CHANGES IN TRANSMITTER SECRETION IN THE RAT NEUROMUSCULAR JUNCTION DUE TO DISTURBANCE OF CALCIUM METABOLISM

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The parathyroid glands are important regulators of calcium metabolism. Inhibition of their function and a decrease in parathyroid hormone production leads to the development of hypocalcemia, accompanied by serious motor disorders [5, 13, 14] and, in particular, by lesions of the peripheral neuromuscular apparatus, which have been well studied in experimental hypoparathyroidism [6]. Although the role of hypocalcemia in the development of these disturbances seems evident, it is not yet clear whether they arise as a result of the well known [1, 11, 12, 15] direct effect of a decrease in the extracellular Ca^{2+} concentration on excitable membranes and synapses or are the result of changes in these structures secondary to hypocalcemia.

Since Ca^{2+} ions are most important for secretion of the synaptic transmitter, the investigation described below was undertaken to study the state of this process in the neuromuscular junction of parathyroidectomized rats.

EXPERIMENTAL METHOD

Male rats weighing 120-150 g were used. Experimental hypoparathyroidism was produced by removing the superficial lobes of the parathyroid glands by electrical coagulation under ether anesthesia. The development of hypoparathyroidism was judged from the severity of the hypocalcemia. The total serum calcium level was determined photometrically in the usual way [6]. Nerve–muscle preparations (phrenic nerve and a strip of diaphragm muscle) were isolated under ether anesthesia and placed in a constant-temperature (35°C) chamber through which flowed Tyrode solution of the following composition (in mM): Na^+ – 150.0, K^+ – 2.7, Ca^{2+} – 2.0, Mg^{2+} – 1.0, Cl – 145.7, HCO_3^- – 12.0, H_2PO_4 – 1.0, glucose 11.0, pH 7.2-7.3, saturated with carbogen (95% O_2 and 5% CO_2). If necessary the Ca^{2+} concentration was reduced to 1 mM or Ca^{2+} was removed from the medium by the addition of 1 mM EGTA. Transmitter secretion was assessed by recording spontaneous intracellular synaptic electrical activity – miniature end-plate potentials (MEPPs) – by means of glass microelectrodes in the usual way. Postsynaptic potentials and, in particular, MEPPs, were preamplified by MZ-4 microelectrode amplifier; next, using dc amplifiers, they were recorded on two channels of an SDR-41 tape recorder. Records with stable membrane potential and a low noise level were chosen and, after reproduction of the process on a 14F 50 U-Visicorder, the signals were measured and subjected to primary processing by the Leitz A. S. M. semiautomatic image analysis system. Since it was shown previously that the distribution of synapses by mean frequencies of MEPP is lognormal [1], numerical values of the statistical parameters also were calculated on a logarithmic scale. Mean values of MEPP frequencies calculated in this way are given below (the asymmetrical 95% confidence interval in parentheses). The goodness of fit test was used to verify the type of distribution and Students' t test for comparing means.

EXPERIMENTAL RESULTS

After removal of the parathyroid glands clinical manifestations of developing hypoparathyroidism in the rats were a reduction of motor activity, a state of stupor, and periodic twitching of the trunk muscles. Electrographic analysis of the neuromuscular defect developing

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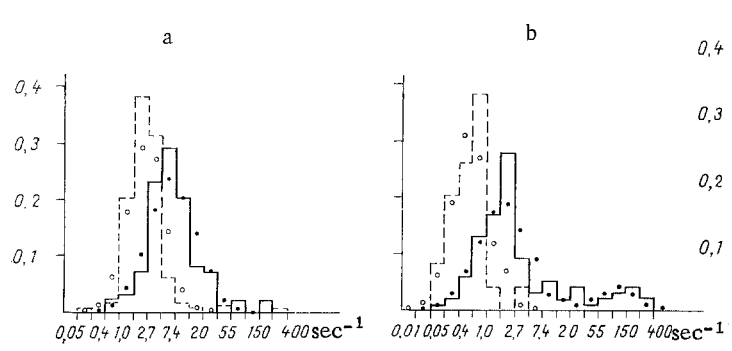

