PHYSIOLOGY

Effect of Blockers of Potential-Dependent and Calcium-Activated K^+-Channels on Facilitation of Neuromuscular Transmission

M. A. Mukhamed'yarov, M. G. Minlebaev, and A. L. Zefirov

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Rhythmic stimulation of nerve-muscle preparation of frog sternal muscle bathed in low-Ca^{2+} saline increased the release of neurotransmitter (facilitation) and modified the shape of extracellular response of nerve terminal (decreased phase III amplitude). Iberiotoxin and 4-aminopyridine modified the dynamics these processes. We conclude that inactivation of potential-dependent K^+-channels and activation of calcium-dependent K^+-channels in frog motor nerve terminals during rhythmic activity modulate Ca^{2+} influx into nerve terminals and contribute into facilitation of neurotransmitter secretion. The degree of these mechanisms depends on the rate of synaptic rhythmic activity.

Key Words: *neuromuscular synapse; facilitation; K^+-channels; iberiotoxin; 4-aminopyridine; residual calcium*

Facilitation, *i.e.* increase in transmitter release during rhythmic activity of the synapse, is a common type of short-term synaptic plasticity [1,4,6,12]. Elevation of calcium concentration in axoplasm of nerve terminal (NT) plays a key role in the development of facilitation [6,12]. According to the hypothesis of "residual calcium" summation of Ca^{2+} ions entering NT during successive stimulations leads to elevation of intracellular calcium concentration and promotes the release of transmitter quanta [1,6,12].

Modulation of Ca^{2+} entry into NT also contributes into the development of facilitation [4,5,7,9-12]. An important factor determining the magnitude of Ca^{2+} current is the duration of action potential. Under natural conditions, the duration of action potential in NT is regulated via outward K^+ currents repolarizing the membrane and shortening the action potentials [2,3]. A major role in these processes is played by potential-dependent K^+_φ channels of NT [2-4,7,9]. Short-term local elevation of intracellular calcium concentration activates Ca^{2+}-dependent potassium channels (K^+_{Ca}). Potassium current though these channels also reduces the duration of action potential in NT [2,3,8].

We previously found that rhythmic stimulation of nerve-muscle preparation bathed with tetraethylammonium chloride, a blocker of all types of potassium channels in NT, produced a less pronounced facilitation effect and changed the rate of modification of the shape of NT responses [4,9]. It was concluded that potassium currents in NT participate in the development of facilitation in the neuromuscular synapse [4,9,10]. Here we studied the effect of K^+_φ and K^+_{Ca} channel blockers on facilitation and the dynamics of the shape of NT responses during rhythmic stimulation in neuromuscular synapse.

MATERIALS AND METHODS

Experiments were carried out on nerve-muscle preparations of sternal muscle from *Rana ridibunda* in

Department of Normal Physiology, Kazan State Medical University. ***Address for correspondence:*** mmarat01@mail.ru. Mukhamed'yarov M. A.

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autumn-winter. The preparation was placed into a bath (6 ml) and perfused with Ringer's solution for cold-blooded animals containing (in mM): 115.0 NaCl, 5.0 KCl, 0.1-0.4 $CaCl_2$, 4.0 $MgCl_2$, and 2.4-2.7 $NaHCO_3$ (pH 7.2). All experiments were carried out at 20°C. K^+_φ and K^+_{Ca} channels were blocked with 4-aminopyridine (0.025 mM, Sigma) and iberiotoxin (100 nM, Sigma), respectively.

The motor nerve was stimulated with series of rhythmic electrical pulses delivered at rates of 1 (low-frequency stimulation), 10, or 50 Hz (high-frequency stimulation). Each series consisted of 500 pulses, the interval between series was no less than 15-20 min.

The synaptic signals were recorded extracellularly on the proximal part of NT using glass microelectrodes with fire-polished tips (diameter 1-2 μ) filled with NaCl (2 M). After stimulation of the motor nerve we recorded integral presynaptic membrane current (NT response) followed by end-plate current (EPC). The NT response consisted of three phases: positive phase I reflecting passive depolarization of NT membrane, negative phase II caused by inward potential-dependent Na^+ current, and positive phase III formed by outward K^+ currents [3].

