with norepinephrine-8- 3 H (Exp. 9) and sacrificed by decapitation, thin slices of the fresh brain were immersed in glutaraldehyde (1 hr) and postfixed in osmium tetroxide (2 hr), or fixed for 45 min in chilled 3% KMnO₄ (30).

For the estimation of the amount of tracer retained in tissue, symmetrical paraventricular fragments of the caudate nuclei, excised prior to postfixation, were dissolved in hyamine and measured for radioactivity by scintillation.

Three regions known to contain catecholaminergic neurons (6, 12) were studied by light and electron microscopic radioautography: the substantia nigra (SN), the substantia grisea periventricularis (SGP) of the midbrain, and the locus coeruleus (LC).

All specimens were dehydrated in ethanol and embedded in Epon, care being taken to preserve comparable orientation of similar tissue samples,

² DL-norepinephrine-7- 3 H, specific activity: 7.27 Ci/mm and DL-normetanephrine-7- 3 H, specific activity: 4.1 Ci/mm, were obtained from New England Nuclear Corp., Boston. DL-norepinephrine-8- 3 H, specific activity: 15 Ci/mm, was obtained from the Commissariat à l'Energie Atomique, Centre d'Etudes Nucléaires de Saclay, France.

from one experiment to another. After polymerization (16 hr at 60°C), 2- μ -thick sections were cut from each block, deposited on glass slides, and coated by dipping (24) in Ilford emulsion K-5 diluted 1:1. After 1 wk of exposure, the radioautographs were processed in D-19, poststained with toluidine blue, and examined by light microscopy.

Precise areas were then chosen for electron microscopic examination. The blocks were retrimmed accordingly, polymerized further (14-40 hr at 60°C), and cut with glass or diamond knives on the LKB Ultratome. Ribbons of regularly thin sections (silver) were deposited on glass slides bearing a celloidin film (19), double stained with uranyl acetate and lead citrate, lightly vaporized with carbon (32), and coated by dipping in emulsion Ilford L-4 diluted 1:4. After 3-8 wk of exposure, the radioautographs were processed in Microdol X (Eastman Kodak Co.), collected on copper grids, and examined with a Siemens Elmiskop 1, after the celloidin membrane had been thinned in isoamyl acetate.

For quantitative evaluation, sections of similar thickness were prepared, exposed, and developed together; 30 labeled nerve cell bodies and/or presynaptic axons were studied in each region examined and at each time interval. They were systematically photographed at \times 4,000 and 8,000, respectively, and enlarged 2.6 times in prints. Silver grains were counted over the labeled neuronal parts, which were cut out of the photographs for the estimation of their surface by weighing. The concentration of the radioactivity was then expressed as the number of silver grains per unit area (Table II).

RESULTS

Distribution of Norepinephrine- 3 H in Glutaraldehyde-Fixed Tissue from Normal and Pretreated Rats

RADIOACTIVITY COUNTS IN THE CAUDATE NUCLEUS: The radioactivity counted in samples of the left and right caudate nuclei, fixed 5 min-18 hr after intraventricular injection of norepinephrine- 3 H, is given in Table I. The retention of the tracer was prominent on the injected left side (see reference 4). Amounts measured at 30 min and 3 hr when MAO was inhibited (Exp. 4 and 5) were roughly similar to those from untreated rats (Exp. 2 and 3). Reserpine markedly lowered the radioactivity retained after 3 hr (Exp. 7).

HIGH-RESOLUTION RADIOAUTOGRAPHY: In the SN, SGP, and LC, reached by the tracer through the third ventricle, the aqueduct of Sylvius, and the fourth ventricle, the distribution

TABLE I
Radioactivity of the Left and Right Caudate Nucleus after Intraventricular Injections of 500 μ Ci of Norepinephrine- 3 H (NE- 3 H)
and Normetanephrine- 3 H (NM- 3 H)

Values given in 10 ³ dpm/mg of tissue.										
Exp. No.	1	2	3	4	5	6	7	8	9	10
Tracer	NE-7- 3 H	NE-7- 3 H	NE-7- 3 H	NE-7- 3 H	NE-7- 3 H	NE-7- 3 H	NE-8- 3 H	NE-8- 3 H	NE-8- 3 H	NM-7- 3 H
Pretreatment	—	—	—	IMAO*	IMAO*	IMAO†	IMAO*+ reserpine	IMAO*	IMAO*	IMAO*
Time after injection	5 min	30 min	3 hr	30 min	3 hr	18 hr	3 hr	3 hr	3 hr	3 hr
Fixative	G§	G	G	G	G	G	G	GF§ or GF + K ₂ Cr ₂ O ₇	G or KMnO ₄	G
Left caudate	103	608	366	730	393	50	75	130	6	610
Right caudate	6	575	200	220	127	25	55	54	5.2	313

*IMAO: Inhibitor of monoamine oxidase; Catron (5 mg/kg) 18 and 2 hr before injection of the tracer.

† Catron (5 mg/kg) 7 hr before, and 5 and 11 hr after NE- 3 H.

§ G, glutaraldehyde; GF, glutaraldehyde + formaldehyde.

|| Fixative used for the samples counted.

of the radioactivity was symmetrical. Three types of reaction were observed in light and electron microscopic radioautographs.

- (1) *A diffuse and discrete reaction* consisted of scattered silver grains, sparingly distributed over all tissue elements except myelinated axons and blood vessel lumina. These silver grains bore no particular relationship with ultrastructural components of the tissue or the intercellular space.

with attenuation of the activity at increasing distance from the ventricular wall. After 3 hr, the number of stray silver grains was reduced, in normal and pretreated animals, but was especially low in the rat given reserpine. No diffuse reaction was visible 18 hr after norepinephrine- ^3H administration.

