NONQUANTAL ACETYLCHOLINE SECRETION AS A FACTOR DETERMINING THE TIME COURSE OF ACETYLCHOLINESTERASE INHIBITION

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The duration of synaptic signals in the neuromuscular synapse, while rising sharply after inhibition of acetylcholinesterase (AChE) [8], nevertheless is not stable but decreases gradually [2]. It has been suggested that this shortening, which is very important for restoration of normal transmission of rhythmic series of impulses through the synapse, is linked with the action of acetylcholine, secreted in nonquantal form, on the postsynaptic membrane [2]. There may perhaps be two mechanisms by which nonquantal acetylcholine (ACh) evokes a shortening effect. The first, based on the ability of "nonquantal" ACh, like exogenous ACh [2, 4, 10, 12], to slow decay of the miniature end-plate currents (MEPC) through postsynaptic potentiation (PSP) [7, 11]. This PSP may decline with time, for nonquantal secretion gradually diminishes and eventually disappears completely [2]. The other mechanism may be based on the phenomenon of desensitization (DS) [9], or a decrease in sensitivity of the postsynaptic membrane to the mediator, for we know that if AChE is inhibited, DS leads to shortening of MEPC [1, 4, 5, 10, 12]. In the present investigation we studied the dynamics of parameters of MEPC when AChE was inhibited, by abolishing nonquantal secretion by means of acute denervation in vitro by ouabain [3] and by magnesium ions [13].

EXPERIMENTAL METHOD

Experiments were carried out on an isolated phrenicodiaphragmatic preparation of albino mice. The muscle was kept in aerated (95% O_2 + 5% CO_2) physiological saline at 20°C. MEPC was derived during voltage clamping by two electrodes. The amplitude and time constant of decay (τ) of MEPC were determined by computer. Averaging was carried out on 64-256 MEPC. AChE was inhibited either by armin (30 min in a solution of $1 \cdot 10^{-5}$ M followed by rinsing to remove the inhibitor) or by neostigmine ($3 \cdot 10^{-6}$ M). Nonquantal secretion was "blocked" by denervation in vitro with ouabain, or by increasing the magnesium ion concentration. In the case of denervation the muscle was kept after removal for 3.5 h in aerated physiological saline, for it is during this time interval that gradual blocking of nonquantal secretion takes place [2]. Ouabain was added to the physiological saline 15 min before inhibition of AChE [3]. The magnesium ion concentration was raised to 3 mM, at which nonquantal secretion also is inhibited [13].

EXPERIMENTAL RESULTS

In the control, after inhibition of AChE by armin, τ increased until the 20th-30th minute from 1.43 ± 0.07 msec (n = 10) to 5.41 ± 0.43 msec (n = 8, $p < 0.05$) (Fig. 1a). The effect of inhibition on amplitude was slight. During subsequent recording, shortening of decay of MEPC was observed, and by the 180th minute τ had fallen to

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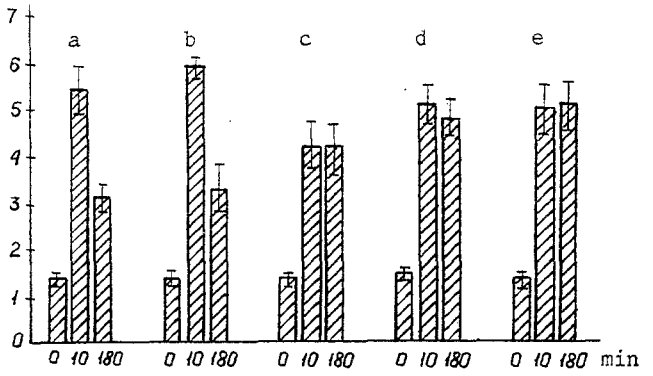

Fig. 1. Changes in values of time constant of decay of miniature end-plate currents after inhibition of acetylcholinesterase by armin (10 μM) (a, c) or neostigmine (3 μM) (b, d, e). a, b) Control, c) ouabain (10 μM), d) denervation in vitro, e) $[Mg^{2+}]_{out}$ = 3 mM. Mouse diaphragm, voltage clamping, 70 mV, 20°C.