The signals were amplified and processed with an original system based on LCARD-1250 digitizer coupled to PC. Parameters of NT responses and EPC amplitude were determined. All parameters obtained with high-frequency stimulation were averaged in groups of 100 runs. The changes were presented as percent of the corresponding parameters obtained during low-frequency stimulation at 1 Hz. The quantum composition of EPC was calculated per each 100 stimuli delivered to motor nerve with so-called fall-out method according to the formula $m=\ln(N/N_0)$, where N is the total number of stimulations and N_0 is the number of stimuli not inducing EPC. The data were analyzed statistically using Student's *t* test.

RESULTS

During perfusion of the nerve-muscle preparation with low-Ca^{2+} solution containing Mg^{2+} the quantum composition of EPC during low-frequency stimulation (1 Hz) varied from 0.04 to 0.34 (n=10). High-frequency stimulation gradually increased quantum composition of EPC (facilitation, Fig. 1). During stimulation at 10 Hz quantum composition by the 40th second (stimuli 401-500) increased more than 3-fold (331.5±61.4%, n=6, p<0.05) compared to baseline (Fig. 1, *a*). At 50 Hz, facilitation was even more pronounced: by the 8th second (stimuli 401-500) quantum composition increased to 1386.0±421.2% of baseline (n=6, p<0.05, Fig. 1, *b*).

In addition to facilitation of the transmitter release, high-frequency stimulation produced progressive changes in the shape of NT response: decrease in phases II and III amplitudes and lengthening of phase II (Fig. 2, *a*). The increase in phase III amplitude is very important, because this phase reflects outward potassium currents. The amplitude of phase III of NT response decreased to 68.9±3.6% from the baseline (n=8, p<0.05) after 40 sec stimulation at a rate of 10 Hz and to 83.3± 3.2% after 8 sec stimulation at a rate of 50 Hz (n=6, p<0.05, Fig. 2, *a*, *b*).

In the following series of experiments, the above changes in quantum composition of EPC and in the shape of NT response produced by high-frequency stimulation were used as the control.

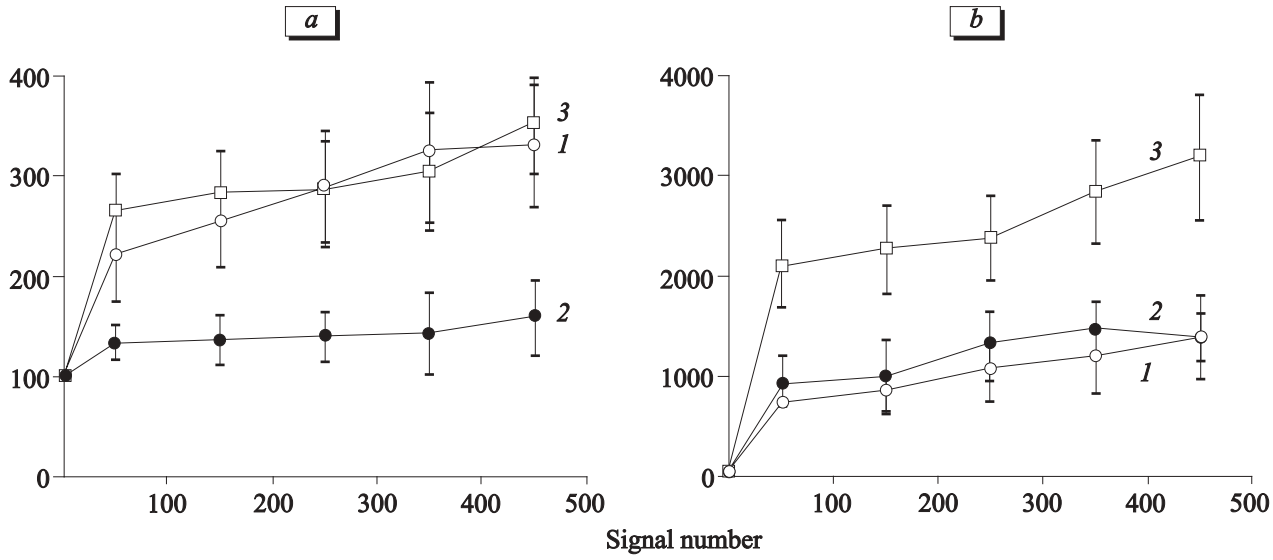

Fig. 1. Facilitation of transmitter secretion during high-frequency stimulation under normal conditions (*1*) and after application of K^+ channel blockers 4-aminopyridine (*2*) or iberiotoxin (*3*). Ordinate: averaged percentage of quantum composition of EPC during stimulation at 10 Hz (*a*) and at 50 Hz (*b*).

