Mechanisms of the Effect of Dimephosphone on Synaptic Transmission in the Frog Neuromuscular Junction

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We studied the influence of dimephosphone, an organophosphorus drug with a broad spectrum of therapeutic effects on the peripheral and central nervous systems, on postsynaptic end-plate currents (EPC) in the frog neuromuscular junction. Dimephosphone was demonstrated to decrease in a voltage-independent manner the EPC amplitude and to prolong the EPC decay. These effects are not related to inhibition of acetylcholinesterase. We propose a theoretical interpretation of the observed phenomena based on the model of blockade of an open ion channel of the acetylcholine receptor and conclude that postsynaptic receptors are one of the most probable targets for the action of dimephosphone.

Keywords: acetylcholine receptor, dimephosphone, blockade of an open ion channel.

INTRODUCTION

Dimephosphone is an organophosphorus compound (OPC), the dimethyl ester of 1,1-dimethyl-3-oxobutylphosphonic acid. It was synthesized at the Arbuzov Institute of Organic and Pharmacological Chemistry [1, 2]. It was demonstrated to exert hypothermic [3], antiacidotic [4], antiinflamatory [5], membrane-stabilizing [6], antiarrhythmic [3, 7], and anticonvulsant [8] effects. It soon became clear that this drug, similarly to other derivatives of 1,1-dimethyl-3-oxobutylphosphonic acid, is deprived of anticholinesterase properties [9]. Some aspects of interaction of other nonanticholinesterase OPC (derivatives of oxophospholanole, which has some structural similarity with dimephosphone) with postsynaptic membranes allowed us to conclude that such drugs modulate functioning of ion channels of the nicotinic cholinoreceptors [10]. The effects of dimephosphone were not subjected to such an analysis. Recently, new data were obtained indicating the possibility that the pharmacological effects of dimephosphone are based on its influence on Ca^{2+} currents through the cell membranes [11]; nonetheless, the molecular and cellular mechanisms of the effects of this drug remain far from final interpretation. Investigation of the neuromuscular junction using modern electrophysiological techniques allows experimenters to analyze in detail the mechanisms responsible for modification of ligand-activated ion channels in the postsynaptic membranes; this is why we examined the effects of dimephosphone on the characteristics of end-plate currents (EPC) in the frog neuromuscular junction.

METHODS

Experiments were carried out on isolated neuromuscular preparations (sciatic nerve – *m. sartorius*) of the frog *Rana ridibunda*. To avoid muscle contractions under conditions of a preserved high level of quantum transmitter release, we transected the muscle fibers under study. An experimental chamber (2.0 ml) with the preparation in it was permanently perfused with Ringer solution for poikilothermic animals (mM): NaCl, 115.0; KCl, 2.5; $CaCl_2$, 1.8; $NaHCO_3$, 11.0; the pH of the solution was maintained at 7.3, and temperature was stabilized within the 20 ± 2ºC range. Evoked and miniature EPC were recorded under two-electrode voltage-clamp conditions. Synaptic signals were stored and averaged using a PC (digitizing period 4 μsec). The parameters and temporal course of decay of miniature and multi-quantum EPC were calculated using corresponding special software. The motor

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nerve was stimulated by rectangular current pulses (0.5-2.0 mV, 0.25 msec); stimulation was programmed with a computer. The significance of differences between the numerical data was estimated with Student's *t*–test.

RESULTS

In the first experimental series, we tested the effects of dimephosphone, when applied in different concentrations, on the amplitude/temporal characteristics of multi-quantum EPC. When the membrane potential was clamped on a –50 mV level, the mean amplitude of such EPC was 94 ± 7 nA (n = 6). The decay of these currents can be fitted with one exponent with a mean time constant of 1.56 ± 0.18 msec (n = 6). Applications of 10 μM, 100 μM, and 1 mM dimephosphone reduced the EPC amplitude by 7 ± 4.8 (n = 12; $P > 0.05$), 25 ± 3.3 (n = 12; $P < 0.05$), and 55 ± 2.8% (n = 12, $P < 0.05$), on average, respectively (Fig. 1A). In addition to a decrease in the EPC amplitude, dimephosphone in all the three tested concentrations noticeably influenced the time constant of the EPC decay. When applied in a 10 μM concentration, the drug increased the decay time constant to 141 ± 11%, as compared with the control (n = 12, $P < 0.05$), but the monophasic pattern of the decay was preserved under these conditions. When dimephosphone was applied in 100 μM and 1 mM concentrations, the decay of postsynaptic currents exhibited a two-phase time course (B), which is typical of the effects of blockers of open channels in the postsynaptic membrane [12]. We should also

