ORGANIC CALCIUM ANTAGONISTS VERAPAMIL AND RIODIPINE PREVENT ELEVATION OF FREE CALCIUM LEVEL IN RAT BRAIN SYNAPTOSOMES DURING METRAZOL KINDLING

M. N. Karpova, I. M. Antonikov, O. Yu. Pankov, and R. N. Glebov

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During the development of epileptic activity (EpA) induced by various methods, intraneuronal Ca^{2+} metabolism is disturbed [2, 8, 11], and this is an important mechanism of the initiation and formation of EpA. Inflow of Ca^{2+} into nerve terminals and axons takes place mainly via voltage-dependent Ca channels. Recently, therefore, to abolish EpA, various calcium antagonists have been widely used [2]. Verapamil, nifedipine, and other calcium antagonists are powerful and selective inhibitors of the calcium current through voltage-dependent Ca channels in smooth-muscle and heart cells [6]. The effect of organic calcium antagonists and, in particular, of the 1,4-dihydropyridines on some neuronal preparations, including synaptosomes, has not yet been adequately studied [4, 9, 10, 12]. Disagreements may be due, on the one hand, to differences in the distribution of dihydropyridine-sensitive and insensitive voltage-dependent Ca channels in neurons in different brain regions, which have been studied experimentally [14], and with the tendency for their preferential localization in the neuron soma rather than in the terminals [13]. On the other hand, investigations have been conducted chiefly on neuronal tissue of normal animals, and not of animals subjected to a certain procedure which somehow or other affects activation of the Ca current [4, 9, 12]. It must also be pointed out that during epileptization the inflow of Ca^{2+} has been observed in dendrites and in the soma of neurons [15], and changes in the Ca^{2+} concentration in nerve endings have been virtually unstudied.

The aim of the investigation described below was to determine the free calcium concentration in the brain synaptosomes of rats subjected to metrazol kindling and of rats receiving calcium antagonists as well as metrazol during the kindling process: verapamil (Finoptin) and riodipine (1,4-dihydropyridine, synthesized at the Institute of Organic Synthesis, Academy of Sciences of Latvia, and marketed under the name "Foridon").

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats. The animals were kept under the ordinary animal house conditions on a standard diet. There were two series of experiments. In the experiments of series 1, kindling was induced by metrazol in a subconvulsive dose of 30 mg/kg, injected intraperitoneally daily for 28 days. The experimental animals of this series were given: verapamil (Finoptin, from Orion, Finland) (10 mg/kg, 10 rats), and riodipine (2 mg/kg, 10 rats) in dimethyl sulfoxide (DMSO). Control animals were given the same volume of sol-

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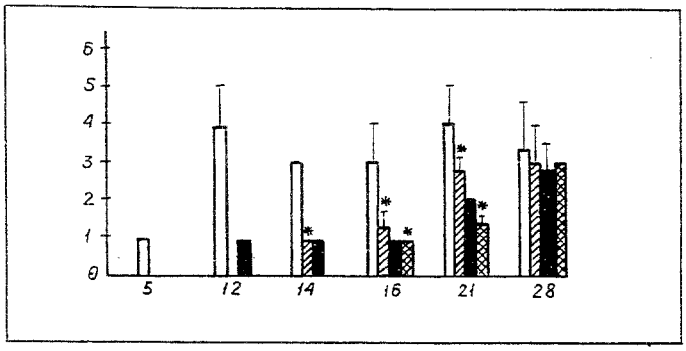

Fig. 1. Severity of convulsive reaction in rats at different times during daily administration of metrazol in a subconvulsive dose, and after preliminary injection of physiological saline and DMSO (control) and of calcium antagonists (verapamil and riodipine). Abscissa, stage of experiment (days); ordinate, average severity of convulsions (in points). Unshaded columns – physiological saline, black columns – DMSO, obliquely shaded – verapamil, cross-hatched – riodipine. *$p < 0.05$.

vent: physiological saline (10 rats) and DMSO (10 rats). Both verapamil and riodipine and the solvents were injected intraperitoneally 15 min before each injection of metrazol. The severity of the seizure reaction to metrazol was assessed daily and expressed in points: 1) head-shaking, 2) isolated clonic convulsions of the whole body, 3) a series of clonic convulsions of the whole body or clonus of the forelimbs, 4) tonico-clonic convulsions with standing up on the hind limbs (the kangaroo posture), 5) clonico-tonic convulsions with the animal falling on to its side, 6) repeated tonico-clonic convulsions and/or death of the animal. The seizure reaction in the animals of each group was evaluated as an averaged score, considering only those animals which in fact developed this reaction.

In series 2 the experimental animals (10 rats) received a single convulsive dose of metrazol (60-70 mg/kg, which caused a seizure reaction rated at 3-5 points, i.e,, similar to that found in the animals of series 1 after the end of kindling. The control animals received the same volume of physiological saline (0.1 ml).