- (2) *A stronger reaction overlaid certain neuronal cell bodies and dendrites*, which exhibited more silver grains than their environment.

TABLE II
Number of Silver Grains per 100 μ^2 , in Radioautographs of Neurons Labeled after Intraventricular Injection of 500 μCi of Norepinephrine- ^3H
1 month of exposure.

Time after injection	Substantia nigra		Locus coeruleus		Substantia grisea periventricularis
	Nuclei*	Perikarya	Nuclei*	Perikarya	Presynaptic axons
5 min	30	26	—	—	647
30 min	65	62	45	51	859
3 hr	19	20	5.5	6	514
Total No. of silver grains counted	1656	2958	831	1861	552

* Nucleoli excluded.

Five min after injection, the diffuse reaction was limited to the SN and SGP, within 200–400 μ of the ependyma. After 30 min, it reached a maximum and spread across all three regions examined,

In untreated rats, such labeled nerve cell bodies were found at every time interval. They were present in the SN as early as 5 min after injection (Fig. 1). Their reaction intensified after 30 min,

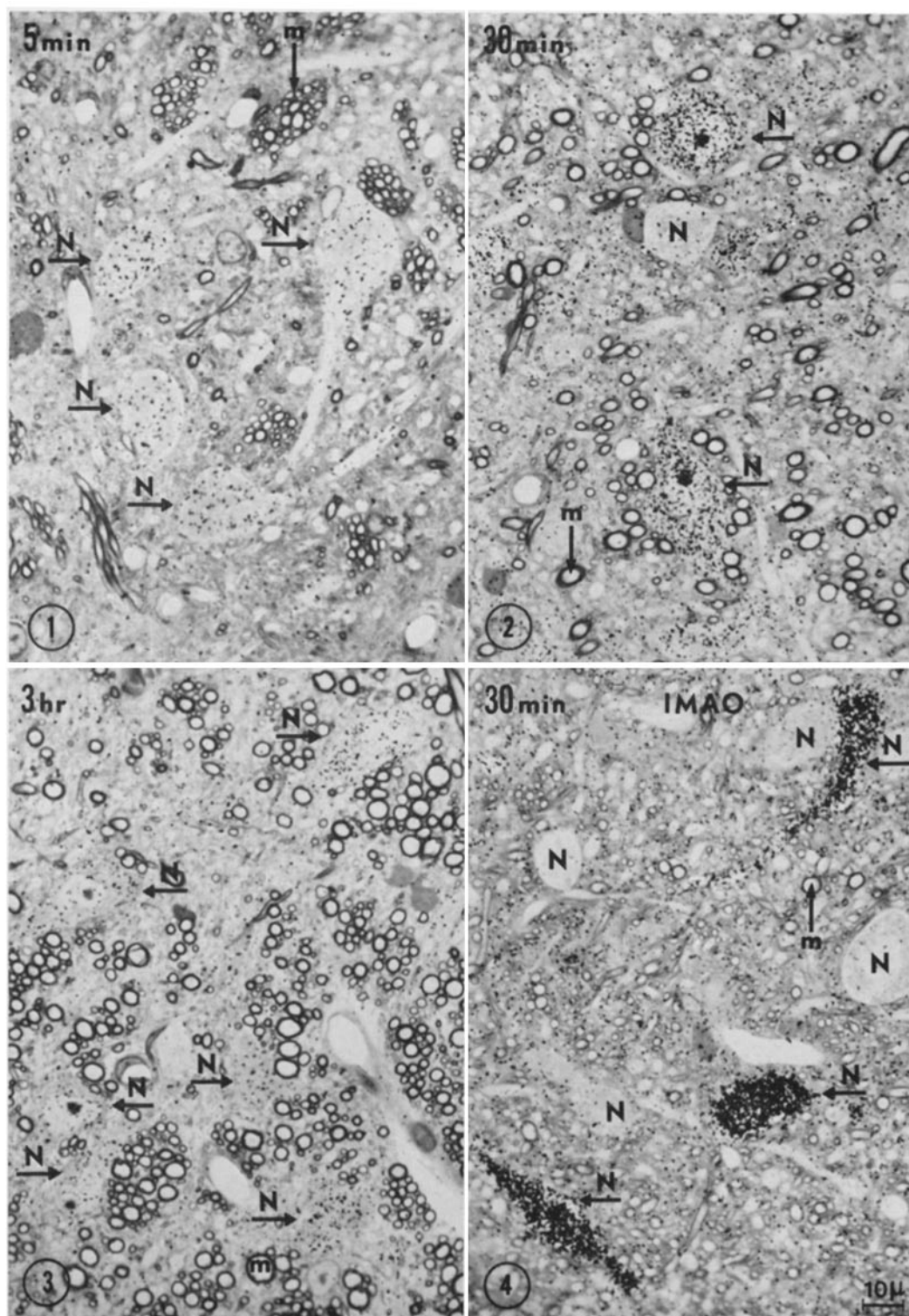
FIGURES 1–4 Light microscopic radioautographs of the substantia nigra (medial portion of the zona compacta) after intraventricular injections of norepinephrine- ^3H . Reactive nerve cell bodies (N), in which the tracer is concentrated, contrast with the surrounding neuropil overlaid by a weak and diffuse reaction; note the absence of reaction over myelinated axons (e.g., m). Exposure time, 1 wk. $\times 600$.

FIGURE 1 5 min after injection. The tracer has already been taken up in the cell body of four neurons, which exhibit more silver grains than their environment.

FIGURE 2 30 min after injection. Two nerve cell bodies are intensely labeled; the silver grains are superimposed upon the perikaryon, the nucleus, and the nucleolus. An adjacent neuron (N) appears unreactive.

