It can be concluded from these results that the action of ethimizole on energy metabolism in the myocardium is effected through adenyl cyclase, with subsequent activation of the slow ionic channels by cyclic AMP-dependent protein kinases [7]. As the result of activation of these channels more extracellular calcium enters the cells during the plateau of the action potential and is retained in the intracellular depots. Meanwhile, there is some evidence [3] that activity of the slow channels is determined to a greater degree by the state of the intracellular energy metabolism and it depends on activity of creatine phosphokinase, located in the plasma membrane. Normalization of calcium metabolism under the influence of ethimizole after neurogenic injury to the myocardium correlates with restoration of the creatine phosphate level, reflecting the close interaction between energy formation processes and activity of the calcium pump in the mechanism of the effect of ethimizole on metabolism of the neurogenically injured heart.

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EFFECT OF ARMIN ON SYNAPTIC TRANSMISSION IN THE FROG NERVE – MUSCLE PREPARATION

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KEY WORDS: armin; synaptic transmission; nerve–muscle preparation.

Many anticholinesterase drugs are considered to have a nonspecific action which complicates purely anticholinesterase effects [3]. Data in the literature on the presynaptic action of anticholinesterase drugs are few in number and contradictory in nature [4, 5]. The mechanisms of the blocking action of the organophosphorus cholinesterase inhibitor armin* on neuromuscular transmission during low-frequency stimulation have not yet been explained.

The object of the present investigation was to study the effects of armin on the various components of the frog neuromotor synapse.

EXPERIMENTAL METHOD

Experiments were carried out on nerve–muscle preparations of the frog pectoralis muscle placed in a transparent plastic chamber with a capacity of 1.5 ml, through which the test solutions flowed. The nerve was stimulated in the solution surrounding the muscle by square pulses 0.1 msec in duration, applied through a "suction" electrode. The frequency of stimulation was 1 Hz and its strength 2-3 thresholds.

The resting membrane potential (MP) of the muscle fibers and spontaneous miniature and evoked end-plate potentials (MEPP and EPP respectively) were recorded intracellularly by the standard microelectrode

*Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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TABLE 1. Effect of Armin on Frog Neuromotor Synapse (M ± m)

Parameter, %	Armin concentration, g/ml		
	10^{-8}	10^{-7}	10^{-6}
t_{asc}	No change	111,3±9,4*	273,8±26,5
$t_{1/2}$EPP	" "	104,4±4,9*	235,1±14,8
A of MEPP	" "	116,4±1,7*	143,2±10,2
A of EPP	152,3±2,0	159,8±18,4	203,0±29,9
QC of EPP	164,1±2,7	160,2±13,2	151,5±25,5*
fMEPP	255,1±11,2	131,8±32,6*	91,9±14,2*

*Differences not statistically significant.

method. To record the end-plate currents (EPC) a voltage clamp method was used [9]. To determine the reversal potential (E_r) of EPC, MP was shifted by depolarizing pulses, 250 msec in duration, from −80 to +40 mV in steps of 20 mV. Stimulation of the nerve was synchronized with the beginning of the depolarizing pulses. The following frequency of the pulses did not exceed once per second.

Ringer's solution containing magnesium, reducing the quantum composition (QC) of the EPP, was used to record EPP. To record EPC, the nerve–muscle preparation were glycerinized beforehand [7].

Solutions of the following composition (in mM) were used as controls: 1) NaCl 112.0; KCl 2.0; $CaCl_2$ 1.9; $NaHCO_3$ 2.9; 2) NaCl 116.2; KCl 2.0; $CaCl_2$ 1.08; $MgCl_2$ 4.0. Test solutions of the same composition contained 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} g/ml of armin; the pH of all solutions was 7.4-7.6 and they were at room temperature. Test solutions were introduced into the chamber in order of rising concentrations. Exposure of the preparations to each solution lasted 10 min.

