POSTSYNAPTIC POTENTIATION OF END-PLATE CURRENTS IN THE RAT DIAPHRAGM DURING VARIATION IN SYNAPTIC ACETYLCHOLINESTERASE ACTIVITY

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Postsynaptic potentiation (PSP) in the neuromuscular synapse is manifested by the fact that an increase in the acetylcholine (ACh) concentration in the synaptic space, brought about in some way or other, leads to delay in the time course of endplate currents (EPC) [5, 8, 11]. In particular, the greater its quantum composition, the slower the decline of EPC. The reason is that in areas of the synaptic space where regions of distribution of ACh molecules of neighboring quanta overlap, diffusion of ACh is delayed and its concentration rises [8]. The probability of release of ACh quanta from neighboring active zones increases with a high quantum composition of EPC. For PSP to be exhibited, ACh molecules must be able to spread and to be held up in the synaptic space. PSP is thus well marked when acetylcholinesterase (AChE) is completely inhibited. PSP is evidently based on a nonlinear dose — response dependence of the postsynaptic membrane, determined by the fact that activation of a single acetylcholine receptor (AChR) requires the consecutive addition of two ACh molecules; this addition, moreover, takes place under cooperative conditions [5, 8, 11]. It has been suggested that due to these properties of the AChR, postsynaptic modulation of synaptic transmission might take place through PSP [6, 8, 13].

The aim of this investigation was to study PSP in the presence of intact and partially inhibited AChE.

EXPERIMENTAL METHOD

Experiments were carried out on isolated phrenic nerve — diaphragm preparations from albino rats in a chamber with a constant flow of physiological saline saturated with carbogen (95% O_2 + 5% CO_2). The composition of the solution (in mM) was as follows: NaCl — 137, KCl — 5, $CaCl_2$ — 2, $MgCl_2$ — 2, NaH_2PO_4 — 1, $NaHCO_3$ — 24, glucose — 11 (the pH of the solution was 7.4-7.5). The temperature was 28°C. EPC evoked by nerve stimulation were recorded in muscle fiber preparations, immobilized by transverse division [7], by the method of voltage clamping using two electrodes. The membrane potential of the fibers was clamped at 30 mV. Miniature EPP (MEPP) were recorded in undivided preparations with the membrane voltage clamped at 100 mV. The nerve was stimulated by single pulses or by series of pulses with frequencies of 20, 50, and 100 Hz. Series of EPC were recorded in different fibers in the presence of intact AChE (control), and later of galanthamine (a tertiary ammonium compound, a competitive AChE inhibitor of reversible type) in concentrations of $0.86 \cdot 10^{-7}$ or $2.7 \cdot 10^{-7}$ M. Under these circumstances AChE activity was depressed according to our estimates, by 25% and 50% respectively [2].

EXPERIMENTAL RESULTS

The amplitude and half-decay time (T_{hd}) of single EPP in the control was 98.7 ± 7.0 nA and 0.70 ± 0.05 msec (number of fibers n = 16), and for MEPP the corresponding values were 6.14 ± 0.22 nA and 0.56 ± 0,02 msec (n = 34).

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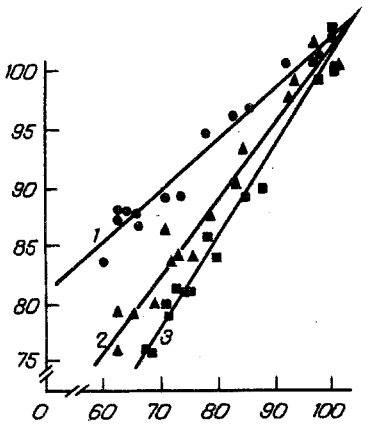

Fig. 1. Amplitude of first 15 EPP as a function of T_{hd} with stimulation of nerve at a frequency of 50 Hz in control (1) and in the presence of galanthamine in a concentration of $0.86 \cdot 10^{-7}$ M (2) and $2.7 \cdot 10^{-7}$ M (3). Abscissa, amplitude of EPP (in %). Ordinate, half-decay time of EPP (in %). Parameters of EPP in each frequency series expressed as percentages of the first EPP and averaged for all fibers studied under these particular conditions. Regression lines are drawn by the method of least squares.

With a correction for different levels of membrane potential when EPP and MEPP were recorded, the quantum composition of the EPP in these experiments could be estimated: it averaged about 50. In the presence of galanthamine in concentrations of $0.86 \cdot 10^{-7}$ and $2.7 \cdot 10^{-7}$ M the value of T_{hd} of EPP rose to 0.84 ± 0.07 msec (n = 9) and 0.97 ± 0.09 msec (n = 13) (120 and 139% of the control values) respectively. The value of T_{hd} for MEPP changed in a similar manner: it increased to 0.66 ± 0.02 msec (n = 19) and 0.80 ± 0.02 msec (n = 17) (118 and 143% of the control) respectively.

