ATYPICAL MINIATURE END-PLATE POTENTIALS IN THE FROG NEUROMUSCULAR JUNCTION AFTER INTERCELLULAR MATRIX MODIFICATION AND OSMOTIC INFLUENCES

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Atypical miniature end-plate potentials (MEPP) or currents, with a wide range of different amplitudes and temporal characteristics and with a frequency independent of Ca^{2+} [15] can be divided into giant and slow [7]. As a formal criterion for classifying atypical MEPP (MEPC) in one or other type, on the suggestion of Professor D. P. Matyushkin, the relative durations of the half-decay and ascending phase of MEPP (MEPC) have been used. It was shown previously that the appearance of slow MEPP is caused by the action of antiserum to galactocerebrosides [3] – components of membranes of Schwann cells, but not of nerve or muscle cells, on frog nerve-muscle preparations. Experiments on the rat diaphragm revealed very low temperature sensitivity of the ascending phase of the slow MEPC, possible evidence of the release of acetylcholine (ACh) quanta in regions remote from the synaptic space and the postsynaptic acetylcholine receptors, during ACh release from Schwann cells into the periaxonal space, for example [4]. Similarity of the characteristics of the slow MEPP in innervated synapses with MEPP created by Schwann cells in denervated synapses has been noted [5, 8]. Taken together, these results indicate a connection between slow MEPP (MEPC) and Schwann cell activity in the neuromuscular synapse, although there is no direct proof of ACh secretion by Schwann cells in normally innervated muscle cells.

Close correlation between function of the Schwann cells and the state of the intercellular matrix [9] and the role of the intercellular matrix in activity of neuromuscular synapses are known [1]. It can be expected that enzymic destruction of the components of the matrix leads to changes in the physiological state of the synaptic Schwann cells and to a change in the frequency of appearance of atypical (slow) MEPP. We also know that swelling caused by the action of a hypo-osmotic medium stimulates ACh secretion by Schwann cells in denervated synapses [8], and also mediator secretion by astrocytes [13]. Treatment of the preparations with solutions of altered tonicity may also be reflected in frequency of the slow MEPP. The aim of this investigation was to test these hypotheses.

EXPERIMENTAL METHOD

MEPP were recorded in the cutaneosternal muscle of the frog by the usual microelectrode technique. Glass microelectrodes filled with 3 M KCl, with a resistance of the tip of about 10 MΩ, were used. Before recording of the MEPP began some of the preparations were treated with a 0.1% solution of collagenase (Fluka) or trypsin (Serva) for 1 h. The action of trypsin was blocked with a 0.1% solution of soy inhibitor (Sigma). To investigate the action of testicular hyaluronidase (Reanal) on MEPP generation, MEPP were recorded after the action of the enzyme for 20-30 min. In some experiments, enzyme treatment was preceded by acetylcholinesterase (AChE) by armin (10^{-5} M) for 10-15 min, followed by rinsing with Ringer's solution for 1 h. For glycerinization the preparations were placed for 45 min in a solution of 800 mM glycerol, and then rinsed again with Ringer's solution. To study the effects of the hypo-osmotic medium MEPP were recorded during the action of a solution with half the normal NaCl concentration.

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TABLE 1. Frequencies (MEPP/sec) of Normal and Atypical MEPP in Preparations Subjected to Various Treatments and with Intact and Inhibited AChE (M ± m)

Conditions	Intact AChE			Inhibited AChE		
	normal MEPP	giant MEPP	slow MEPP	normal MEPP	giant MEPP	slow MEPP
Ringer's solution	0,98±0,21 (15)	0,015±0,002 (5; 33 %)	0,020±0,006 (11; 73 %)	1,04±0,12 (22)	0,038±0,012 (12; 55 %)	0,031±0,006 (14; 64 %)
Collagenase	0,90±0,15 (26)	0,044±0,015 (9; 35 %)	0,055±0,009 (22; 85 %)	0,65±0,10 (23)	0,040±0,013 (14; 61 %)	0,50±0,010 (20; 90 %)
Hyaluronidase	0,84±0,35 (5)	0,019 (1; 20 %)	0,014±0,005 (3; 60 %)	0,74±0,14 (9)	0,009±0,002 (4; 44 %)	0,028±0,013 (6; 67 %)
Trypsin	0,31±0,03 (16)	0,005±0,001 (2; 12,5 %)	0,032±0,008 (13; 81 %)			
Glycerinization	0,93±0,32 (6)	0,011±0,003 (3; 50 %)	0,036±0,011 (5; 83 %)			
Hypo-osmotic solution	0,38±0,06 (12)	0,008±0,002 (3; 25 %)	0,34±0,009 (11; 93 %)			

