ATYPICAL MINIATURE END-PLATE CURRENTS IN THE NORMAL RAT NEUROMUSCULAR SYNAPSE AND AFTER ACETYLCHOLINESTERASE INHIBITION AND COOLING

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In recent years the attention of many research workers has been drawn to quantal secretion independent of Ca^{2+} [14]. This type of secretion is usually linked with the appearance of miniature end-plate potentials (currents) (MEPP and MEPC respectively) in neuromuscular synapses. Atypical MEPC are characterized by an amplitude and duration which vary within very wide limits, and a nonexponential decay. The probability of appearance of atypical MEPC or MEPP is increased by the action of botulinus toxin, vinblastine, acetylcholinesterase (AChE) inhibitors, and certain other agents [8, 9, 13, 14]. The mechanisms of appearance of these postsynaptic responses are unknown, nor have they any precise classification. The exception to this rule is given by publications [4, 5] in which atypical MEPC are divided into giant, corresponding to those described in [9], and slowly rising. In the investigation described below, at Professor D. P. Matyushkin's suggestion we subdivided atypical MEPC into two groups: giant, having an abnormally large amplitude, and slow, distinguished by the ratio of their half-decay time (T_{hd}) to the duration of the ascending phase (T_{ap}) of MEPC. In normal and giant MEPC this ratio is about 2, whereas in slow MEPC it is about 1 (Table 1).

EXPERIMENTAL METHOD

Experiments were carried out on preparations of phrenic nerve and hemidiaphragm of albino rats in a chamber through which a solution of the following composition (in mM): NaCl – 137, KCl – 5, $CaCl_2$ – 2, $MgCl_2$ – 2, NaH_2PO_4, 1, $NaHCO_3$ – 24, glucose – 11 (the pH of the solutions was 7.4-7.5) at 28°C, aerated with 95% O_2 + 5% CO_2, flowed continuously. In some experiments the temperature was lowered to 18°C. AChE was inhibited by the organophosphorus AChE inhibitor armin (ethyl-*p*-nitrophenyl ester of ethylphosphinic acid) in a concentration of $4 \cdot 10^{-6}$ M. Nicotinic acetylcholine receptors (AChR) were partially blocked with *d*-tubocurarine (*d*-TC) in a concentration of $(1.3\text{-}6.5) \cdot 10^{-8}$ M.

MEPC were recorded intracellularly with the membrane voltage clamped by two microelectrodes at the –100 mV level. In some experiments MEPP were recorded. Mean values and errors of the mean are given in the text, Table 1, and the figures.

EXPERIMENTAL RESULTS

With intact AChE slow MEPC (MEPP) were found more often than giant: 5.1% and 2.9% respectively of the total number of MEPC (Figs. 1 and 2; Table 1). Positive correlation was present between the frequencies of the giant and slow MEPC ($r = 0.54$, $p < 0.01$). After inhibition of AChE by armin the frequency of the slow MEPC was unchanged but the frequency of the giant MEPC increased significantly by 3.2 times (Fig. 2; Table 1). Correlation between the frequencies of atypical MEPC weakened and ceased to be significant ($r = 0.27$). Addition of *d*-TC against the background of inhibited AChE did not affect the frequency of the slow MEPC but reduced the frequency of the giant type to its initial value when AChE was intact (Fig. 2, Table 1). Correlation between the frequencies of the atypical MEPC, disturbed by inhibition of AChE, was restored ($r = 0.67$, $p <$

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TABLE 1. Parameters of MEPC of Different Types in Synapses of Rat Diaphragm under Different Conditions (28°C)

Parameters of MEPC	Type of MEPC					
	normal		giant		slow	
	intact AChE	inhibited AChE	intact AChE	inhibited AChE	intact AChE	Inhibited AChE
Frequency, sec^{-1}	0,91±0,08 (43)	2,45±0,35 (22)	0,033±0,007 (43)	0,106±0,020 (22)	0,060±0,008 (43)	0,055±0,11 (22)
Amplitude, nA	6,49±0,19	6,77±0,25	12,72±1,05	14,37±0,74	6,65±0,41	8,37±1,24
T_{ap}, msec	0,27±0,01	0,32±0,01	0,58±0,06	1,19±0,19	1,45±0,08	2,14±0,20
T_{hd}, msec	0,56±0,02	1,54±0,06	1,05±0,07	4,48±0,72	1,39±0,06	3,21±0,33
T_{hd}/T_{ap}	2,07	4,81	1,81	3,76	0,96	1,50
Number of fibers	30	42	17	14	37	15

Legend. Values of frequency also include data obtained in experiments with recording of MEPP (total number of fibers shown in parentheses), but amplitudes and temporal characteristics are given only for MEPC.