To estimate PSP, we made use of the fact that during repetitive stimulation of a motor nerve the amplitude of the EPP changes as a function of frequency. Although a postsynaptic component of this change is possible, connected with reduction of the potassium gradient due to accumulation of potassium ions in the synaptic space [4], basically the change in amplitude of EPP is due to an increase (facilitation) and a decrease (depression) of the quantum composition of EPP [10]. In our experiments, as a rule, depression was observed immediately, and only in some fibers was the depression preceded by slight facilitation. The duration of individual EPP in a frequency series changed in accordance with the change in their amplitude; the relationship between these parameters of EPP, moreover, was close to linear (Fig. 1). As the criterion of PSP we therefore used the coefficient of linear correlation between the value of T_{hd} of the first 15 EPP in the series and their amplitude ($r_{T/A}$), and ESP was characterized quantitatively by the linear regression coefficient (R) of this dependence. During calculations the parameters of EPP were always normalized (in percentages) relative to the first EPP in the given frequency series.

In the control correlation between T_{hd} and the amplitude of EPP was observed at all frequencies of nerve stimulation. The average value of $r_{T/A}$ varied from 0.635 to 0.765 (Table 1); in 32 of the 38 fibers (84%), moreover, correlation was significant ($p < 0.05$). The value of R, which is the tangent of the angle of slope of the dependence of T_{hd} on amplitude of EPP, amounted on average to between 0.353 and 0.458 (angle of slope 19-24°). The mean values of $r_{T/A}$ and R in the control, incidentally, were somewhat reduced with an increase in the frequency of stimulation of the nerve (Table 1). This may perhaps be connected with the fact that if the rhythm is fast enough, the effect of ACh remaining in the synaptic space after preceding activity may be felt. This ACh may also participate in PSP [11], delaying the time course of the subsequent EPP a little, even despite a reduction in their quantum composition. The effect of the accumulated potassium in this case is unlikely, for an increase in the potassium concentration in the solution, according to our observations, does not alter the duration of EPP.

TABLE 1. Parameters Characterizing Postsynaptic Potentiation Depending on Acetylcholinesterase Activity

Experimental conditions	Frequency of nerve stimulation, Hz					
	20		50		100	
	$r_{T/A}$	R	$r_{T/A}$	R	$r_{T/A}$	R
Control (AChE – 100%)	0,765±0,047 (13)	0,458±0,058 (13)	0,730±0,057 (14)	0,412±0,048 (14)	0,635±0,082 (11)	0,353±0,061 (11)
Galanthamine, $0.86 \cdot 10^{-7}$ M (AChE – 75%)	0,773±0,056 (9)	0,763±0,125* (9)	0,829±0,069 (8)	0,736±0,128* (8)	0,793±0,045 (8)	0,612±0,105* (8)
Galanthamine, $2.7 \cdot 10^{-7}$ M (AChE – 50%)	0,806±0,060 (8)	0,602±0,115 (8)	0,922±0,015** (12)	0,796±0,057*** (12)	0,851±0,040* (9)	0,670±0,096* (9)

Legend. Mean values and their errors (in parentheses — number of fibers studied) are indicated in Table 1, just as in the text also. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control.

In the presence of galanthamine in a concentration of $0.86 \cdot 10^{-7}$ or $2.7 \cdot 10^{-7}$ M, at all frequencies of nerve stimulation a tendency was noted for the mean value of $r_{T/A}$ to increase (Table 1). Under these circumstances correlation between T_{hd} and the amplitude of EPP was significant ($p < 0.05$) in 96 and 100% of fibers respectively. In all versions of the experiments (except a frequency of 20 Hz and a concentration of $2.7 \cdot 10^{-7}$ M) a significant increase was observed, and the higher the galanthamine concentration, the greater this increase (Table 1). The value of R varied from 0.602 to 0.796 (angle of slope of the regression line 31-38°).

The data showing close correlation between the duration of decay of EPP and their amplitude are evidence that PSP may be exhibited if AChE is completely active. This means that even if AChE is intact, binding sites of individual ACh quanta with AChR cannot be regarded as spatially completely separate, i.e., repeated bindings of ACh with AChR and the spread of ACh (within certain limits) along the synaptic space are possible. Consequently, from this point of view AChE is not superfluous, as other data also demonstrate [1, 3]. The existence of PSP in the presence of intact AChE suggests that PSP plays a role in the modulation of neuromuscular transmission. Of course, a small change in the duration of individual EPP, coupled with an adequate guarantee factor cannot by itself affect synaptic conduction in a fast muscle fiber. However, the chemical sensitivity of the postsynaptic membrane can be modulated through PSP in the course of rhythmic activity [13]. Another possibility is that conformational changes in the macromolecule of activated ChR may serve as the signal for membrane and (or) intracellular mechanisms related to the regulation of synaptic efficiency, and thus dependent on the conditions of activation of the AChR.

In our experiments PSP was significantly intensified even in the presence of a comparatively small (by 25%) reduction of AChE activity. We know that activity of synaptic AChE can be regulated by nerve and muscle cells [9, 12]. In that case modulation of synaptic transmission through PSP may take place with the participation of AChE, whose activity changes under the influence of several different endogeneous factors.

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