FIGURE 3 3 hr after injection. The intensity of the reactions has considerably diminished, but reactive nerve cell bodies are still recognizable.

FIGURE 4 30 min after injection. Monoamine oxidase inhibited (*MAO*). Reactive neurons are so heavily laden with tracer that their cell body and dendrites are entirely masked by silver grains. Unlabeled nerve cells (N) are still present among them.



in both SN (Fig. 2) and LC (Fig. 5); it diminished markedly after 3 hr (Figs. 3 and 6). In the SN, reactive cells were interspersed among numerous unlabelled neurons (Fig. 2), whereas, in the LC, most visible nerve cell bodies and their dendrites crowding the neuropil, were laden with tracer (Fig. 5). Within labeled nerve cell bodies, the silver grains were seen over the perikaryon, the nucleus, and the nucleolus (Figs. 2, 5, and 9). The concentration of the radioactivity in the perikaryon and the nucleus was roughly the same, at each given time interval (Table II).

In rats pretreated with MAO inhibitor, the reaction of the nerve cell bodies and dendrites was enhanced, at 30 min and 3 hr; it, nevertheless, spared a great number of neighboring neurons in the SN (Fig. 4). On the contrary, in the animal treated with reserpine, few nerve cell bodies of the SN and LC reacted barely more than the surrounding neuropil. 18 hr after injection, no labeled nerve cell body persisted.

The patterns of fine structural distribution of the tracer in reactive nerve cell bodies were comparable, at all time intervals studied, in normal and pretreated rats. In the nucleus, the silver grains were often clumped over the nucleolus; in the cytoplasm, organelles of all types were labeled (Fig. 9). Grouping of silver grains was occasionally found over mitochondria or multivesicular bodies, but rarely over the Golgi zone or its vicinity.

(3) *Intense accumulations of tracer restricted to presynaptic axonal enlargements* were visible, in the light microscope, as small and dense aggregates of silver grains, confined to minute areas of the neuropil (Figs. 7 and 8).

In normal animals, these aggregates were prominent in the SGP and LC, 30 min after injection (Fig. 7). Most often, they lay adjacent to dendrites (Fig. 7, horizontal arrow) and, rarely, at the periphery of a nerve cell body. In electron microscope radioautographs, axonal bulbs, filled with synaptic vesicles, were the only structure of the neuropil in which the tracer accumulated (Figs. 10, 12); such labeled bulbs were not found in the SN.

The labeled presynaptic axons were the site of a reaction much more intense than that of labeled nerve cell bodies (compare Figs. 10–12 and 9). As early as 5 min after injection (Fig. 10), the radioactivity concentration measured in the labeled axonal enlargements was 25 times greater than that of the reactive nerve cell bodies; at later time intervals (Figs. 11 and 12), it was 15–100 times higher (Table II). After 3 hr (Fig. 12), a few presynaptic axons were still heavily laden with tracer.

When MAO was inhibited, the number of labeled axonal bulbs was the same after 30 min as in the untreated animal. However, after 3 hr, this number doubled and the radioactivity concentration reached 845 grains/100 μ^2 , instead of 514 grains/100 μ^2 in the untreated rat. On the contrary, in the animal given reserpine 30 min after norepinephrine- ^3H , no accumulation of radioactivity was detected in presynaptic axons, 3 hr after injection of the tracer. In the rat pretreated with MAO inhibitor and examined after 18 hr, a few presynaptic enlargements remained intensely labeled (Fig. 16).

Among 225 labeled axonal bulbs, from the

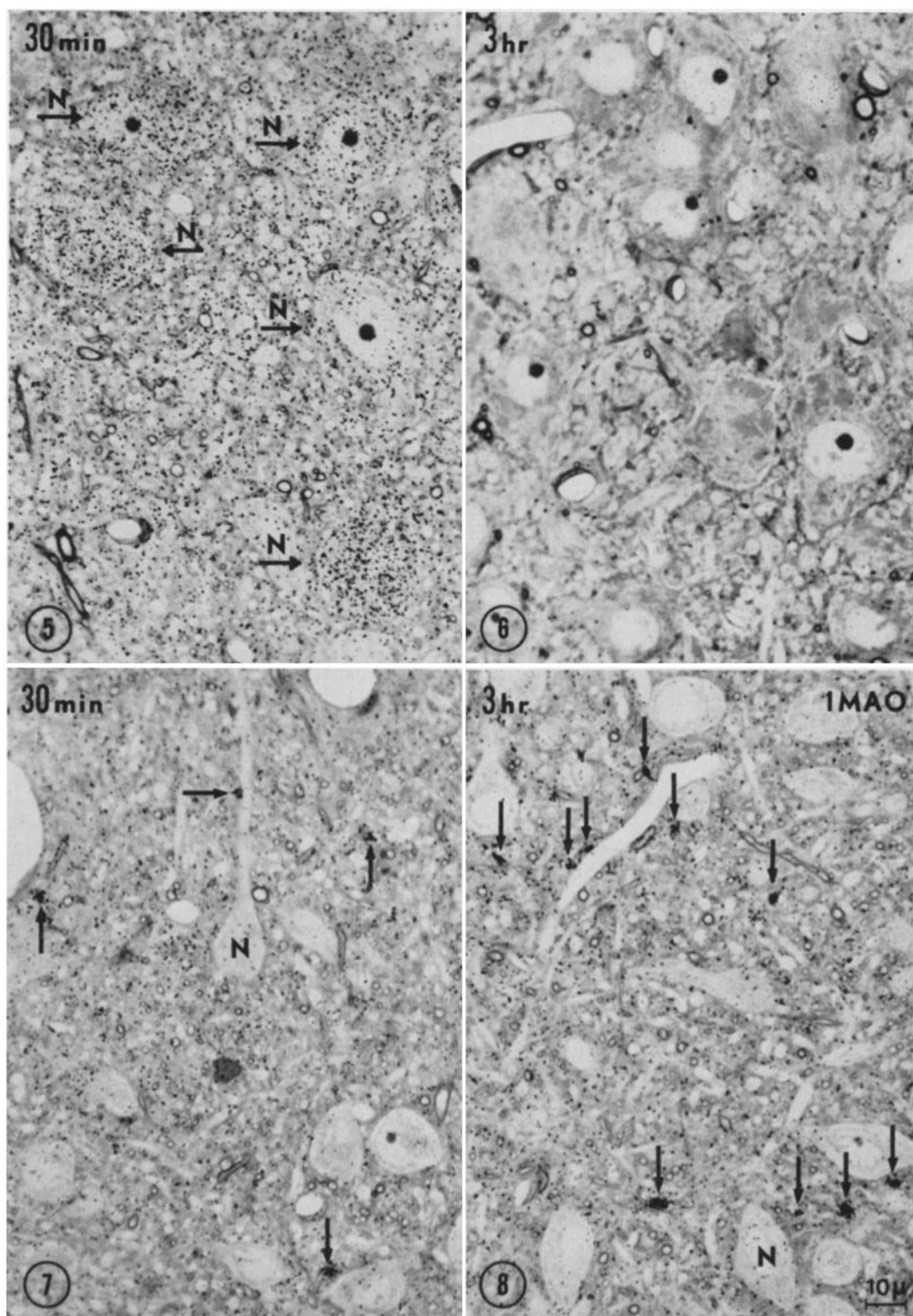
FIGURES 5–8 Light microscopic radioautographs of the locus coeruleus, above, and substantia grisea periventricularis, below. Exposure time, 1 wk. $\times 600$.

