Serotonin Modulates Neuromuscular Transmission in Frogs

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The effect of 5-hydroxytryptamine (serotonin) on neuromuscular transmission in frog skeletal muscle was studied using voltage clamp technique. Serotonin produced no effect on end-plate currents during low frequency electrical stimulation of the motor nerve, but increased the amplitude depression of multiquantal currents during high-frequency stimulation similar to motor commands fired by motoneurons. It was shown that the inhibitory effect of serotonin on neuromuscular transmission is determined by slow potential-dependent block of open ionic channels in the postsynaptic membrane accumulating during rhythmic activation of the synapse.

Key Words: *neuromuscular transmission*; *acetylcholine*; *serotonin*; *ionic channels*

Serotonin (5-hydroxytryptamine, 5-HT) possesses intrinsic neurotransmitter activity in the central and peripheral nervous system and affects the peripheral motor apparatus by modulating the function of the neuromuscular synapse [11,13]. However, the mechanism of action of 5-HT on synapses in skeletal muscle remains unclear. It was previously hypothesized that 5-HT promoted acetylcholine secretion from presynaptic motor nerve terminals [5]. However, 5-HT inhibits postsynaptic acetylcholine-induced response [7]. Moreover, this agent can modify the potential dependence of the amplitude and decay time of the end-plate current (EPC) [4,8]. The latter effect can be explained by interaction of 5-HT with ionic channel of acetylcholine receptor. This interaction can be either a simple plug-in block of the open channel or a trap block, in which the blocker is trapped within the closed channel after dissociation of the agonist [9]. Our aim was a detailed study of the mechanisms of 5-HT action on synaptic transmission in skeletal muscle.

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MATERIALS AND METHODS

Two-electrode voltage clamp experiments were performed on neuromuscular preparation (sciatic nerve—sartorius muscle) isolated from *Rana ridibunda.* The muscle was perfused with physiological saline containing (in mM): 113.0 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 2.4 $NaHCO_3$ (pH 7.2-7.4). Muscle contractions were prevented either by transverse cutting [1] or by decreasing Ca^{2+} concentration with simultaneous addition of Mg^{2+} ions (0.9 mM Ca^{2+}, 4.0 mM Mg^{2+}). The nerve was stimulated at rates of 0.03, 10, and 60 Hz. EPC recording and analysis were performed using original software.

The data were processed statistically using nonparametrical Wilcoxon test.

RESULTS

Under the control conditions of infrequent nerve stimulation (0.03 Hz) at a holding potential of -40 mV, the amplitude of multiquantal EPC was 265±37 nA (n=11). The EPC rise and decay time constants were 0.53±0.07 and 1.42±0.20 msec (n=11), respectively. 5-HT (10^{-5}-10^{-4} M) produced no significant changes

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in evoked EPC after 20-min exposure. In the presence of 10^{-4} M 5-HT, EPC amplitude was 107±4% of the control value (n=12). The EPC rise and decay time constants did not change significantly (104±6 and 97±8% of the control, respectively, n=12). Similar results were obtained in the analysis of the effect of 5-HT on miniature EPC. 5-HT in a concentration of 10^{-4} M had not effect on the amplitude of miniature EPC (94±5% of the control after 20-min application, n=5). The fact that 5-HT produced no effect on the amplitude of EPC and miniature EPC suggests that this agent changed neither the number of neurotransmitter quanta released in response to nerve impulse nor the sensitivity of the postsynaptic membrane. This conclusion is corroborated by the fact that 5-HT produced no marked changes in the frequency of miniature EPC (1.58±0.06 and 1.32±0.07 Hz in the control and in the presence of 5-HT, respectively, n=5). Therefore, 5-HT produced no marked pre- and postsynaptic effects during low-frequency stimulation of the neuromuscular apparatus.

More interesting results were obtained when we studied the effect of 5-HT on conduction of rhythmic impulses similar to motor neuron discharge. In this case, the motor nerve was stimulated with a short trains of pulses (20 pulses in a train) at the rate of 10 or 60 Hz.

At a stimulation rate of 10 Hz, 5-HT (10^{-4} M) slightly but significantly changed the dynamics of successive EPC. In the control, the amplitude of the 20th EPC in the train was 102±4% of the first EPC (n=12), while in the presence of 5-HT it decreased to 92±2% (n=15; p<0.05). More pronounced changes were observed when the rate of stimulation was increased to 60 Hz. Depression of EPC observed after initial facilitation increased in the presence of 5-HT, which attests to pre- or postsynaptic frequency-dependent depression. In the control, the amplitude of 20th EPC in the train was 117±3% (n=12), while in the presence of 5-HT it decreased to 94±5% (n=14; p<0.05).

