# Mechanisms of the Effect of 1,1-Dimethyl-3-Hydroxybutyl Phosphonic Acid Derivatives on Synaptic Transmission in Neuromuscular Junction

**E. G. Pryazhnikov, A. I. Skorinkin*, R. S. Garaev, R. A. Giniatullin, A. O. Vizel'**, and L. I. Shchukina****

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We studied the effect of homologues derivatives of 1,1-dimethyl-3-hydroxybutyl phosphonic acid on synaptic transmission in frog neuromuscular junction. Here we reviewed general mechanisms of inhibition of the postsynaptic current.

**Key Words:** *dimephosphon; cholinoceptor; allosteric modulation; mathematical modeling*

1,1-Dimethyl-3-hydroxybutyl phosphonic acid esters synthesized at the A. E. Arbuzov Institute of Organic and Physical Chemistry (Kazan Research Center) are organophosphorus compounds producing a variety of pharmacological effects not mediated by the anticholinesterase mechanism [1]. The dimethyl ester of 3-hydroxybutyl phosphonic acid (dimephosphon) is used in clinical practice as a drug normalizing functions of the central and peripheral nervous system [2], and therefore it seems important to study the effects of this agent synaptic transmission. The effects of dimephosphon can be evaluated using a model of blocker of open ion channel of cholinoceptor, considering the preparation as a blocker with intermediate rate of action [3]. Dimephosphon is soluble in lipids. It was hypothesized that the channel-blocking effect of dimephosphon can be related to the interaction with not only ion channels of the cholinoceptor, but also with lipid regions of the membrane adjacent to the cholinoceptor. It is interesting to evaluate the effect of homologues derivatives of 1,1-dimethyl-3-hydroxybutyl phosphonic acid (D-$(OH)_2$) differing from dimephosphon by the degree of lipophilicity. Here we studied the effects of D-$(OH)_2$ and dimethyl (D-$(OMe)_2$), diethyl (D-$(OEt)_2$), dipropyl (D-$(OPr)_2$), and dibutyl esters (D-$(OBu)_2$) on major characteristics of the endplate current in frog neuromuscular junction.

## MATERIALS AND METHODS

The coefficients of distribution of compounds between octanol and water were estimated by gas-liquid chromatography at 20°C. We measured the content of compounds in each phase.

Experiments were performed on neuromuscular junction isolated from the sciatic nerve (sartorius muscle) of lake frogs using two-electrode patch clamp technique [6]. The test substance was routinely added to the bathing solution, and after attaining the maximum effect the neuromuscular junction was washed with Ringer's solution. Washing was considered as 100% when amplitude-temporal characteristics of the multiquantum (EPC) and miniature endplate current (MEPC) retuned to the baseline level.

The results were analyzed by Student's *t* test.

## RESULTS

The distribution coefficients (ratio between the concentration of compounds in the octanol and aqueous

Kazan State Medical University; *Kazan Institute of Biochemistry and Biophysics, Kazan Research Center, Russian Academy of Sciences; **Institute of Organic and Physical Chemistry, Kazan Research Center, Russian Academy of Sciences, Kazan. ***Address for correspondence:*** ask@ksu.ru. R. S. Garaev

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phase) for D-$(OMe)_2$, D-$(OEt)_2$, D-$(OPr)_2$, and D-$(OBu)_2$ were 1.09±0.09, 8.64±0.34, 89.36±5.13, and >1000, respectively. These data suggest that lipophilicity increased with increasing the length of ester radicals in the homologous compounds.

At a membrane potential of -45 mV, the mean amplitude of multiquantum currents under control conditions was 93.7±3.0 nA ($n$=6). The test compounds (except polar D-$(OH)_2$) in concentration of 10, 100, and 500 μmol/liter decreased the amplitude and modulated the decay time for EPC and MEPC (Table 1). Treatment with D-$(OMe)_2$ in concentrations of 100 and 500 μmol/liter was accompanied by a biphasic decrease in EPC. The rapid phase was followed by the slow phase. These changes were not observed under the influence of other analogues (Fig. 1). The effects of dimephosphon decreased by 66±4% after 20-min washout ($n$=10). The effectiveness of washing decreased with increasing the length of molecules of the homologues compounds. For example, during treatment of the neuromuscular preparation with D-$(OBu)_2$ the effectiveness of washout was 45±5% ($n$=6). In case of discontinuation of periodic stimulation of the motor nerve within 15 min after treatment with D-$(OMe)_2$ and D-$(OBu)_2$, their effects decreased by 40±5% compared to experiments with constant stimulation.

