EFFECT OF DIPHENHYDRAMINE ON FUNCTION OF THE NEUROMUSCULAR SYNAPSE AND ACTION POTENTIAL GENERATION BY MOTOR NERVE FIBERS

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Diphenhydramine is widely used in medical practice as an antihistamine which also possesses sedative properties. In connection with the analysis of its sedative effect, its action on transmission in nonhistamine synapses also is interesting, but data on this subject could not be found in the electrophysiological literature.

The aim of this investigation was to study the action of diphenyhydramine on transmission in the frog neuromuscular synapse, an object which can be used as a model of central cholinergic synapses, and also on the parameters of action potentials (AP) of frog motor nerve fibers.

EXPERIMENTAL METHOD

The standard microelectrode technique was used to record synaptic potentials in the frog cutaneopectoral muscle. Microelectrodes were filled with 3M KCl (impedance 10-20 MΩ) and the tip potential did not exceed 5 mV. Signals were led to the ac channel of a UBP1-02 amplifier with transmission band of 10-10,000 Hz and were recorded from the screen of an S1-18 oscilloscope. The nerve was stimulated with single pulses of twice the threshold amplitude (for the synapse under investigation) and with a duration of 0.1 msec from ESU-1 stimulator, and with bursts of such stimuli with 50 pulses per burst (in this case the frequency of stimulation was 50 Hz. The quantum composition (QC) of transmission was determined from the ratio of the mean amplitudes of end-plate potentials (EPP) and minature EPP (MEPP): $QC_1 = A_{epp}/A_{mepp}$, and from the coefficients of variation of EPP amplitudes: $QC_2 = 01/(CV)^2$. Martin's correction was not introduced because the amplitudes of EPP were small. The probability of release of quanta p was estimated as $p = (1 - QC_1/QC_2)$, the reserves of available mediator n as $n = QC_1/p$ [1]. The background solution consisted of magnesium Ringer's solution of the following composition (in mM): NaCl — 108, KCl — 2.0, $CaCl_2$ — 1.0, MgCl — 4.0, $NaHCO_3$ — 2.5 (pH 7.3-7.4). The solution in the experimental chamber was changed after 2 min and recording of potentials began at the 10th minute of action of the substance.

Investigation of the action of diphenhydramine onthe Ranvier node membrane of fibers isolated from the ventral spinal roots was carried out by the Tasaki—Staempfli method, using constant hyperpolarization of the nodes to —90 mV from an external source. The resistance of the nodes was determined from the potential shift in response to a linearly increasing change of current with a gradient of 0.1 nA/10 msec. The solution perfusing the node was changed after 30 sec and AP were recorded at the 3rd, 5th, and 7th minutes of action of diphenhydramine. The composition of the Ringer's solution for the nodes (in mM) was: NaCl — 112.0, KCl — 2.5, $CaCl_2$ — 2.0, $NaHCO_3$ — 2.9, (pH 7.3-7.4). Diphenhydramine was dissolved in the corresponding solution immediately before the experiment to obtain final concentrations of 4×10^{-7}, 4.6×10^{-6}, and 7.9×10^{5} M.

EXPERIMENTAL RESULTS

The results of the action of diphenhydramine on neuromuscular transmission, averaged for nine fibers investigated throughout the period of analysis, are given in Table 1. Diphenhydramine (7.9×10^{-5} M) caused a decrease in amplitude of the MEPP, reduced to the

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TABLE 1. Effect of Diphenhydramine on Parameters of Synaptic Transmission in Frog Cutaneopectoral Muscle

Parameter	Background value	Diphenhydramine ($7.9 \cdot 10^{-5}$ M), % of background value
MP, mV	—84,0±2,1	89,1±1,98*
f_{mepp}, sec^{-1}	2,11±0,48	87,9±10,6
A_{mepp}, mV	0,540±0,08	80,5±2,8*
A_{epp}, mV	2,39±0,35	71,5±3,3*
QC_1	5,31±1,49	82,2±4,1*
QC_2	18,9±4,2	109,1±8,3*
p	0,686±0,061	118,2±6,5*
n	8,63±3,77	76,9±5,58

Legend. Here and in Table 2 mean values and errors of means are given. *P = 0.95.