Legend. Number of fibers in which MEPP of the given type were recorded and percentage of number of fibers in which giant or slow MEPP were recorded are given between parentheses.

All test solutions were made up with normal Ringer's solution of the following composition (in mM): NaCl – 110.5; KCl – 2.0; $CaCl_2$ – 2.0; $NaHCO_3$ – 1.9, pH 7.2-7.4.

The period of recording of MEPP varied from 1 to 3 min depending on the frequency of the atypical MEPP.

EXPERIMENTAL RESULTS

The results are given in Table 1, and examples of MEPP under different conditions are shown in Fig. 1. In synapses with intact AChE the frequency of the slow MEPP and the relative percentage of fibers in which these MEPP were recorded were higher than the corresponding values for giant MEPP. Frequencies of giant and slow MEPP did not correlate with the frequency of normal MEPP ($r = 0.26$ and 0.33 respectively), and did not correlate with each other ($r = 0.29$). Inhibition of AChE did not affect the frequency of the slow MEPP but increased the frequency of the giant MEPP and the percentage of fibers in which they were found (Fig. 1). Inhibition of AChE was not accompanied by the appearance of correlation between the frequency of giant or slow MEPP and the frequency of normal MEPP ($r = 0.39$ and 0.13 respectively) or between the frequencies of the two types of atypical MEPP ($r = 0.02$).

Collagenase, acting on synapses with intact AChE, increased the frequency of the giant MEPP (Fig. 1), possibly in connection with its anticholinesterase effect, for collagenase did not cause any additional increase in the frequency of the giant MEPP in preparations with inhibited AChE (Table 1). Collagenase caused an increase in frequency of the slow MEPP in synapses with both intact and inhibited AChB ($0.99 < p < 0.999$ and $0.8 < p < 0.9$ respectively). Hyaluronidase did not affect the frequency of atypical MEPP in preparations with intact AChE, but in preparations with inhibited AChE it reduced the frequency of the giant but did not affect the frequency of the slow MEPP. Destruction of noncollagen proteins by trypsin caused an approximately threefold decrease in the frequency of normal and giant MEPP, together with a small and not significant increase in the frequency of slow MEPP. In preparations subjected to the action of a hyper- and hypo-osmotic solution the frequencies of the slow MEPP did not differ and coincided with the frequency of slow MEPP in preparations treated with trypsin. Under these circumstances, in preparations subjected to the action of a hypoosmotic solution, a decrease was observed in the frequency of both normal [11] and giant MEPP. Incidentally, under hypo-osmotic conditions slow MEPP were recorded in virtually all the fibers studied (Table 1).

The results confirm the view that giant and slow MEPP are generated by different mechanisms and, in particular, that the appearance of giant MEPP during inhibition of AChE is connected with accumulation of unhydrolyzed ACh [4]. Thus, hyaluronidase, by increasing losses of ACh by diffusion from the cleft [2], on inhibition of AChE reduces the frequency of giant but does not affect the frequency of slow MEPP.

