possesses stronger antihypoxic activity than orthofen, is a weaker inhibitor of PG biosynthesis than orthofen, whereas neither aspirin nor butadione exhibited any antihypoxic activity. However, we know that in some cases positive correlation does not exist between the activity of inhibitors of various enzymes in vitro and their action in vivo [7]. The view that the antihypoxic action of ibuprofen may be linked with inhibition of the arachidonic acid cascade (at least one of the possible mechanisms of its action) is supported by the protective action of ibuprofen against the lethal effect of sodium arachidonate in rabbits [4].

The results are evidence that the new NSAIA orthofen and ibuprofen, which are widely used at the present time, possess antihypoxic properties and that the study of the presence of these properties in other antiinflammatory agents influencing PG biosynthesis is indicated.

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DIPYROXIME AS A BLOCKER OF ACETYLCHOLINE-ACTIVATED IONIC CHANNELS IN RAT SKELETAL MUSCLE

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Dipyroxime (TMB-4) is a classical representative of the oximes, which are widely used in the treatment of poisoning by organophosphorus compounds which inhibit acetylcholinesterase (AChE) [1]. It has been suggested that the fundamental molecular mechanism of the antidotal action of the oximes is their ability to reactivate AChE, including skeletal muscular AChE [5]. At the same time, we know that the mechanism of the antitoxic action of these substances in vivo is much more complex and involves several components [1]. One such component is the cholinolytic action, which is manifested in skeletal muscles with both inhibited and intact AChE [4].

EXPERIMENTAL METHOD

Experiments were carried out in November on a rat phrenic nerve — diaphragm preparation with uninhibited AChE. The isolated muscle was placed in a bath with capacity of 1.5 ml, with continuously flowing Ringer–Krebs solution of the following composition (in mM): NaCl — 137, KCl — 5, $CaCl_2$ — 2, $MgCl_2$ — 1, $NaHCO_3$ — 11, NaH_2PO_4 — 1.0, glucose 11.0. The solution, which was aerated beforehand for 1 h with carbogen (95% oxygen, +5% carbon dioxide) had pH 7.3. The experiments were carried out at 21 ± 0.1°C. The membrane potential of the muscle fiber in the region of the synapse was clamped by a two-electrode method. The amplitude and temporal parameters of the miniature end-plate currents (MEPC) were analyzed by computer with 29 μsec signal quantization interval.

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EXPERIMENTAL RESULTS

The mean amplitude of MEPC in the control, when the membrane potential of the muscle fiber was clamped at the −70 mV level, was 2.17 ± 0.20 nA (n = 8). The fall of MEPC was described by a single exponent with time constant (τ_{mepc}) of 1.40 ± 0.09 msec (n = 8). Under the influence of dipyroxime the amplitude of MEPC was reduced, and their fall was more protracted. Both effects were observed starting with a concentration of dipyroxime of 10^{-4} M, and they were potentiated by an increase in concentration of the drug. For instance, when dipyroxime was used in a concentration of $5 \cdot 10^{-4}$ M the amplitude of MEPC fell to 1.08 ± 0.07 nA ($p < 0.05$; n = 5), and the fall was lengthened to 2.30 ± 0.20 msec ($p < 0.05$; n = 5), although it still remained exponential. The effects of dipyroxime were easily abolished by rinsing with physiological saline and were reproduced again by a second dose of the drug. The effect of dipyroxime on both the amplitude and the time course of MEPC depended on the membrane potential of the muscle fiber, as shown by changes both in the current-voltage characteristic of MEPC and the dependence of τ_{mepc} on membrane potential. In the control the current-voltage characteristic of MEPC was linear from −50 to −120 mV (Fig. 1, 1), but in the presence of dipyroxime not only was this dependence nonlinear, but there was also a region of negative conductance, when the amplitude of MEPC fell with an increase in membrane potential (Fig. 1, 2). In the control, τ_{mepc} was an exponential function of membrane potential, and it increased by e times during hyperpolarization by 80 mV (Fig. 2, 1). Dipyroxime ($5 \cdot 10^{-4}$ M) strengthened this dependence (Fig. 2, 2) so that a change of τ_{mepc} by e times took place in response to hyperpolarization by only 35 mV. Whereas lengthening of MEPC under the influence of dipyroxime was 30% at a transmembrane potential difference of −50 mV, it was 278% at −110 mV. Under these circumstances the fall of MEPC was described by a single exponent at all levels.