QC of synaptic transmission was estimated from the results of comparison of the mean amplitudes (A) of EPP and MEPP and the coefficient of variation of A of EPP [11]:

$$QC_1 = \bar{A}_{EPP}/\bar{A}_{MEPP} \quad (1)$$

$$QC_2 = I/CV^2_{EPP} \quad (2)$$

If the initial values of A of EPP were high (over 2 mV) a correction was made for nonlinear summation of single potentials in EPP [11]. Changes in QC of EPP and EPC under the influence of armin were analyzed by equation (1). Comparison of QC of EPC, calculated by equations (1) and (2), enabled the probability of liberation of a quantum of mediator P to be estimated indirectly [2, 12]:

$$QC_1 = QC_2(I - P)$$

whence

$$P = I - (QC_1/QC_2).$$

All results were subjected to statistical analysis. The significance of differences was estimated by Student's t-test. Mean values with their errors, based on results for 10 experiments, are given in Table 1.

EXPERIMENTAL RESULTS

The anticholinesterase action of armin, during an exposure for 10 min, was exhibited in concentrations of over 10^{-7} g/ml. In a concentration of 10^{-6} g/ml the action was well marked, as shown by lengthening of time parameters and an increase in the mean values of A of MEPP and EPP. No significant change in MP of the fibers took place in the presence of armin.

Low concentrations of armin (10^{-8} g/ml) had a positive effect on spontaneous and evoked secretion of the mediator: the frequency (f) of MEPP and QC of EPP were increased to 255 ± 11 and 164 ± 3% respectively. Since there was no change under these conditions in the mean value of A of MEPP, the increase in the mean value of A of EPP (to 152.2 ± 2%) must be explained by an increase in QC of EPP. This positive influence remained with armin in a concentration of 10^{-7} g/ml, although the increase in fMEPP was no longer significant. In a concentration of 10^{-6} g/ml, fMEPP and QC of EPP no longer differed significantly from the control (Table 1).

In armin in a concentration of 10^{-6} g/ml, fMEPP in some fibers increased suddenly and very considerably (by about 40 times). Giant MEPPs appeared in nearly all fibers.

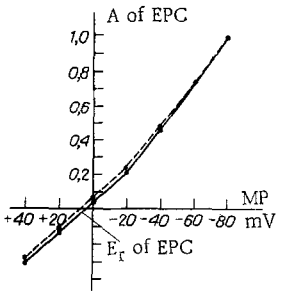

Fig. 1. A of EPC as a function of MP of muscle fiber. Abscissa, MP (in mV); ordinate, ratio of A of EPC at different MP levels to initial value of A at −80 mV. Continuous line − control; broken line − armin in a dose of 5×10^{-7} g/ml.

These results suggest that presynaptic disturbances of mediator liberation play an important role in the mechanism of the functional disturbances in the motor system under the influence of high concentrations of armin during low-frequency stimulation.

Relationships between the peak A of EPC and MP were obtained in preparations glycerinized beforehand. The mean A of EPC in the control at MP = −80 mV was 183 ± 24 nA (Fig. 1). Mean values of peak A of EPC at different MP levels, divided by the initial A of EPC at MP = −80 mV, are plotted on the graph in Fig. 1. The character of the relationship in the control and during exposure to armin (5×10^{-7} g/ml) was thus unchanged. E_r of EPC also was unchanged in the presence of armin. E_r in the control was 5.0 ± 4.4 mV and in the presence of armin it was 5.1 ± 4.6 mV. The anticholinesterase action of armin was relatively high − the half-decay time of the EPC ($t_{1/2}$) was 291 ± 30% compared with the control.

The action of a high concentration of armin (10^{-5} g/ml) on QC of synaptic transmission was investigated under voltage clamp conditions, for against the background of the powerful anticholinesterase action of armin, it is difficult to analyze EPP amplitudes [8]. QC of EPC during 10-min exposure in armin fell significantly to 69.5 ± 5.7%, fMEPP fell to 44.0 ± 8.4%, $t_{1/2}$ of EPC rose to 270 ± 55%, and under these circumstances A of EPC was lowered by about 20%, whereas A of MEPP exceeded the control on average by 40%. The duration of the ascending phase of the MEPP and EPP did not change significantly.

