EFFECT OF FLUACIZINE ON THE UPTAKE OF EXOGENOUS NORADRENALIN

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The effect of the original Soviet antidepressant fluacizine compared with imipramine on the ability of adrenergic nerve fibers of the rat vas deferens to accumulate exogenous noradrenalin in vitro after depletion of the endogenous reserves of the mediator by reserpine was studied by methods of fluorescence histochemistry, spectrofluorimetry, and cytochemical electron microscopy. Fluacizine (1 μg/ml), like imipramine (1 μg/ml), was found to inhibit the uptake of noradrenalin by the sympathetic fibers. This effect is considered to be attributable to blocking of the axonal cell membrane.

Imipramine and certain other antidepressants of this group have the property of inhibiting the ability of adrenergic nerve fibers to accumulate exogenous noradrenalin (NA) from the external medium [1, 5].

The object of this investigation was to discover whether the original Soviet antidepressant fluacizine (10-[β-diethylaminopropionyl]-2-trifluoromethylphenothiazine hydrochloride) possesses this property, and to compare its effect with that of imipramine. For this purpose, the ability of presynaptic adrenergic fibers to assimilate and accumulate exogenous NA after depletion of the endogenous reserves of the mediator by reserpine was studied by spectrofluorimetric and fluorescence histochemical methods. An electron-microscopic analysis of the NA content in the synaptic vesicles was carried out in the course of these experiments.

EXPERIMENTAL METHOD

The vas deferens from rats weighing 200-300 g, richly innervated with sympathetic nerve fibers, was used. To deplete the catecholamine reserves, reserpine was injected in a dose of 5 mg/kg 20 h before the experiment. After isolation the vas deferens was incubated under conditions (Krebs' solution, 32° C, aeration) permitting its functional activity to be monitored during electrical transmural stimulation throughout the experiment. After free incubation of the organ in the medium for 1.5 h, in some cases NA (0.5 μg/ml) or NA and iproniazid (0.1 mM) were added for 30 min, while in other cases the antidepressants fluacizine (1 μg/ml) or imipramine (1 μg/ml) were added 15 min before the NA and iproniazid. In all the experiments the vas was divided into three parts: in one part the total NA content was determined spectrofluorimetrically by the method of Euler and Lishajko [3], the second part was treated for fluorescence-histochemical study of NA in the nerve fibers by the method of Falck and Owman [4], and the content of mediator in the synaptic vesicles was studied in the third part by cytochemical electron microscopy [10]. The number of granular synaptic vesicles containing catecholamines per μ^2 of section through the nerve fiber was counted, using at least 20 electron micrographs from each specimen.

EXPERIMENTAL RESULTS

In the control series the total NA content in the vas was 9.7 μg/g wet weight of tissue. The fluorescence-histochemical investigation showed that the NA was contained almost entirely in adrenergic nerve fibers, which formed a dense plexus of terminal branches of axons giving green fluorescence. Along their

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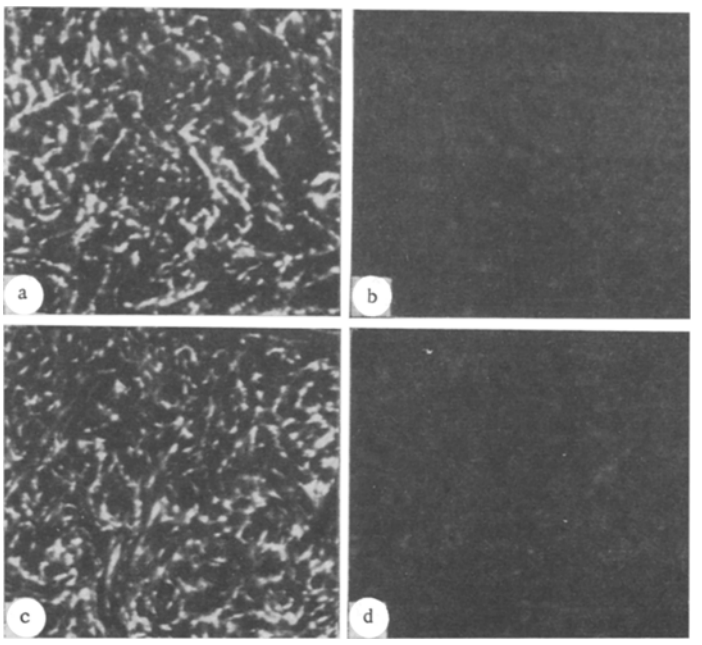

Fig. 1. Fluorescence of monoamines in adrenergic nerves of the rat vas deferens (200×): a) control; b) 20 h after injection of reserpine (5 mg/kg); c) 20 h after injection of reserpine (5 mg/kg) and incubation with NA (0.5 μg/ml, 30 min) and with iproniazid (0.1 mM, 30 min); d) 20 h after reserpine (5 mg/kg) and incubation with fluacizine (1 μg/ml, 15 min before NA), NA (0.5 μg/ml, 30 min), and iproniazid (0.1 mm, 30 min).