TABLE 2. Effect of L-Histidine and Diphenhydramine Preceded by Histidine on Parameters of Synaptic Transmission in Frog Cutaneopectoral Muscle

Parameter	Background value	Histidine (2 mM), % of background value	Histidine (2 mM) + diphenydramine ($7.9 \cdot 10^{-5}$ M), % of background value
MP, mV	—77,4±4,3	97,3±3,2	91,8±4,3
A_{mepp}, mV	0,431±0,142	90,4±5,3	89,3±9,97
A_{epp}, mV	1,24±0,29	105,6±7,6	166,4±44,8
QC_1	3,58±0,88	120,2±7,3*	163,2±43,9
QC_2	9,44±1,85	98,7±8,0	237,6±35,3*
p	0,548±0,115	97,0±7,8	
n	11,79±5,73	148,6±18,7*	Not estimated

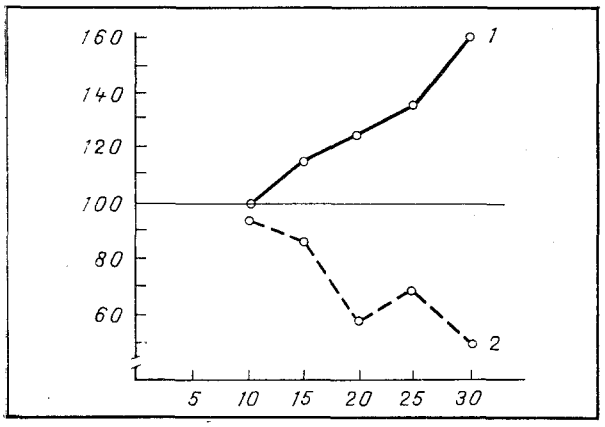

Fig. 1. Time course of changes in probability of transmitter release p (1) and reserves of accessible transmitter n (2) during the action of 7.9×10^{-5} M of diphenhydramine. Abscissa, time from beginning of action of diphenhydramine (in min); ordinate, changes (in % of value in Ringer's solution). n = 7.

initial level of membrane potential (MP) and in the absence of significant changes in the frequency of MEPP, i.e., it had a cholinolytic action. Diphenhydramine also casued a decrease in the mean QC_1 without any significant change in QC_2 of transmission. Analysis of the distribution of MEPP amplitudes in the background and under the influence of diphenhydramine and the absence of significant changes in the coefficient of variation of amplitudes of MEPP under the influence of diphenhydramine together suggest that the fall in QC_1 of EPP was not the result of overestimation of the mean amplitude of MEPP under conditions of the cholinolytic action ("MEPP losses in noise"), but it reflected a real decrease in evoked release of the transmitter. Calculation showed that during the action of diphenhydramine in a concentration of 7.9×10^{-5} M there was an increase in p and a decrease in n (Fig. 1).