Fig. 1

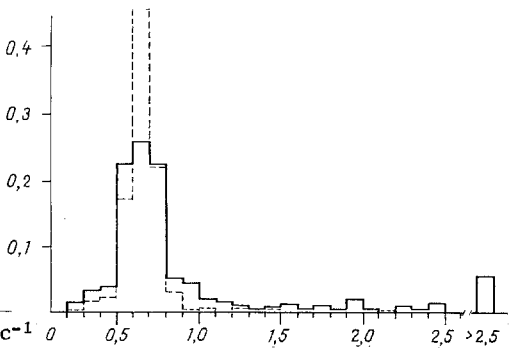

Fig. 2

Fig. 1. Shift of spontaneous transmitter secretion level in neuromuscular junctions in hypoparathyroidism. Distribution of neuromuscular junctions by mean MEPP frequencies. Continuous line — experiment; broken line — control; empty and filled circles — theoretical lognormal distributions for control and experiment respectively. Rat diaphragm, 35°C. a) Medium with normal calcium level (2 mM); b) calcium-free medium (1 mM EGTA). Ordinate, relative frequency of occurrence; abscissa, mean frequencies of MEPPs (sec^{-1}) on scale of natural logarithms.

Fig. 2. Disturbance of character of spontaneous exocytosis of transmitter in neuromuscular junction in hypoparathyroidism. Distribution of MEPP by amplitudes. Rat diaphragm, medium with normal calcium level, 35°C. Continuous line — experiment (n = 394 MEPPs, $\bar{a}$ = 0.8 mV, σ = 0.7 mV, extreme bin on right side of histogram contains "spontaneous EPPs" — MEPPs, with an amplitude of over 2.5 mV; broken line — control (n = 990 MEPPs, $\bar{a}$ = 0.6 mV, σ = 0.1 mV). Ordinate, relative frequency of occurrence; abscissa, amplitude of MEPPs (in mV).

after the operation, and the dynamics of changes in the serum Ca^{2+} concentration, described previously [6], enabled the times of stabilization of the changes (5th-8th day) to be selected for investigation. The Ca^{2+} concentration in the rats' blood serum before isolation of the neuromuscular preparation was 2.75 ± 0.07 mM in the control and 1.50 ± 0.9 mM in the experiment.

The distribution of mean frequencies of MEPP for individual fibers in nerve–muscle preparations obtained from parathyroidectomized rats and placed in Tyrode solution with normal (2 mM) calcium concentration showed no significant change compared with that in the control (healthy animals) and also was lognormal (Fig. 1). However, the mode of distribution in the experiment was shifted toward higher values and comparison of the mean values of mean MEPP frequencies in the two synapse populations revealed a significant ($P < 0.01$) increase in the MEPP frequency in the experiment compared with the control: 6.42 (5.48-7.53) sec^{-1} (n = 124) and 2.56 (2.37-2.77) sec^{-1} (n = 221), respectively. If the Ca^{2+} concentration in the surrounding medium was reduced by half (1 mM) — to a value approximately the same as the serum Ca^{2+} level of parathyroidectomized rats, the mean value of the mean frequencies in the experiment was 4.76 (2.49-9.11) sec^{-1} (n = 20) significantly higher ($P < 0.01$) than that in the control for normal medium. As regards amplitudes of MEPPs observed in medium with normal calcium concentrations, the first point to note is an increase in the number of fibers with high-amplitude MEPPs in the experiment. Potentials of this kind were found also in the control but only in a few fibers (under 10%, period of investigation 10-30 sec), where they transformed somewhat the approximately normal appearance of MEPP distribution by amplitude. In experimental hypoparathyroidism the number of these fibers increased to 50-80%, and the region of the curve of MEPP distribution by amplitude for them, corresponding to the principal mode of distribution, was approximately normal in appearance, just as in the control. In many of these fibers, however, the marked increase in the number of high-amplitude MEPPs led to appreciable asymmetry of the curve as a whole (Fig. 2). With a greater sweep it could be seen that these spontaneous high-amplitude EPPs led to local or spreading action potentials (Fig. 3).

In calcium-free medium the distribution of mean MEPP frequencies among separate fibers in nerve–muscle preparations isolated from parathyroidectomized rats did not obey the lognormal rule ($P > 0.05$), unlike the control in the same medium. In this case the distribution of mean MEPP frequencies experimentally was correctly described by the sum of two lognormal

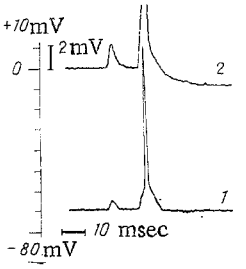

Fig. 3. Appearance of spontaneous action potential (AP) in muscle fiber in hypoparathyroidism. 1) Transmembrane potential, large MEPP and AP of above zero level, developing against the background of "spontaneous EPP" can be seen; 2) the same, with higher amplification (ac amplifier). Rat diaphragm, medium with normal calcium concentration, 35°C.

distributions (Fig. 1). Comparison of the mean value of mean frequencies in the experiment, corresponding to the principal (n = 88) peak of the distribution, with the mean in the control reveals a shift of the first, principal peak also toward higher frequencies compared with the control ($P < 0.01$): 1.77 (1.45-2.16) sec^{-1} (n = 88) and 0.54 (0.45-0.64) sec^{-1} (n = 50), respectively. Consequently, not only did a considerable number of high-frequency fibers appear, which could be distinguished as a separate population (the second peak on the histogram of mean frequencies), but discharge of transmitter in all fibers was activated.

