EFFECT OF NONACHLAZINE ON ADRENERGIC NEUROTRANSMISSION IN THE RAT VAS DEFERENS

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The effect of a new antianginal preparation, nonachlazine, on adrenergic neurotransmission in the isolated rat vas deferens was studied by recording contractions of the duct in response to transmural stimulation of postganglionic sympathetic nerves by the electric current or by application of noradrenalin (NA) or $BaCl_2$. The effect of nonachlazine on the NA content was studied spectrofluorometrically and the ability of the preparation to block the uptake of exogenous NA by the tissues also was investigated. Nonachlazine was found to have a moderate sympatholytic action, which was combined with its spasmolytic effect. Nonachlazine also has a well marked ability to block the reaccumulation of NA in the tissues.

KEY WORDS: nonachlazine; adrenergic neurotransmission; content and uptake of noradrenalin.

Nonachlazine – [10'β-(1,4-diazabicyclo(4,3,0)-nonany-4)propionyl-2-chlorphenothiazine dihydrochloride] – is a new antianginal preparation used clinically for the treatment of ischemic heart disease. The experimental study of nonachlazine has shown [2] that the mechanism of its positive effect on the circulation and cardiac activity is connected with its ability to interfere with peripheral adrenergic processes. However, this problem has been inadequately studied.

In this investigation the effect of nonachlazine on adrenergic neurotransmission was studied in the isolated rat vas deferens, a convenient object with which to study these problems because of its abundant supply of adrenergic nerves, its marked contraction response, and so on.

EXPERIMENTAL METHOD

The isolated vas deferens of rats weighing 180-250 g was used. The ducts were placed in aerated Kreb's solution at 32°C and incubated for 1.5 h, the incubation solution being changed every 15 min. The effect of nonachlazine was then studied on contractions induced by various methods depending on the purpose of analysis of adrenergic neurotransmission. The response of the vas was recorded by means of a frontally writing lever on the paper strip of a kymograph.

Contractions were studied in response to transmural electrical stimulation of the postganglionic sympathetic fibers. Stimuli were applied through circular platinum electrodes for 3 sec every 2 min, using conditions (0.1 msec, 100 V, 30 Hz) under which only adrenergic nerve fibers were stimulated [1]. To discover whether nonachlazine had adrenolytic or adrenomimetic properties, its effect on contractions induced by addition of noradrenalin (NA) was studied. The spasmoytic action of the preparation was studied from its ability to reduce contractions in response to conditions of $BaCl_2$.

The total NA content in the tissue was determined spectrofluorometrically by the method of Euler and Lishajko [3].

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TABLE 1. Effect of Nonachlazine on Contractions of Isolated Rat Vas Deferens Induced by Transmural Electrical Stimulation or by Addition of NA and $BaCl_2$ (M ± m)

Nonachlazine concentration (μg/ml)	Transmural stimulation (amplitude of contractions, % of control)	Contractions after addition of 1 μg/ml NA (% of control)	Contractions after addition of 1 mg/ml $BaCl_2$ (% of control)*
Control	100±9†	100±10	100±7
0,01	98±7	120±11	106±9
0,1	92±15	195±76	127±16
0,5	98±8	213±84	146±38
1,0	86±4	251±90	193±40
5,0	64±17	316±95	226±47
10,0	48±6	224±80	101±34
25,0	29±3	73±25	49±19
50,0	19±5	15±7	26±17

*Experiment with $BaCl_2$ carried out in Krebs-Ringer solution (difference between results not statistically significant).
†Confidence limits on the mean given for P=0.05.

TABLE 2. Effect of Nonachlazine on Content and Uptake of NA by Isolated Rat Vas Deferens

Substances and concentration (μg/ml)	Content of NA	
	μg/g wt. of tissue (M ± m)	% of control
Control*	9,7±0,7†	100
Nonachlazine 0,95‡	9,6±0,8	100
9,5**	10,0±0,6	102
NA 1,0††	14,3±1,8	147
Nonachlazine + NA 0.95	11,5±1,3	119
Nonachlazine + NA 9.5	9,9±1,0	102

*Incubation in Krebs' solution.
†Confidence limits of mean given for P=0.05.
‡ 0.1 ED_{50} for inhibition of contractions of vas by transmural electrical stimulation.
** ED_{50} for inhibition of contractions of vas by transmural electrical stimulation.
†† Incubation with NA in a concentration of 1 μg/ml lasted for 30 min in all experiments.

To study the effect of nonachlazine on the uptake and accumulation of exogenous NA the preparation was added 15 min before the addition of NA. The results were subjected to statistical analysis and confidence limits of the mean were determined for P=0.05.