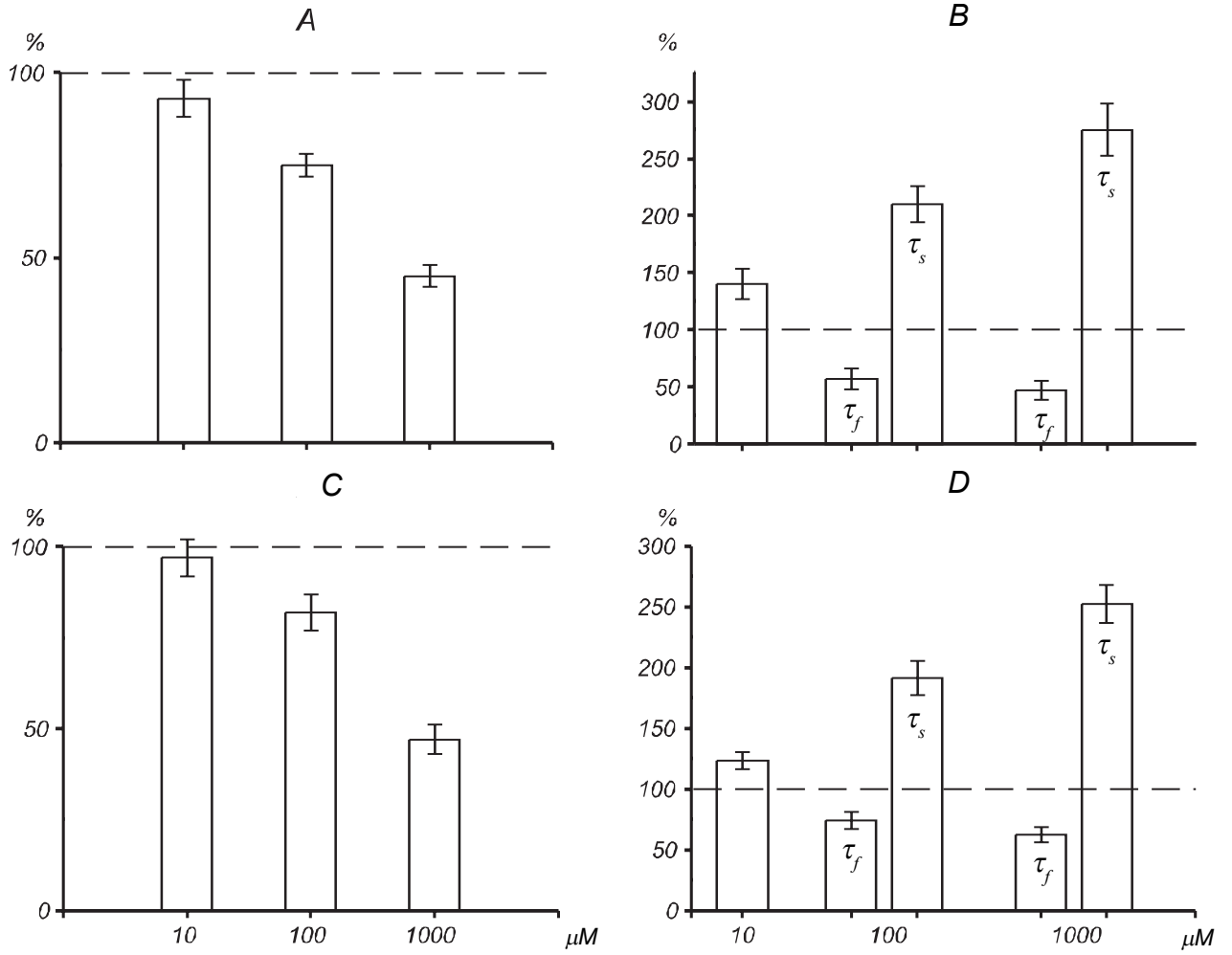

Fig. 1. Effects of different concentrations of dimephosphone on the characteristics of end-plate currents (EPC) in the frog neuromuscular junction (number of experiments n = 6; stimulation frequency 0.05 sec^{-1}). A) Changes in the EPC amplitude; B) those in the time constant of EPC decay; C and D) the same, respectively, but against the background of application of 5 μM proserine. Normalized values of the parameters are shown; values in the control are taken as 100%. Concentrations of dimephosphone, μM, are shown below the columns. In B and D, values of the time constants for fast and slow decay components (τ_f and τ_s, respectively) are shown.

mention that, with a change in the concentration of the drug from a smaller to a greater value, the difference between slopes of the decay phases increased, the rate of decay within the slower component decreased, and, because of this, the total duration of postsynaptic signals increased (Fig. 2C, D). All effects of dimephosphone were eliminated after washing off the preparation with the normal Ringer solution.

Prolongation of the decay of postsynaptic signals is a feature typical of the effects of cholinesterase inhibitors [13]. This is why we checked out in the next experimental series whether dimephosphone influences the activity of synaptic cholinesterase. We used an inhibitor of cholinesterase, proserine. Under control conditions, we