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ROLE OF ACETYLCHOLINE RECEPTOR DENSITY IN MECHANISMS PROLONGING POSTSYNAPTIC CURRENT DECAY

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The duration of end-plate currents (EPC) has virtually no effect on transmission of a single signal through the myoneural junction, but plays a decisive role during passage of repetitive series of impulses through a synapse [3]. One approach to the discovery of factors determining the duration of the EPC is to study sensitivity of EPC decay to a change in the density of free acetylcholine receptors (AChR) of the postsynaptic membrane. A decrease in the density of the free AChR due to α-bungarotoxin (α-BT), which binds irreversibly with AChR, quickens EPC decay in tonic muscles [3], and also in phasic muscles, if acetylcholinesterase (AChE) in the latter is inhibited [8]. It has been suggested that α-BT abolishes, and in that way brings to light, the unsynchronized opening of ionic channels present in both cases and associated with repeated activation of AChR by the transmitter during generation of a single signal [8]. However, decay of EPC can be prolonged not only through inhibition of AChE, but also through other influences, such as hyperpolarization of the postsynaptic membrane [6, 11], cooling of the muscle [6], and the action of ethanol [2, 7, 10] and dipyroxime* — a rapid blocker of ionic channels of the postsynaptic membrane [1]. The problem of whether their effect, which conjecturally is realzied through a change in the kinetics of AChR function, may be accompanied by disturbance of synchronization of ionic channel opening, in much the same way as that occurs after inhibition of AChE, has not been investigated systematically.

To solve this problem, it was decided to study the effect of α-BT on the duration of EPC decay under the influence of the various factors mentioned above.

EXPERIMENTAL METHOD

Experiments were carried out on isolated nerve-muscle preparations consisting of the frog sciatic nerve and sartorius muscle. The temperature was measured by a miniature transducer, located next to the muscle. Its mean value was 20.0 ± 0.5°C (except in experiments with cooling). In the course of a single experiment the temperature drift did not exceed 0.1°C. Muscle contractions were abolished by the use of Ringer's solution of the following composition (in mM): NaCl 115.0, KCl 2.5, $CaCl_2$ 0.9, $MgCl_2$ 6.0, $NaHCO_3$ 2.5, pH 7.3. EPC evoked by nerve stimulation were recorded under voltage clamp conditions by the use of two electrodes. Signals were analyzed by a system consisting of digital analyzer and microcomputer, with interrogation frequency of 1 point in 60 μsec, in accordance with special programs (author V. A. Snetkov) for determining the amplitude of the signal, the exponential nature of EPC decay, and the time constant of EPC decay (τ_{epc}). Drugs were used in the follow-

*Trimedoxime bromide.

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ing concentrations (in M): neostigmine $3 \cdot 10^{-6}$, dipyroxime $3 \cdot 10^{-3}$, ethanol 0.17. The experimental procedure was as follows. After control values of EPC amplitude and τ_{epc} had been recorded α-BT (10^{-6} g/liter) was injected and caused a progressive decline of EPC amplitude, until complete disappearance of the signals. During the action of α-BT values of EPC amplitude and of τ_{epc} were recorded continuously. To compare the results of different series of experiments, values of EPC parameters corresponding to a fall of amplitude of EPC under the influence of α-BT to 50% and 25% of the initial level were used (Table 1).

EXPERIMENTAL RESULTS

In control experiments with voltage clamping at −70 mV and at a temperature of 20.0 ± 0.5°C the amplitude of EPC was 24 ± 7 nA (n = 4) and decay of EPC was monoexponential with τ_{epc} = 1.0 ± 0.1 msec (n = 4). The decrease in amplitude of EPC under the influence of α-BT was accompanied by a tendency for quickening of EPC decay (by 5-10%), but these changes were not significant ($p > 0.05$; n = 4) whether the amplitude of the signals was reduced by 50% or by 75%.

In experiments with cooling of the muscle to 10.0 ± 0.5°C, as the temperature fell the amplitude of EPC decreased and τ_{epc} increased to 3.6 ± 0.3 msec (n = 6), decay remaining exponential. With the voltage clamped at −150 mV, τ_{epc} was 2.6 ± 0.2 msec (n = 9). During reduction of the amplitude of EPC under the influence of α-BT, there was no significant change in τ_{epc} (Table 1). Dipyroxime was used as a substance delaying EPC decay by blocking ionic channels of the postsynaptic membrane. Dipyroxime has been described as a very rapid blocker of ionic channels in rat muscle [1]. In the present investigation diproxime also exhibited the properties of a very rapid blocker of the open ionic channel of AChR in frog synapses: the fall of amplitude of EPC under the influence of dipyroxime was accompanied by delay of EPC decay, while retaining its exponential nature; τ_{epc} increased up to 3.4 ± 0.3 msec (n = 3). Reduction of the AChR density by α-BT against the background of dipyroxime led to reduction of the amplitude of EPC, but just as in the previous series, no significant change in τ_{epc} was found under these circumstances (Table 1). In experiments with inhibition of AChE by neostigmine τ_{epc} was 3.1 ± 0.1 msec (n = 3). By contrast with all previous procedures, reduction of the AChR density by α-BT accompanied by inhibition of AChE led both to a decrease in amplitude of EPC and to more rapid EPC decay (Table 1). The same result as with inhibition of AChE was obtained with ethanol. In the concentration which we used ethanol delayed EPC decay, while preserving its exponential nature; τ_{epc} was 2.2 ± 0.1 msec (n = 5). Reduction of the amplitude of EPC under the influence of α-BT in the presence of ethanol, just as against the background of neostigmine, was accompanied by more rapid EPC decay.

