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UPTAKE AND ACCUMULATION OF CATECHOLAMINES IN PERIPHERAL ADRENERGIC NEURONS OF RESERPINIZED ANIMALS, STUDIED WITH A HISTOCHEMICAL METHOD

B. HAMBERGER, T. MALMFORS, K-A. NORBERG and CH. SACHS

Department of Histology, Karolinska Institutet, Stockholm, Sweden

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Abstract—A recent fluorescence histochemical method for certain monoamines has been applied to a study of some aspects of the uptake and storage of exogenous catecholamines in adrenergic neurons. It was found, that the whole adrenergic neuron has the property to take up and concentrate noradrenaline and dopamine. This uptake was not inhibited if the amines were given to animals previously depleted of their endogenous catecholamines by reserpine. Since there is good evidence that the storage mechanism in the amine granules is inhibited by reserpine, it is likely that the amines accumulated in this way are present mainly in an extra-granular pool. The concentration of the amines was concluded to be much higher if the potent monoamine oxidase inhibitor nialamide was given before the administration of the amines. This suggests that monoamine oxidase is present in the neuron, and that the amines exist free or loosely bound in the cytoplasm, readily accessible to this enzyme. The mechanism for the accumulation of the amines seems to be localized to the cell membrane or the Schwann cell covering the axon.

THERE is good evidence that sympathetic adrenergic nerves can take up and store catecholamines.[1–3] The uptake and storage mechanisms and the effects of drugs on these mechanisms have been studied extensively.[4–6] Although many important results have been obtained in these experiments with labelled catecholamines, it is obviously a great advantage to be able to study these mechanisms directly on the cellular level. The present paper reports experiments showing that the uptake of administered noradrenaline (NA) and dopamine (DA) in the various parts of the adrenergic neuron can be studied in an entirely new way by means of a recent fluorescence microscopical method.[7–9] This method has recently been used in the study of noradrenaline uptake in vascular adrenergic nerve terminals.[10]

MATERIAL AND METHODS

Male albino rats with a body weight of about 150 g were used. The uptake of administered DA and NA was examined in normal animals, animals depleted of their endogenous NA by reserpine, and in reserpinized animals treated with the monoamine oxidase inhibitor nialamide some hours before the administration of the catecholamines. Fairly high doses of the amines were given to obtain optimal conditions for their histochemical demonstration. Each group consisted of at least 5 animals. No catecholamines were given to three control groups of normal, reserpinized,

and reserpinized and nialamide treated animals, respectively. On the basis of preliminary pilot experiments[11] there were chosen the following doses and intervals between the administration of the drugs and death of the animals.

Drug	Dose (mg/kg body wt.)	Time before death (hr)
Reserpine	10	18–22
Nialamide	100	3–5
Dopamine	1–10	1
Noradrenaline	0·5–10	1

All drugs were given intraperitoneally. The animals were anaesthetized with ether, killed by bleeding out and dissected at once. The following tissues were taken for freeze-drying: vas deference, submandibular gland, sup. cervical and stellate ganglia, chorioid with ciliary body and iris. The last-mentioned tissue was also prepared as a stretch-preparation mounted as a whole on a microscopical slide and dried for about 1 hr in a desiccator containing phosphorus pentoxide. After drying, all tissues were treated with formaldehyde gas at +80° for 1 hr in a closed vessel containing paraformaldehyde of optimum water content, prepared according to Hamberger *et al.*[12] After formaldehyde treatment, the freeze-dried material was embedded in paraffin, sectioned and the sections mounted for fluorescence microscopy mainly a.m. Falck.[9]

RESULTS

On formaldehyde treatment, primary catecholamines are readily converted to 3,4-dihydroisoquinolines which show an intense green to yellow-green fluorescence.[13] In this way the intraneuronal distribution of the adrenergic transmitter can be studied directly.[9, 14, 15] In untreated animals, most cell bodies of the adrenergic neurons display a weak to moderate fluorescence and the non-terminal axons a very weak or no fluorescence[9, 14, 15] (Figs. 1 and 2). The nerve terminals, however, show a strong fluorescence along their entire length, and especially in the varicosities, indicating a very high concentration of NA (Figs. 1 and 2). The differences in concentration of catecholamines as between the different parts of the neuron seem to be pronounced, since the terminals, which are very thin, have an intense fluorescence, while the cell bodies, which occupy the whole thickness of the tissue section show a moderate or weak fluorescence. In the tissues examined, typical varicose nerve terminals were present in abundance in the walls of blood vessels (especially the arteries and arterioles), in the smooth muscle layers of vas deferens, in the iris and around the acini in the submandibular gland. No specific fluorescence developed after treatment with reserpine. When nialamide was given after reserpine, a faint fluorescence developed in some ganglion cell bodies, while the non-terminal axons and terminals were still devoid of specific fluorescence. Exogenous catecholamines (NA and DA) given to normal animals, give rise to a generally more intense fluorescence in non-terminal axons and terminals.