Under control conditions the amplitude of signals linearly depended on the membrane potential (voltage-current characteristics of the postsynaptic signals). After treatment with the test compounds this dependence remained linear with coefficients similar to those in the control. The time constant for current decay exponentially depended on the membrane potential under control conditions. This parameter increased by 197±7 mV during hyperpolarization ($n$=5). A similar dependence was observed after addition of D-$(OH)_2$. The sensitivity of slow decay to variations in the membrane potential upon treatment with D-$(OMe)_2$ was 502±13 mV ($n$=6). Moreover, rapid decay did not depend on the membrane potential under these conditions ($n$=5). After treatment with lipophilic analogues of D-$(OMe)_2$ the EPC decay time did not depend on changes in membrane potential ($n$=5 in each series).

The effect of dimephosphon on the main characteristics of ECP and MECP can be evaluated on the model of open cholinoceptor ion channel blocker [3]. Changes in the potential dependence for ECP decay time constant under the influence of D-$(OEt)_2$, D-$(OPr)_2$, and D-$(OBu)_2$, as well as incomplete washout, are not typical of electrically neutral blockers of slow-acting open ion channels [4,7]. This effect of compounds is not necessarily associated with direct blockade. Probably, they produce an allosteric effect on cholinoceptors and modulate kinetic characteristics of channels and lipid layer after partial dissolution of esters in the postsynaptic membrane. This can be accompanied by changes in receptor affinity for the agonist, rate of channel opening and closing, and potential dependence of these parameters. The effect of the test compounds on the lipid membrane can explain variations in the type of ion channel blockade with homologous derivatives of D-$(OH)_2$. This effect becomes more significant with increasing the length of carbohydrates radicals and lipophilicity of compounds. The amplitude-temporal characteristics of EPC and MEPC remained practically unchanged after treatment with D-$(OH)_2$ characterized by the lowest lipophilicity. The