On the basis of data on sensitivity to the action of α-BT, the methods of slowing EPC decay without changing its exponential character, used in the present investigation, can thus be divided into those dependent on and those independent of AChR density. The action of hyperpolarization, temperature, and dipyroxime was found to be independent of AChR density, in agreement with the view that these factors delay EPC decay by modifying the kinetics of activated AChR. For instance, it has been suggested that hyperpolarization and temperature affects predominantly the velocity constant of transition of ionic channels from the open into the closed state [6, 11], whereas diproxime converts the open ionic channel into a new (blocked) state. Meanwhile it must be pointed out that against the background of hyperpolarization, cooling, and the action of dipyroxime, the same tendency as in the control for decay to be accelerated by the action of α-BT was observed, evidently reflecting the minor contribution of nonsynchronization of activation of ionic channels which is found in the presence of active AChE [3].

Slowing of EPC decay under the influence of ethanol and neostigmine was found to be dependent on AChR density. The ability of α-BT to accelerate EPC decay against the background of the action of neostigmine has been described previously [3, 8]. It was suggested in this case that after inhibition of AChE rebinding of acetylcholine (ACh) with the AChR is intensified, leading to marked desynchronization of ionic channel opening [3] and to slowing of EPC decay. Reduction of the AChR density, however, makes rebinding less probable and accelerates EPC decay [8].

Slowing of EPC decay under the influence of ethanol can be explained most easily also by the anti-AChE effect of this agent. However, there is evidence against regarding an anti-AChE mechanism as the leading cause of delay of EPC decay under the influence of ethanol: 1) analysis of ACh noise in the presence of ethanol shows equally effective slowing of decay

TABLE 1. Effect of α-BT on Time Constant of Decay of End-Plate Currents (τ_{epc}) on Control and under the Influence of Factors Prolonging EPC Decay

Conditions of injection of α-BT	τ_{epc}, msec		
	amplitude of EPC		
	initial (before injection of α-BT)	after reduction of action of α-BT	
		by 50%	by 75%
Control (20 ± ±0,5 °C, —70 mV)	1,0±0,1 (4)	0,9±0,1 (4)	0,9±0,1 (4)
Cooling (10 ± ±0,5°C)	3,6±0,3 (6)	3,3±0,3 (6)	3,0±0,4 (4)
Hyperpolarization (—150 mV)	2,6±0,2 (9)	—	2,4±0,1 (9)
Dipyroxime (3 × ×10^{-3} M)	3,4±0,3 (5)	3,3±0,3 (4)	3,2±0,3 (5)
Neostigmine (3 × ×10^{-6} M)	3,1±0,1 (3)	2,4±0,2* (3)	2,2±0,2* (3)
Ethanol (0,17 M)	2,2±0,1 (5)	1,6±0,1* (4)	1,5±0,1* (4)

Legend. Amplitude of EPC before action of α-BT taken as 100%. Number of experiments given in parentheses. Asterisk indicates that values of τ_{epc} differ significantly ($p < 0.05$) from τ_{epc} with EPC amplitude of 100%.

of miniature EPC (MEPC) and an increase in the duration of the open state of ionic channels [7, 10]; 2) ethanol slows EPC decay even after inhibition of AChE [2, 10].

Consequently, ethanol delays EPC decay through the intervention of a complex mechanism, which differs both from the action of AChE inhibitors and from that of other agents and procedures such as dipyroxime, hyperpolarization, and cooling of the muscle. Ethanol may perhaps increase the functional importance of kinetic states of the AChR which, under normal conditions, do not make any significant contribution to the time course of the EPC (for example, complexes of AChR with one ACh molecule).

Slowing of EPC decay in the neuromuscular synapse may thus be based on different kinds of postsynaptic mechanisms, some dependent on, other independent of AChR density. Among mechanisms dependent on AChR density, moreover, there may be some which are realized both through a change in AChE activity and through a change in the kinetics of function of receptor-channel complexes.

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