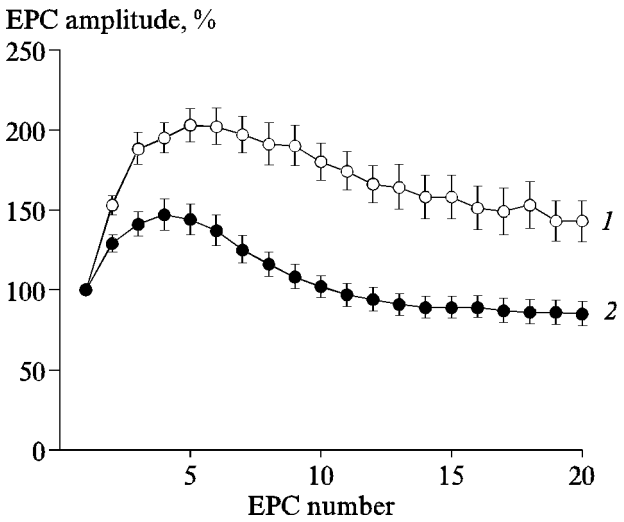

Fig. 1. Potentiation of depression of end-plate currents (EPC) in the presence of serotonin (10^{-4} M) after inhibition of acetylcholine esterase with neostigmine (5×10^{-5} M). The curves show the averaged amplitudes of EPC (n=17) recorded in response to stimulation of the nerve at 60 Hz in the control (*1*) and in the presence of 5-HT (*2*).

In further experiments we used inhibitors of acetylcholine esterase (AChE) to distinguish between pre- and postsynaptic effect of 5-HT on the action of high-frequency trains of nerve impulses. It was hypothesized that increasing the concentration and life-time of acetylcholine in the synaptic cleft potentiates the post-

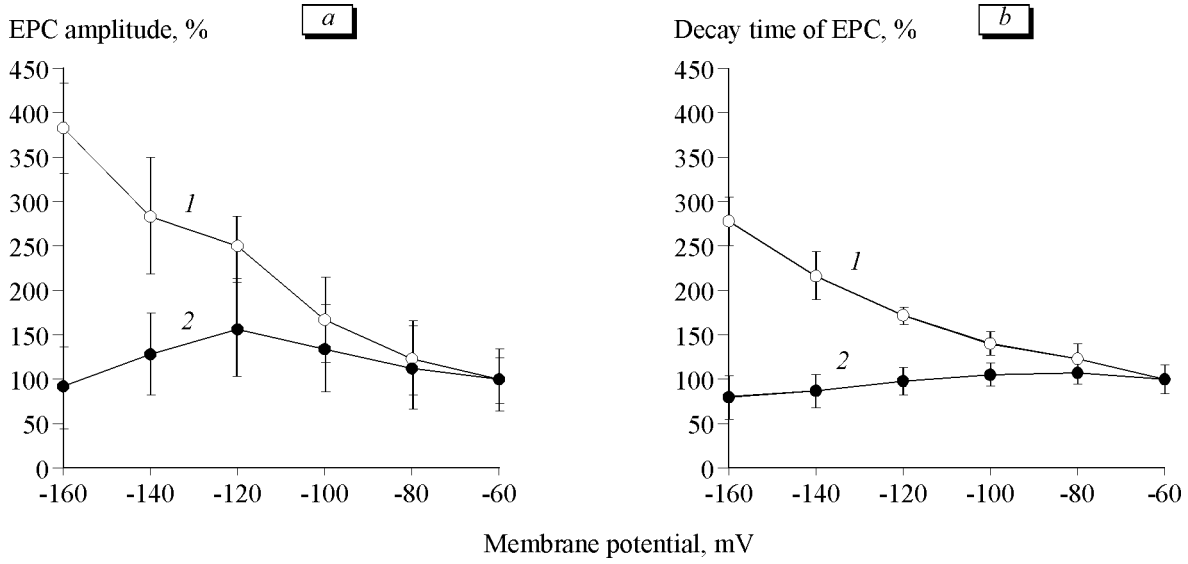

Fig. 2. Effect of membrane potential on averaged amplitude (*a*) and decay time constant (*b*) of EPC (n=14) in control (*1*) and in the presence of 10^{-4} M 5-HT (*2*).