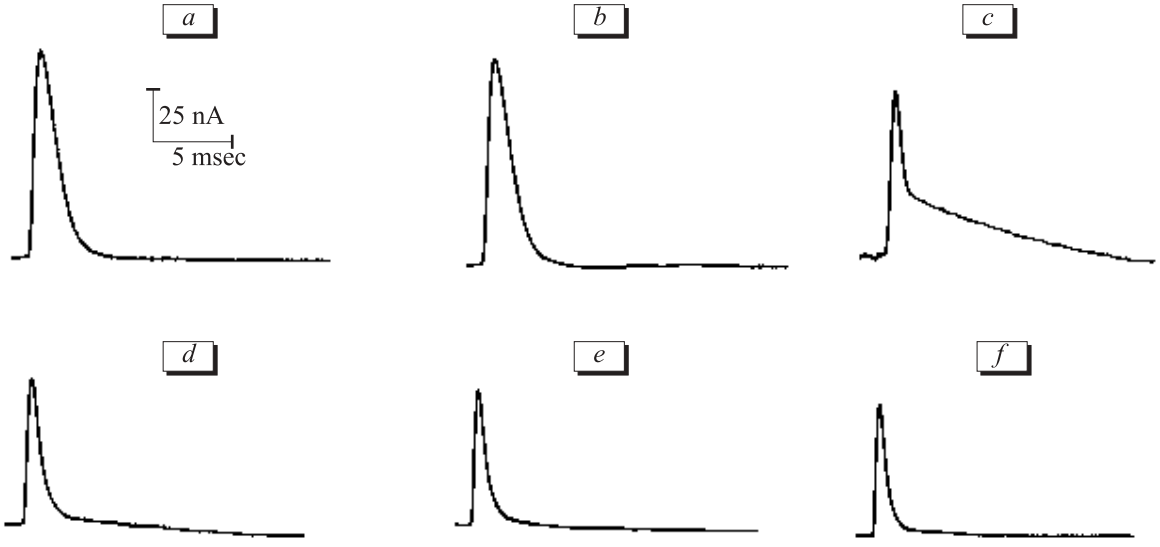


**Fig 1.** Typical multiquantum endplate currents under control conditions and after treatment with 1,1-dimethyl-3-hydroxybutyl phosphonic acid derivatives: control (*a*); D-$(OH)_2$, 100 μmol/liter (*b*); D-$(OMe)_2$, 100 μmol/liter (*c*); D-$(OEt)_2$, 100 μmol/liter (*d*); D-$(OPr)_2$, 100 μmol/liter (*e*); and D-$(OBu)_2$, 100 μmol/liter (*f*).

effects of compounds were studied upon termination of stimulation accompanied by a significant decrease in the opening rate for ion channels of cholinoceptors. Some effects of compounds are associated with modulation of the synaptic membrane, while others are related to the direct influence on open ion channels. These effects rapidly developed over the first minutes after the start of stimulation. Our assumption is confirmed by partial washout of the effects produced by study compounds. The effectiveness of washing decreased with increasing lipophilicity of the test compounds. It can be hypothesized that rapid washout is characteristic of compounds not dissolved in membrane lipids.

The effects of D-$(OH)_2$ derivatives on the amplitude-temporal characteristics of EPC (*e.g.*, type of decay) can be illustrated by the scheme, which includes the mechanism of open ion channel blockade and phenomenon of allosteric modulation:

$$A+R \underset{k_1^-}{\overset{k_1^+}{\rightleftarrows}} AR+R \underset{k_2^-}{\overset{k_2^+}{\rightleftarrows}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftarrows}} A_2R^*+S \underset{k_b^-}{\overset{k_b^+}{\rightleftarrows}} A_2R^*S,$$

$$A_2R^*+S \underset{\tilde{k}^-}{\overset{\tilde{k}^+}{\rightleftarrows}} \tilde{R},$$

$$A+\tilde{R} \underset{\tilde{k}_1^-}{\overset{\tilde{k}_1^+}{\rightleftarrows}} A\tilde{R}+A \underset{\tilde{k}_2^-}{\overset{\tilde{k}_2^+}{\rightleftarrows}} A_2\tilde{R} \underset{\alpha}{\overset{\tilde{\beta}}{\rightleftarrows}} A_2\tilde{R}^*+S \underset{\tilde{k}_b^-}{\overset{\tilde{k}_b^+}{\rightleftarrows}} A_2\tilde{R}^*S,$$

**TABLE 1.** Effect of D-$(OH)_2$ Derivatives on Characteristics of EPC and MECP

| Compound | Change in ECP amplitude | | Change in ECP decay time | | Change in MECP amplitude | | Change in MECP decay time | |
|---|---|---|---|---|---|---|---|---|
| | C, μmol/liter | % | C, μmol/liter | % | C, μmol/liter | % | C, μmol/liter | % |
| D-$(OH)_2$ | 10 | 0 | 10 | -3 | 10 | 0 | 10 | 0 |
| | 100 | -5 | 100 | $-11^+$ | 100 | -7 | 100 | -3 |
| | 500 | -6 | 500 | -5 | 500 | 6 | 500 | 5 |
| D-$(OMe)_2$ | 10 | $-7^+$ | 10 | $-8/41^+$ | 10 | -2 | 10 | $-0/39^+$ |
| | 100 | $-25^+$ | 100 | $-27^+/90^+$ | 100 | $-25^+$ | 100 | $-12^+/83^+$ |
| | 500 | $-32^+$ | 500 | $-35^+/125^+$ | 500 | $-40^+$ | 500 | $-25^+/117^+$ |
| D-$(OEt)_2$ | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |
| | 100 | $-23^+$ | 100 | $-15^+$ | 100 | $-9^+$ | 100 | $-10^+$ |
| | 500 | $-39^+$ | 500 | $-20^+$ | 500 | $-16^+$ | 500 | $-16^+$ |
| D-$(OPr)_2$ | 10 | $-10^+$ | 10 | 0 | 10 | -6 | 10 | 0 |
| | 100 | $-30^+$ | 100 | $-21^+$ | 100 | $-15^+$ | 100 | $-17^+$ |
| | 500 | $-47^+$ | 500 | $-34^+$ | 500 | $-30^+$ | 500 | $-26^+$ |
| D-$(OBu)_2$ | 10 | $-14^+$ | 10 | -3 | 10 | $-11^+$ | 10 | 0 |
| | 100 | $-34^+$ | 100 | $-19^+$ | 100 | $-26^+$ | 100 | $-17^+$ |
| | 500 | $-56^+$ | 500 | $-39^+$ | 500 | $-51^+$ | 500 | $-30^+$ |

