MECHANISM OF NORADRENALIN LIBERATION FROM THE RAT BRAIN UNDER THE INFLUENCE OF NICOTINE

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Definite relationships were found between the noradrenalin concentration and Mg^{++}-ATPase activity in the rat brain during the action of nicotine. It is suggested that Mg^{++}-ATPase regulates the storage of noradrenalin in the tissue depots.

KEY WORDS: *ATPase; noradrenalin; nicotine.*

It has now been shown that the storage and liberation of catecholamines in presynaptic structures depends on the ATP content and the presence of Mg^{++}, Ca^{++}, Na^{+}, and K^{+} ions [10, 11, 13, 14]. Meanwhile the role of membrane-bound enzymes in these processes has been inadequately studied, although data have been obtained to show that membranes of presynaptic formations contain Ca^{++}- and Mg^{++}-dependent ATPases [6, 7].

The effect of nicotine on Mg^{++}-ATPase activity and the noradrenalin (NA) concentration in the rat brain was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-200 g, which received an intraperitoneal injection of nicotine in a dose of 0.4 mg/kg in the form of 0.1 ml of solution/100 g body weight; control animals received water. In experiments in vitro nicotine was added up to a final concentration of 3×10^{-4}-3×10^{-6} M. The animals were decapitated and the brain removed and freed as much as possible from blood. The NA level was determined spectrofluorometrically [4]. ATPase activity was determined in the membrane fraction [8]; the concentration of sodium deoxycholate was 0.2% and the incubation time 30 min. In experiments in vitro, the duration of preincubation of the synaptosomes [15] and the deoxycholate extract was 15 min, after which ATP was added to the mixture, which was incubated for 15 min. The reaction was stopped by the addition of an equal volume of 10% TCA. The samples were centrifuged and inorganic phosphate (P_i) determined in the supernatant by the use of ammonium molybdate and 1% ascorbic acid. The protein concentration was determined by Lowry's method [9].

Mg^{++}-ATPase activity in all cases was determined in the presence of Na^{+} (10^{-1} M), K^{+} (10^{-2} M), and Mg^{++} (10^{-3} M) ions, with the addition of strophanthin K (ouabain, 10^{-4} M). The control samples contained all the ingredients except Na^{+}, K^{+}, and Mg^{++} ions.

EXPERIMENTAL RESULTS

When nicotine was injected in a dose of 0.4 mg/kg the NA content in the brain

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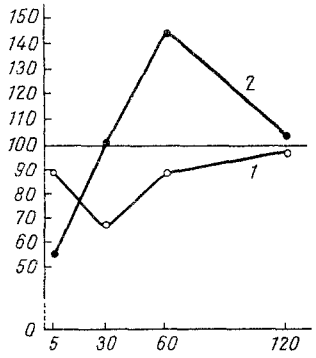

Fig. 1. Effect of nicotine on noradrenalin concentration and Mg^{++}-ATPase activity in rat brain. Abscissa, time after injection of 0.4 mg/kg nicotine (in min); ordinate, deviation of values from control, taken as 100% (in%). 1) Noradrenalin concentration, 2) Mg^{++}-ATPase activity.

TABLE 1. Mg^{++}-ATPase Activity of Synaptosomes and of Deoxycholate Extract of Rat Brain following Action of Nicotine (in μmoles P_i/mg protein/30 min), M ± m

Experimental conditions	Mg^{++}-ATPase activity (n = 4) of	
	synaptosomes	deoxycholate extract
Control	24,1±1,8	0,305±0,025
Nicotine		
$3 \cdot 10^{-6}$ M	17,0±1,1	0,295±0,03
P	<0,05	>0,5
$3 \cdot 10^{-5}$ M	12,9±1,0	0,290±0,035
P	<0,05	>0,5
$3 \cdot 10^{-4}$ M	16,4±1,4	0,287±0,03
P	<0,05	>0,5

homogenate fell after 5 min to 89% and after 30 min to 68% of the control level. The brain NA level returned to normal after 2 h. The Mg^{++}-ATPase activity 5 min after injection of nicotine was 55% of the control, 30 min after injection at the time of the greatest decrease in the NA concentration in the brain it was at the control level again, and 1 h after the injection it was significantly higher than the control, at a time when the NA reserves in the brain were beginning to be restored. The ATPase activity returned to its initial level 2 h after the injection, i.e., at about the time when the normal NA concentration was restored (Fig. 1).

The main site of NA storage in the brain is the nerve endings (synaptosomes), which contain numerous synaptic vesicles [12, 15] and in which ATPase activity is high [5]. The corresponding experiments showed that 5 min after injection of nicotine Mg^{++}-ATPase activity of the synaptosomes was reduced by 22% (24.1 1.5 in the control and 18.7 1.8 μmoles P_i/mg protein/30 min in the experimental series), but after 1 h it was increased by 40% (27.5 2.6 in the control and 38.9 2.3 μmoles P_i/mg protein/30 min in the experimental series), i.e., the same effect was observed as in the whole brain.

The results indicate that the decrease in Mg^{++}-ATPase activity coincided in time with the liberation of NA from the presynaptic structures, whereas its increase preceded the beginning of NA accumulation in them.

Experiments in vitro showed that through the action of nicotine (3×10^{-6} M) the NA concentration in synaptosomes previously saturated with NA was reduced by 45% (0.22 ± 0.004 in the control and 0.12 ± 0.006 μg/ml in the experimental series) and Mg^{++}-ATPase activity was inhibited (Table 1).

All these results point to the presence of a connection between the liberation and restoration of the stocks of NA in the rat brain and the activity of membrane-bound Mg^{++}-ATPase.

The change in ATPase activity probably takes place as a result of interaction between nicotine and the lipid components of the membranes of the nerve endings, for nicotine is highly lipophilic [2].

Later the nicotine–lipid complex is hydrolyzed and the activity of the enzyme is restored. This hypothesis is supported by experiments with a deoxycholate extract of rat brain (Table 1), in which the activity of the enzyme was unchanged by the action of nicotine. The membranes probably lose their native structure on treatment with sodium deoxycholate [13] and the bond between nicotine and the membrane lipids is broken.

The results of these experiments suggest that Mg^{++}-ATPase regulates the processes of NA storage in the tissue depots.

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