applied 5 μM proserine, which was enough for a 95% inhibition of the activity of synaptic cholinesterase [13]. This procedure resulted in a slow increase in the EPC amplitude (by 17 ± 6% for 30 min, $n = 4$, $P < 0.05$), and the time constant of decay of EPC increased by 146 ± 2%, on average ($n = 4$, $P < 0.05$). Addition of dimephosphone in 10 μM, 100 μM, or 1 mM concentrations against the background of application of proserine decreased the EPC amplitude by 3 ± 5 ($n = 6$, $P > 0.05$), 18 ± 5 ($n = 6$, $P < 0.05$), and 53 ± 4% ($n = 6$, $P < 0.05$), respectively (Fig. 1C). These data differed insignificantly ($P > 0.05$) from those obtained when dimephosphone was applied with an unchanged activity of cholinesterase, and the increase in the duration of EPC decay remained practically identical to that observed at active cholinesterase ($n = 6$, $P < 0.05$; Fig. 1D). When proserine was applied together with 100 μM or 1 mM proserine, a typical two-phase pattern of the EPC decay, with an initial fast and subsequent slow components, also appeared; such a two-phase shape was not observed when proserine was applied separately. The above results allow us to conclude that the effects of dimephosphone are preserved against the background of the action of proserine; in other words, dimephosphone demonstrated no anticholinesterase properties.

Testing of the voltage dependence of the effects of one pharmacological agent or another provides