**Note.** For D-$(OMe)_2$: change in the decay time of a rapid/slow component; $^+p<0.05$.

**TABLE 2.** Rate Constants for Blockade and Unblocking of the Cholinoceptor Channel with D-$(OH)_2$ Derivatives

| Parameter | D-$(OMe)_2$ | D-$(OEt)_2$ | D-$(OPr)_2$ | D-$(OBu)_2$ |
|---|---|---|---|---|
| $k^+_b$ | 13 $mM^{-1}\times msec^{-1}$ | 3 $mM^{-1}\times msec^{-1}$ | 3 $mM^{-1}\times msec^{-1}$ | 6 $mM^{-1}\times msec^{-1}$ |
| $k^-_b$ | 0.75 $msec^{-1}$ | 0.29 $msec^{-1}$ | 0.2 $msec^{-1}$ | 0.375 $msec^{-1}$ |

where A is acetylcholine; R is free cholinoceptor; AR is cholinoceptor with closed ion channel bound to single agonist molecule; $A_2R$ is cholinoceptor with closed ion channel bound to 2 molecules of the agonist; $A_2R^*$ is the cholinoceptor with open ion channel; $A_2R^*S$ is blocked non-conducting cholinoceptor; S is open channel-blocking active substance; and $\tilde{S}$ is the same substance dissolved in the lipid membrane and operating as an allosteric modulator (transition of the cholinoceptor from state R to state $\tilde{R}$ with other kinetic constants, superscript ~). The rate of receptor transition from state R to state $\tilde{R}$ and vice versa is much lower compared to activation/deactivation of receptors. Therefore, modeling of postsynaptic currents was

performed by the results of studying activation/deactivation of receptors (ratio between these states). Dynamic processes were assayed as described elsewhere [5]. We used the following constants for the major chain of cholinoceptor activation [8]: $k^+_1=2\times k^+_2$, $k^+_2=80$ $mM^{-1}\times msec^{-1}$, $k^-_1=18$ $msec^{-1}$, $k^-_2=2\times k^-_1$, $\beta=36.7$ $msec^{-1}$, and $\alpha(0)=2.66$ $msec^{-1}$:

$$\alpha(V)=\alpha(0)\times e^{V/156\ mV},$$

where V is the membrane potential (mV). Mathematical modeling was performed taking into account this scheme of the mechanism of dual effects. The described effects were best reproducible at the following constants: $\tilde{k}^+_1=120$ $mM^{-1}\times msec^{-1}$, $\tilde{k}^-_1=18$ $msec^{-1}$, $\tilde{k}^+_2=60$ $mM^{-1}\times msec^{-1}$, $\tilde{k}^+_2=36$ $msec^{-1}$, $\beta=32$ $msec^{-1}$, $\alpha=1.7$ $msec^{-1}$, and $\tilde{k}^+/\tilde{k}^-=100$. As differentiated from $\alpha$, $\tilde{\alpha}$ does not depend on the membrane potential. It explains our findings that the time constant for EPC decay does not depend on the potential. Table 2 shows the rate constants for blockade and unblocking upon treatment with various substances. During modeling we assumed that D-$(OH)_2$ has no effect on cholinoceptors, and the concentration of esters in the membrane is proportional to their solubility in lipids. The equilibrium ratio of $\tilde{R}$ in the total number of receptors for D-$(OMe)_2$, D-$(OEt)_2$, D-$(OPr)_2$, and D-$(OBu)_2$ was 0.01, 0.08, 0.474, and 0.91, respectively.

Our results suggest that the dual-effect model (blockade and modulation) can be used to describe the mechanism of the channel-blocking action of D-$(OH)_2$ derivatives on postsynaptic currents.

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