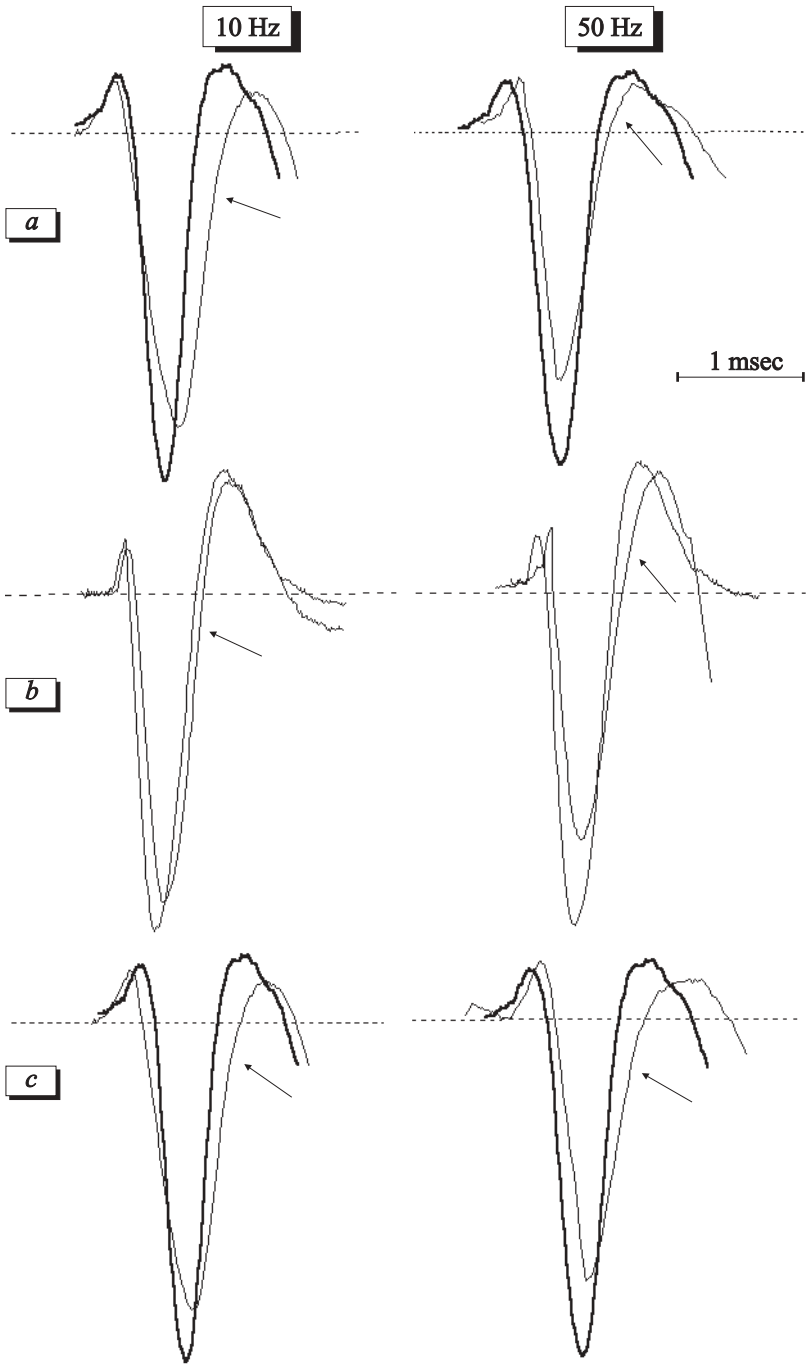

Fig. 2. Effect of high-frequency stimulation on the shape of the response of nerve terminal under normal conditions (*a*) and in the presence of K^+ channel blockers 4-aminopyridine (*b*) or iberiotoxin (*c*).

Application of 4-aminopyridine (K^+_φ channel blocker) potentiated secretion of the transmitter and induced repeated activity. Since the degree of facilitation depends on the initial (baseline) level of secretion, in these experiments we decreased concentration of extracellular Ca^{2+} to 0.10-0.15 mM to equalize quantum composition of EPC before and after application of the blocker.