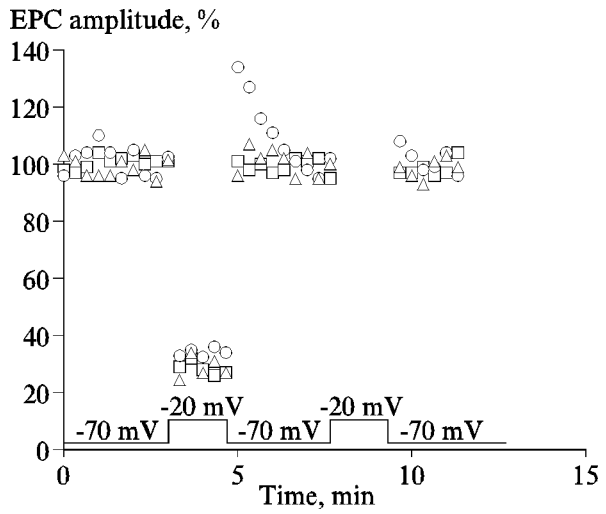

Fig. 3. Effect of transient depolarization (from -70 to -20 mV) on EPC amplitude in stimulated (0.03 Hz) and non-stimulated preparation. In the control (squares) the amplitude of EPC did not change after depolarization in both cases. In the presence of trapped blocker mecamylamine (2×10^{-5} M, circles) EPC amplitude increased by 35% after nerve stimulation and membrane depolarization; by contrast, in the presence of 5-HT (10^{-4} M, triangles) EPC amplitude did not change significantly.

synaptic mechanisms of frequency-dependent depression.

Inhibition of AChE with neostigmine (5×10^{-6} M) markedly potentiated EPC depression in the presence of 5-HT (Fig. 1). In neostigmine-treated preparation stimulated at a rate of 60 Hz, the amplitude of the 20th EPC in the train was 144±5% of the first EPC, while in the presence of 5-HT it was only 84±4% (n=17; p<0.05). The decay time constant in 5-HT-treated preparation stimulated at a rate of 60 Hz also decreased: 290±11% of the first EPC in the control and 235±7% of the first EPC in the presence of 5-HT (n=17; p<0.05).

Thus, 5-HT induces a frequency-dependent postsynaptic depression of successive EPC, which results either from blockade of open ionic channels of the cholinergic receptor [10], or from desensitization of the postsynaptic membrane [3,14,15].

To differentiate between these two putative mechanisms of 5-HT action on postsynaptic cholinergic receptors, we analyzed the dependence of the amplitude and decay time of EPC on membrane potential, which is one of the most informative tests for revealing blockers of open ionic channel [12]. This experimental series was carried out on non-cut nerve-muscle preparations, which allowed wide-range variation of the membrane potential. Muscle contractions were prevented by partial substitution of Ca^{2+} with Mg^{2+} ions (0.9 mM Ca^{2+}, 4 mM Mg^{2+}).

In the control, hyperpolarization of the postsynaptic membrane from -70 mV to -160 mV led to linear increase in the amplitude and exponential lengthening of EPC decay time (Fig. 2). In the presence of 5-HT (10^{-4} M), we observed a phenomenon typical of open channel blockers: the sensitivity of EPC amplitude to hyperpolarization decreased and a "negative conductance region" appeared (a decrease in the current amplitude after increasing hyperpolarization). In the presence of 5-HT, the decay time decreased pronouncedly during hyperpolarization in comparison with the control value, while the time course of the decay phase remained monoexponential. This phenomenon is typical of slow potential-dependent blockers of open channels. The rate of blockade increased during hyperpolarization, which results in potentiation of blockade and lengthening of the decay phase of ionic current [6].

To reveal whether 5-HT is a trapping-type blocker interacting with deep sites of open channels and persisting within the channel after closing of it, we used the depolarization test developed for mecamylamine, a trapping-type blocker [9]. Experiments showed that in contrast to mecamylamine, 5-HT did not increase EPC after transient depolarization of the postsynaptic membrane combined with activation of cholinoreceptors (Fig. 3). This indicates that 5-HT molecule is not trapped within the channel. Therefore, 5-HT binds to the outer site of channel-forming portion of acetylcholine receptor-channel complex.

Thus, potential-dependent block of open ionic channels in postsynaptic membrane underlies the frequency-dependent depression produced by 5-HT on the effect of rhythmic stimulation in neuromuscular preparation. By its kinetic parameters, this block is slow, and it can accumulate during frequent stimulation of postsynaptic membrane. This frequency-dependent block of cholinoreceptors in postsynaptic membrane by 5-HT can decrease reliability of neuromuscular transmission during allergic diseases or under other pathological conditions characterized by appearance of this bioactive substance in the cleft of motor synapses in skeletal muscle.

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