EFFECT OF CARBIDINE ON CONTENT AND DEPOSITION OF ADRENERGIC NEUROTRANSMITTER IN SYNAPTIC VESICLES

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The effect of the original Soviet psychotropic agent carbidine on the content and deposition of adrenergic mediator in the synaptic vesicles of sympathetic nerve fibers of the rat vas deferens was studied by electron-microscopic cytochemistry. The results showed that carbidine can reduce the reserves of monoamines deposited in synaptic vesicles but does not affect the uptake and accumulation of exogenous noradrenalin by synaptic vesicles.

KEY WORDS: carbidine; synaptic vesicles; noradrenalin.

An important role in the mechanisms of action of carbidine (3,6-dimethyl-1,2,3,4,4a,9a-hexahydrocarboline dihydrochloride), an original Soviet psychotropic agent with pharmacological properties of a neuroleptic and antidepressant [3], is played by the effect of the compound on adrenergic processes [4]. However, the effect of carbidine on the content of adrenergic mediator and on its deposition in synaptic vesicles (SVs) has not yet been investigated.

Accordingly, in the investigation described below, the effect of carbidine on integrity of the monoamines in SVs of adrenergic nerves and the penetration of exogenous noradrenalin (NA) into the SVs were studied.

EXPERIMENTAL METHOD

The NA content of SVs of adrenergic nerve fibers of the rat vas deferens was determined by a combined electron-microscopic and cytochemical method (fixation in a mixture of glutaraldehyde and paraformaldehyde, pH 7.4, potassium bichromate, pH 4.2, and osmium tetroxide), by means of which catecholamines can be specifically demonstrated in SVs as granules [5].

The material was examined and photographed on the JEM-100V (Japan) electron microscope. The number of granular SVs per μ^2 of section through the nerve fiber was counted in not less than 30 electron micrographs from each specimen. The experimental results were subjected to statistical analysis and the confidence limits of the means were determined at the $P = 0.05$ level.

The number of granular SVs in vivo was determined 4, 12, and 24 h after administration of carbidine in doses of 5 and 20 mg/kg. The accumulation of exogenous NA in the SVs was studied after exhaustion of the endogenous reserves of mediator by tyramine. For this purpose, the tyramine was injected intraperitoneally (50 mg/kg) 5 times, with an interval of 1 h between injections. Exogenous NA (0.5 mg/kg) was then injected intravenously, and material was taken for examination 30 min later. In some experiments, the animals were given carbidine in the same doses 2 h before the injection of NA.

The vas deferens was incubated in vitro in aerated Krebs' solution at 32°C. The experiments were carried out after preliminary incubation of the vas in Krebs's medium for 1.5 h. The number of SVs was

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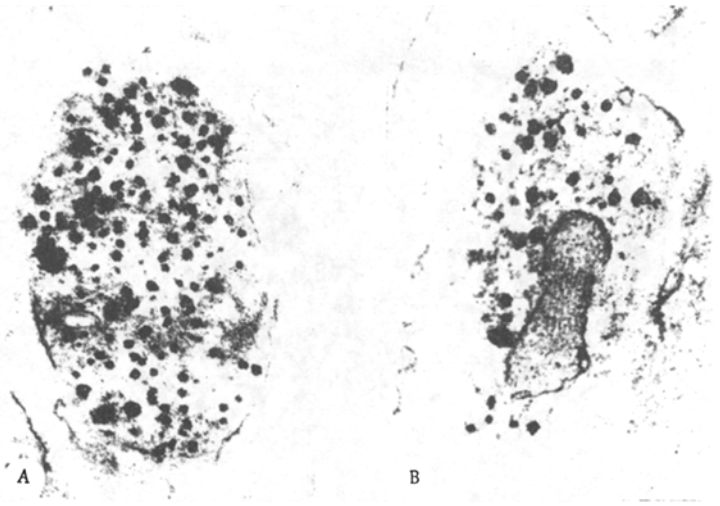

Fig. 1. Electron-microscopic demonstration of adrenergic mediator in SVs of sympathetic nerve fibers of rat vas deferens: A) content of mediator in SP of intact animal, 51,000 ×; B) 24 h after injection of carbidine in dose of 20 mg/kg, 55,500 ×.

TABLE 1. Effect of Carbidine on NA content in SVs of Adrenergic Nerve Fibers of Rat Vas Deferens in vivo

Dose of carbidine (in mg/kg)	Time (in h)	No. of granular SVs	
		per μ^2	% of control
Control		158±29*	100
5	4	111±19	70
	12	106±17	67
	24	101±20	64
20	4	107±17	68
	12	103±19	65
	24	96±16	61

* Confidence limits of means given for P = 0.05.