In the animals depleted of their endogenous NA by reserpine, the administration of catecholamines (NA or DA) caused a weak to moderate fluorescence to appear in the

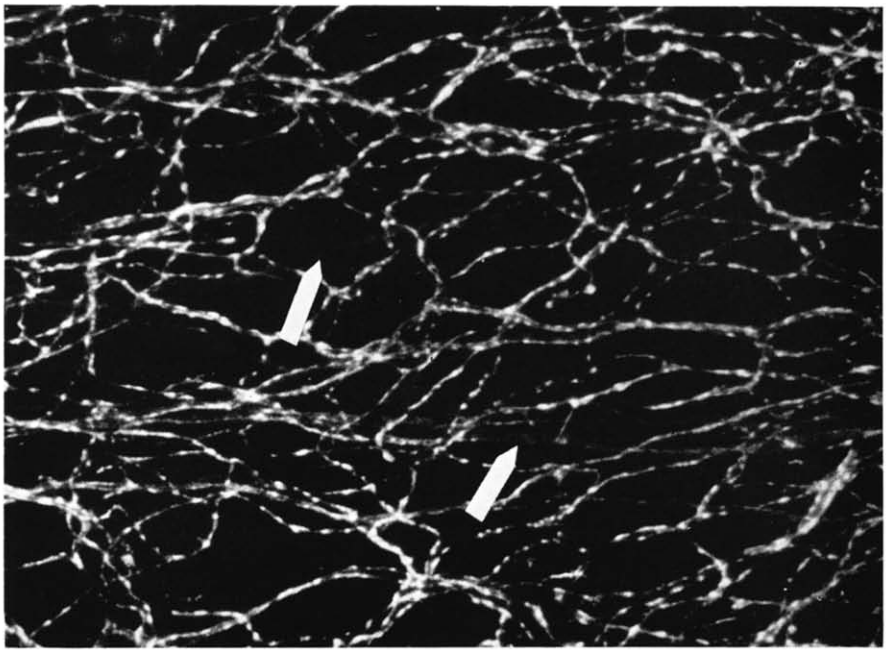

FIG. 1.

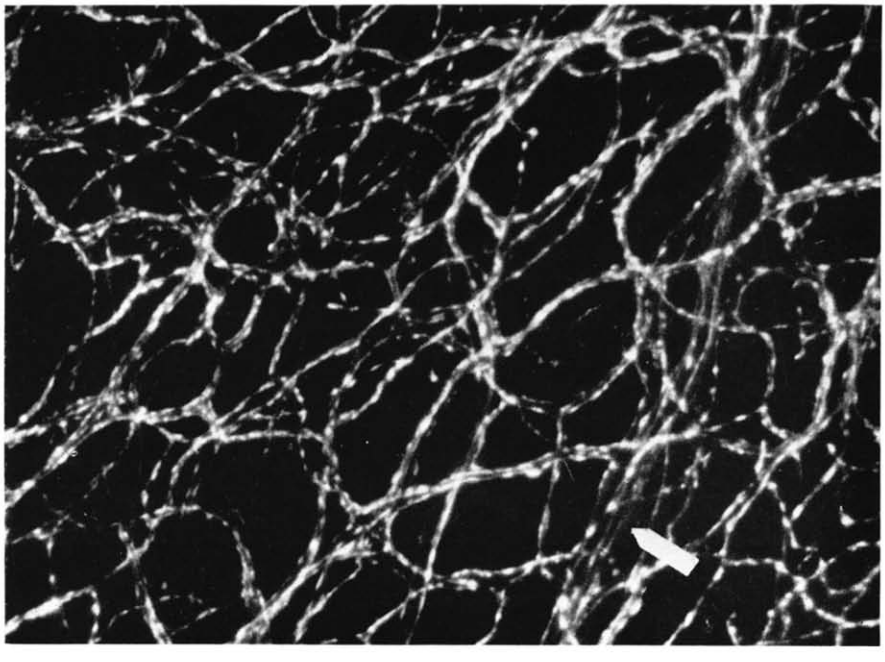

FIG. 2.

FIGS. 1 and 2. Iris from untreated animals prepared as stretch-preparations mounted as a whole. The adrenergic ground plexus with numerous varicosities shows a strong fluorescence. Nonterminal axons with a very faint fluorescence are seen at the arrows. Magnification: Fig. 1 320×, Fig. 2 500×. HBO 200 high-pressure mercury-lamp with a Schott BG 12 (4 mm) filter. Kodak Wratten filter number 15 and Schott OG 4 (3 mm) in the tube. Dark field condensor. Photographs taken with Scopix G (Gevaert).