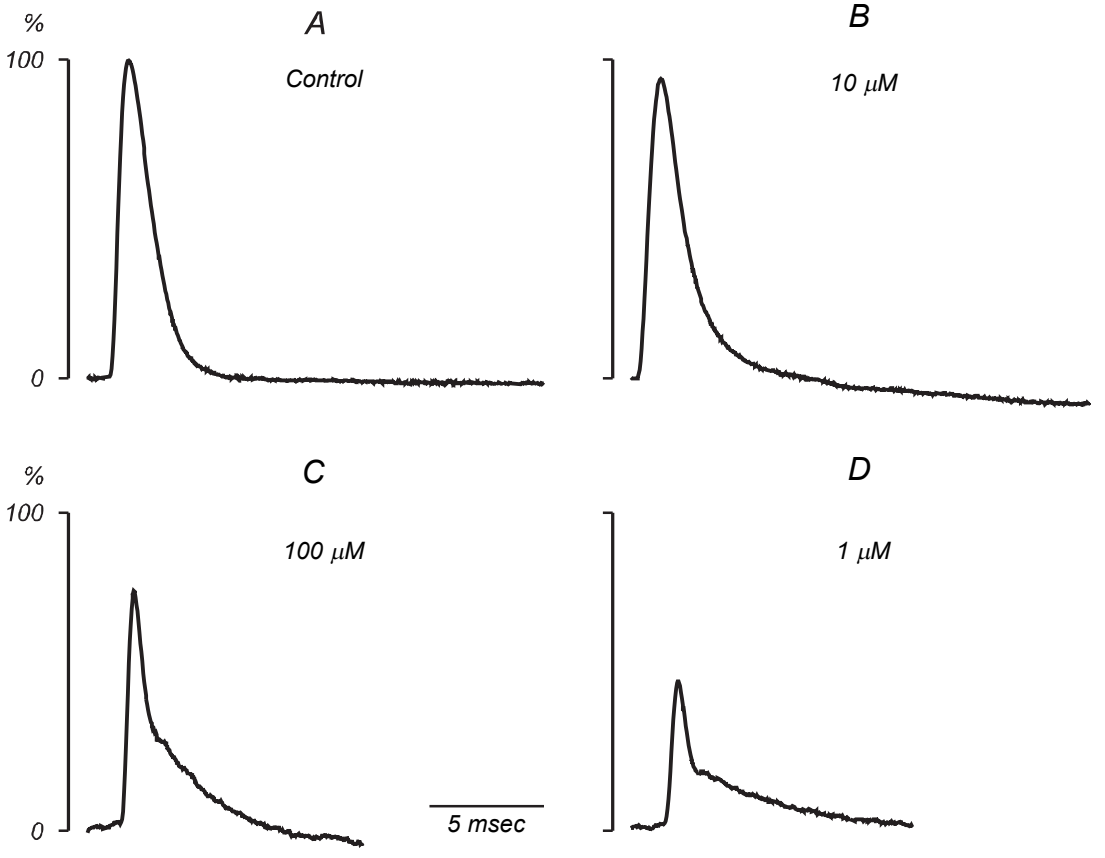

Fig. 2. End-plate currents (EPC) in the control and under the influence of dimephosphone. A) A typical EPC in the control; B-D) EPC under conditions of application of dimephosphone in different concentrations (shown at the upper right). Amplification in A-D is the same; EPC amplitude in the control is taken as 100%.

one with substantial information on the type of action of the drug on ion channels. The dependence of changes in the EPC amplitude on the level of the membrane potential (current-voltage relation for this postsynaptic signal) was linear in the control, with the slope of 0.73 ± 0.03 nA/mV. After application of 100 μM dimephosphone, this linear pattern was preserved, while the mean slope became, on average, 0.68 ± 0.04 nA/mV (Fig. 3A). Thus, dimephosphone evoked practically no changes in the current-voltage relation for EPC. In the control, the time constant of decay of the currents depended on the membrane potential in an exponential manner; extrapolation demonstrated that the above parameter should increase by *e* times for hyperpolarization to 197 ± 7 mV. Application of 100 μM dimephosphone for 20 min resulted in an increase of the above index, characterizing the sensitivity of the slow decay component to the membrane potential, to 502 ± 13 mV (n = 5); the fast decay component under these conditions showed practically no voltage dependence (Fig. 3B).

Therefore, dimephosphone in the three tested concentrations significantly influences the EPC parameters. Their amplitude drops, while the time constant of decay increases. In addition, application of 100 μM of the agent results in the appearance of a two-component pattern of the EPC decay (the latter begins to include a fast phase followed by a slow phase). Changes in the EPC parameters are greater when dimephosphone is used in 1 mM concentration, i.e., the effect demonstrates a clear concentration dependence. We should emphasize that the effect of dimephosphone is specific only with respect to the synaptic cholinoreceptors. Our parallel study showed that dimephosphone does not influence currents mediated by activation of the ionotropic P2X2 purine receptors and activated by application of 1 μM to 1 mM ATP.