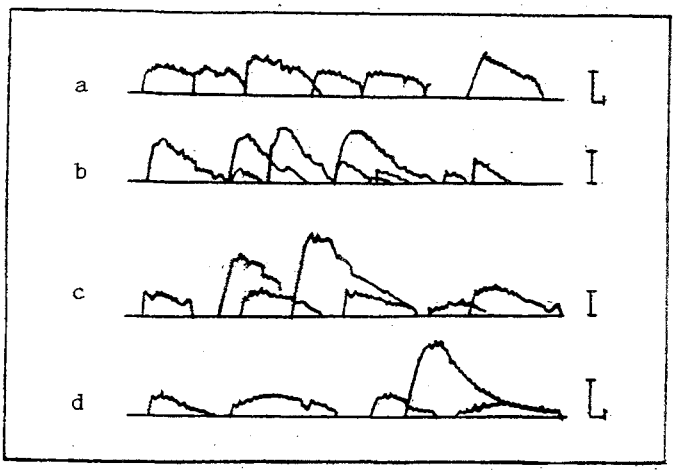

Fig. 1. Examples of traces of MEPP under different conditions: a) in a fiber with intact AChE; b) in a fiber with inhibited AChE; c) after collagenase treatment of preparation with intact AChE; d) after collagenase treatment of preparation with inhibited AChE. Calibration: amplitude 500 μV, duration 1 msec (a) and 5 msec (b, c, and d).

Of all the procedures used, only treatment with collagenase caused an increase in the frequency of slow MEPP. The effect was evidently unconnected with disturbances of the structure of the neuromuscular synapse, for it has been shown [14] that only by combined action with other proteases can collagenase cause changes in structure of the synapse and of the active zones. If it is assumed that slow MEPP arise as a result of ACh secretion by Schwann cells, and assuming that they form type IV collagen [10], it can be tentatively suggested that destruction of collagen and the appearance of its enzymic degradation products caused a compensatory increase in collagen secretion, which, by virtue of some aspects of Schwann cell metabolism, may be coupled with ACh release.

Destruction of noncollagen proteins by trypsin and of a certain part of the glycosaminoglycans of the intercellular matrix by hyaluronidase did not lead to changes in frequency of the slow MEPP in preparations of intact AChE Unlike collagen, these components of the extracellular matrix of the synaptic cleft are formed mainly by nerve endings and muscle fibers [12]. The reason for the sharp fall in the frequency of normal MEPP after trypsin treatment is not known, but attention is directed to the fact that this fall is accompanied by a decrease in the frequency of giant, but not of slow MEPP. A similar picture was observed during the action of the hypo-osmotic solution. These findings are further confirmation of the view that giant and slow MEPP are generated by different mechanisms.

Under the influence of a hypo-osmotic solution, leading to swelling of the cells and a decrease in the intracellular ion concentration, including of Ca^{2+}, the familiar [11] decrease in the frequency of normal MEPP, and it our experiments also, of giant MEPP was observed. The absence of any change in the frequency of slow MEPP can be regarded as an illustration of the insensitivity of their frequency to the intracellular Ca^{2+} concentration [15]. The absence of a stimulating effect of the hypo-osmotic solution in our experiments on the frequency of slow MEPP can be explained by the action of the hypotonic solution, described in denervated neuromuscular synapses [8], in which an increase in the frequency of ACh secretion by Schwann cells was rapidly replaced by return to its initial level. The fact that slow MEPP were recorded in virtually all synapses tested under the influence of the hypotonic solution can be regarded as an indication of the stimulating action of such a solution on the frequency of the slow MEPP.

The absence of any changes in frequencies of normal and atypical MEPP in glycerinized preparations most probably reflects rapid restoration of spontaneous secretion after hyperosmotic shock. The disturbance of axon-Schwann relations [6] taking place during glycerinization also may be partially or completely reversible. We also know that the frequency of ACh release from Schwann cells in denervated synapses is unchanged by the action of hyperosmotic solutions [8].

The results likewise do not contradict the view that atypical MEPP (MEPC) arise during secretion of ACh, independent of Ca^{2+}, outside the active zones of the synapse [15], but they do not permit a direct link between the appearance of slow MEPP and ACh secretion by Schwann cells in innervated synapses. However, the relative specificity of the effect of collagenase combined with data in the literature [3-5, 8] allows such a possibility. The final answer to the question of the origin of slow MEPP and their possible link with Schwann cell activity will be obtained only after development of a method of selective destruction of synaptic Schwann cells.

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