These results are evidence that in the rat myoneural junction, in the presence of intact AChE, dipyroxime gives a postsynaptic effect, reducing the amplitude of MEPC and increasing their duration. The intensity of the effect not only on the amplitude, but also on the time course of the synaptic signals in this case depends on the membrane potential of the muscle fiber. A change in the current-voltage characteristic of MEPC, observed in the presence of dipyroxime, is characteristic of many charged blockers [3, 10], and this fact suggests blockade of acetylcholine-activated ionic channels by this agent. Meanwhile charged blockers of end-plate channels as a rule induce monoexponential acceleration of decay of the postsynaptic responses or convert their decay into biexponential [7]. Monoexponential slowing of the decay of MEPC observed under the influence of dipyroxime could be explained by the anticholinesterase action of this agent. However, the high potential-dependence of the effect of dipyroxime on τ_{mepc} is an argument against this hypothesis, for inhibition of AChE either leaves dependence of τ_{mepc} on membrane potential unchanged, or actually reduces it [6, 8].

Slowing of decay of MEPC caused by dipyroxime can also be explained in terms of the blockade of open end-plate ionic channels by the drug To describe interaction of a blocking agent with an open ionic channel, the following sequential model of blockade is frequently used [9]:

$$A + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AR \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AR^* + B \underset{k_b}{\overset{ck_f}{\rightleftharpoons}} A_{RB},$$

where k_1, k_{-1} are the velocity constants of formation and breakdown of the complex of agonist (A) and receptor (R); β, α the velocity constants of opening and closing of the ionic channels; k_f, k_b the velocity constants of blocking and unblocking of the channels by the blocking agent B; c the concentration of the substance B.

According to this model, monoexponential slowing of the decay of MEPC combined with a reduction in amplitude of the signal may be observed during frequently repeated and very rapid transitions from the state of the acetylcholine receptor with an open ionic channel (AR*) into the blocked state (ARB) and vice versa. This evidently corresponds to a much higher level of k_f and k_b than the values of these constants for blockers causing biexponential decay of the end-plate current or their monoexponential acceleration. This combination of the kinetic parameters of the block of the channels indicates that dipyroxime belongs to a virtually unstudied group of blockers of "very rapid type" [10]. The high voltage dependence of the rate of decay of MEPC in the presence of dipyroxime can accordingly be explained on the grounds that the blocking constants are more sensitive to the electric field on the membrane than α, which determines the voltage-dependence of decay of MEPC under normal conditions [6, 8].

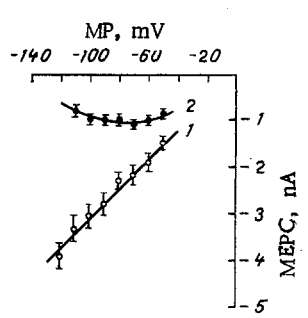

Fig. 1

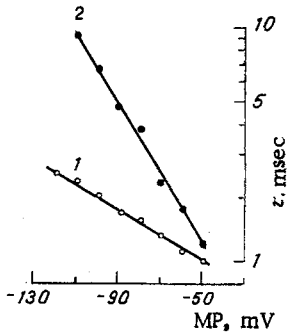

Fig. 2

Fig. 1. Current-voltage characteristic of MEPC in control (1) and in the presence of dipyroxime (2).

Fig. 2. Dependence of time constant of fall of MEPC on potential in control (1) and in the presence of dipyroxime (2).

It can thus be concluded from all the data obtained in this investigation that the lead-mechanism of the cholinolytic effect of dipyroxime is not its curare-like action but interaction of this drug with an acetylcholine-activated ionic channel. The kinetic parameters of this block established in the course of this study may be helpful with the elucidation of the effects of dipyroxime on the postsynaptic membrane when AChE is inhibited, and also during the transmission of repetitive series of impulses through a synapse.

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