FIGURE 5 LC. 30 min after injection. Most nerve cell bodies react intensely (N), but to a varying degree. The neuropil, in which labeled dendrites are numerous, also shows a great number of silver grains.

FIGURE 6 LC. 3 hr after injection. The label has virtually disappeared.

FIGURE 7 SGP. 30 min after injection. Small and dense aggregates of silver grains, visible in the neuropil between unreactive neurons (e.g., N), are often adjacent to dendrites (horizontal arrow) and occasionally located near a neuronal cell body (lower right). These sites of accumulation of the tracer consist of presynaptic axons (arrows).

FIGURE 8 SGP. 3 hr after injection. Monoamine oxidase inhibited (*IMAO*). Numerous labeled presynaptic axons (arrows), in which norepinephrine- ^3H and/or its metabolites are stored, remain laden with tracer, between unreactive neurons (e.g. N).



SGP and LC, examined with the electron microscope, 26 were seen in synaptic junction with a dendrite; 19 and 7 resembled type I and II synapses of Gray's classification (20), respectively. Only one lay in close contact with a nerve cell body (Fig. 13); another was degenerating. In some cases, a single presynaptic enlargement was found to be labeled among two or three axons terminating on the same dendrite (Figs. 14 and 15). In consecutive thin sections, the accumulations of tracer were invariably confined to the same nerve ending (Figs. 17–21).

Within the labeled axonal bulbs, the patterns of the radioautographic reaction were similar in animals pretreated or not with MAO inhibitor. The silver grains, often distributed in clusters, were superimposed upon all components of the axon terminal: groups of synaptic vesicles of various sizes, shapes, and contents, smooth endoplasmic reticulum, axoplasm, and the axonal membrane (Figs. 10–21).

Complementary Experiments in Pretreated Rats

In tissue fixed with glutaraldehyde-formaldehyde and potassium dichromate, 3 hr after injection of norepinephrine-8-³H, the radioactivity measured in the caudate nucleus was high (Table I, Exp. 8). With or without treatment in K₂Cr₂O₇, the intensity and the distribution of the radioautographic reactions, the appearance and the content of the labeled presynaptic axons did not

differ from those observed after injection of norepinephrine-7-³H and fixation with glutaraldehyde.

After potassium permanganate fixation, also performed 3 hr after injection of norepinephrine-8-³H, the amount of tracer retained in the caudate nucleus was noticeably low (Table I, Exp. 9). No significant radioautographic reaction was visible either in 2- μ -thick or in thin sections, in which only rare silver grains were scattered at random. In electron micrographs of the SGP, a few unlabeled axons exhibited small, dense-core vesicles (300–400 Å in diameter). When normetanephrine-³H was injected instead of norepinephrine-³H, the radioactivity measured in the caudate nucleus, 3 hr after injection, was elevated (Table I, compare Exp. 10 and 5). However, in radioautographs of glutaraldehyde-fixed tissue, the reaction was of the diffuse type only: no nerve cell body or presynaptic axon contained more silver grains than its environment.

DISCUSSION

Conditions Required for the Radioautographic Detection of Exogenous Norepinephrine-³H

Reliable radioautographic detection of exogenous norepinephrine in the central nervous system implies that several conditions be fulfilled: (1) bound norepinephrine-³H must be preserved *in situ* during the course of fixation and subsequent tissue processing; (2) free norepinephrine-³H must be extracted by the preparative procedure; (3) labeled metabolites must also be removed.