DISCUSSION

When one studies the pharmacological activity of OPC (dimephosphone belongs to this class of the compounds), the first question is: Are the observed phenomena related to the anti-cholinesterase effect or not? In our experiments, both dimephosphone and proserine increased the duration of the EPC decay. When dimephosphone was applied against the background of proserine, the time constant of decay increased in a similar way. Such an effect of dimephosphone as the appearance of the two-phase mode of the EPC decay was preserved, while under conditions of isolated application of proserine it was not

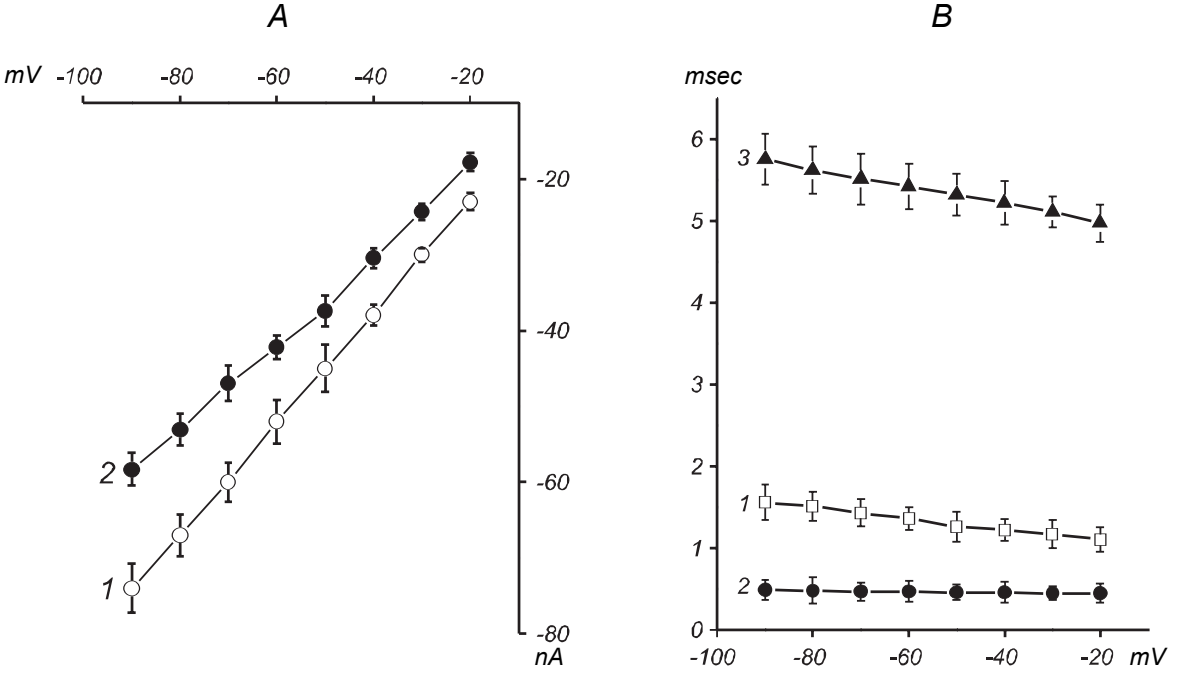

Fig. 3. Dependence of the amplitude (A, nA) and time constant of decay (B, msec) of end-plate potentials on the membrane potential (mV) in the control and after application of dimephosphone (n = 5). 1) Control; A2 and B2, 3) upon application of 100 μM dimephosphone. 2 and 3 in B are dependences for the fast and slow phases of decay, respectively.

observed. If we take into account that proserine was used in a concentration sufficient for nearly complete inhibition of the acetylcholinesterase activity (95%), summation of the effects of the tested drugs cannot be explained by a deeper suppression of the enzyme activity.

