Bradykinin Effect on Conduction of Rhythmic Series of Pulses through the Neuromuscular Synapse

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Bradykinin did not change the amplitude and duration of growth and decrease of individual currents of the terminal plate, but potentiated the depression of amplitudes of consecutive currents during stimulation of the motor nerve at a frequency of 10-60 Hz under conditions of two-electrode fixation of potential on a frog neuromuscular preparation. The origin of this effect is presynaptic, because it does not depend on the activity of acetylcholinesterase and is associated with a longer increase in the terminal plate currents which are synchronous with the amplitude depression. The deconvolution method revealed a slower kinetics of the mediator secretion under the effect of bradykinin. Bradykinin impairs the conduction of rhythmic pulse series through the synapse by augmenting the asynchronism of the transmitter quantum secretion from the motor nerve endings, which may underlie the development of muscular asthenia observed in inflammatory and allergic reactions.

Key Words: *synapse; plasticity; bradykinin; secretion; acetylcholine*

Bradykinin (BK) is a humoral factor whose concentration increases in damaged tissues and during allergic and inflammatory reactions [12]. BK effects on smooth muscular cells and sympathetic neurons, which it activates through the second messengers diacylglycerol and inositol trisphosphate with subsequent increase in the concentration of intracellular calcium, are studied in detail [4]. There are no published data on the effect of BK on the peripheral neuromuscular system; experiments in a cell culture of the glioma synaptically connected to the skeletal muscular fibers revealed an inhibitory effect of this agent on synaptic transfer because of suppressed acetylcholine (AC) secretion [13]. If a similar effect of BK develops in the neuromuscular system *in vivo*, it may lead to myasthenia in inflammatory and allergic diseases. Therefore we investigated the effect of BK on the amplitude and time course of currents in the terminal plate (TPC) during rhythmic stimulation of the motor nerve under conditions of intact high (close to the normal) level of induced secretion of AC.

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MATERIALS AND METHODS

Experiments were carried out on a neuromuscular sciatic nerve—*musculus sartorius* preparation of *Rana temporaria* at 20-21°C. The muscle was perfused with Ringer's solution containing (in mmol/liter): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and 2.4 $NaHCO_3$, pH 7.3. In order to preserve induced secretion of the mediator at the level close to the normal, muscular contractions were blocked by transverse section of the muscle. TPC were recorded using two-electrode fixation of the membrane potential with a numbering of 5-20 μsec/point. The TPC parameters were calculated on a PC. The time course of secretion during a single synaptic transfer was evaluated by the deconvolution method [8,14]. The methods were described in detail previously [1-3].

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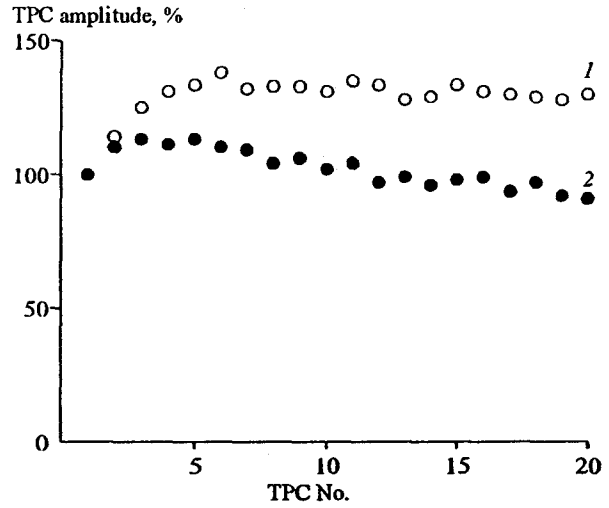

Fig. 1. Effect of bradykinin on the amplitude of the terminal plate currents (TPC) during rhythmic stimulation of the motor nerve at 60 Hz. *1*) time course of TPC amplitude in the control; *2*) after injection of bradykinin in a concentration of 10^{-5} mol/liter.

RESULTS

In the first series of experiments, the effects of BK on the amplitude, duration of decrease, and time of individual TPC were studied under conditions of a rarefied (0.03 Hz) stimulation of the motor nerve. In control experiments at a fixation potential of -40 mV, the mean amplitude of multiquantum TPC was 146±12.8 nA (8 synapses). The addition of BK to a final concentration of 10^{-6}-10^{-5} mol/liter did not change the amplitude of TPC. Twenty minutes after the addition of 10^{-5} mol/liter BK, the amplitude of TPC was 115.8±19.3 nA (n=8, p>0.05).

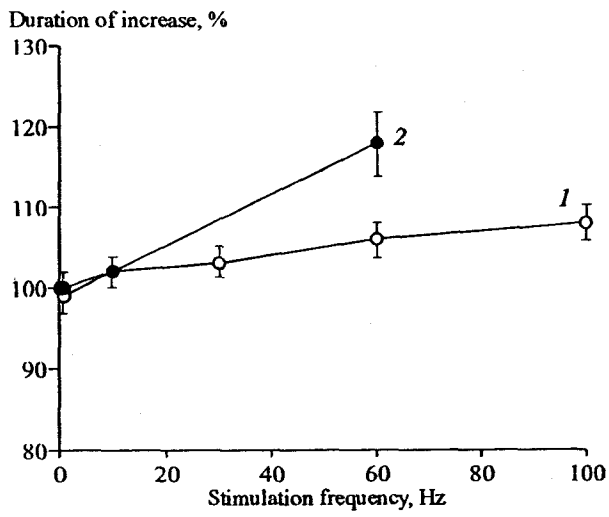

Fig. 2. Effect of bradykinin on the relationship between the duration of the increase in the terminal plate currents and the frequency of the motor nerve stimulation. *1*) changes in the duration of the increase in the control; 2) changes in the duration of the increase in the presence of bradykinin in a concentration of 10^{-5} mol/liter.