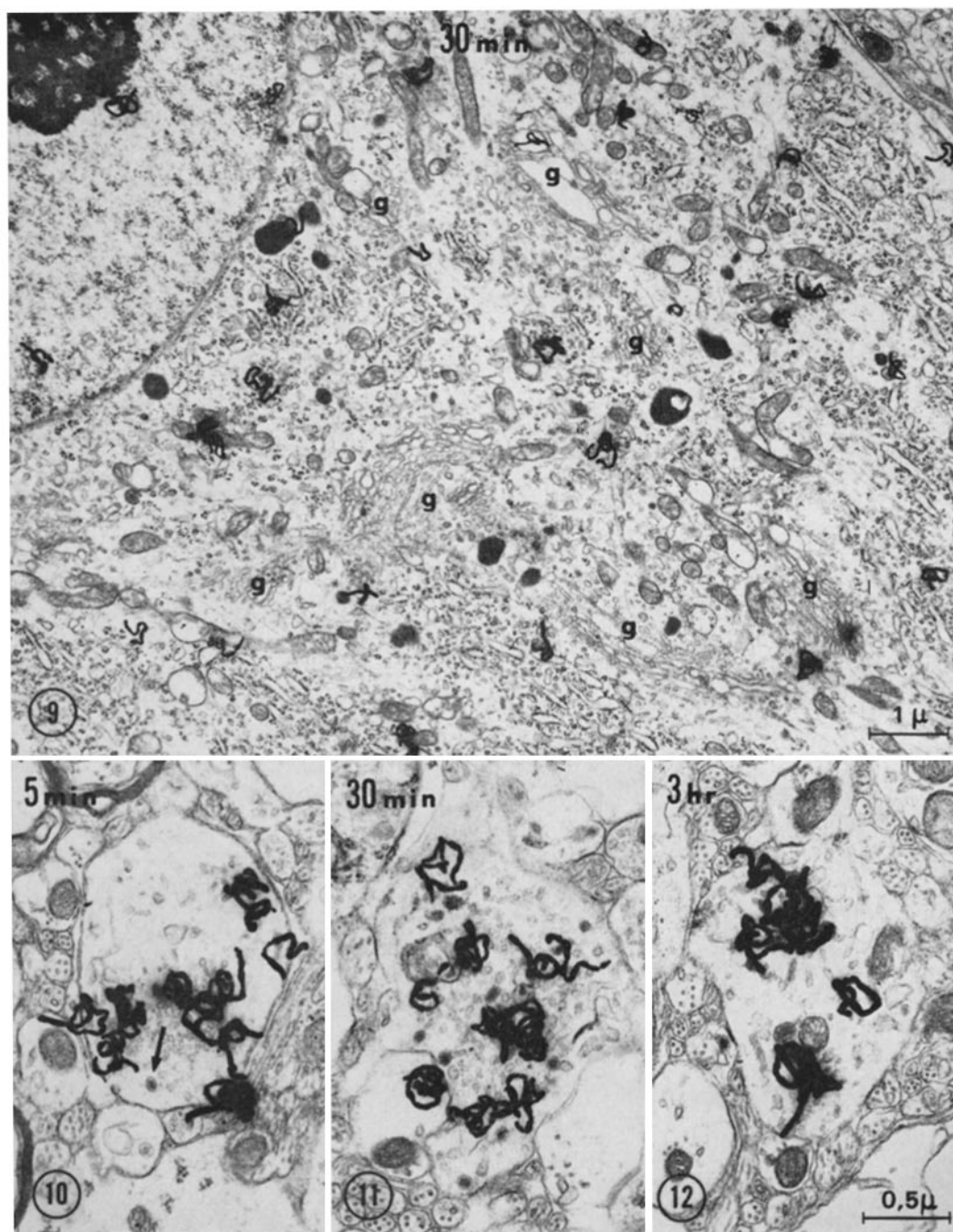
FIGURES 9–12 Electron microscopic radioautographs of a reactive nerve cell body (above) and three labeled presynaptic axons (below), after an identical exposure of 3 wk. The average concentration of the tracer within labeled axonal bulbs is 15–100 times that of reactive nerve cell bodies.

