PHYSIOLOGY

Localization of Sites Where Abnormal Miniature End-Plate Currents Arise at the Myoneural Junction

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 9, pp. 235-238, September, 1995
Original article submitted April 12, 1994

Frog cutaneous-sternal muscle preparations were used to examine mechanisms by which extracellularly recorded abnormal miniature postsynaptic signals are generated. The frequency of these signals was found to increase after denervation as well as after the addition of 4-aminoquinolinic acid (0.25 mmol/liter) and emetine (10 mmol/liter) to the solution perfusing the preparation. Graphic delineation, using a 3-electrode technique, of the sites where signals were arising in the nerve terminal showed that neurotransmitter (acetylcholine) quanta responsible for the generation of postsynaptic signals emerged in limited areas of the myoneural junction between terminal portions of the active zones instead of being released from the latter. It is concluded that the source of such quanta is most likely to be the Schwann cell and that the mechanism via which the neurotransmitter is released from this cell is distinct from the mechanism of its release from the nerve terminal.

Key Words: *myoneural junction; abnormal miniature signals; Schwann cell; active zone*

As demonstrated in a number of studies where temporal characteristics of the miniature end-plate currents (MEPC) normally arising at myoneural junctions of coldblooded and warmblooded animals were analyzed, there exist MEPC whose rise times and decay half-times are much greater than those of ordinary MEPC (o-MEPC) [7,9]. These MEPC have been variously designated as "giant," "slow," or "abnormal" MEPC (a-MEPC). The frequency of their occurrence is normally not high (0.1 min^{-1}) but may substantially rise under certain circumstances - for example, when the concentration of sodium in the surrounding medium is lowered or when the muscle has been treated with the neurotoxin botulin [10], is strongly stimulated at low temperature, or has been placed in a hypertonic solution [11]. a-MEPC do not change their frequency in response to agents or procedures that alter the frequency of o-MEPC (e.g., ouabain, ethanol, or elevation of the Ca^{2+} concentration in the solution) [7,8]. a-MEPC have been shown to arise in the same region of the nerve terminal where o-MEPC are generated.

It has been suggested that the rise times of a-MEPC and their decay half-times may be prolonged as a result of repeated binding of acetylcholine molecules to postsynaptic membrane receptors because of their slowed diffusion or due to a change in the number and properties of the acetylcholine receptors. a-MEPC are presumed to be produced as a consequence of acetylcholine release in large amounts from a nerve terminal area between the active zones far from the postsynaptic membrane [9,10]. The present study was designed

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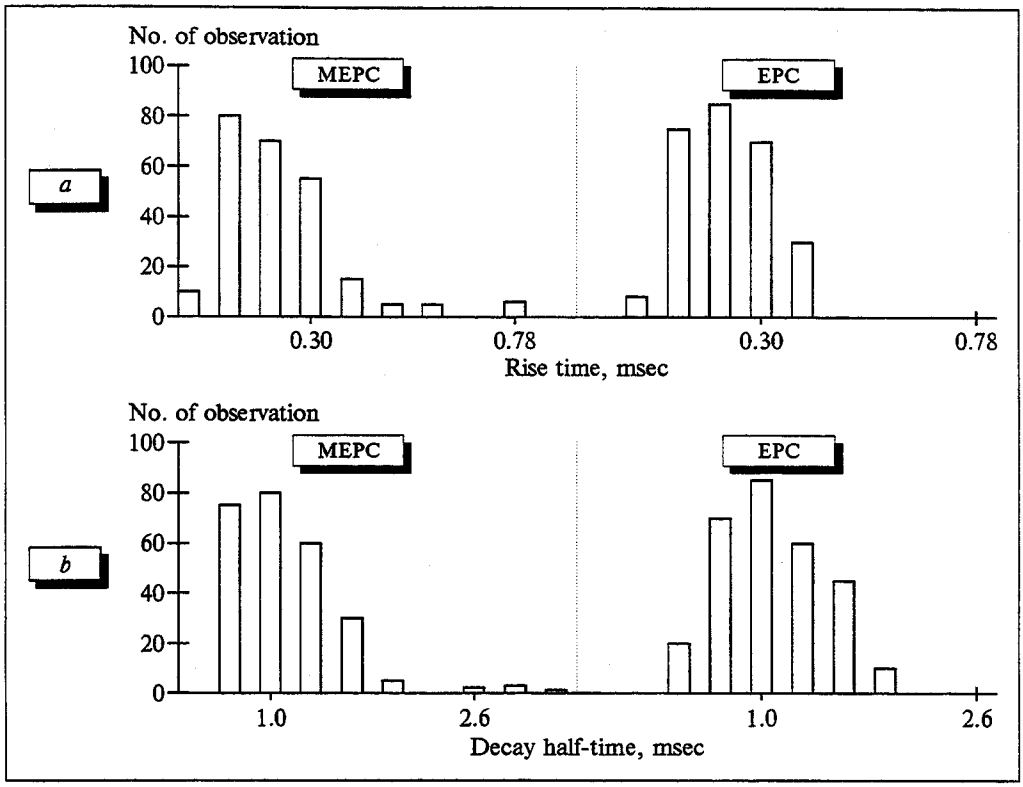