The effects of dimephosphone on the amplitude/temporal characteristics of EPC (in particular, on the time course of its decay) can be interpreted using the scheme of the mechanism of blocking of the open ion channel, which was proposed earlier [10, 14]:

$$A + R \underset{k_1^-}{\overset{k_1^-}{\leftarrow\!\!\rightarrow}} AR + R \underset{k_2^-}{\overset{k_2^+}{\leftarrow\!\!\rightarrow}} A_2R \underset{\alpha}{\overset{\beta}{\leftarrow\!\!\rightarrow}} A_2R^* + B \underset{k_b^-}{\overset{k_b^+}{\leftarrow\!\!\rightarrow}} A_2R^*B,$$

where A is acetylcholine, R is a free cholinoreceptor, AR is a cholinoreceptor with closed ion channel and connected with one molecule of the agonist, A_2R is a cholinoreceptor with closed ion channel but connected with two agonist molecules, A_2R^* is a cholinoreceptor with open ion channel, A_2R^*B is a blocked cholinoreceptor in the nonconducting state, and B is a blocker. When connected to a receptor–channel complex, blockers of the open ion channel change the configuration of such a channel and transform it from an open to an intermediate nonconducting state; the channel can leave the latter state only via the open state. According to the equations of this model, application of blockers of this type should be followed by changes in the pattern of the EPC decay, and the mode of these changes depends on the rate of development of blocker-induced effects. In the case where blockers of fast [14] and slow [15] types were used, only speeding up and slowing down of the decay are observed, respectively; transformation of the decay into a two-exponent one occurs when blockers of the so-called intermediate type are used [12]. Another consequence of the above model of the blocking mechanism is an increase in the rate of decay of the slow EPC component and speeding-up of the fast component with an increase in the concentration of the blocker. This phenomenon was observed in our experiments; it was manifested as an increase in the difference of slopes of two decay phases when the highest tested concentration of dimephosphone (1 mM) was used (Fig. 2B, C).

According to Ogden et al. [16], when electrically neutral blockers of the open ion channel are used, the linear pattern of the current-voltage relation of the postsynaptic effect is preserved and does not differ from the control one, while the voltage dependence of the rate of decay of the fast EPC component becomes significantly smaller than that of the control EPC. This statement agrees with our results. Theoretically, the rate of decay of the slow component should demonstrate the same dependence on the membrane potential as the rate of decay of the control EPC. According to our findings, the voltage dependence of the slow component decreases somewhat, but, nonetheless, this dependence is manifested to a much greater extent, than that of the fast component (Fig. 3B).

Mathematical simulation based on the above-described scheme of the mechanism of blocking the open channel showed that the observed effects of dimephosphone can be adequately reproduced at $k_1^+ = 2 \cdot k_2^+$, $k_2^+ = 80$ mM$^{-1} \cdot$ msec^{-1}, $k_1^- = 18$ msec^{-1}, $k_2^- = 2 \cdot k_1^-$, $\beta = 36.7$ msec^{-1}, $\alpha = 1.7$ msec^{-1} [17], $k_b^+ = 16$ mM$^{-1} \cdot$ msec^{-1} and $k_b^- = 0.74$ msec^{-1}, at a –70 mV membrane potential and voltage dependence of two constants

$$\alpha(V) = \alpha(0) \cdot e^{\frac{V}{156\ \text{mV}}} \quad \text{and} \quad k_b^-(V) = k_b^-(0) \cdot e^{\frac{-V}{350\ \text{mV}}},$$

where V is the membrane potential, mV.

Therefore, the model of sequential blocking of the open channel can be considered acceptable for interpretation of the effects of dimephosphone on postsynaptic currents. We believe that dimephosphone, an electrically neutral blocker, does not interact with a cholinoreceptor *per se* but influences sites of the postsynaptic membrane adjusting to this receptor. This effect modified the lipid–protein interactions of the chemoreceptor molecule and the lipid molecules surrounding the receptor [10]. Such an interpretation possesses an obvious practical aspect because a few effects of dimephosphone, in particular antiarrhythmic and antinociceptive, cannot be explained as based exclusively on antihypoxic and membrane-stabilizing properties of this agent [7]. It seems probable that interaction between dimephosphone and the postsynaptic membrane can play in these cases a rather significant role because it is well known that the effects of a number of antiarrhythmic drugs and anesthetics are determined just by the

influence of these agents on the permeability of ion channels in the membranes of target cells.

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