BK did not alter the constant of decline duration (τ_{TPC}) and the duration of TPC increase. In the control these parameters were τ_{TPC}=1.27±0.12 msec (n=8, p>0.05) and the increase duration 0.42±0.01 msec(n=8, p>0.05). After BK administration in a concentration of 10^{-5} mol/liter these parameters did not change: τ_{TPC} was 1.09±0.14 msec (n=8, p>0.05) and the increase duration was 0.41±0.02 msec (n=8, p>0.05). The lack of the effect on the amplitude and τ_{TPC} of multiquantum TPC indicated the absence of channel-blocking and desensitization [5,6,9].

Study of BK effects on the conduction of rhythmic pulse series yielded the most interesting results. In the control, rhythmic stimulation by short packs of pulses at a frequency of 10-60 Hz similar to the motoneuron motor command led to an initial increase in TPC amplitude, resulting from presynaptic facilitation, which was then replaced by slowly developing depression of the TPC amplitude (Fig. 1). This depression essentially increased in the presence of BK. In the control the amplitude of the 20th TPC in the rhythmic series was 109±1% at a stimulation frequency of 60 Hz (n=6), 100% being the amplitude of the first current; without BK it decreased to 86±2% (n=7, p<0.05).

Acetylcholinesterase inhibition by proserin in a concentration of 3×10^{-6} mol/liter, which almost completely blocks AC activity [10] and amplifies the manifestations of the postsynaptic mechanisms of TPC depression in the rhythmic series [5], did not increase the depression of TPC in the presence of BK. At a stimulation frequency of 10 Hz, the depression of amplitudes in the presence of active acetylcholinesterase after BK injection in a concentration of 10^{-5} mol/liter was 89.0±3% (n=9, p>0.05) vs. 94.0±4% (n=10, p>0.05) after inhibition of this enzyme. These data indicate a presynaptic nature of the BK effect.

This effect was confirmed by a prolonged increase in the number of multiquantum TPC in the rhythmic series, which was particularly obvious at a stimulation frequency of 60 Hz (Fig. 2). The effect was very weak in the control and reached 18% in the presence of BK (n=7, p<0.05).

The effect of TPC growth prolongation was analyzed using the convolution method [8,14]. This helped us to evaluate the changes in the time course of AC secretion, which is slower in the presence of BK than in the control (Fig. 3). The 1st and the 20th TPC, which were modeled on the basis of these transmitter secretion patterns of standard area, differed only by the degree of synchronism of AC quantum secretion. This difference reproduced the depressive effect of BK in packs at a stimulation frequency of 60 Hz (Fig. 3).

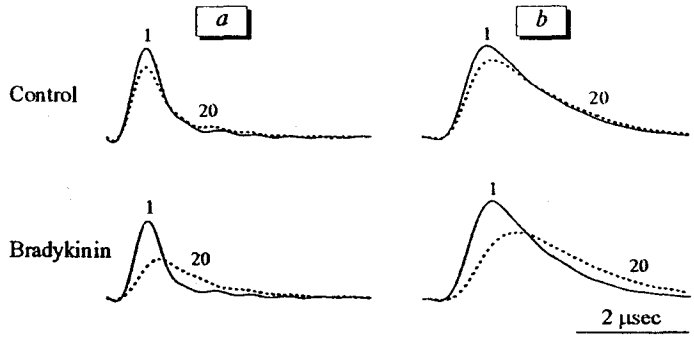

Fig. 3. Time course of transmitter secretion derived by the convolution method (*a*) and reconstructed terminal plate currents (*b*) in the control and after bradykinin injection. The numbers of signals in rhythmic series at a stimulation frequency of 60 Hz are shown in the figure.

Thus, experimental findings and data of stimulation experiments indicate that the effect of BK is realized at a presynaptic level. The increased asynchronism of AC quantum secretion may be the main mechanism of depression induced by BK. Under normal circumstances the effect of asynchronism amplification plays a negligible role [7,8]. Our study showed that its functional significance increases considerably at high BK concentrations in the neuromuscular environment. The effect of BK on the kinetics of AC quantum secretion seems to be based on the ability of this agent to increase the concentration of the second messengers diacylglycerol and inositol trisphosphate [12]. This activates protein kinase C and increases the level of intracellular calcium [4]. These intracellular processes can modify the kinetics of a complex cascade of reactions that determine the time course of the transmitter secretion [11].

Previously we showed that another inflammation mediator, histamine, depresses the neuromuscular transfer, but its mechanism is different: postsynaptic [2]. The effect of BK described in this report — suppression of rhythmic pulses transfer — may contribute to the development of myasthenia during allergic and inflammatory diseases.

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