EXPERIMENTAL BIOLOGY

Effect of Magnesium-Free Solution on Spontaneous Excitatory Postsynaptic Potentials of *Helix lucorum* L. Identified Neurons

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The contribution of synaptic potentials to the development of epileptiform activity is a current topic in studies of epileptogenesis. Changes in synaptic activity may induce or anticipate epileptic attacks [6]. A nervous system preparation of *Helix lucorum* L. in magnesium-free medium represents an adequate model for studies of the mechanisms of development of epileptiform activity. To detect the contribution of excitatory synaptic inputs to the development of epileptiform activity in identified cells, we tested the effects of magnesium-free solution on the spontaneous synaptic input of RPa2, RPa3, LPa2, and LPa3 neurons of *Helix lucorum* L. The studied neurons are command elements of the defense reflex (retraction into the shell). Cells of a semiintact preparation are silent, but excitatory postsynaptic potentials (EPSP) are recorded in them which arise synchronously in all command neurons [1,8]. Command neuron EPSP are classified as slow and fast [7]. We examined changes only of fast EPSP.

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MATERIALS AND METHODS

A simplified variant of CNS semiintact preparation, the mantle, was used in the experiments [9]. Neuronal bioelectric activity was recorded intracellularly by glass capillary microelectrodes filled with 2.5-3 M KCl, with a resistance of 15-20 MOhms. Electrical stimulation of the nerves and pneumo-

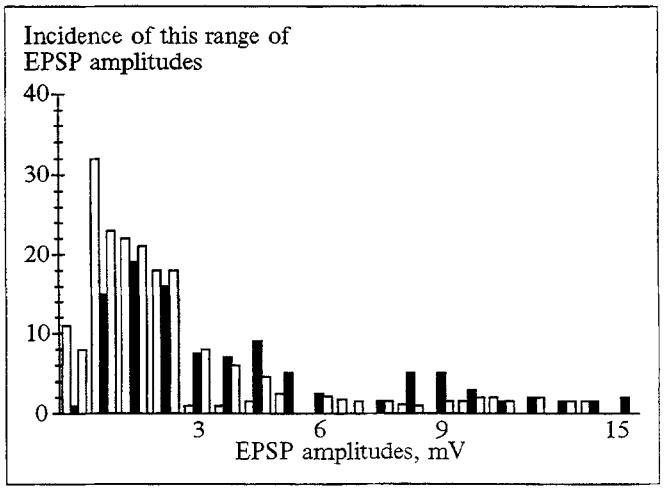

Fig. 1. Distribution histograms of EPSP amplitude in RPa3 neuron. Light bars: in solution with 4 mM $MgCl_2$; black bars: in Mg-free solution; cross-hatched bars: in normal Ringer's solution.

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stome was carried out using a Multistim stimulator. Tactile stimulation of the pneumostome and other viscera was made with a probe. Normal salines were applied to the whole preparation with a Zalimp water-jet pump. Solutions of the following composition (in mmol/liter) were used: 111 NaCl; 1.19 $NaHCO_3$; 0.9 KCl; 1 $CaCl_2$; 4 $MgCl_2$; 3.9 $C_6H_{12}O_6$; tris-HCl, 1.5 mM in normal solution; 111 NaCl; 1.19 $NaHCO_3$; 0.9 KCl; 1 $CaCl_2$; 0 $MgCl_2$; 3.9 $C_6H_{12}O_6$; tris-HCl, 5.5 mM in magnesium-free solution.

Data were processed using an IBM PC AT perosnal computer with INTERGRAPH and MICROSTAT software. Frequency histograms of the experimental values and the dynamics of the means in the normal and magnesium-free solutions were plotted. The reliability of the sampling differences was assessed using the Wilcoxon-Mann nonparametric test [2,5].

RESULTS

EPSP amplitude and duration, the time needed to attain the maximum, and the intervals between individual EPSP were analyzed. Background EPSP amplitude distribution, distribution in Mg-free solution, and on a washed preparation reliably differed from each other ($\alpha \leq 0.01$) (Fig. 1). The mean EPSP amplitude increased by 160% over the background value. The intervals between individual EPSP also changed. The distribution of intervals between background excitatory synaptic potentials and those in Mg-free solution differed significantly ($\alpha \leq 0.01$) (Fig. 2). The arithmetic mean of the intervals between EPSP decreased by 81%. Analysis of EPSP duration and time needed to attain the maximum in the background, in Mg-free solution, and on a washed preparation showed no significant changes in the distribution of these parameters under the given conditions. Hence, the absence of magnesium ions enhanced the efficacy of excitatory synaptic transmission. A synaptic nature of the epileptiform discharges in the examined neurons may thus be deduced.

Our previous studies [11] revealed in the *Helix lucorum* L. nervous system cells which react to the removal of Mg ions from the extracellular solution by paroxysmal depolarization shifts of the membrane characteristic of epileptiform activity. Initially silent RPa2, RPa3, LPa2, and LPa3 cells generated in Mg-free solution depolarization shifts culminating in bursts.

At least three possible mechanisms are known which may lead to the development of paroxysmal depolarization shifts in *Helix lucorum* neurons in magnesium-free medium: 1) enhancement of input

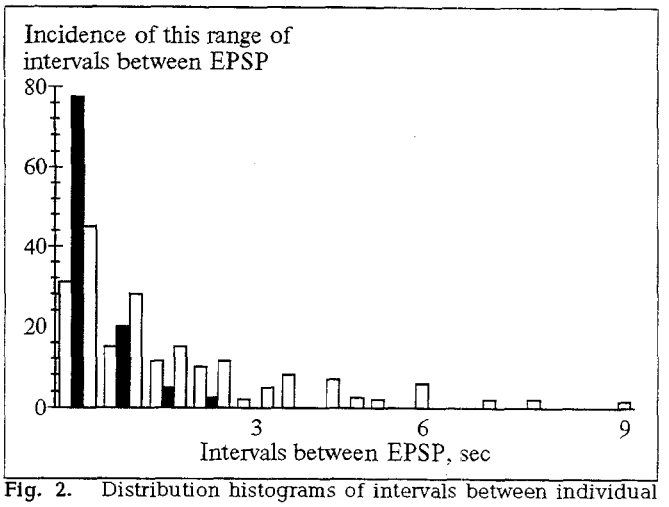

Fig. 2. Distribution histograms of intervals between individual EPSP of RPa3 neuron. Other notation as in Fig. 1.

currents, sodium or calcium, either by the removal of the blocking effect of Mg ions on the Ca channels [13], or by the reduction of the shielding effect of the Mg ions [4]; 2) enhancement of excitatory synaptic currents [12]; 3) simultaneous realization of both possibilities: enhancement of excitatory synaptic transmission efficacy paralleled by enhancement of potential-dependent input currents [10]. Blocking or attenuation of the output currents does not seem to occur [3]. An increase in EPSP amplitude was observed during application of Mg-free solution. It may be assumed that for cells with an abundant synaptic input and without background activity RPa2, RPa3, LPa2, LPa3 and LPa5 cells) the increased efficacy of excitatory synaptic transmission is the cause of the paroxysmal depolarization shifts.

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