Stimulation at a rate of 10 Hz increased quantum composition of EPC, but less markedly than in the control: by the 40th second of stimulation it attained 158.7±38.6% of baseline (n=5, p<0.05, Fig. 1, *a*). At the same time, by the 8th second of stimulation at 50 Hz quantum composition of EPC was 1386.7±236.0% (n=5, p<0.05), *i.e.* did not significantly differ from the corresponding values in the control (Fig. 1, *b*). It should be noted that at both rates of stimulation repeated activity disappeared after 4-10 pulses and had no effect on the development of facilitation.

Application of 4-aminopyridine changed the dynamics of modification of the shape of NT response during high-frequency stimulation. Stimulation at 10 Hz

did not change the amplitude of phase III: 94.2± 6.8% of baseline value by the 40th second (n=5, p<0.1). By the 8th second of stimulation at 50 Hz this amplitude decreased to 88.4±2.3% (n=5, p<0.05), which corresponded to the control values (Fig. 2, *b*).

In the next experimental series we used K^+_{Ca} channel blocker iberiotoxin. This toxin produced no significant changes in secretion of transmitter and the shape of NT response (n=4).

Rhythmic stimulation (10 Hz) of iberiotoxin-treated preparation increased quantum composition of EPC to 321.5±47.4% (n=5, p<0.05), which did not significantly differ from the control value (Fig. 1, *a*). At 50 Hz, facilitation in the iberiotoxin-treated preparations was more pronounced than in the control: by the 8th second quantum composition of EPC increased to 2645.4± 543.3% from the baseline (n=5, p<0.05, Fig. 1, *b*).

Rhythmic stimulation of iberiotoxin-treated preparation at a rate of 10 Hz decreased phase III amplitude of NT response to 76.7±8.2% and 89.3±2.6% of baseline value after 10 and 40 sec, respectively (n=5, p<0.05). Stimulation at 50 Hz produced significantly greater decrease in phase III amplitude in comparison with the control value: to 63.1±6.5% of baseline value (n=5, p<0.05, Fig. 2, *c*).

Thus, during stimulation at 10 Hz in the control experiments facilitation was accompanied by pronounced decrease in the amplitude of phase III of NT response reflecting inactivation of K^+ channels [3,9]. The fact that facilitation and changes in the shape of NT response during high-frequency stimulation were less pronounced in solutions containing tetraethylammonium [9] and 4-aminopyridine suggests that inactivation of K^+_φ channels is the key mechanism of facilitation during stimulation at 10 Hz. Experiments with iberiotoxin confirmed that K^+_{Ca} channels are not involved into this process.

During stimulation at 50 Hz facilitation was more pronounced, while shape of NT response changed to a lesser extent compared to stimulation at 10 Hz. Rhythmic stimulation of iberiotoxin-treated preparation at ae rate of 50 Hz induced more pronounced facilitation and decrease in the amplitude of phase III of NT response in comparison with the control values. Evidently, this high-frequency stimulation induced accumulation of "residual" Ca^{2+} ions in NT axoplasm, which potentiates secretion and activates K^+_{Ca} channels in the immediate proximity to Ca^{2+} channels [2,8]. Ionic current across K^+_{Ca} channels shortens action potentials in NT, reduces Ca^{2+} entry, and facilitates secretion of the transmitter during rhythmic stimulation.

The degree of facilitation decreases with increasing the intervals between stimuli. This dependence can be described as the sum of two exponentially fading processes [1,6]. The first component is characterized by higher amplitude and shorter time course than the second component, the corresponding decay times were 50 and 300 msec [1,6, 12]. During rhythmic stimulation, both components are added linearly [1,6].

Our findings suggest that facilitation in the neuromuscular synapse is determined by different mechanisms depending on the rate of stimulation. During high-frequency stimulation at 50 Hz we observed accumulation of "residual" Ca^{2+} ions in NT axoplasm and gradual decrease of Ca^{2+} entry into NT due to activation of K^+_{Ca} channels. These processes determine the magnitude and duration of the first facilitation component. At lower stimulation rate (10 Hz), facilitation is predominantly caused by increased Ca^{2+} entry into NT due to prolongation of action potentials in NT resulting from inactivation of K^+_φ channels. By its kinetics, this process is similar to the second component of facilitation and can play a role in its development.

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