Physiological Role of Desensitized Cholinoceptors in Skeletal Muscle

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The effect of desensitized cholinoceptors on the time course of end-plate currents was evaluated in frog skeletal muscle at a high (physiological) level of acetylcholine secretion in the presence of active acetylcholinesterase, with two-electrode recording of the membrane potential. When the number of cholinoceptors was small so that they did not appreciably affect the amplitude of end-plate currents or the parameters of one-quantum responses (miniature currents), the decay of multiquantum currents was significantly accelerated. Moreover, the presence of cholinoceptors drastically reduced the ability of acetylcholinesterase inhibitors to prolong the decay of end-plate currents. It is suggested that desensitized cholinoceptors in a synapse with a physiological level of acetylcholine secretion and active acetylcholinesterase may bind free acetylcholine with high affinity and thus supplement the well-known physiological role of acetylcholinesterase in limiting the reactivation of postsynaptic membrane cholinoceptors.

Key Words: *synapse; acetylcholine; desensitization*

Desensitization of a muscle cell membrane, i.e., its reduced sensitivity to the neurotransmitter acetylcholine (ACh), may be induced by exogenous cholinomimetics, including depolarizing myorelaxants [10], and by the endogenous ACh released from nerve endings [5,6,12]. How desensitization affects the parameters of synaptic signals and what the functional role of this phenomenon is in skeletal muscle have not been ascertained. Desensitization, if strong, appears to be manifested in lowered amplitudes of synaptic signals as a result of a decrease in the number of receptors capable of opening ion channels when ACh is active. It has also been suggested that desensitized receptors may play a functional role in the redistribution of free ACh in the synaptic cleft through high-affinity binding of the transmitter [13]. Evidence in support of this hypothesis has so far been obtained only for muscles with inhibited acetylcholinesterase and low levels of ACh secretion. In view of this, we tried in this study to elucidate the functional role of desensitized receptors in the presence of active acetylcholinesterase and a high (physiological) level of ACh secretion, i.e., under conditions that, like acetylcholinesterase inhibition, increase the local concentration of this transmitter in the synaptic cleft as compared to its level in the case of one-quantum responses.

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MATERIALS AND METHODS

The experiment was conducted on neuromuscular preparations of the sciatic nerve-sartorius muscle from *Rana ridibunda* frogs, with two-electrode recording of the membrane potential. The muscle was continuously perfused with physiological solution of the following composition (mmol/liter): NaCl 113, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 2.4; pH 7.2-7.4. To maintain the induced ACh secretion at a high level close to the physiological one, muscle contractions were prevented by transecting the muscle [1]. The nerve was stimulated at a fre-

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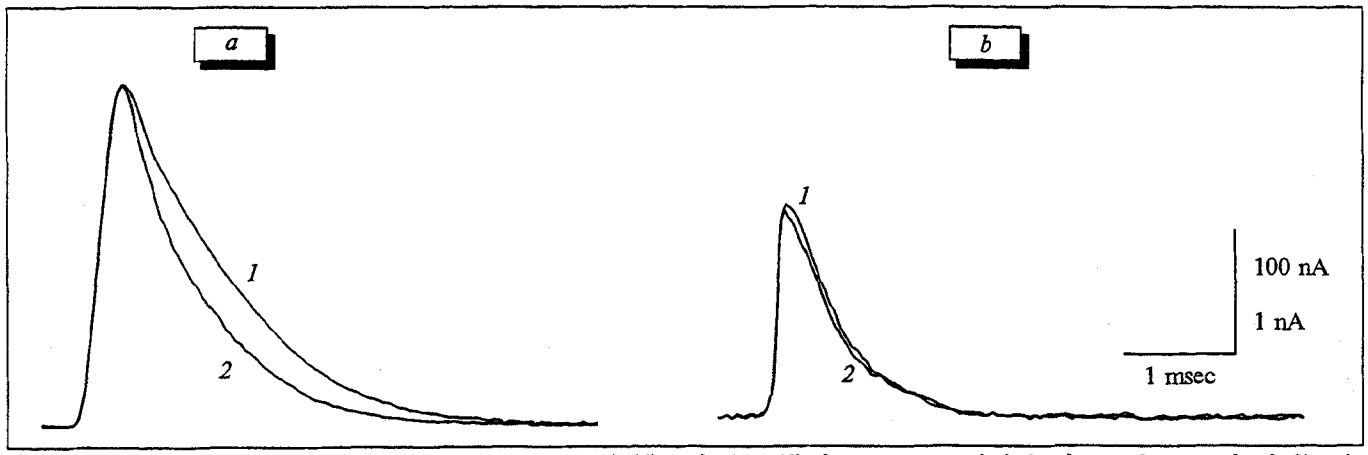

Fig. 1. End-plate currents (EPC) before (control tests) (*1*) and after (*2*) the exposure of skeletal muscle to carbacholine in a concentration of 10 μmol/liter. *a*) multiquantum EPC; recorded potential, −40 mV at 20°C. *b*) MEPC; recorded potential, −70 mV at 20°C.

quency of 0.03 Hz. Parameters of end-plate currents (EPC) and miniature end-plate currents (MEPC) were calculated by computer at a conversion frequency of 1/20 μsec.