0.01). The frequency of normal MEPC also was increased (by 2.7 times) on inhibition of AChE and was returned to its initial value by the presence of *d*-TC.

One of the reasons for the appearance of giant MEPC is evidently the release of clusters of normal synaptic vesicles from the nerve terminal [10, 13]. This is indicated also by the multiplicity of the amplitudes of the giant and normal MEPC (Table 1). When AChE was inhibited, the contraction of acetylcholine (ACh) released by the nonquantal route into the synaptic space rises to 10^{-8}-10^{-7} M [7]. Blockers of postsynaptic AChR accelerate the diffusion of ACh from the synaptic space [6], thereby preventing this accumulation. It can therefore be tentatively suggested that the increase in the frequency of giant MEPC when AChE is inhibited, which can be abolished by *d*-TC, is linked with the presynaptic action of unhydrolyzed ACh. This may perhaps also account for the increase in frequency of normal MEPC after inhibition of AChE, which is reversible in the presence of *d*-TC [12]. Under these circumstances the effect of *d*-TC can be explained not only by a decrease in the ACh concentration in the synaptic space, but also by the direct blocking of presynaptic ACh binding sites.

Cooling to 18°C reduced the frequency of the slow MEPC in the presence of both intact and inhibited AChE (in both cases Q_{10} = 1.4). The temperature sensitivity of the frequency of giant MEPC with intact AChE was a little higher (Q_{10} = 2.2), and it was very high when AChE was inhibited (Q_{10} = 8.8; see Fig. 2). Incidentally, after cooling, inhibition of AChE did not cause an increase in frequency of the giant MEPC (Fig. 2). Thus the process responsible for the increase in frequency of the giant MEPC after inhibition of AChE is strongly dependent on temperature (it is inhibited by cooling), but the high value of Q_{10} = 8.8 indicates that this process may perhaps consist of several temperature-sensitive stages.

The existence of positive correlation between frequencies of the giant and slow MEPC is not necessarily evidence that the sources of these signals are the same. The frequency of atypical MEPC may depend simultaneously on certain common factors, creating correlation between them, and independent factors, masking this correlation. When AChE is inhibited, a factor influencing only the frequency of the giant MEPC is evidently activated, and this therefore begins to correlate less strongly with the frequency of the slow MEPC.

After inhibition of AChE, the temporal course of both normal and atypical MEPC was slowed (Table 1). In other words atypical, including slow MEPC, are the result of the postsynaptic action of ACh and not of any other substances [14].

The duration of the ascending phase of MEPC is determined by several factors, including the velocity of the process of release of the ACh quantum, the time of diffusion of ACh to AChR, and AChE activity [2]. It might be supposed that the slow MEPC arise in those regions of the synaptic space where, for some reason or other, AChE activity is low. However, the value of T_{ap} of the slow MEPC, with AChE intact, is 4.5 times greater than the value of T_{ap} of normal MEPC when AChE is inhibited (Table 1). Moreover, the observed increase in the amplitude of T_{ap} and T_{hd} of the slow MEPC observed after inhibition of AChE (by 1.26, 1.48, and 2.31 times respectively), compared with the anticholinesterase effect for normal MEPC in other objects [3], characterizes the sufficiently high original activity of synaptic AChE at sites of generation of slow MEPC. However, with active AChE and rapid release of the ACh quantum, prolonged diffusion of the mediator into the synaptic space is unlikely, for hydrolysis of quantal ACh takes about 0.2 msec [11]. Consequently, the long duration of T_{ap} of the slow MEPC (1.45 msec) with intact AChE most probably reflects a slow process of appearance of ACh in the synaptic space, but the low temperature coefficient of T_{ap} of the slow MEPC (1.0 when AChE is intact, 1.6 when it is inhibited) indicates the diffusion nature of this process. In other words, during generation of slow MEPC there is either an abnormally slow release of ACh quanta from the

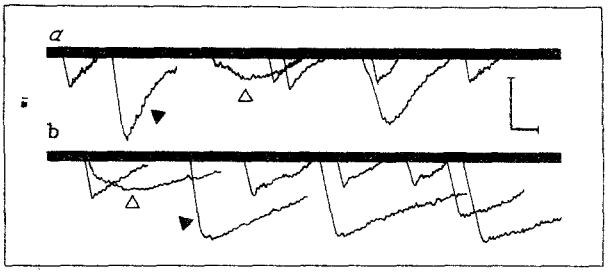

Fig. 1. Examples of traces of MEPC in control (a) and after inhibition of AChE (b). Temperature 28°C. Empty triangles show characteristic slow MEPC, filled triangles show giant MEPC. Calibration: amplitude 10 nA, duration 2 msec.