Analysis of the probability (P) of liberation and storage (n) of quanta of accessible mediator showed that n fell significantly in armin to 63.3 ± 8.2 whereas P remained unchanged. Rinsing the preparation for 10 min caused no change in the time parameters of EPC, but QC and f of MEPP were partially restored.

The results are evidence of the powerful anticholinesterase action of armin, as reflected in the considerable lengthening of synaptic potentials and currents, and which was well marked in armin in concentrations of 10^{-6} g/ml or more. The presynaptic action of armin, manifested as changes in QC of synaptic transmission and fMEPP, is two-directional. In low concentrations (10^{-8} g/ml) armin has a positive action, but in high concentrations (10^{-5} g/ml) a negative action on both spontaneous and evoked secretion of mediator. The presynaptic effects of armin are probably unconnected with inhibition of synaptic cholinesterase activity, for in a concentration of 10^{-8} g/ml, when the positive presynaptic action was well marked, no changes were observed in the time parameters of MEPP and EPP. With a further increase in the armin concentration (10^{-7} g/ml), despite the absence of significant signs of anticholinesterase action, fMEPP was already reduced to the control level. This suggests that armin has a direct and nonspecific action on quantum secretion processes.

Analysis of the parameters P and n in the presence of armin showed that the decrease in QC of EPP at a concentration of 10^{-5} g/ml took place because of a decrease in the quantity of mediator accessible for liberation. This is also confirmed by the fact that changes in spontaneous and evoked liberation of mediator were in the same direction, for both fMEPP and QC of EPP are known to be connected positively with the value of n. On the one hand, it has been shown that in synaptic contacts with a higher value of fMEPP, QC of EPP also is higher, and on the other hand, positive correlations have been found between fMEPP and n. It can thus be postulated that armin affects the formation of complexes of discharge point and vesicle, the number of which,

in the modern view, determines the reserves of available mediator in a nerve ending. This is in agreement with the decrease in the number of synaptic vesicles observed under the influence of armin, obtained during a study of the ultrastructure of motor endings in the rat diaphragm [1].

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LUMINESCENCE-HISTOCHEMICAL STUDY OF THE EFFECT OF ATROPINE AND DIMETHOXYDICHLOROVINYL PHOSPHATE ON THE CATECHOLAMINE CONCENTRATION IN SOME RAT BRAIN STRUCTURES

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KEY WORDS: luminescence histochemistry; brain; catecholamines; atropine; dimethoxydichlorovinyl phosphate; interaction between mediators.

Facts indicating a close functional connection between the cholinergic and catecholaminergic mediator systems of the brain have now been obtained [2, 3, 9, 10]. However, not all aspects of this phenomenon have been equally well studied. In particular, further investigation is required into the effect of substances exciting and blocking conduction of the nervous impulse in cholinergic synapses on catecholamines (CA), for this is hindering our understanding of the mechanisms which lie at the basis of their action on the CNS.

The object of this investigation was a quantitative luminescence-histochemical study of the effect of the cholinolytic atropine and the anticholinesterase agent dimethoxydichlorovinyl phosphate (DDVP) on the content of dopamine (DA) and noradrenalin (NA) in various rat brain structures.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male albino rats weighing 180-200 g. Atropine sulfate or DDVP was injected in doses of 5 and 10 mg/kg respectively, into the animals intraperitoneally. Physiological saline was injected into control rats. CA were detected by the method of Loren et al. [13], for which purpose the brain of the animal, anesthetized with hexobarbital, was perfused 30 min after the injection with 150 ml of a solution (0-4°C) of the following composition: glyoxylic acid 2%, paraform 1%, magnesium sulfate 25%, in 0.1 M phosphate buffer, pH 7.4. After the end of perfusion the brain was quickly removed and frozen in solid CO_2. Sections 30 μ thick were dried in a jet of warm air (90°C) for 5 min and mounted in polystyrene. The sections were examined in the "Luman-I" luminescence microscope (the light source was a DRSh-250-2M

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