TABLE 2. Effect of Carbidine on NA Content in SVs of Adrenergic Nerve Fibers of Rat Vas Deferens in vitro

Concentration of carbidine (in M)	No. of granular SVs	
	per μ^2	% of control
Control	123±17*	100
$3{,}4 \cdot 10^{-4}$ †	108±15	88
$3{,}4 \cdot 10^{-3}$	87±11	71

* Confidence limits of means given for P = 0.05.
† Incubation with carbidine for 30 min in all experiments.

determined 30 min after addition of carbidine in concentrations of $3.4 \cdot 10^{-4}$ and $3.4 \cdot 10^{-3}$ M, equivalent to 0.1 and 1 ED_{50}, calculated in preliminary experiments to study the effect of inhibition by carbidine of contractions of the vas in response to transmural electrical stimulation of postganglionic sympathetic nerves [1].

Accumulation of exogenous NA in SVs was studied after exhaustion of the reserves of mediator by tyramine (0.2 mM, 2 h) and subsequent incubation of the tissue with exogenous NA ($3 \cdot 10^{-6}$ and $9 \cdot 10^{-5}$ M, 30 min). In some experiments, carbidine was added to the medium in the same concentration 15 min before determination of NA.

EXPERIMENTAL RESULTS

On the electron micrograph the adrenergic nerve fibers of the vas deferens appeared as circular, oval, or elongated profiles depending on the direction of the fiber in the plane of section. In intact animals the adrenergic fibers contained numerous SVs, mainly 600-800 Å in diameter (sometimes 1200-2000 Å)

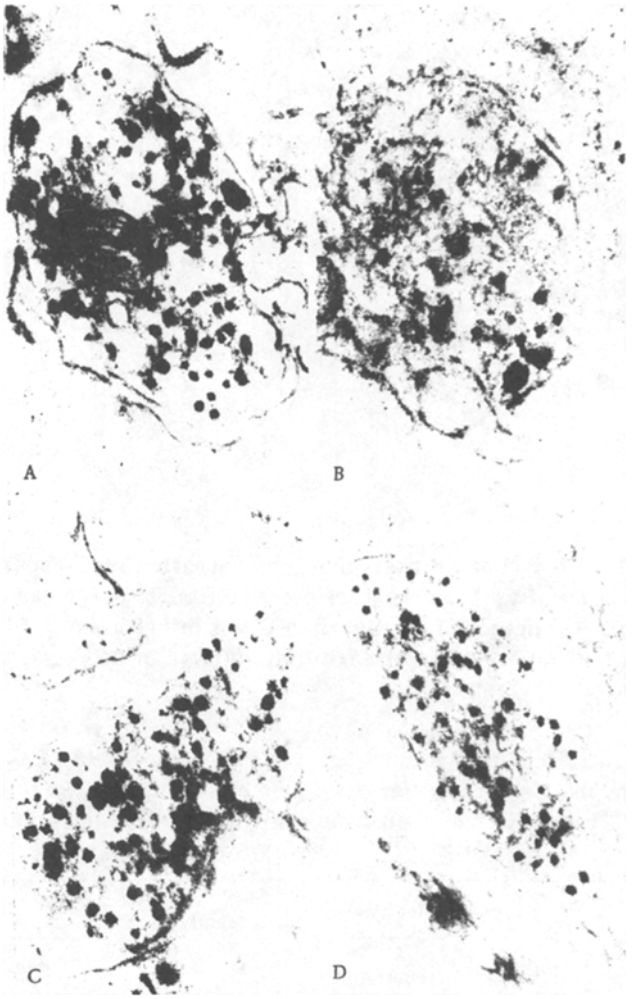

Fig. 2. Electron-microscopic demonstration of adrenergic mediator in SVs of sympathetic nerve fibers of rat vas deferens: A) control, 50,500 ×; B) after exhaustion of reserves of mediator by tyramine, 56,000 ×; C) after exhaustion of reserves of mediator by tyramine and subsequent injection of NA (0.5 mg/kg, 30 min), 52,000 ×; D) after exhaustion of reserves of mediator by tyramine and injection of carbidine (20 mg/kg) 2 h before injection of NA, 50,000 ×.

with dense inclusions in the center, consisting of the product of the cytochemical reaction of catecholamines deposited in the SVs (Fig. 1A). The mean number of granular SVs calculated per μ^2 of section through the nerve fiber in the control animals in vivo was 158±29 (P = 0.05).

The effect of carbidine on the number of SVs storing adrenergic mediator is shown by the data in Table 1. Clearly after injection of carbidine in doses of 5 and 20 mg/kg the number of granular SVs fell to about 60-70%, whereas 4 h after injection of the compound in a dose of 5 mg/kg, their mean number showed only a tendency to fall. In all other cases the decrease in number of granular SVs differed significantly from the mean value in the control experiments (Fig. 1B).