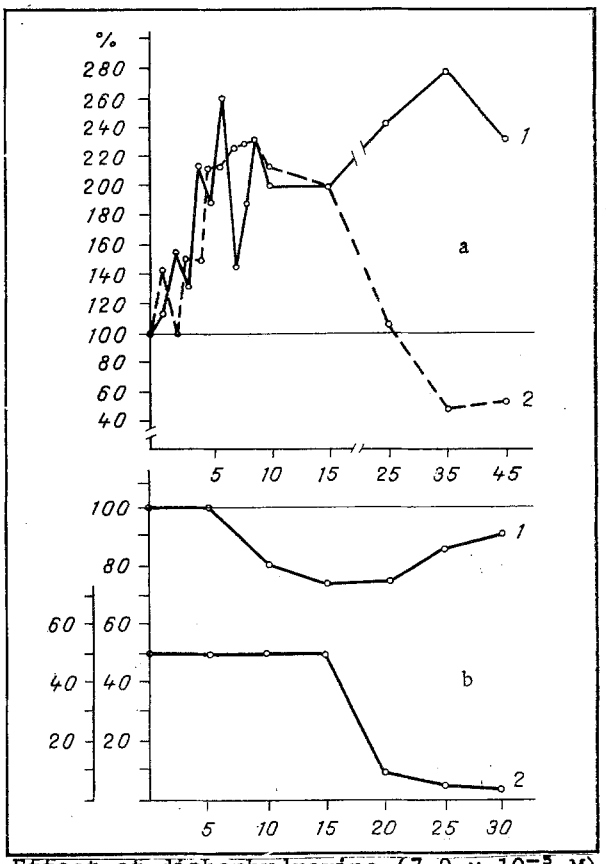

Fig. 2. Effect of diphenhydramine (7.9×10^{-5} M) on working of neuromuscular synapse under frequency stimulation. Abscissa, serial No. of EPP; ordinate, ratio of amplitude of N-th EPP to amplitude of first EPP in series. a) Course of facilitation of EPP during stimulation with frequency of 50 Hz in Ringer's solution (1) and at 15th minute of action of diphenhydramine (2). b) Change in amplitude of MEPP (1) relative to amplitude in Ringer's solution, in %, with time and change in number of EPP before first omission (2) during frequency stimulation.

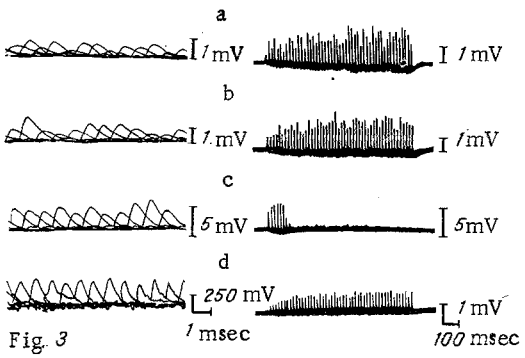

Fig. 3

Fig. 3. Traces of single EPP (left) and bursts of EPP during stimulation with a frequency of 50 Hz (on right): a) background, b) action of 2 mM histidine, c) action of mixture of 2 mM histidine and 7.9×10^{-5} M diphenhydramine, d) after rinsing to remove mixture of histidine and diphenhydramine.

Data obtained during frequency stimulation of the preparations also point to exhaustion of the transmitter reserves n (Fig. 2). During stimulation with a burst of stimuli with a frequency of 50 Hz, synaptic depression was observed in diphenhydramine solution. Besides depression, diphenhydramine also caused presynaptic losses of EPP, and these losses appeared before the development of the cholinolytic effect (see Fig. 2b). All the effects described were reversible.

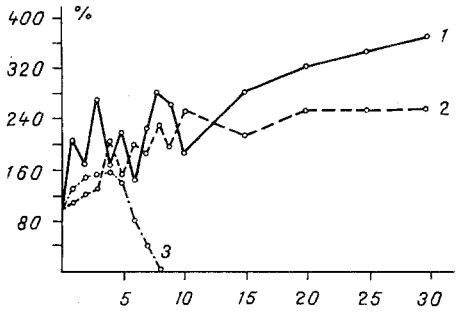

Fig. 4

Fig. 4. Changes in amplitude of EPP during frequency stimulation. Abscissa, serial No. of EPP; ordinate, ratio of amplitude of N-th EPP to amplitude of first EPP in series. 1) Background, 2) action of 2 mM histidine, 3) action of mixture of histidine and diphenhydramine.

The effect of diphenhydramine on AP of the Ranvier nodes, whose membrane was regarded as a model of the electrogenic membrane of the nerve ending, depended on concentration: diphenhydramine in a concentration of 4×10^{-7} M increased the amplitude of AP of the nodes by 74.2 ± 19.3%, in a concentration of 4.6×10^{-6} M it did not affect the value of AP, but in a concentration of 7.9×10^{-5} M it caused reversible block of AP generation. In all concentrations studied diphenhydramine caused an increase in the input resistance and lengthening of the leading and trailing edges of the node AP. One result of the change in temporal parameters of AP was an increase in the area described by AP, to reach 159 ± 57% when the drug was used in a concentration of 7.9×10^{-5}M. Hence it could be concluded that the increase in probability of quantal release observed in diphenhydramine solutions was linked with an increase in the inflow of Ca^{++} ions into the terminal during the AP.

