The Influence of Magnesium-Free Saline on the Activity of *Helix lucorum* Neurons

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Paroxysmal depolarization shifts (PDS) are a common feature of neuron intracellular activity in a wide range of clinical and experimental epileptic foci created by the application of epileptogenic agents (penicillin, picrotoxin, etc.) or by electrical current (electroshock, kindling stimulation) [2]. Walter *et al.* [7] revealed that epileptic bursts can be induced in mammalian hippocampal slices by lowering the extracellular magnesium ion concentrations. The intrinsic mechanisms of convulsant action on neurons of vertebrates and invertebrates are similar in many cases [3]. The idea of using simple nervous systems for investigating the mechanisms of epileptogenesis is of obvious theoretical and practical importance. This paper explores for the first time the possibility of creating an epilepsy model by application magnesium-free solution to a snail brain preparation.

MATERIALS AND METHODS

The experiments were performed on semiintact CNS-mantle preparations from *Helix lucorum L.* [1]. Intracellular recordings were made with conventional glass capillary microelectrodes filled with 2.5-3 M KCl; tip resistance varied from 15 to 20 MOhm. The nerves and pneumostoma were stimulated with silver electrodes hooked up to a Multistim stimulator. Peripheral organs were stimulated manually with a brush. The nervous system was perfused with salines applied to the entire preparation with a Zalimp pump. The salines had the following composition, in mM: 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; and 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 saline.

The data were processed on IBM PC AT computer with MICROSTAT software.

RESULTS

Thirty-three neurons were studied, 9 of them identified (cells RPa2, RPa3, LPa2, LPa3, LPa5, V1, RPa6, V4, V6). One hundred twenty intracellular recordings were made. We found that for removal of magnesium ions from the external solution, some neurons exhibited epileptic discharges with delayed depolarizations in their action potentials. Other nerve cells in the magnesium-free solution were not involved in epilepsy manifestations. The neurons studied were classified on the basis of their responses to low extracellular magnesium as active or passive after Matsumoto and Marsan [4].

In the active neurons (cells V4, V6, unidentified spontaneously active cells) a massive sustained membrane depolarization as well as typical spontaneous PDS were observed. Such PDS consisted of a rapid positive shift of the membrane potential to 15-30 mV, which triggered a train of ac-

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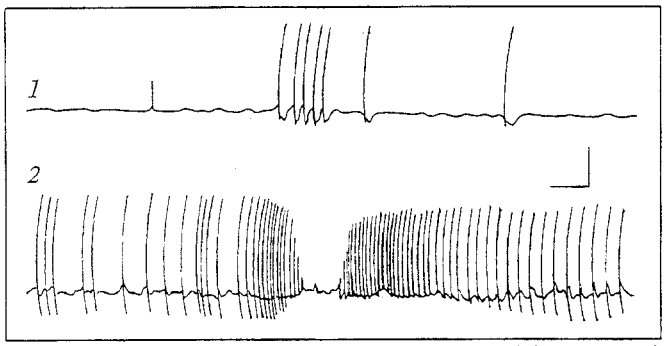

Fig. 1. Paroxysmal depolarization shifts induced by intestinal nerve stimulation. *1*) RPa3, *2*) unidentified neuron of visceral ganglion. Stimulation amplitude 15 V. Calibration: horizontal 1 sec, vertical 30 mV.

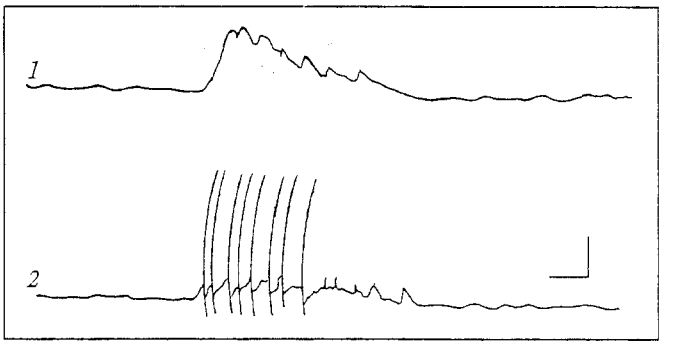

Fig. 2. Synchronized depolarization shifts in neurons *1*) LPa3 and *2*) LPa5. Calibration: horizontal 1 sec, vertical 30 mV.

tion potentials, the plateau, characterized by complete inactivation of the spike-generating mechanism, and a repolarizing phase, which in the majority of cases ended with a hyperpolarizing shift and recovery of the spike-generating mechanism and pre-PDS level of the membrane potential. The duration of PDS varied from 0.4 to 20 sec and its arithmetic mean was 2.7 sec. The frequency of spontaneous epileptic discharges was 1-5/min.

In addition, PDS in the active neurons developed in response to stimulation of both the skin surface and nerves (Fig. 1). The latent period of its appearance varied from 3 to 21 sec, and its arithmetic mean was 12.1 sec. The structure of induced paroxismal bursts was similar to the spontaneous one.

The responses of passive neurons (RPa2, RPa3, LPa2, LPa3, LPa5, and unidentified neurons) to removal of magnesium ions from the external solution differed from those of active cells. Like active neurons, passive neurons generated delayed depolarizations in their action potentials and paroxysmal depolarization shifts in their spontaneous activity. In the passive neurons depolarization shifts developed, resulting in the generation of single or multiple spikes by initially silent neurons and in a spike frequency increase in spontaneously discharging cells. However, the magnitude of these shifts was not sufficient to inactivate the mechanism of spike generation (Fig. 2). Similar epileptic bursts were recorded in the responses of passive neurons to the stimulation of their receptive field and magistral nerves. The amplitude of PDS varied from 15 to 30 mV; its arithmetic mean was 13 mV. The duration of PDS varied from 0.8 to 11.6 sec, the arithmetic mean being 5.6 sec.

The spontaneous as well as induced PDS were synchronized in neurons where synaptic connectivity has proved to be absent (Figs. 1, 2). The results obtained show that bathing the snail brain in magnesium-free solution results in the development of synchronised epileptiform discharges in synaptically unconnected nerve cells, which is a characteristic feature of epilepsies. Snail CNS preparation immersed in magnesium-free solution is a suitable model of experimental epilepsy, since *Helix* nerve cells are easy to identify.

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