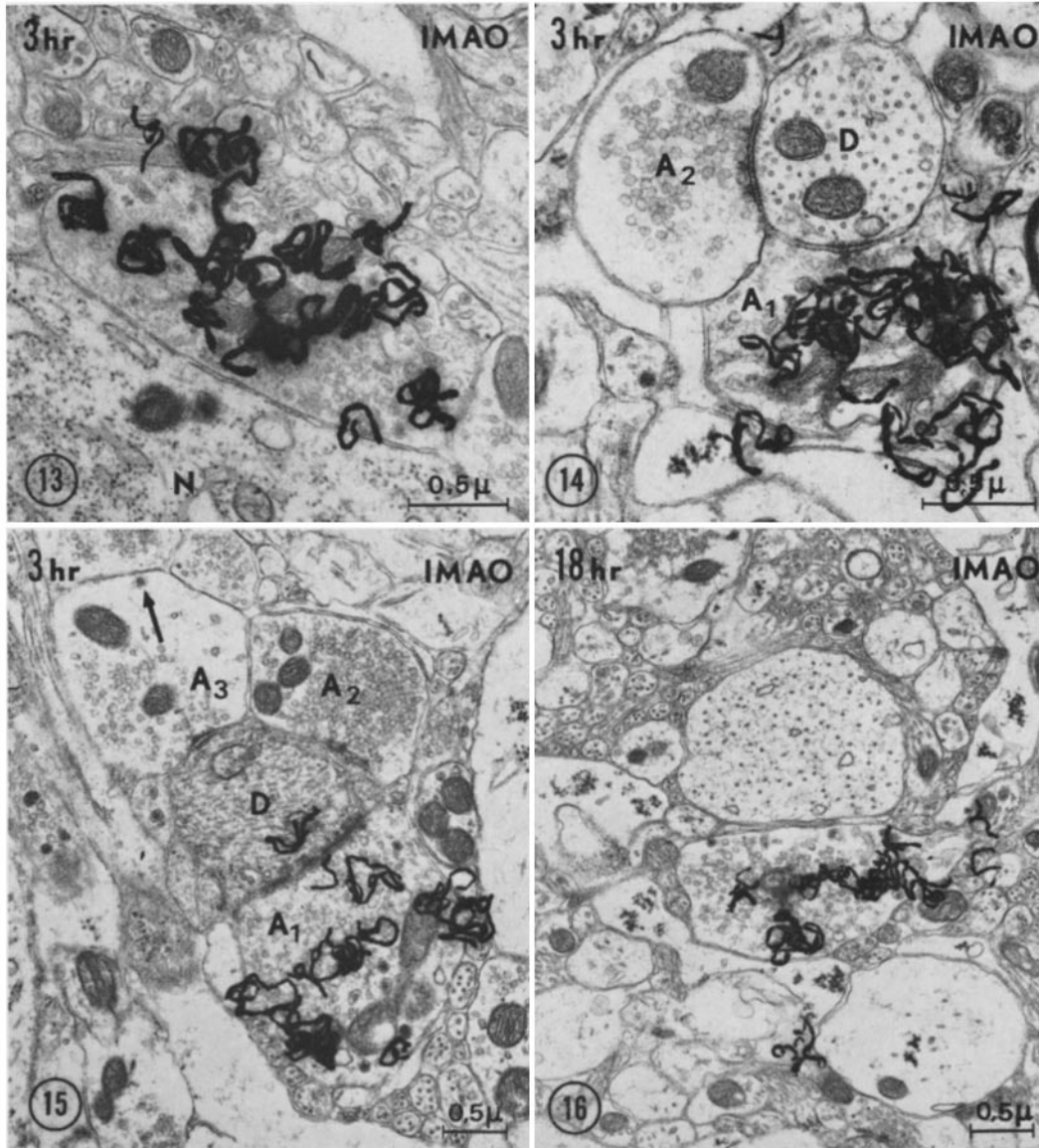
FIGURE 9 LC. 30 min after injection. The distribution of the radioactivity in the neuronal cell body is ubiquitous. Silver grains are present over the perikaryon, the nucleus, and the nucleolus. In the cytoplasm, they are superimposed upon the endoplasmic reticulum, mitochondria, dense bodies, Golgi zones (*g*), and the neuroplasm. $\times 11,200$.

FIGURE 10 SGP. 5 min after injection. The tracer is already accumulated within an axonal bulb, in which most synaptic vesicles are small and clear; only one large granular vesicle is visible (arrow). $\times 25,000$.

FIGURE 11 SGP. 30 min after injection. Numerous silver grains overlie a presynaptic axon, in which several large granular vesicles are present. $\times 25,000$.

FIGURE 12 SGP. 3 hr after injection. An intense reaction persists over an axonal enlargement, in which the synaptic vesicles vary in size, shape, and content. $\times 25,000$.





FIGURES 13-16 Labeled axonal bulbs in the SGP of rats pretreated with monoamine oxidase inhibitor (*IMA0*).

FIGURE 13 3 hr after injection. An axonal enlargement, laden with tracer, is adjacent to a neuronal cell body (*N*). Silver grains overlaid mitochondria as well as synaptic vesicles of various types. 4-wk exposure. $\times 26,200$.

FIGURE 14 3 hr after injection. One of two axons (*A*₁ and *A*₂) making synapse on the the same dendrite (*D*) is intensely labeled. The reactive bulb (*A*₁) contains several large granular vesicles, whereas the unlabeled terminal (*A*₂) exhibits only small and clear vesicles. 6-wk exposure. $\times 30,400$.

FIGURE 15 3 hr after injection. One of three axons (*A*₁-*A*₃) in synaptic contact with the same dendrite (*D*) is labeled; it contains several large granular vesicles. One of the two unlabeled terminals (*A*₂) shows only clear vesicles, but a granular vesicle is visible in the other (*A*₃, arrow). 3-wk exposure. $\times 17,000$.

FIGURE 16 18 hr after injection. A presynaptic axon remains intensely labeled, over a region in which most synaptic vesicles are small and clear. 3-wk exposure. $\times 16,800$.

The preservation of bound norepinephrine- ^3H was assessed after fixation with chilled 3% potassium permanganate, a technique which reveals small, dense-core vesicles (300–500 Å in diameter) in certain axon terminals of the central nervous system (22). Contrary to previous suggestions (23), permanganate fixation failed to retain significant amounts of radioactivity in central nervous tissue containing norepinephrine- ^3H (Table I, Exp. 9); under the action of this strong oxidizing agent, the exogenous amine was probably destroyed and lost (see also reference 34). After glutaraldehyde-formaldehyde fixation followed by potassium dichromate treatment, the pattern of the radioautographic reactions and the fine structure of the labeled neurons were comparable with those observed after glutaraldehyde alone, although the general preservation of the tissue was rather less satisfactory. Thus, glutaraldehyde appeared to be the fixative of choice for visualization of norepinephrine- ^3H in the sites in which it was taken up and presumably bound.

