DEVELOPMENT OF DESENSITIZATION DURING RHYTHMIC SYNAPTIC ACTIVITY AND DURING SINGLE SIGNAL GENERATION

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Desensitization, or a decrease in the sensitivity of the end plate to activating agents, is observed in neuromuscular synapses during sustained or repeated action of acetylcholine (ACh) on the postsynaptic membrane [5, 10]. The question whether this phenomenon, which is easily reproduced with the aid of pharmacologic ACh, can develop in response to the action of ACh isolated from motor nerve endings, has been discussed for a long time in the literature [1, 2, 8, 11-13, 15]. Development of desensitization as a result of accumulation of endogeneous ACh was shown most convincingly in experiments with sufficiently long (30-60 sec) continuous stimulation of the motor nerve [2, 11]. Desensitization is considerably accelerated by membrane hyperpolarization [9], by a rise of temperature [10], by an increase in the Ca^{++} concentration [9], and in the presence of various pharmacologic agents, the most specific and effective of which is proadifen [3].

By using these agents to facilitate conversion of the cholinoceptive membrane into the desensitized state, we attempted to establish the possibility of development of desensitization in the course of short bursts of end-plate current (EPC), similar to those with which the synapse responds to the motor command of the motoneuron. We also tested the hypothesis according to which, after inhibition of acetylcholinesterase (AChE), when the action of ACh is considerably prolonged, desensitization may arise in the course of single signal generation [4].

EXPERIMENTAL METHOD

Experiments were carried out on a frog neuromuscular preparation (sciatic nerve – sartorius muscle) at temperatures of 20-21 and 25-26°C. The preparation was placed in a bath through which there was a continuous flow of Ringer's solution with the following composition (in mM): NaCl – 115, KCl – 2.5, $CaCl_2$ – 1.8, $NaHCO_3$ – 2.4, pH 7.2-7.4. To maintain a high (near-physiological) level of evoked mediator secretion, muscular contractions were blocked by transverse division of the muscle fibers or by keeping the muscle in hypertonic glycerol solution, followed by rinsing. The results obtained by two methods were in agreement. EPC and miniature EPC (MEPC) were recorded under voltage clamp conditions by a two-electrode method. Signals were analyzed by a system consisting of a digital analyzer and computer. AChE was inhibited by the use of neostigmine (3×10^{-6} M).

EXPERIMENTAL RESULTS

Control experiments were undertaken on the divided and glycerinized preparation against the background of inhibited AChE. With a membrane potential of −40 to −60 mV and a temperature of 20-21°C, only very slight depression of the amplitude of EPC was observed in the course of a short (15-20 signals) burst of multiple-quantum EPC with a following frequency of 10 Hz (Figs. 1 and 2). In the presence of proadifen depression was enhanced. The effect increased with an increase in the concentration of this agent from 10^{-6} to 10^{-5} M (Fig. 1). The same result could be produced by reducing the sensitivity of the postsynaptic membrane or inhibiting mediator release from the motor nerve endings to an increasing degree in the course of the burst. To discover whether depression was pre- or postsynaptic, two approaches were used. First, dependence of this depression on membrane potential was analyzed, for we

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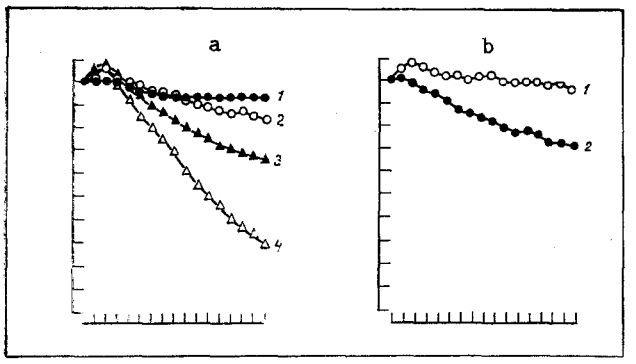

Fig. 1. Effect of motor nerve stimulation with frequency of 10 Hz on amplitude of EPC in presence of proadifen (a) and during a rise of temperature, of Ca^{++} concentration, and change of membrane potential (b). a: 1) Control, 2, 3, 4) proadifen in concentrations of 10^{-6}, 5×10^{-6}, and 10^{-5} M respectively. Membrane potential from −40 to −60 mV, temperature 20-21°C, Ca^{++} concentration 1.8 mM. b: 1) Membrane potential from −60 to −100 mV, 2) membrane potential from +30 to +40 mV, temperature 25°C, Ca^{++} concentration 2.5 mM. Abscissa, serial No. of EPC in burst; ordinate, amplitude of EPC relative to first EPC in burst.