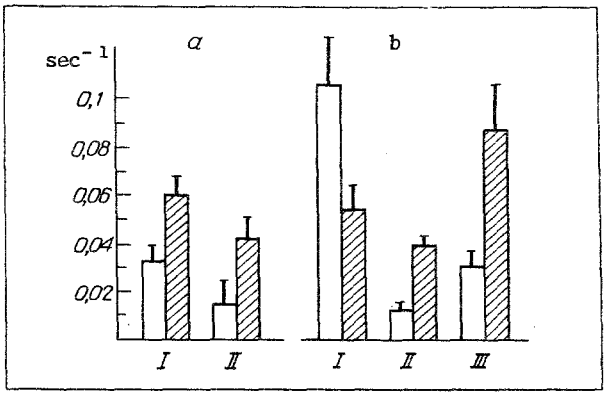

Fig. 2. Frequencies of atypical MEPC with AChE intact (a) and inhibited (b). Temperature 28°C (I, III) and 80°C (II), in presence of *d*-tubocurarine (III). Unshaded columns represent giant MEPC, shaded columns slow MEPC.

nerve terminal (possibly outside active zones), or sites of secretion of ACh quanta are remote from the synaptic space and the postsynaptic AChR. The latter is possible in the case of release of ACh quanta from the Schwann cell into the periaxonal space, as has been indirectly confirmed by the increase in the frequency of slow MEPC in the neuromuscular synapse of the frog after "attack" of the Schwann cell by antibodies to galactocerebrosides [1].

Inhibition of AChE and cooling thus have a significant effect on the frequency of giant MEPC but do not significantly change the frequency of slow MEPC. In our view, some presynaptic mechanism connected with generation of giant MEPC exists, which activates ACh and is highly temperature dependent. This mechanism is perhaps triggered by activation of presynaptic nicotinic and/or muscarinic AChR and it is related to membrane proteins, enzymes, and the cytoskeleton of the presynaptic nerve terminal.

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EFFECT OF NORADRENALIN ON RESISTIVE AND CAPACITIVE FUNCTION OF THE GASTRIC VESSELS

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Of all the different parts of the gastrointestinal tract, the stomach is that which has received the least study from the standpoint of adrenergic control of its interlinked vascular functions [3, 5], for most research on this problem has been done on the small intestine [1, 3, 4, 6]. Progress in the elucidation of humoral regulation of the gastric vessels has largely been impeded by the multicomponent nature of its blood supply and the consequent difficulty in using perfusion techniques to study its vascular functions [3]. Such research as has been published so far on this question [6, 7, 9, 10] has been distinguished by the contradictory and fragmentary nature of the results.

The aim of this investigation was to develop a physiologically adequate method of perfusing the vascular bed of the stomach and of studying the effect of noradrenalin on the resistive and capacitive functions of its vessels.

EXPERIMENTAL METHOD

Experiments were carried out on 15 male and female cats weighing 3-5 kg. The stomach was isolated in situ from other parts of the gastrointestinal tract by ligating it proximally at the esophageal level and distally on the boundary with the hepatoduodenal ligament. Collateral vessels and also parts of the omentum along the greater and lesser curvature of the stomach were ligated. The left gastric and gastroepiploic arteries and the right gastric vein were left intact, whereas the right gastric and gastroepiploic arteries and the right gastroepiploic and gastrosplenic veins were ligated. Heparin (1000 U/kg) was used to prevent clotting. The stomach, isolated hemodynamically in this manner, was perfused by the method described previously [3, 5]. Blood was taken from the animal's left femoral artery and reinjected into the left gastric artery by means of a constant delivery pump. Venous blood from the right gastric vein was directed through a catheter inserted into it into the extracorporeal venous reservoir, from which it was returned to the femoral vein by the second channel of the pump at constant volume, equal to the perfusion volume. The pressure of the venous outflow was set, on the basis of data in the literature [2], at 8 mm Hg. The volume velocity of perfusion in these experiments averaged 25 ml/min/100 g with a perfusion pressure of 100-110 mm Hg. Considering that the volume blood flow in the gastric vessels agreed with values in the literature [2, 6], it can be considered that under the conditions of hemodynamic isolation, no significant changes took place in its blood supply. In each experiment the completeness of blocking of anastomoses between the hemodynamically isolated vascular bed of the stomach and other abdominal organs was monitored.

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