The extraction of free norepinephrine- ^3H by fixatives had been tested in earlier experiments (10), in which thin slices of fresh caudate nucleus were incubated at 4°C in a medium containing norepinephrine- ^3H at a high concentration (approximately 100 $\mu\text{Ci}/\text{mg}$ dry weight of tissue). At this temperature, the specific uptake of catecholamines was prevented (21), while the tracer could freely diffuse in tissue. After fixation, the radioactivity retained in the samples, measured by scintillation, was found to be 10 times greater with glutaraldehyde than formaldehyde. Thus, glutaraldehyde had a lower capacity to extract free norepinephrine- ^3H . Radioautography confirmed this result: the diffuse reaction, probably due to an artefactual retention of free labeled amine (28, 3, 27) and/or metabolites, was more pronounced after glutaraldehyde than formaldehyde fixation. However, in formaldehyde-fixed tissue, all specific and reproducible accumulations of tracer, localized in the axonal bulbs, had vanished. In contrast, glutaraldehyde preserved norepinephrine- ^3H accumulations.

The possible retention of labeled metabolites was also considered. In normal rats, the radioautographic reaction could indeed be due to exogenous norepinephrine- ^3H and deaminated or *O*-methylated metabolites. However, when the oxidative deamination of norepinephrine was inhibited (Exp.

4–10), norepinephrine could only give rise to normetanephrine, under the action of catechol-*O*-methyltransferase. Since this enzyme appears to be located outside of neurons (15), it could be postulated that newly formed normetanephrine- ^3H was also taken up by catecholaminergic neurons and contributed to the radioautographic reactions. This possibility was investigated by injecting normetanephrine- ^3H (Exp. 10) into the brain ventricle of a rat pretreated with MAO inhibitor: no accumulation of tracer was observed in any part of catecholaminergic neurons. Therefore, the intraneuronal accumulation of radioactivity, in animals pretreated with MAO inhibitor, probably resulted from norepinephrine- ^3H proper. Nevertheless, in these conditions, the exogenous labeled norepinephrine was taken up and retained by nerve cell bodies which presumably contained endogenous dopamine, instead of norepinephrine (e.g. SN, Figs. 1–4), owing to the common mechanism of uptake for both amines (21, 16). Neurons specifically labeled after norepinephrine- ^3H injections were, therefore, referred to as catecholaminergic, rather than noradrenergic.

The Intraneuronal Distribution of Exogenous Norepinephrine- ^3H

A radioautographic signal unequivocally designates axonal enlargements or nerve endings as the site of an intense accumulation of exogenous norepinephrine. A similar conclusion has been reached by the use of other techniques: the subcellular fractionation of brain injected with labeled norepinephrine has shown that the exogenous amine is mainly distributed in the “nerve ending fraction” and, to a lesser extent, in the supernatant and the microsomal layer (33, 17). The use of the fluorescence method has demonstrated that exogenous norepinephrine may be retained, in addition to endogenous catecholamines, in the “axonal varicosities” (14). Light microscope radioautography after intraventricular injection of norepinephrine- ^3H has indicated a close correlation between the presence of label and the specific fluorescence observed in the same neurons (13). The first results obtained by electron microscopic radioautography (1, 2, 25), in spite of the weak reactions recorded, suggested that exogenous norepinephrine was stored mainly in certain nerve endings. In the above analysis, quantitative data, obtained from

normal rats, confirmed that exogenous norepinephrine was at least 15–100 times more concentrated in presynaptic axons than in nerve cell bodies (Table II).³

A high concentration of labeled amine was found in synaptic axons of various types: with a prominent synaptic density or with short and less pronounced dense bands in the postsynaptic cytoplasm (corresponding to types I and II of Gray, respectively); with round or ovoid synaptic vesicles, or vesicles of both types; with numerous or, more rarely, no dense-core vesicle of large diameter (800–1200 Å). Although these large granular vesicles were usually present within the labeled bulbs, they could also be seen in unlabeled synaptic terminals (Figs. 15 and 17–21). Thus, no morphological feature could be specifically ascribed to presynaptic axons which stored exogenous norepinephrine-³H.

Frequently, a dendritic trunk received several axonal endings, only one of which was labeled (Figs. 14 and 15). This finding could indicate that the surface of a dendrite bears specific receptors for more than one neurotransmitter.

In labeled nerve cell bodies, the exogenous norepinephrine was scattered throughout the perikaryon and also the nucleus; it was also present in the main dendritic trunks (9). The labeled amine, ubiquitously distributed, could not be related to any given cell organelle. Special attention was paid to vesicles similar in shape and size to synaptic vesicles and located in the Golgi region (Fig. 9), but no definite reaction could be observed

³ The statistical probability of radiations hitting already exposed silver grains is low in the perikaryon (Fig. 9), where the concentration of radioactivity must be directly proportional to the number of silver grains per unit area (37). This condition is probably not met in the axonal bulbs (Figs. 10–12), for which the values obtained would represent lower limits.