In a small series of experiments diphenhydramine (7.9×10^{-5} M) acted on the nerve-muscle preparation against the background of L-histidine (2 mM), which increases QC on account of an increase in n [3]. Data on the action of histidine are given in Table 2 (N = 6) and, on the whole, they do not differ from those obtained previously. The action of histidine developed rapidly and was stable in time. In the experiments with diphenhydramine the maximal increase of p was 300%, and in the experiments with histidine the maximal increase of n was 240%. Assuming independence of the parameters p and n, a six-sevenfold increase in QC might be expected during the combined action of the substances. In one experiment, at the 20th minute of action of the mixture of substances, a sixfold increase in QC_1 was in fact observed, but on average under these conditions QC_1 increased by only 67%; moreover, during further combined action QC_1 of transmission fell. Rinsing out the mixture of substances led to a decrease of QC_1 to 56.5 ± 12.8% (Figs. 3 and 4). The absence of multiplication of the effects during the action of histidine and diphenhydramine could be the result of exhaustion of the reserves due to intensification of transmitter release on account of the increase in p caused by diphenhydramine, and also on account of a disturbance of replenishment of the reserves by diphenhydramine. Acting against the background of histidine, diphenhydramine also caused blocking of presynaptic AP.

The effect of diphenhydramine on synaptic transmission can be regarded from two standpoints: as a specific effect, linked with blocking of H_1-receptors, and as a nonspecific nonantihistamine effect. We know that histamine, in high concentrations (10^{-4}-10^{-3} M) causes a decrease in MP of nerve fibers [5] and a decrease in the quantum composition of EPP [6]. However, we do not know how specific this action may be. Possibly diphenhydramine abolishes the negative action of endogenous histamine, released by mast cells or muscle fibers during activity, but this effect, under our experimental conditions, was evidently masked by a direct effect on processes of transmitter release and AP generation by nerve fibers. The ability of diphenhydramine to influence ionic channels of the excitable membrane is evidently determined by the presence of benzene groups [2] and a tertiary nitrogen atom, which interacts with K channels [4], in its molecule. The increase in integral depolarization of the ending and in the inflow of Ca^{++} into it, which leads to an increase in QC because of an increase in p, is determined by partial blocking of the K channels of the terminal. Dephenhydramine interferes with replenishment of n evidently, simultaneously and independently of blocking of the K channels, and this leads to exhaustion of the reserves. The second ef-

fect is more important than the first, and it ultimately leads to a fall of QC and depression of EPP during frequency testing. In turn, the change in permeability of the nerve endings for sodium causes lengthening of the relative refractory period of the nerve fibers, and during frequency stimulation this leads to omissions of AP and, correspondingly, of EPP.

We do not know whether diphenhydramine causes exhaustion of transmitter reserves in synapses of noncholinergic nature in the CNA. However, the sedative action of diphenhydramine may be partly associated with a decrease in the reliability of conduction along central nerve fibers as a result of blocking of ionic channels.

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INHIBITION OF PLATELET AGGREGATION BY ANTIOXIDANTS

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One way of influencing thrombus formation in the blood vessels is by controlling platelet aggregation with the aid of chemicals. In this connection interest in new compounds with antiaggregating activity *in vitro* is increasing. In the modern view, platelet aggregation is the final stage of a complex sequence of reactions, triggered by the action of a stimulus on the plasma membrane. The molecular mechanism of the platelet aggregation process has not yet been finally established. It is considered that an important role in the aggregation process is played by cyclo-oxygenase (COG), which catalyzes the formation of prostaglandin endoperoxides. Evidence in support of this view is given by the high ability of specific inhibitors of COG, namely aspirin and indomethacin, to inhibit platelet aggregation both *in vitro* and *in vivo* [6, 7]. It has frequently been suggested that substances capable of terminating free-radical stages in reactions of oxidation of biological substrates may be inhibitors of the COG reaction. It has in fact been shown that some antioxidants have an inhibitory action of platelet aggregation [2, 3]. However, no direct proof of a connection between the antiaggregating activity of antioxidants and the COG reaction has yet been obtained.

The aim of the investigation described below was to determine whether the antiaggregating activity of antioxidants is connected with their possible effect on COG activity or whether their effect is realized by other mechanisms.

EXPERIMENTAL METHOD

Platelets were isolated from the blood of healthy donors by the method in [5]. Aggregation was recorded on an aggregometer (Chronolog Corp., USA). Aggregation was initiated with arachidonic acid (50 μM), thrombin (1.5 unit/ml), and the Ca^{++} ionophore A23187 (1.5 μg/ml). The substances for testing were added to a platelet suspension in the form of a

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