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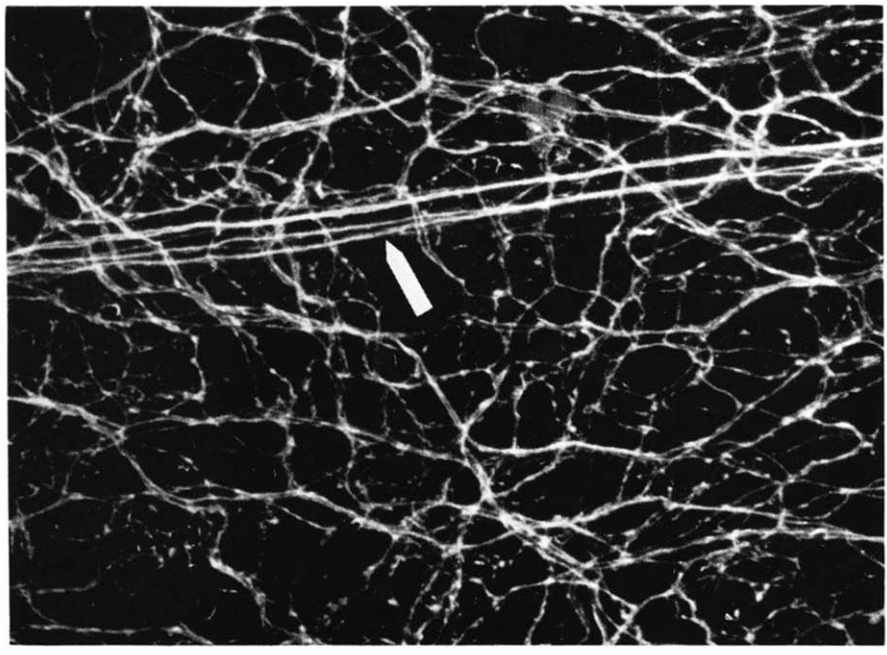

FIG. 3.

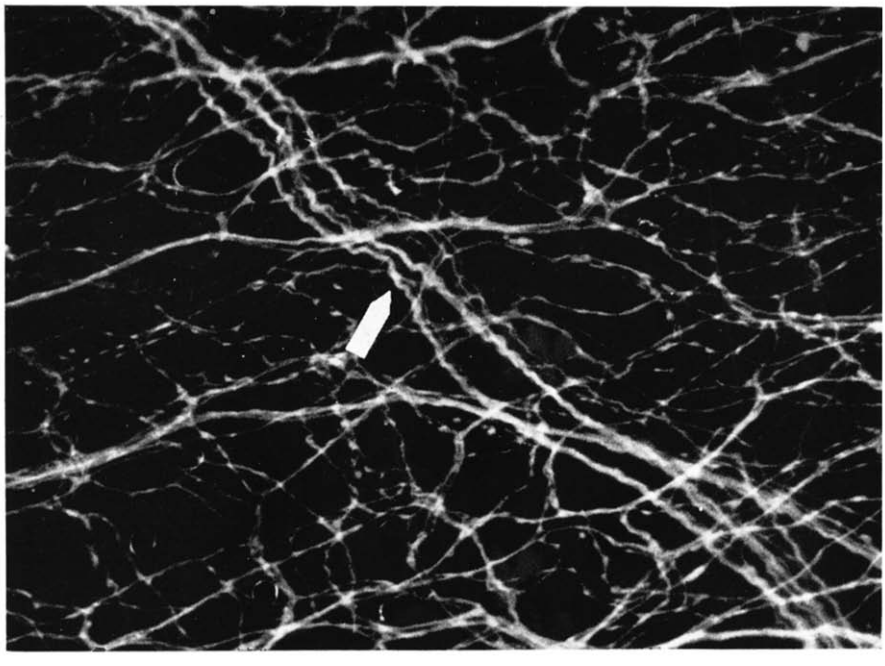

FIG. 4.

FIGS. 3 and 4. Iris prepared as stretch-preparations mounted as a whole from animals treated with reserpine, nialamide and noradrenaline. The nerve terminals of the adrenergic ground plexus have the same distribution and intensity of fluorescence as in the untreated animal, but look smoother because of the less pronounced varicosities. The nonterminal axons (at the arrows) exhibit a very strong fluorescence as compared with those of the untreated animal. Magnification: Fig. 3 320×, Fig. 4 500×. Optical conditions as for Figs. 1 and 2.