EFFECT OF CARBIDINE ON ADRENERGIC NEUROTRANSMISSION

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Methods of physiological analysis were used to study the effect of the original psychotropic agent carbidine on adenergic neurotransmission on the isolated rat vas deferens as the model. The content of noradrenalin (NA) was determined spectrofluoremetrically in the same ducts and the ability of the neuroleptic to block the uptake of exogenous NA by the tissue also was investigated. Carbidine was found to possess an adrenomimetic effect, due to its ability to liberate NA, leading to a decrease in the endogenous reserves of the neuromediator.

KEY WORDS: vas deferens; carbidine; adrenergic neurotransmission; adrenomimetics.

Carbidine (3,6-dimethyl-1,2,3,4,4a,9a-hexahydro-γ-carboline) is an original Soviet psychotropic agent with the properties of a neuroleptic and antidepressant [3]. An important role in the mechanism of its action is ascribed to its effect on adrenergic structures [4], although this problem has so far received little investigation.

In this investigation the effect of carbidine was studed on adrenergic neurotransmission on the isolated rat vas deferens, a convenient model with which to study these processes because of its rich innervation with sympathetic nerve fibers [2, 6]. In addition, the noradrenalin (NA) content was studied in this test object, and the ability of carbidine to block the reutilization of exogenous NA after exhaustion of the NA reserves by tyramine also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated vas deferens of rats weighing 180-250 g. The ducts were incubated in aerated Krebs' solution at 32°C for 1.5 h, with the nutrient solution changed every 15 min. Contractions of the vas were then studied in response to 1) transmural electrical stimulation of postganglionic sympathetic fibers, 2) addition of NA, and 3) addition of $BaCl_2$. Contractions were recorded on paper on a kymograph drum by means of a frontal-writing lever. Transmural stimulation was applied for 3 sec every 2 min by means of circular platinum electrodes, using pulses of 0.1 msec, 100 V, and 30 Hz, capable of stimulating only postganglionic sympathetic nerve fibers [1].

In order to detect the adrenolytic or adrenomimetic properties of the preparation its effect was studied on contractions induced by the addition of NA (1 μg/ml); the spasmolytic effect of carbidine was studied by investigating its effect on contractions induced by the addition of 1 mg/ml $BaCl_2$.

The total NA content in the ducts was determined by spectrofluorometry [5]. To study the action of carbidine on the uptake of exogenous NA the preparation was administered 15 min before the addition of the neurotransmitter, after preliminary exhaustion of the endogenous NA reserves by incubating the vas with tyramine (0.2 mM, 2 h).

EXPERIMENTAL RESULTS

In the first stage the effect of carbidine was studied on contractions of the vas evoked by transmural

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TABLE 1. Effect of Carbidine on Contractions of Rat Vas Deferens Evoked by Transmural Electrical Stimulation of Sympathetic Fibers or by Addition of NA and $BaCl_2$

Carbidine concentration (in μg/ml)	Transmural stimulation		Contractions after administration of NA	Contractions after addition of $BaCl_2$ (1 mg/ml)
	amplitude of contractions	contracture		
	In % of control			
Control	100±9*	—	100±10	100±7
0,1	83±12	—	99±8	—
1	124±21	—	97±9	—
5	131±28	—	94±11	—
10	129±26	15±6	178±31	—
50	158±9	20±7	342±86	—
100	168±20	26±10	369±134	—
250	145±18	30±8	371±127	235±74
500	93±6	34±9	366±105	102±45
1000	47±21	41±13	229±58	58±38
1500	32±14	46±12	212±62	47±22

*Significant intervals of means for P = 0.05.

TABLE 2. Effect of Carbidine on Noradrenalin Content in Rat Vas Deferens

Carbidine concentration (in M)	NA concentration	
	in μg/g weight of tissue	in % of control
Control	9,7±0,7*	100
$3,4.10^{-4}$**	8,9±1,1	92
$3,4.10^{-3}$	6,7±0,7	69

*Significant intervals of means for P = 0.05.
†Incubation with carbidine in all experiments was carried out for 30 min.

electrical stimulation of the sympathetic nerves. In this series of experiments carbidine was added in concentrations of 0.1-1500 μg/ml and 15 min later, the test object was rinsed with the incubation solution until the initial level of contractions was restored. After each dose of carbidine the mean value of the response was determined over a period of 15 min and was expressed as a percentage of the amplitude of the contraction in the control series. The results are given in Table 1.