RESULTS

In the first series of tests, the muscle was acted upon with the cholinomimetic carbacholine in order to establish how desensitization of cholinoceptors would affect the parameters of multiquantum EPC elicited by secretion of 100-200 ACh quanta. In control tests, at a recorded membrane potential of -40 mV, the mean EPC amplitude was 214.2±15.0 nA (n=6, where n is the number of synapses) and the constant of decay time (τ_{EPC}) was 1.43±0.08 msec (n=6), exceeding by 25% the duration of MEPC decay and indicating reactivation of cholinoceptors [7]. After a 20-minute exposure to carbacholine in a concentration of 10 μmol/liter, the EPC amplitude and τ_{EPC} decreased to 178.6±19.4 nA and 1.10±0.11 msec, respectively (n=6, p<0.05). The decay of EPC in the presence of the cholinomimetic was thus more rapid than in the control. This effect could be due to the emergence of desensitized cholinoceptors on the postsynaptic membrane and to their ability to serve as high-affinity "traps" for ACh [13]. However, analysis of the functional role of desensitized receptors under these conditions was complicated by the presence of a constant concentration of carbacholine which could saturate the traps, thereby preventing them from binding quantum ACh. For this reason, we proceeded to analyze the time course of carbacholine washing off, as the postsynaptic membrane recovers its sensitivity very slowly with such delivery of the agonist [13]. Complete restoration of EPC amplitudes to the control values (213.4±14.8 nA; n=6) then occurred by minute 15 of the washing process. However, although the EPC amplitudes were restored, the τ_{EPC} was 1.09±0.12 msec, or only 74.8±5.5% of its initial value (n=6, p<0.05; Fig. 1, *a*). This can probably be taken as evidence that desensitized receptors, a few of which still remained after the washing, shortened the period of EPC decay by reducing the probability of ACh receptor reactivation.

In the next test series, exogenous ACh was used to elicit the appearance of desensitized cholinoceptors on the postsynaptic membrane. The results were very similar to those obtained with carbacholine. In control tests, the mean EPC amplitude was 215.6±23.8 nA and the mean τ_{EPC} was 1.78±0.13 msec (n=5). After 20 min of exposure to ACh in a concentration of 100 μmol/liter, the EPC amplitude decreased to 131.6±32.4 nA and the τ_{EPC} to 1.31± 0.14 msec (n=5). After the ACh was washed off, the EPC amplitude was completely restored and equaled 229.8±32.6 nA, while τ_{EPC} increased to 1.35±0.15 msec (75.4±5.3% of its initial value; n=5, p<0.05).

The time course of MEPC, unlike that of multiquantum EPC, remained unchanged as desensitization developed. In control tests, at a recorded membrane potential of -70 mV, the MEPC amplitude was 2.92±0.2 nA (200-300 signals were averaged for each synapse) and the τ_{MEPC} was 1.01±0.09 msec (n=4). After a 20-minute exposure to carbacholine (10 μmol/liter), the mean MEPC amplitude was 2.47±0.10 nA and the mean τ_{MEPC} was 0.85±0.09 msec, n=4). After a 15-minute washing off of the agonist, the MEPC amplitude increased to 2.82±0.5 nA and the τ_{MEPC} to 0.97±0.05 msec, i.e., it was the same as in the control tests (n=4; Fig. 1, *b*). This result agrees well with the fact that the decay of MEPC is determined solely by the rate at which ion channels are closed and virtually does not depend on receptor reactivation by

the transmitter [3,9]. Desensitized receptors, therefore, affect the time course only of physiological multiquantum responses.

In order to exclude other possible effects of agonists on the time course of synaptic signals (apart from the formation of desensitized receptors), we used the desensitizing agent SKF-525A (proadifen) for accelerating the development of desensitization. Although it is incapable of opening ion channels, this substance stabilizes receptors in the desensitized state [4,11]. In this test series, the control values of EPC and τ_{EPC} were 252.7±78.3 nA and 1.16±0.08 msec (n=5). Exposure to proadifen in a concentration of 5 μmol/liter, at which it affects neither the amplitude and temporal characteristics of MEPC [5,11] nor the operating parameters of ion channels [14], shortened the τ_{EPC} to 0.83±0.08 msec, n=5, p<0.05). As in the case of its action on MEPC when acetylcholinesterase was inhibited [5], proadifen initially shortened EPC without altering signal amplitudes. These results corroborate the conclusion that desensitized cholinoceptors are capable of limiting receptor reactivation by the transmitter.

The emergence of desensitized cholinoceptors had more striking consequences when ACh hydrolysis was eliminated. In control tests, the EPC decay was prolonged from τ_{EPC}=1.43±0.08 msec to 7.81±0.51 msec as a result of acetylcholinesterase inhibition with proserine in a concentration of 3 μmol/liter (n=8, p<0.05). The main cause of this prolongation was receptor reactivation by the transmitter that had remained in the synaptic cleft for a long time [9]. The presence of even a small number of desensitized cholinoceptors almost completely abolished the prolongation effect when acetylcholinesterase was inhibited (Fig. 2). Thus, the inhibition with proserine in the presence of proadifen (5 μmol/liter) increased the τ_{EPC} only to 1.78±0.38 msec, n=6, p>0.05).