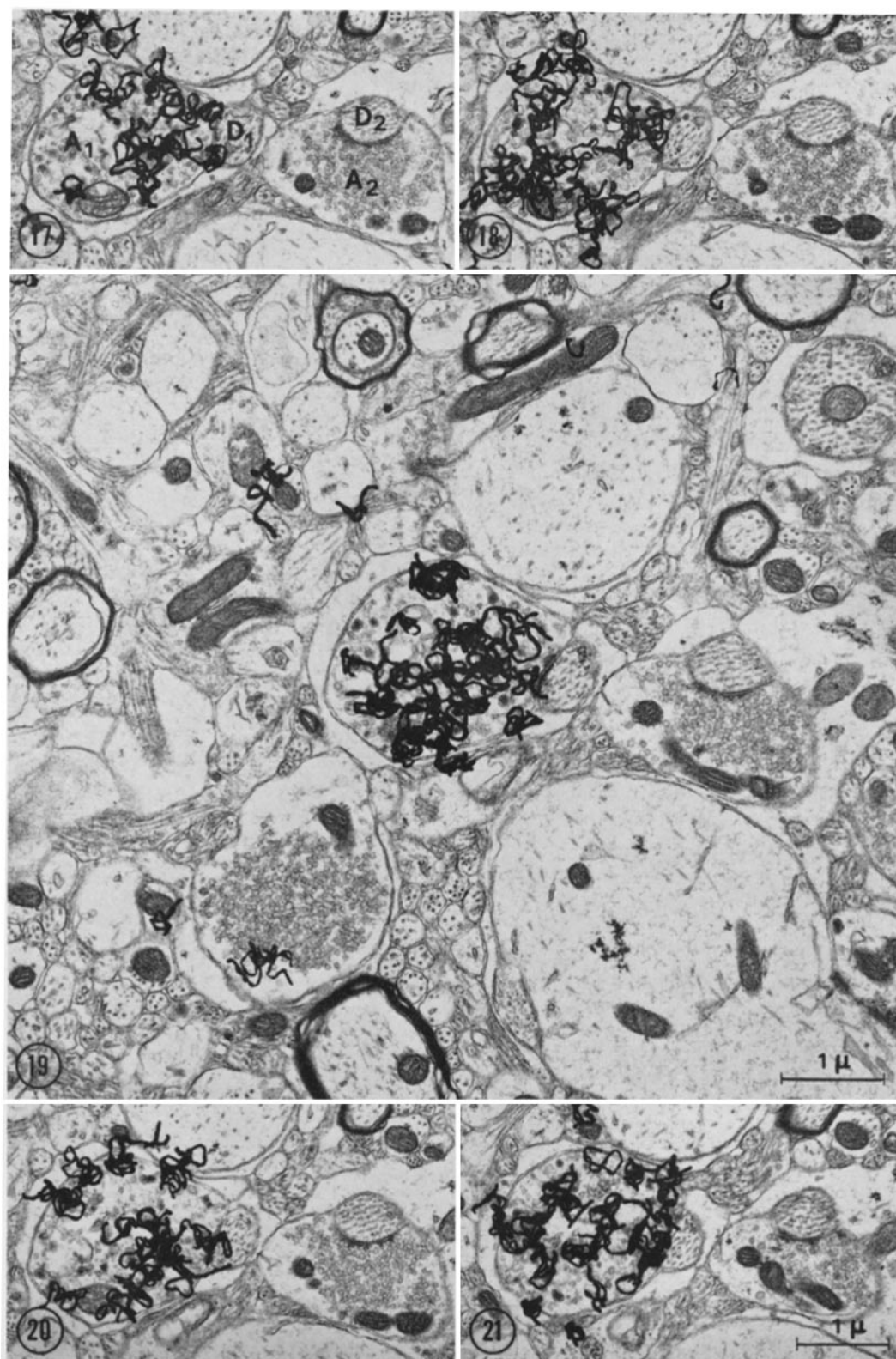
in their vicinity. Therefore, the postulate that norepinephrine present in nerve cell bodies is also stored in vesicles (5, 6) may be ruled out for exogenous norepinephrine.

Two possibilities may account for the presence of labeled amine in the nerve cell bodies and dendrites: an artefactual retention or binding to a specific macromolecular complex (8). After treatment with reserpine, radioautography shows that norepinephrine-³H disappears from all parts of the neuron. Since reserpine does not impair the neuronal uptake of norepinephrine, but depletes bound catecholamines (21), this finding indirectly favors the second possibility.

The exogenous norepinephrine is found to turn over at different rates in the nerve cell bodies and presynaptic axons of normal rats (Table II). The rate of disappearance of the labeled norepinephrine is three to five times faster in the nerve cell bodies of the SN and LC ($t_{1/2}$: 0.8 and 1.3 hr) than in the nerve endings of the SGP ($t_{1/2}$: 4 hr). Thus, exogenous norepinephrine appears to be more protected against intraneuronal attack by MAO in preterminal axons than in nerve cell bodies. However, when MAO is inhibited, and intraneuronal norepinephrine-³H persists at a higher concentration, the radioactivity still disappears earlier from the nerve cell body than the presynaptic axon. This could result either from a local degradation of norepinephrine-³H by MAO, eventually resuming its catabolic activity, or from a passage of bound norepinephrine-³H from the nerve cell body into the axon.

An axonal transport of catecholamines, sequestered in synaptic vesicles produced in the nerve cell body, and carried in that form from the perikaryon towards the nerve endings, has been postulated by Dahlström et al. (6, 7). The ubiquitous distribution of exogenous norepinephrine, which is not exclusively confined to vesicular structures in reactive nerve cell bodies, gives support to

FIGURES 17–21 SGP of a rat pretreated with monoamine oxidase inhibitor, 3 hr after injection. The same presynaptic axon (A_1 in Fig. 17), visualized in five adjacent thin sections, is the site of an intense and selective reaction. The labeled bulb contains a mixed population of synaptic vesicles and makes synaptic contact on a small dendrite (see D_1 in Fig. 19). The neighboring axon (A_2 in Fig. 17), which makes a synapse on another dendrite (D_2 in Fig. 17), shows several large granular vesicles, but remains unlabeled throughout the series of radioautographs. Within the labeled axonal bulb, silver grains are distributed in clusters over all types of organelles. 4-wk exposure. Figs. 17, 18, 20, 21, $\times 14,000$; Fig. 19, $\times 16,000$.



an alternate hypothesis. In catecholaminergic neurons, a macromolecular complex (protein), elaborated in the perikaryon, could constitute the binding site for norepinephrine. Macromolecular complexes, rather than preformed synaptic-like vesicles, would migrate from the perikaryon into the axon and accumulate within presynaptic terminals. Such complexes might assemble as constituents of the synaptic vesicles when reaching a sufficient local concentration in the axonal bulbs.

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