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EFFECT OF METRAZOL ON TIME COURSE OF MEMBRANE-BOUND CALCIUM LEVEL IN THE CEREBRAL CORTEX

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In the modern view calcium ions play an important role in the mechanisms of hyperexcitability and injury of brain neurons under the influence of convulsants [1, 7-9]. An increase in the inflow of extracellular Ca^{++} into cerebral cortical nerve cells has been found under the influence of several convulsants. Changes in intracellular Ca^{++} exchange arising under these circumstances evidently exert some influence primarily on the disturbance of regulation of ionic permeability of the plasma membrane, and activity of enzymes involved in neurotransmitter synthesis and secretion, and energy production.

Ca^{++} ions are known [2, 5, 6] to act as regulators of intracellular processes, mainly on binding with components of neuron membranes. It can be postulated that a disturbance of this process may play an essential role in the pathogenesis of convulsive states. However, no information is yet available on this problem.

The aim of this investigation was to study the time course of the membrane-bound calcium (Ca^{++}_{mb}) in structures of the cerebral cortex under the influence of metrazol, a typical convulsant.

EXPERIMENTAL METHOD

Structural elements (bodies of neurons and the surrounding neuropil) from layers II and III of a living preparation of the motor cortex taken from cats anesthetized with pentobarbital (30 mg/kg), with artificial ventilation of the lungs, served as the test object.

Intravital spectrophotometry of microregions of the cerebral cortex (diameter 60 μ), stained with chlorotetracycline (50 μM) was carried out with the LYUMAM KF contact microscope. The time course of the Ca^{++}_{mb} concentration was estimated as a change in the intensity of fluorescence of chlortetracycline–Ca^{++}–membrane complex (CTC-Ca^{++}-MC) [4, 6] and expressed as a percentage of the initial level.

Metrazol was applied to the microregions for microscopy by means of an iontophoretic current (70 nA) through one barrel of a multibarreled micropipet for various periods of time (5-120 sec). Electrical activity of the neuron was recorded extracellularly through another channel. The techniques for combined morphological and physiological study of structures of the cortical preparation with microiontophoretic application of biologically

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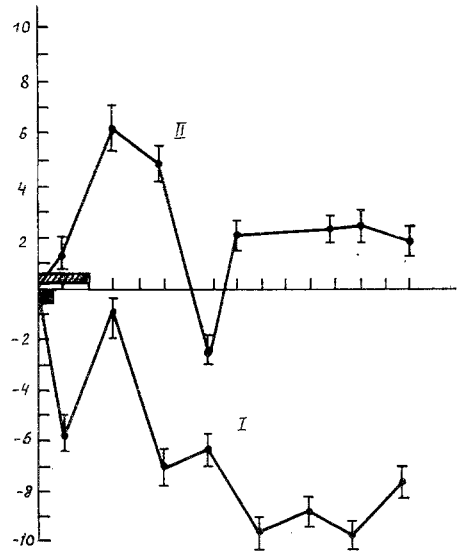

Fig. 1

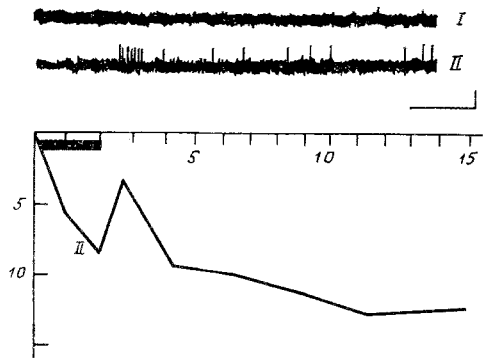

Fig. 2

Fig. 1. Time course of intensity of fluorescence of CTC-Ca^{++}-MC in cortical microregions after microiontophoretic application of metrazol for 30 sec (I) and 120 sec(II). Ordinate, change in intensity of fluorescence (in % of initial value); abscissa, time of observation (in min). Periods of application of metrazol (30 and 120 sec) denoted by black and obliquely shaded rectangles respectively. Short vertical lines on graphs indicate standard error of the mean, p = 0.05; for graph I, n = 17, for II, n = 12.

Fig. 2. Changes in intensity of fluorescence of CTC-Ca^{++}-MC (below) and unit activity recorded from a microregion of cortical area III after application of metrazol for 120 sec, preceded by application of EGTA. I) Unit activity before application; II) after action of metrazol for 2 min. Calibration: horizontally 0.1 sec, vertically 100 μV. Arrow on graph indicates time of appearance of bioelectrical response. Legend as to Fig. 1.

active substances, and also for investigating the time course of their Ca^{++}_{mb} levels were described in detail previously [3, 4].

EXPERIMENTAL RESULTS

Application of metrazol for 15-30 sec or longer as a rule induced the development of "seizure" activity in the cortical microregions studied during the first few minutes of its after-effect. Marked changes in their Ca^{++}_{mb} concentrations were recorded.

In experiments of series I (Fig.; 1, I) a progressive decrease in the intensity of fluorescence of CTC-Ca^{++}-MC was discovered after exposure to metrazol for 30 sec, evidence of a fall of Ca^{++}_{mb} in the course of 10-15 min, with a phase of relative elevation of its level with a maximum at the 3rd minute. In series II (Fig. 1, II), after a fourfold increase in the dose of metrazol applied (120 sec, iontophoresis) phasic changes in the Ca^{++}_{mb} concentration were found. Initially there was a sharp increase in the intensity of fluorescence of CTC-Ca^{++}-MC with a maximum at the 3rd minute, and this was followed by a relative decline from the 3rd to the 7th minutes, followed by a second rise.

On the basis of the results of these series of experiments it can be concluded that in response to metrazol two opposite processes of change in Ca^{++}_{mb} develop in nerve cells, and their intensity depends on the dose of the convulsant and the duration of its after-effect. To estimate the possible contribution of extracellular Ca^{++} to the time course of the Ca^{++}_{mb} concentration during the action of metrazol, in the experiments of series III this substance was applied after application of EGTA (1 mM), a specific Ca-chelating agent, to the cortex, preventing Ca^{++} from entering the cells.

One typical example of the response of cortical structures after application of metrazol for 120 sec under these conditions is illustrated in Fig. 2. In this case, unlike the results

recorded in experiments of series II (Fig. 1, II), a marked decrease in the intensity of fluorescence of CTC-Ca^{++}-MC was recorded during the period of after-effect of metrazol, with a phase of a relative increase with a maximum at the 3rd minute. It is important to note that under these experimental conditions metrazol led to the development of "seizure" activity of the neurons, the beginning of which coincided with appreciable release of Ca^{++}_{mb} (Fig. 2). Comparison of the results of the experiments of series II and III showed that the metrazol-dependent sharp increase in fluorescence of CTC-Ca^{++}-MC which was due mainly to binding of Ca^{++}, entering from the extracellular medium, with the membranes.

Thus, according to these results, intraneuronal exchange of Ca^{++} is disturbed by the influence of metrazol, due to a change in the Ca^{++}_{mb} concentration and more intensive penetration of extracellular Ca^{++} into the cells. The primary effect of metrazol is to induce rapid release of a certain part of the Ca^{++}_{mb}, which correlates with the onset of "seizure" activity of the neurons. At the same time adsorption of Ca^{++} by intracellular membranes develops, and is most clearly manifested if the dose of the convulsant is increased. It is evident that functionally different membranes are involved in these opposite processes. It must be considered that the changes discovered in the Ca^{++}_{mb} concentration are an important mechanism for the initiation and formation of cortical neuronal responses to the action of convulsants.

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