This study showed that even a small number (judging from the absence of a decrease in signal amplitudes) of desensitized cholinoceptors may have a strong influence on postsynaptic membrane activation in a neuromuscular synapse. The impact of such receptors is primarily manifested in an altered duration of synaptic signals under conditions of a high (physiological) level of ACh secretion in the presence of active acetylcholinesterase and is much less pronounced when one-quantum responses are recorded. The amplitudes of signals (both multiquantum and one-quantum) begin to decrease only when the desensitization is more profound.

The ability of desensitized cholinoceptors to shorten the postsynaptic current decay appears to be

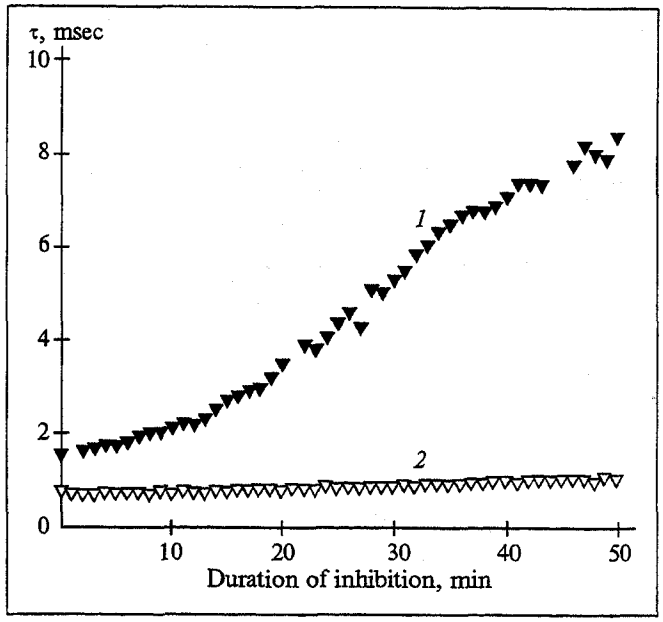

Fig. 2. Effect of the acetylcholinesterase inhibitor proserine (3 μmol/liter) on the duration of end-plate current decay in control tests (*1*) and in the presence of the desensitization promoter proadifen in a concentration of 5 μmol/liter (*2*). Recorded potential, −40 mV at 20°C.

based on their high affinity for ACh, a view which is supported by biochemical evidence [4,8]. A functional consequence of the high affinity shown by desensitized receptors is prevented reactivation of free receptors by the transmitter. Desensitized receptors may appear on the postsynaptic membrane in the course of rhythmic activity [2,5,12] and in the presence of ACh released in the nonquantum form [6]. This suggests that the effects of desensitized receptors that we have described may develop during functioning of the neuromuscular apparatus *in vivo*. The activity of desensitized receptors as high-affinity traps for ACh may contribute to increased reliability of synaptic transmission as high-frequency commands are sent to the muscle, for this will ensure a short duration of the synaptic signals. When the level of acetylcholinesterase activity is normal, the desensitized receptors supplement the well-known functional role of this enzyme. When it is low, the above-described method of eliminating free ACh from the synaptic cleft through its high-affinity binding to desensitized receptors may become a leading mechanism for limiting the excessive activity of synaptic signals.

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On the Possibility of Ventral Root Fibers Undergoing Ephaptic Excitation under Conditions of Very Severe Spinal Hyperreflexia

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Monosynaptic discharges by ventral roots were studied in rats under conditions of pronounced spinal hyperreflexia 5 days after simultaneous transection of the sciatic nerve and spinal cord. In 40% of tests with such rats, an enhanced monosynaptic discharge of a ventral root was found to be followed by a synchronized and high-amplitude discharge similar in shape and amplitude to the response of the ventral root to electrical stimulation of its fibers. The threshold amplitude for elicitation of these extra discharges was close to the amplitude of the ventral root's monosynaptic discharges at which high-amplitude discharges occurred. It is concluded that when the excitability of spinal reflex arcs is excessively high, ephaptic transmission of excitation probably occurs in ventral roots from fibers involved in the enhanced reflex discharge to unexcited fibers.

Key Words: *spinal cord; hyperreflexia; ventral root; ephaptic excitation*

Excitability of spinal cord neurons in rats has been shown to be highest on days 3-5 after nerve transection [5,7]. After cordotomy, stably enhanced excitability of spinal cord reflex arcs was reported in rats 3 days postsurgery and persisted for a long time [4,6]. We assumed, therefore, that an operation combining nerve transection with cordotomy would lead 5 days later to the formation in the spinal cord of a focus of enhanced excitability exceeding that observed after separate nerve or spinal cord transection. Characteristics of the reflexes from the spinal cord after such a combined operation are of interest for studies designed to gain better insight into how a generator of pathologically enhanced excitation forms in the spinal cord [3].

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