As Table 1 shows, in concentrations of 5-250 μg/ml the neuroleptic, after addition, led to a marked increase in the amplitude of contractions in response to electrical stimulation. In addition, after addition of carbidine the appearance of a tonic contraction (contracture) of the vas was observed. For instance, in concentrations of 5 and 10 μg/ml, carbidine increased contractions up to 129-131%, and in concentrations of 50, 100, and 250 μg/ml—up to 145-168%. Contracture of the vas increased to 15, 20, and 30% respectively. With a further increase in the neuroleptic concentration to 500-1500 μg/ml, the tonic concentration continued to increase (to 34-46%) and the response to electrical stimulation gradually decreased. The effective concentration in which carbidine reduced contractions of the vas by 50% (ED_{50}) in response to electrical stimulation was 940 μg/ml ($3.4 \cdot 10^{-3}$ M).

The effect of carbidine on contractions induced by the addition of NA also reflected the data given in Table 1. The neuroleptic potentiated the NA effect. After its addition, carbidine also caused the vas to contract independently. This adrenomimetic action could also be found in cases when the pharmacological agent is able a) to activate adrenergic receptors, b) to liberate mediator from nerve fibers or, and c) to block the uptake of NA by sympathetic fibers.

To analyze these hypotheses, 30 min after the addition of carbidine the NA content was determined in the ducts, and the ability of the neuroleptic to block the uptake of exogenous NA by the tissue of the vas and to accumulate it after exhaustion of the endogenous NA reserves by tyramine also was investigated. The results are given in Tables 2 and 3.

As Table 2 shows, after the addition of carbidine in a concentration of 0.1 ED_{50} ($3.4 \cdot 10^{-4}$ M) a tendency was observed for the NA concentration to diminish, whereas after the addition of carbidine in a concentration of 1.0 ED_{50} ($3.4 \cdot 10^{-3}$ M) the NA content fell appreciably (to 69%). It will be clear from Table 3 that after preliminary incubation of the vas with tyramine (0.2 mM, 2 h) the NA level in the tissue fell to 57%. The subsequent addition of NA (1 μg/ml) led to an appreciable increase in the content of the mediator in the tissue (to 112%).

Carbidine, added 15 min before the neurotransmitter in concentrations of 0.1 ED_{50} and 1.0 ED_{50}, did not block the uptake and accumulation of exogenous NA in the tissue. The small accumulation of NA in the case when carbidine was added in a concentration of 1.0 ED_{50}, can be explained by the ability of carbidine to liberate the neuromediator.

The spectrofluorometric data thus indicates that the neuroleptic can liberate NA but cannot block the uptake and accumulation of the exogenous mediator. The problem of the ability of carbidine to excite α-adrenergic receptors directly remains open. However, facts obtained in the course of this investigation (contraction of the vas in response to addition of a preparation potentiating the effect of NA, the appearance

TABLE 3. Effect of Carbidine on Uptake of Exogenous Noradrenalin by Isolated Rat Vas Deferens

Substances	Carbidine concentration (in M)	NA content	
		in μg/g weight of tissue	in % of control
Control		9,7±0,7*	100
Tyramine†		5,5±0,6	57
Tyramine + NA‡		10,9±1,7	112
Tyramine + carbidine + NA	$3,4.10^{-4}$	9,8±0,9	100
Tyramine + carbidine + NA	$3,4.10^{-3}$	7,1±0,6	73

*Confidence intervals of mean when P = 0.05.

†Incubation with tyramine (0.2 mM) in all experiments carried out for 2 h.

‡Incubation with NA (1 μg/ml) carried out in all experiments for 30 min.

of contracture, and an increase in the contractions in response to electrical stimulation) can be explained by the property of the neuroleptic to liberate NA from nerve endings.

The reduction in the amplitude of the contractions during stimulation of adrenergic fibers after the addition of carbidine in concentrations of 500-1500 μg/ml is presumably the result of a sympatholytic effect. For instance, it follows from Table 1 that inhibition of the contractions in response to transmural stimulation cannot be explained by the adrenolytic or the slight spasmolytic action of carbidine (manifested in concentrations of 1000-1500 μg/ml). The decrease in amplitude of the contractions may also be connected with a decrease in the number of free adrenergic receptors capable of interacting with the NA liberated during stimulation. This hypothesis is based on the fact that carbidine, when administered in increasing concentrations, leads to an increase in the liberation of NA, which occupies an ever-increasing number of adrenergic receptors and potentiates the tonic contraction of the vas.

It can accordingly be concluded from these results that the adrenomimetic effects of carbidine are connected with its well-marked ability to liberate NA from nerve endings. These qualities of the neuroleptic may evidently play an important role in the mechanisms of its pharmacological action.

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