Fig. 1. Histograms showing the distribution of times of rise to the maximum (*a*) and of decay half-times (*b*) for MEPC and EPC. The arrows indicate signals with prolonged temporal parameters.

to identify the source of released acetylcholine quanta responsible for a-MEPC generation and the site(s) of their release.

MATERIALS AND METHODS

Muscle-nerve preparations made of cutaneous-sternal muscles from *Rana ridibunda* were used. Preparations of isolated muscles were each placed in a 7 ml glass bath and continuously perfused with Ringer's solution for coldblooded animals. The solution had the following composition (mmol/liter): 118.0 NaCl, 5.0 KCl, 0.3 $CaCl_2$, 2.0 $MgCl_2$, and 2.4 $NaHCO_3$ (20°C). A polarizing interference microscope (magnification 400) was used to visualize the superficially located nerve terminals and to pass microelectrodes to these. The microelectrodes used to record MEPC and one-quantum end-plate currents (EPC) had tips with an inner diameter of 1-3 μ and were filled with NaCl solution (2 mmol/liter). Synaptic signals (400-600 MEPC and/or 200-400 EPC) were recorded with one or three extracellular microelectrodes. In the latter case, the microelectrode tips had a triangular arrangement such that the nerve terminal was located between them, and the signal led off by each microelectrode was amplified and measured with an IBM PC-based automated system. The coordinates of the points at which the neurotransmitter was released were then defined and graphic representations of its release in the nerve terminal were produced by comparing the spatial distribution patterns of sites where o-MEPC and a-MEPC had arisen. The procedure used to pass the microelectrodes and the method of calculating the coordinates of those points are described in another article [2]. In a number of experiments, 4-aminoquinolinic acid (0.25 mmol/liter) and emetine (10 mmol/liter), which markedly increase the frequency of a-MEPC generation [5,9], were added to the perfusing solution. In some other experiments, preparations of denervated muscles were used and MEPC were recorded starting on day 4 postdenervation [1]. The motor nerve was stimulated by rectangular pulses of suprathreshold amplitude and 0.15 to 0.3 msec duration.

RESULTS

By analyzing the distribution histograms constructed for the temporal parameters of MEPC recorded using one electrode (their rise times and decay half-times), we identified signals that had values of these parameters exceeding their average values two or more times (Fig. 1). Normally, these signals appeared at a frequency of not more than 0.2 min^{-1}. It is noteworthy that there was no evidence of such signals on similar distribution histograms constructed for one-quantum EPC recorded in the same area (Fig. 1). When emetine and 4-aminoquinolinic acid were added to the perfusing solution, the incidence