Significant changes in secretory function of the synaptic endings in the neuromuscular junction were thus found. In the first place this was due to intensification of spontaneous synaptic activity (an increase in MEPP frequency) which, in the modern view, depends both on the operative reserves of transmitter and on the Ca^{2+} concentration in the terminal [1, 7, 9, 11]. This last parameter is determined by inflow of Ca^{2+} from outside, which depends on the Ca^{2+} concentration gradient. Under conditions of natural activation of the synapse, the value of the Ca^{2+} current, which is controlled by the depolarization wave, will be determined essentially also by the portion of synchronously released transmitter in response to the nervous impulse and the corresponding EPPs [1, 7, 9, 11]. In low-calcium medium the decrease in the Ca^{2+} concentration gradient and, correspondingly, in Ca^{2+} inflow leads to a decrease in EPP that does not go on to produce an appreciable disturbance of synaptic conduction purely because of the high safety factor. The reduction in expenditure of transmitter, and, consequently, the increase in its operative reserves, can themselves lead to an increase in spontaneous secretion after a certain moment. Meanwhile transformation of function of synaptic endings in the neuromuscular junction of parathyroidectomized rats can be linked with actual disturbance of endocrine regulation of calcium metabolism at the cell level and reorganization of the Ca^{2+} transport systems in cell membranes [8]. One result of these effects may be a decrease in Ca^{2+} outflow from the cytoplasm which, even if its uptake by the mitochondria or other intracellular organelles which accumulate Ca^{2+} is increased, will lead to an increase in MEPP frequency. An increase in the internal Ca^{2+} concentration may also cause a decrease in the flow of Ca^{2+} from outside [10], i.e., further impairment of synaptic conduction.

Judging from assessment of the level of secretion with different Ca^{2+} concentrations in the external medium, the secretory apparatus in the neuromuscular synapse cannot thus be regarded as intact in hypoparathyroidism. In this case, if consideration is paid to amplitude characteristics of postsynaptic potentials and, in particular, the appearance of "spontaneous EPPs," not only the mechanism determining the level of transmitter secretion will be affected, but also the actual method of its exocytosis, just as takes place in other disturbances of transmitter secretion [2, 3, 4]. This assessment of its state is in good agreement with data on the character of neuromuscular transmission as a whole obtained in situ in parathyroidectomized rats [6]. They likewise cannot be explained purely by the direct effect of hypocalcemia on synaptic and electrogenic processes. In particular, effects of repetitive stimulation are more likely to be connected with an increase in the intracellular Ca^{2+} reserves [7, 9].

The results suggest that in experimental hypoparathyroidism there is not only a direct effect of hypocalcemia on transmitter exocytosis, but there are also substantial changes in the apparatus of secretion and, possibly, of accumulation of transmitter and (or) Ca^{2+} by the terminal, secondary to the hypocalcemia. Correlation discovered between disturbances of calcium homeostasis at organism and cell level under these conditions is particularly interesting.

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CHANGES IN AREA OF MYOCARDIAL DAMAGE DURING POSTISCHEMIC REPERFUSION

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The change in area of myocardial injury during postischemic reperfusion is not only of theoretical, but also of practical interest because of the introduction of thrombolytic and revascularization methods of treatment of ischemia and infarction of coronary origin into clinical practice. Data in the literature on this question are contradictory. Some workers state that after ischemia ranging in duration from 14-30 min [7] to 1-6 h [6] reperfusion leads to a decrease in size of the infarct, whereas others [4] state that reperfusion after ischemia for 5 h causes an increase in size of the infarct in most cases. To test which of these conclusions is correct the investigation described below was carried out.

EXPERIMENTAL METHOD

Experiments were carried out on 88 noninbred male albino rats weighing 180-200 g. At each stage of the experiment 5-8 rats were used. Permanent and transient ischemia were simulated by ligating the left coronary artery 2-3 mm below the left inferior angle of the

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