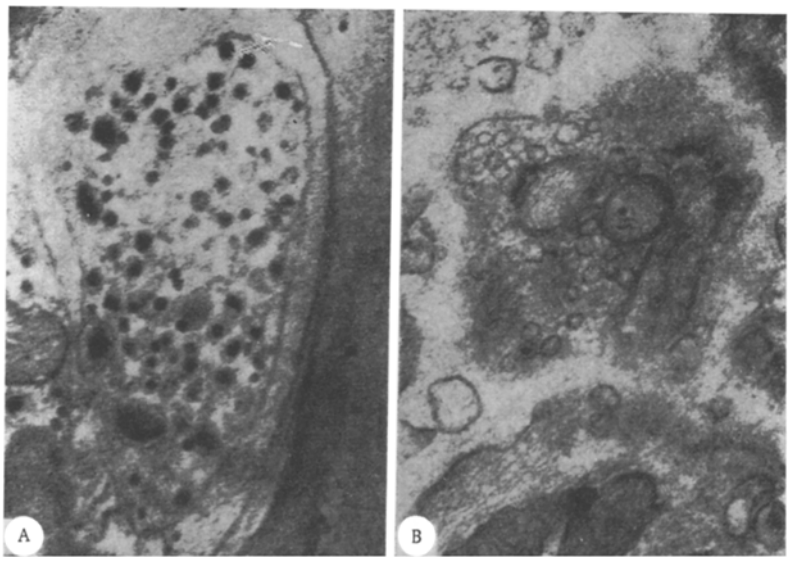

Fig. 2. Electron-microscopic study of mediator in synaptic vesicles of adrenergic nerve fibers of the rat vas deferens: A) control (80,000×); B) 20 h after injection of reserpine (5 mg/kg) and incubation with NA (0.5 μg/ml, 30 min) and iproniazid (0.1 mM, 30 min) (70,500×).

length the fibers contained brightly fluorescent swellings or varicosities, which were richest in NA (Fig. 1a). Electron-microscopic study of the same tissue revealed numerous terminal or preterminal axons containing synaptic vesicles, mainly 600-800 Å in diameter, with dense inclusions in the center (Fig. 2A); these consisted of the cytochemical reaction product of catecholamines stored in the vesicles [2, 8-10]. The mean number of granular synaptic vesicles per μ^2 section through the nerve fiber in the control series was 130 ± 12.

TABLE 1. Inhibition of Uptake of Exogenous NA and Its Storage in the Synaptic Vesicles of Nerves of the Rat Vas Deferens by Antidepressants after Depletion of the NA Reserves by Reserpine

Treatment	Content of NA in μg/g wet wt. of tissue	Content of NA in % of control	Intensity of fluorescence[2]	Content of granular vesicles (in % of control)
Control	9,7±0,7[1]	100	++++	100
Reserpine[3]	2,0±0,8	21	+	9
Reserpine + NA[4]	2,5±0,9	26	+	10
Reserpine + NA + iproniazid[5]	7,9±1,2	81	+++(+)	11
Reserpine + fluacizine + iproniazid	2,2±0,6	23	+	10
Reserpine + imipramine[6] + NA + iproniazid	2,4±0,5	25	+	9

[1]Confidence limits of the mean for P = 0.05.
[2]++++Bright fluorescence; +++ moderate; ++ weak; + very weak.
[3]Reserpine in a dose of 5 μg/kg was injected 20 h before sacrifice.
[4]Incubation with NA (0.5 μg/ml) lasted 30 min in all experiments.
[5]In this and subsequent experiments 0.1 mM iproniazid was added to the medium.
[6]Fluacizine (1 μg/ml) or imipramine (1 μg/ml) was added 15 min before NA.

After depletion of the catecholamine reserves with reserpine the total NA content in the vas fell to 2 μg/g (Table 1). The fluorescence-histochemical study revealed the almost total extinction of fluorescence of the adrenergic fibers, except in single variscosities (Fig. 1b; Table 1). Electron-microscopic investigation showed a sharp decrease in the number of axons containing granular synaptic vesicles and the number of these vesicles in the axons also was considerably reduced (Table 1). The subsequent addition of NA (0.5 μg/ml) led to marked recovery of the mediator reserves in the tissue and nerve fibers but only in those cases in which a monoamine oxidase inhibitor (iproniazid, 0.1 mM) was present in the medium. It will be clear from Table 1 that the NA content in the tissue in this case rose to 81%. Restoration of fluorescence of the adrenergic fibers was observed under these circumstances (Fig. 1c, Table 1), indicating the accumulation of NA inside the axons. If NA (and iproniazid) were added to the medium after incubation of the vas with fluacizine (1 μg/ml) or imipramine (1 μg/ml), the recovery of the reserves and accumulation of NA in the fibers did not take place. As Table 1 shows, during incubation with the antidepressants the total content of the mediator in the tissue and the fluorescence of the nerve fibers (Fig. 1d) remained the same as in the experiments with reserpine. The electron-microscopic study showed that the number of granular synaptic vesicles in the experiments in which NA was added and in the experiments with the antidepressants and NA was not increased (Fig. 2B; Table 1). It must also be pointed out that the observed effects of fluacizine and imipramine were comparable.

The results of the spectrofluorimetric and fluorescence-histochemical tests to detect the mediator thus showed close correlation between the total content of NA in the tissue and its content in the adrenergic nerve fibers. Hence it follows that the main reserves of NA revealed biochemically were localized in the nerve fibers. The accumulation of mediator in the sympathetic fibers after incubation of the vas with NA and iproniazid demonstrates the ability of axons of the vas deferens to assimilate exogenous NA after inhibition of monoamine oxidase. In this case it can be supposed that NA accumulates in the extravesicular space of the axons, for the electron-microscopic investigation showed synaptic vesicles containing hardly any catecholamines (Table 1), in agreement with the observations of Van Orden et al. [11]. The absence of NA accumulation in the synaptic vesicles can be explained in all probability by the property of reserpine not only to deplete the catecholamine reserves, but also to block the reentry of NA into the synaptic vesicles [6, 11]. Inhibition of the ability of the presynaptic fibers to accumulate exogeneous mediator by fluacizine resembles the effect of the antidepressants of the imipramine group.

It can be concluded from the results of this investigation that the mechanisms of this inhibition are located at the level of the outer axon membrane [7].

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