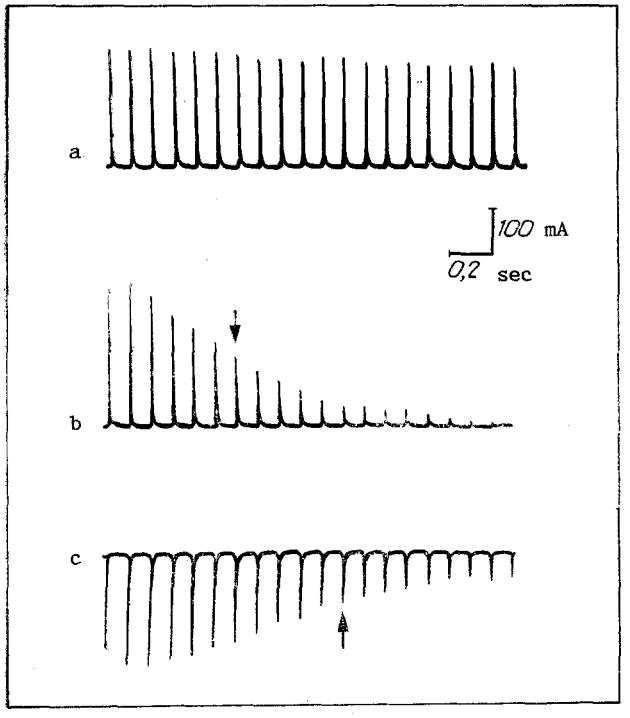

Fig. 2. Effect of motor nerve stimulation with frequency of 10 Hz on amplitude of EPC in presence of proadifen, and at different membrane potentials. a) Control, membrane potential −50 mV; b and c) proadifen (10^{-5} M), membrane potential −70 and −50 mV, respectively.

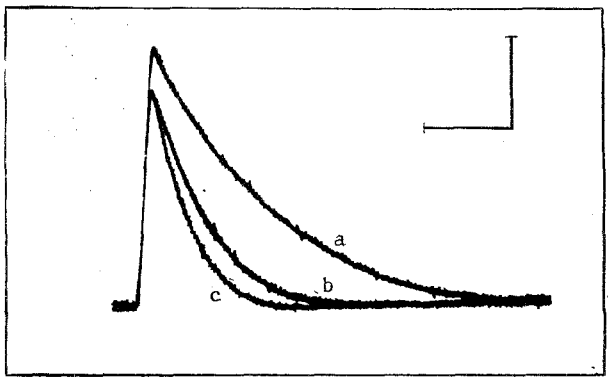

Fig. 3. Effect of proadifen on miniature end-plate currents during inhibition of AChE by neostigmine. a) Control, b) neostigmine (3×10^{-6} M), c) proadifen (5×10^{-6} M) in the presence of neostigmine (3×10^{-6} M). Membrane potential −70 mV.

know that desensitization is enhanced during hyperpolarization [9]. It was found that at a negative potential (from −50 to −70 mV) in the presence of proadifen (5×10^{-6}-1×10^{-5} M) the decrease in amplitude of successive EPC took place significantly faster than at a positive potential (from +30 to +50 mV; see Fig. 2). Second, MEPC (n = 16-32) were recorded immediately after passage of 15-20 EPC in the presence of proadifen (5×10^{-6} M). Under these circumstances the amplitude of the MEPC fell to 64 ± 5% of the initial values ($p < 0.05$; n = 5). In the control, before the action of proadifen, no such decrease in the amplitude of MEPC was observed. Marked voltage-dependence of depression of EPC and a decrease in the amplitude of MEPC after a burst of EPC were evidence of the postsynaptic nature of the decrease in amplitude of successive EPC.