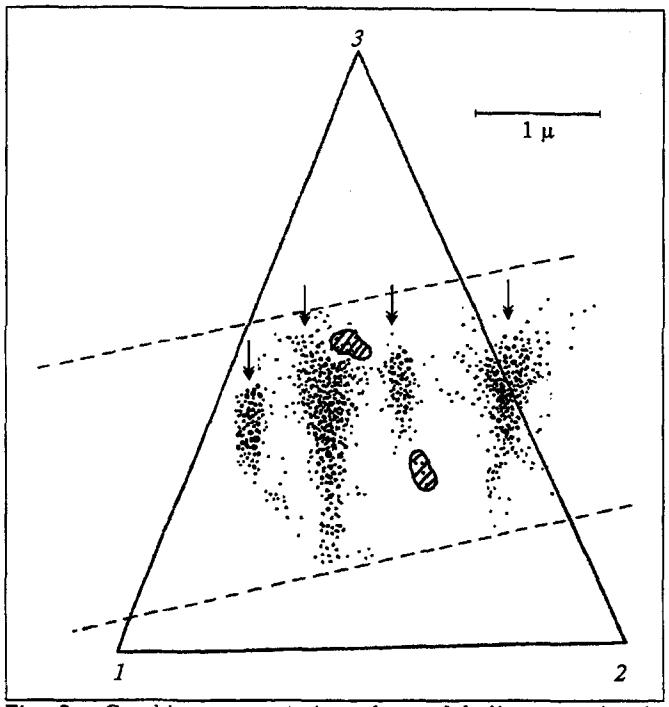

Fig. 2. Graphic representation of acetylcholine secretion in a nerve terminal (one separate experiment). The groups of release points reflecting o–MEPC liberation are indicated by arrows. Here and in Fig. 3: the dashed lines mark terminal boundaries and the hatched areas, the sites of release of acetylcholine quanta responsible for a–MEPC generation. *1*, *2*, and *3* are the numbers and sites of microelectrodes.

of a-MEPC increased 5- to 8-fold without there being an appreciable change in that of o-MEPC. Denervation led to a sharp fall in the o-MEPC frequency and severalfold (4 to 5 times) increases in the number of signals with long rise times and decay half-times on day 4 or 5 postdenervation.

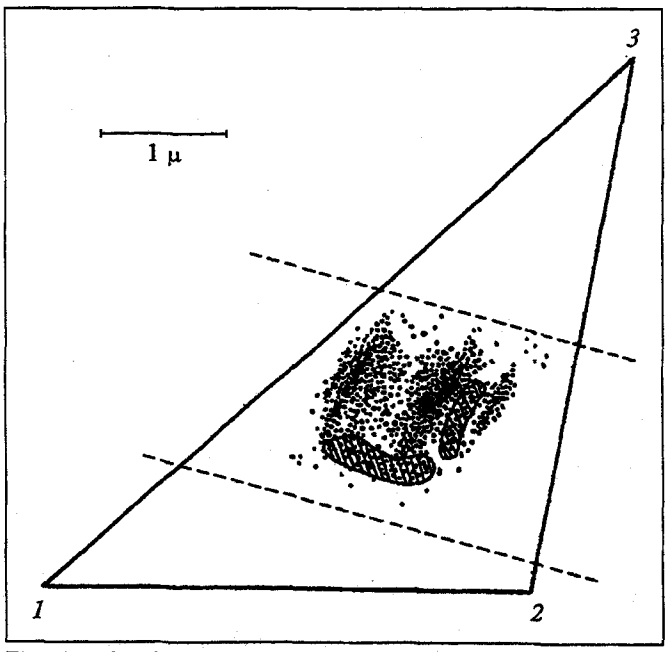

Fig. 3. Graphic representation of acetylcholine secretion in a nerve terminal on day 5 after denervation.

All graphic representations of acetylcholine secretion in the nerve terminals under normal conditions contained discrete groups of points at which o-MEPC were generated. These groups were 2.5-3.5 μ long and 1.2-1.5 μ wide and were separated from each other by distances of 0.6 to 1.5 μ. In an earlier study we presumed that such groups reflect acetylcholine secretion in the active zone of the nerve terminal and that this zone lies across the latter, in the center of the respective group [3]. The sites of a-MEPC generation were found not to coincide with the groups of points where o-MEPC were generated and to be arranged in a strictly definite pattern relative to these groups, namely between their terminal areas. The results of one experiment are graphically depicted in Fig. 2. Note that the points of a-MEPC generation lie, as a rule, in close proximity to each other. In the nerve terminal area whose activity was recorded, we usually observed not more than 1 or 2 compact groupings of a-MEPC generation points, and these did not change their location throughout the period of signal recording (60-90 min).