3.11 ± 0.50 (n = 8, $p < 0.05$) without any appreciable changes in the amplitude of MEPC. Similar results were obtained on inhibition of AChE by neostigmine: in this case τ reached the value of 5.93 ± 0.22 (n = 6, $p < 0.05$) immediately after inhibition of AChE, and by the 180th minute of recording shortening was observed to 3.33 ± 0.56 msec (n = 6, $p < 0.05$). This agreement between results obtained with an irreversible AChE inhibitor, not present in the solution during the shortening process, and a reversible inhibitor, neostigmine, present constantly in the solution, compels rejection of such possible mechanisms of MEPC shortening as spontaneous AChE reactivation, and also a cholinolytic effect of the inhibitor itself, in particular, of neostigmine. It seems a more likely suggestion that the process of shortening of decay of MEPC is connected with the effect of nonquantally secreted ACh, capable of evoking PSP and DS.

In the next series of experiments we attempted to determine which of the two processes, DS or PSP, is connected with the effect of MEPC shortening.

To test the hypothesis that nonquantally secreted ACh can induce PSP and delay decay of MEPC, in the same way as exogenous ACh, we compared the degree of slowing of τ in the control (in the presence of nonquantal secretion) and after different methods of blocking. The first important question was to decide whether the procedures which we used as instruments to modulate nonquantal secretion are capable of direct action on the postsynaptic membrane. To solve this problem, we studied the effect of these factors on amplitude and τ of MEPC when AChE was active. These results are given in Table 1. They show that neither denervation nor ouabain nor magnesium ions have any effect, in the concentrations used, on amplitude–time parameters of MEPC in the presence of active AChE. If nonquantal secretion does in fact lead to the development of PSP, it would be expected that the increase in τ as a result of inhibition of AChE would be smaller when nonquantal secretion was blocked. The results of these experiments are given in Fig. 1. They show that when nonquantal secretion was blocked by all three methods (denervation, ouabain, magnesium ions) there was a tendency for the increase in τ to be reduced as a result of inhibition of AChE, and the tendency was particularly marked when ouabain was used. However, this effect was not significant ($p > 0.05$). Also, the values of τ immediately after inhibition of AChE in the presence of all the factors mentioned above, which abolished nonquantal secretion, was considerably greater than values of τ for signals recorded in the control toward the 180th minute after removal of the muscle preparation from the animal.

Whereas in the control, the shortening of the decay of MEPC amounted to 43% in the experiments with armin and 44% in the presence of neostigmine, after abolition of nonquantal secretion of ACh the effect of shortening of MEPC under the influence of the various factors listed above completely disappeared (Fig. 1). This result agrees with data obtained after acute denervation in vivo [2], and confirms the hypothesis that the shortening effect is in fact connected with nonquantal secretion of ACh, whereas the mechanism of the decrease in τ is based primarily on the development of DS of the postsynaptic membrane.

TABLE 1. Miniature End-Plate Currents in Control and After Procedures Blocking Nonquantal Secretion of Acetylcholine

Experimental conditions	Amplitude of MEPC, nA	Time constant of decay of MEPC,
Control	3,9±0,5 (10)	1,43±0,07 (10)
Denervation in vitro	4,5±0,9 (3)	1,48±0,08 (3)
Mg^{2+} (3 mM)	3,7±0,4 (4)	1,43±0,09 (4)
Ouabain (10 μM)	4,4±0,6 (4)	1,42±0,08 (4)

Thus nonquantal secretion of ACh is a factor determining the consequences of inhibition of AChE and, in particular, the time course of synaptic signals. Since the principal pathogenetic consequences of inhibition of AChE are linked with long-term exposure of the acetylcholine receptors to unhydrolyzed ACh, it is particularly important to discover the mechanisms leading to normalization of the duration of synaptic signals.

The present investigation has shown that nonquantal secretion evokes ultimately shortening of the decay of MEPC, mainly through a mechanism of desensitization. The PSP evoked by nonquantal ACh is weak in the first stages after inhibition of AChE, although the role of this factor may increase with time, for the PSP is strengthened as DS develops [6] and may mask the manifestations of desensitization [1, 4]. The possibility cannot be ruled out that this mechanism explains the "latent" period of 60-80 min of a stable value of τ, preceding shortening and the H-effect before the beginning of its fall [2]. Later the PSP falls, for the level of nonquantal secretion diminishes sharply toward the 180th minute [2]. DS, however, under these conditions, unlike PSP, is abolished extremely slowly [2, 10], and this explains shortening of the decay of MEPC after blocking of nonquantal secretion. It follows from these ideas that the DS which develops under the influence of "nonquantally" secreted ACh can be regarded as a mechanism compensating the deficient AChE activity, whereas factors accelerating DS can be regarded as possible pathogenetic agents in cases of poisoning by AChE inhibitors.

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