EXPERIMENTAL RESULTS

The effect of nonachlazine on contractions of the vas evoked by transmural electrical stimulation of the adrenergic nerves is shown in Table 1. The vas was rinsed out 15 min after the addition of the preparation by repeated changes of the incubation solution until the original level of contractions was restored. In each case after addition of the preparation the mean amplitude of the contraction was calculated for a period of 15 min and expressed as a percentage of the original reaction. It will be clear from Table 1 that nonachlazine, starting with a concentration of 1-5 μg/ml, caused inhibition of contractions in response to sympathetic nerve stimulation. For instance, after addition of the preparation in a concentration of 1 μg/ml a tendency for the amplitude of the contractions to be reduced was found. After addition in a concentration of 5 μg/ml, nonachlazine caused inhibition of the contractions by up to 64%, in a concentration of 10 μg/ml up to 48%, and in concentration of 25 and 50 μg/ml, of up to 29 and 14% respectively. Calculation of the half effective concentration (ED_{50}), at which nonachlazine lowered the level of contractions by 50% in response to electrical stimulation, gave a mean value of ED_{50}=9.5 μg/ml.

A decrease in amplitude of the contractions could be observed if the substance studied possessed: 1) a spasmolytic action, 2) adrenolytic properties, or 3) sympatholytic properties.

In order to determine which of these properties nonachlazine in fact possesses experiments were carried out to study the action of the preparation on contractions induced by addition of $BaCl_2$ for NA. The experiments with $BaCl_2$ (1 mg/ml) showed that nonachlazine has a sympatholytic action starting with a concentration of 25 μg/ml (Table 1). Investigation of the effect of nonachlazine on contractions induced by the addition of NA showed that it does not possess α-adrenoblocking properties. It will be clear from Table 1 that nonachlazine, on the other hand, has a well-marked ability to potentiate the action of NA, starting from very low concentrations (0.01 μg/ml).

It can be concluded from a comparison of these data that inhibition of contractions of the vas by nonachlazine (in concentrations of 1-10 μg/ml) in response to stimulation of the adrenergic nerves cannot be explained by the existence of spasmolytic or adrenolytic properties of the preparation, but is the result of its moderately strong sympatholytic action. On addition of the preparation in higher concentrations

(25-50 μg/ml) this effect was supplemented by the spasmolytic action of nonachlazine. The decrease in the contractions in response to addition of NA after the addition of nonachlazine in concentrations of 25-30 μg/ml also was connected with the appearance of a spasmolytic action of the preparation in these concentrations (Table 1). The well-marked ability of nonachlazine in a concentration of 0.01-10 μg/ml to potentiate contractions induced by NA (Table 1) cannot be explained by the ability of the preparation to activate α-adrenoceptors directly or through liberation of mediator from nerve endings. In that case, during transmural stimulation nonachlazine would not inhibit but, on the contrary, would increase the amplitude of response of the organ by causing it to contract tonically. The ability of nonachlazine to potentiate the action of NA could be linked with its ability to block the reassimilation of NA by sympathetic fibers, with a consequent increase in the concentration of the mediator at the receptors.

To test this hypothesis the NA content in the vas was determined spectrofluorometrically after the addition of nonachlazine or of exogenous NA, or the combined addition of nonachlazine and exogenous NA. It will be clear from Table 2 that nonachlazine after addition in concentrations equal to ED_{50} (inhibition of contractions in response to transmural stimulation) and 0.1 ED_{50} had no appreciable effect on the content of NA reserve. The subsequent addition of exogenous NA in a concentration of 1 μg/ml led to a considerable increase in the content of neuromediator in the tissue (up to 147%). Addition of nonachlazine before NA prevented the effect of NA accumulation. As Table 2 shows, addition of nonachlazine before NA in a concentration of 0.95 μg/ml (0.1 ED_{50}) considerably reduced the uptake and accumulation of NA, the concentration of which in the tissue did not rise above 119%. When added in a concentration of 9.5 μg/ml (ED_{50}), the preparation completely blocked NA accumulation.

Results obtained by different methods of investigation thus supplement each other and lead to the conclusion that nonachlazine, with its effect on adrenergic neurotransmission, has a moderately strong sympatholytic action (in concentrations of 1-10 μg/ml), which is supplemented by its spasmolytic effect (in concentrations of 25-30 μg/ml). Nonachlazine also has a well-marked property of blocking the reaccumulation of exogenous NA by the tissue, and thus of potentiating the action of NA.

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