Besides proadifen, a combination of the following conditions also was used to accelerate desensitization: a rise of temperature to 25-26°C; an increase in the Ca^{++} concentration to 2.5 mM; hyperpolarization of the end plate between −60 and −100 mV. According to data in the literature [8, 9] each of these procedures accelerates densensitization. In these experiments depression of EPC was found to take place more rapidly than in the control. In this case, just like depression induced by proadifen, the effect was voltage-dependent, i.e., when the potential was positive the decrease in amplitude of the EPC in the burst was much less (Fig. 1). This directly points to a leading role of a postsynaptic mechanism of reduction of the amplitude of EPC under the conditions stipulated above.

When conditions favorable for the development of desensitization are created, even several consecutive EPC can leave a clear trace on the postsynaptic membrane, leading to a more marked reduction of its sensitivity to the mediator. This is evidently possible only when the probability that quanta of ACh will reach that part of the postsynaptic membrane which has become activated in the course of generation of the preceding EPC is high (with maintenance of the physiological level of secretion).

To discover the possibility that densitization may develop during single signal generation when AChE is inhibited and the action of the mediator prolonged, we analyzed the effect of proadifen on the amplitude-time characteristics of MEPC. Control experiments show that if AChE was intact, proadifen in a concentration of 5×10^{-6} M affected neither the amplitude nor the duration of MEPC. For instance, with a membrane potential of −70 mV the amplitude was 2.93 ± 0.20 nA (n = 7), and the fall was exponential, with a time constant of fall (τ MEPC) of 1.20 ± 0.05 msec (n = 7). After the action of proadifen the amplitude of MEPC was 2.63 ± 0.05 nA ($p > 0.05$; n = 7), and τMEPC was 1.19 ± 0.07 msec ($p > 0.05$; n = 7). Besides, when AChE was intact, normal sensitivity of τMEPC to changes of membrane potential was preserved. Thus the coefficient of voltage-dependence H in the control was 88 ± 6 mV (n = 11), and after the action of proadifen it was 95 ± 12 mV ($p > 0.05$; n = 3). This result agreed with data in the literature, according to which proadifen in this concentration does not induce a cholinolytic (including channel-blocking) effect [10]. If AChE was inhibited before the action of proadifen the amplitude of MEPC was 3.37 ± 0.22 nA (n = 6), and the fall, just as in the control, was approximated by a single exponent with τMEPC = 3.59 ± 0.15 msec

(n = 6). After the action of proadifen 5×10^{-6} M, the amplitude of MEPC was very slightly reduced (2.71 ± 0.22 nA; $p < 0.05$; n = 8), and this was accompanied by marked shortening of the decay of the signals to τMEPC = 1.69 ± 0.09 msec ($p < 0.05$; n = 8). The results of one typical experiment was given in Fig. 3, and they show that in the presence of proadifen the effect of an increase in τMEPC, induced by inhibition of AChE, is largely lost, and this can be explained by repeated binding of ACh with the ACh receptor [5]. Moreover, the absence of changes in the amplitude of MEPC, of τMEPC, and of voltage-dependence of the decay of MEPC when AChE is inhibited can be interpreted as a special kind of loss of sensitivity of the end plate in the course of a single signal. The same result is given by strengthening of desensitization following administration of desensitizing concentrations of exogenous ACh against the background of inhibited AChE [2]. Most probably, after disturbance of mediator hydrolysis a situation arises when, in the course of signal generation, some of the ACh receptors, after activation, may pass into a desensitized state, i.e., they may cease to respond to the subsequent action of the mediator on them. In that case the ability of proadifen to accelerate decay of MEPC when AChE is inhibited can be easily explained by potentiation of such a change of state. We know from experiments with single channels that signs of desensitization are observed in the presence of a micromolar range of ACh concentrations [14], whereas the ACh concentration during MEPC formation may reach 3×10^{-4} M [7]. In addition, when AChE is inhibited the role of repeated binding with ACh receptors in the course of a single signal is sharply enhanced [5]. This binding evidently takes place mainly with the same ACh receptors. Despite the relatively short duration of the response to one ACh quantum (when the AChE is inhibited), a very high ACh concentration can be created and may act repeatedly on the postsynaptic membrane, i.e., under the same conditions as are essential for the onset of desensitization.

The results of this investigation are thus evidence that, in principle, densensitization can develop during generation of short bursts of EPC and even of single signals, although special conditions had to be created to detect this phenomenon. This suggests that desensitization may play a functional role, especially, in synapses with deficient AChE activity and against the background of the action of certain pharmacologic agents [10], which, like proadifen, can modify the rate of desensitization.

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