The location of a-MEPC generation points following the addition of emetine and 4-aminoquinolinic acid in micromolar concentrations was identical to that under physiological conditions.

In the graphic representations of acetylcholine secretion on day 4 or later after denervation, groups of o-MEPC generation points were often indistinguishable or were less compact and less well defined than those seen before denervation, although acetylcholine secretion between the groups was more pronounced. a-MEPC were mostly located in lateral portions of the degenerating nerve terminals, but were also seen in their centers. The points where the neurotransmitter quanta responsible for a-MEPC generation were released after denervation were arranged in a more diffuse manner and occupied a larger area than such points before denervation (Fig. 3).

The results of these experiments led us to conclude that a-MEPC arise as a result of acetylcholine being released by a mechanism distinct from the one resulting in the generation of o-MEPC. This conclusion is supported by the observations that a-MEPC are not involved in the formation of EPC and that their incidence does not depend on the Ca^{2+} concentration and rises following denervation. Also, the frequency of a-MEPC has been shown to be more temperature-dependent (Q_{10}=12) than that of o-MEPC [10].

The 3-microelectrode technique of recording we used is based on the assumption that the point at

which an acetylcholine quantum is released on the presynaptic membrane is spatially coincident with the site of postsynaptic current generation. This coincidence is attributable to the small geometric dimensions of the synaptic cleft [2,3]. The points of MEPC generation can therefore provide an indication of where the acetylcholine quanta responsible for o-MEPC and a-MEPC generation are released. Thus, our findings warrant the conclusion that the acetylcholine quanta responsible for o-MEPC generation are liberated neither from the active zone nor from the nerve terminal. They may originate either in the invaginations of the Schwann cell that extend into the synaptic cleft or in the Schwann cell processes enwrapping the nerve terminal. Our ultrastructural studies [4] showed that Schwann cell processes occur at an average frequency of 1 for every 2 to 4 active zones. Since the graphic reconstructions of our method make it possible to judge at one time the function of a nerve terminal area containing 2 to 6 active zones, the presence of 1 or 2 compact groupings of points where a-MEPC are released agrees well with morphological data on the arrangement of Schwann cell processes.

On day 4 or 5 postdenervation, the nerve terminal gradually begins to undergo degeneration, which is primarily manifested in disorganization of the active zones and associated calcium channels [6]; the Schwann cell then invades the synaptic cleft and enfolds the nerve terminal. This may explain why the groups of points where o-MEPC are released lose their orderly arrangement and characteristic shape. Increases in the number of a-MEPC and in the territory where they arise are indications that the Schwann cell exhibits elevated activity and has more processes than before.

REFERENCES

1. S. N. Zemskova, K. Eder, R. A. Ginniatullin, *et al.*, *Fiziol. Zh. SSSR*, **77**, № 2, 57 (1991).
2. A. L. Zefirov, T. V. Benish, and N. V. Fatkullin, *Neirofiziologiya*, **22**, № 3, 310 (1990).
3. A. L. Zefirov, T. V. Benish, N. V. Fatkullin, and S. Yu. Cheranov, *Ibid.*, 319.
4. A. L. Zefirov, E. N. Bezgina, L. A. Kashapova, *et al.*, *Dokl. Akad. Nauk SSSR*, **290**, № 5, 1277 (1986).
5. K. A. Alkadhi, *Brain Res.*, **447**, 293 (1988).
6. C. P. Ko, *J. Physiol. (London)*, **321**, 627 (1981).
7. M. T. Lupa, N. Tabti, S. Thesleff, and F. Vyskocil, *J. Physiol. (London)*, **381**, 607 (1986).
8. J. Molgo, S. Gomez, R. L. Polak, and S. Thesleff, *Acta Physiol. Scand.*, **115**, 201 (1982).
9. J. Molgo and S. Thesleff, *Proc. R. Soc. Lond. [Biol.]*, **214**, 229 (1982).
10. S. Thesleff, J. Molgo, and H. Lundh, *Brain Res.*, **264**, 89 (1983).
11. S. Thesleff, *Int. Rev. Neurobiol.*, **28**, 59 (1986).