The number of granular SVs in the control specimens in vitro was less than in the experiments in vivo, probably on account of loss of some of the catecholamine reserves. In these experiments a decrease in the number of granular SVs could also be seen after incubation of the tissue with carbidine in a concentration of $3.4 \cdot 10^{-3}$ M (Table 2).

It can be concluded from analysis of these data that the decrease in number of granular SVs after administration of carbidine in experiments in vivo and in vitro can be attributed to the ability of the compound to lower the reserves of catecholamines in the depot vesicles. Similar results have previously been recorded in experiments on the vas deferens with reserpine and tyramine [2, 6].

TABLE 3. Effect of Carbidine on Uptake of Exogenous NA by SVs of Adrenergic Nerve Fibers of Rat Vas Deferens in vivo

Substance and dose (in mg/kg)	Time (in h)	No. of granular SVs per μ^2	No. of granular SVs % of control
Control		158±20*	100
Tyramine†	5	54±14	34
Tyramine + NA 0,5	0,5	119±20	75
Tyramine + carbidine 5 + NA 0,5	2 0,5	107±19	68
Tyramine + carbidine 20 + NA 0,5	2 0,5	101±15	64

* Confidence limits of means given for P = 0.05.

† Tyramine added 5 times (50 mg/kg) at hourly intervals between injections in all experiments.

TABLE 4. Effect of Carbidine on Uptake of Exogenous NA by SVs of Adrenergic Nerve Fibers of Rat Vas Deferens in vitro

Substance and dose (in mg/kg)	No. of granular SVs per μ^2	No. of granular SVs % of control
Control	123±17*	100
Tyramine $0{,}2 \cdot 10^{-3}$†	22±9	18
Tyramine + NA $2 \cdot 10^{-6}$‡	49±10	40
Tyramine + NA $9 \cdot 10^{-5}$	105±14	85
Tyramine + carbidine $3{,}4 \cdot 10^{-4}$ + NA $3 \cdot 10^{-6}$	37±6	30
Tyramine + carbidine $3{,}4 \cdot 10^{-3}$ + NA $3 \cdot 10^{-6}$	34±7	28
Tyramine + carbidine $3{,}4 \cdot 10^{-4}$ + NA $9 \cdot 10^{-6}$	100±13	81
Tyramine + carbidine $3{,}4 \cdot 10^{-3}$ + NA $9 \cdot 10^{-6}$	91±12	74

* Confidence limits of means given for P = 0.05.

† Incubation with tyramine (0.02 mM) for 2 h in all experiments.

‡ Incubation with NA for 30 min in all experiments.

The effect of carbidine on penetration of exogenous NA into SVs and the accumulation of mediator in them was studied after exhaustion of the catecholamine reserves by tyramine. These results are given in Tables 3 and 4 for experiments in vivo and in vitro respectively. It will be clear from Table 3 that the content of granular SVs in vivo after administration of tyramine fell to 34% (Fig. 2A, B), but 30 min after injection of NA (Fig. 2C) it had risen again appreciably (to 75%). Carbidine did not prevent the penetration of exogenous NA into SVs (Fig. 2D), and the number of granular SVs was increased to 64-68% after administration of carbidine and NA. Similar effects were obtained in experiments in vitro (Table 4). It will be clear from Table 4 that after incubation of the tissue with tyramine the number of SVs containing granules fell more (down to 18%) than in the experiment in vivo. Subsequent restoration of the mediator reserves in SVs took place differently depending on the concentration of added NA. For instance, after incubation for 30 min with NA in concentrations of $3 \cdot 10^{-6}$ and $9 \cdot 10^{-5}$ M the number of granular SVs increased to 40 and 85% respectively. In experiments in which carbidine was injected before the addition of NA, the number of granular SVs also rose, although the results of these experiments depended on the concentration of NA and carbidine.

The increase in the number of granular SVs was particularly great if NA was present in the medium in a higher concentration ($9 \cdot 10^{-5}$ M), and carbidine in a lower concentration ($3.4 \cdot 10^{-4}$ M). Preincubation with carbidine in a higher concentration ($3.4 \cdot 10^{-3}$ M), on the other hand, resulted in a small decrease in the number of granular SVs. This fact, in all probability, is connected with the ability of the preparation to reduce the catecholamine reserves in the SVs, in agreement with the results of experiments reported above (Table 2).

The following conclusions can accordingly be drawn from these facts: 1) Carbidine reduces the reserves of adrenergic mediator stored in SVs; 2) it does not prevent the penetration of exogenous NA into SVs, and the accumulation and storage of NA depend on the concentration of extraneuronal mediator.

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