The free Ca^{2+} concentration in synaptosomes isolated from the cerebral cortex [7] was determined with the aid of the fluorescent probe Quin 2 [1] 7 days after the last injection of metrazol during kindling (series 1) and after the single convulsive dose of metrazol (series 2) the free Ca^{2+} concentration was determined in synaptosomes isolated from the cerebral cortex [7] with the aid of the fluorescent probe Quin 2 [1]. The experimental results were subjected to statistical analysis by the method of paired comparisons and by Student's test.

EXPERIMENTAL RESULTS

In the control animals in the experiments of series 1 daily injection of metrazol and physiological saline caused the appearance of convulsions rated at 1 point on the 5th day of the injections, and in animals receiving metrazol and DMSO, on the 12th day. In the experimental animals of series 1 which received verapamil, convulsions rated at 1 point occurred after 14 injections of metrazol, and in animals receiving riodipine, after 16 injections (Fig. 1). Later the severity of the convulsions in animals of all groups increased, but the average point rating in animals receiving calcium antagonists was less than that for the corresponding group of control animals. A difference was observed until the 21st-23rd day. On the following days no difference was found in the groups of control (metrazol) and experimental (metrazol + calcium antagonists) animals (Fig. 1). Thus preliminary injection of calcium antagonists significantly delayed the development of convulsions in the presence of kindling induced by chronic administra-

TABLE 1. Free Calcium Concentration in Brain Synaptosomes of Control Rats, in Rats Subjected to Metrazol Kindling, and in Animals Receiving Verapamil or Riodipine Together with Metrazol

Experimental conditions	$[Ca^{2+}]_i$, nM	Changes, %	Number of experiments	p
Physiological saline (control)	152 (53—250)	100	12	
Metrazol	238 (78—380)	156,6	12	<0,02
Metrazol + Physiological saline (control)	257 (193—301)	162,5	6	
Metrazol + verapamil	168 (121—301)	110,5	6	<0,02
Metrazol + DMSO (control)	249 (121—366)	163,8	9	
Metrazol + riodipine	143 (100—184)	94,1	9	<0,01

Legend. $[Ca^{2+}]_i$ free calcium concentration. Range of changes in values given in parentheses

tion of a subconvulsive dose of metrazol, and it reduced the severity of the convulsions during kindling significantly. in the course of 21-23 days.

After the end of kindling an increase in the free Ca^{2+} concentration in the synaptosomes on average by 60% was found in the control animals of series 1, whereas in the experimental animals injection of verapamil and riodipine prevented this increase – the free Ca concentration virtually corresponded to its level in intact animals not subjected to kindling (Table 1).

In the experimental animals of series 2, in the postictal period after a single injection of metrazol the free Ca^{2+} concentration in the rat brain synaptosomes was indistinguishable from the control values (231 ± 37 in the experiment, 260 ± 32 nM in the control respectively).

Thus the results of this investigation indicate that organic calcium antagonists (verapamil and riodipine) lower the free Ca^{2+} concentration in brain synaptosomes of rats subjected to metrazol kindling. This effect was obtained when calcium antagonists of different nature were used (belonging to the phenylalkylamine group – verapamil, and to the 1,4-dihydropyridine group – riodipine). Our results showing an increase in the calcium concentration in the brain synaptosomes of rats subjected to metrazol kindling agree with those obtained by other workers who studied the kinetics of $^{45}Ca^{2+}$ uptake in rat brain slices after electrical kindling of the hippocampus [11].

The mechanism of the increase in the free Ca^{2+} concentration in synaptosomes of the cerebral cortex requires special study. The results are interesting in the sense that during development of a state of enhanced readiness of the brain to respond by seizures during metrazol kindling an increase in the free Ca^{2+} concentration is observed in nerve endings, and this effect, moreover, is found 7 days after the end of metrazol administration. It is thus not connected with the direct action of metrazol, as is also shown by the fact that this effect is absent after a single injection of metrazol, even in doses 2-2.5 times greater than subconvulsive. This effect is not connected with the convulsive process itself, for it does not arise after convulsions induced by a single injection of metrazol. It can be concluded from all these particular features that stable accumulation of free Ca^{2+} in synaptosomes is connected with plastic structural changes in nerve tissue, arising during chronic exposure to an epileptogen in subconvulsive doses (kindling), and maintaining a steady state of enhanced readiness of the brain to respond by convulsions. As a secondary intracellular messenger, Ca^{2+} participates in the mechanism of the most important intracellular processes, including synthesis of structural and functional proteins, and it thus makes its own contribution to the realization of pathological plastic processes which lie at the basis of chronic epileptogenesis. We know that blockers of protein synthesis (cycloheximide etc.) prevent the development of proneness to convulsions during electrical kindling of the hippocampus [5] and also prevent the preservation of enhanced proneness to convulsions during repetitive electrical stimulation of the cerebral cortex [3].

Meanwhile the Ca^{2+} antagonists studied. at least under the above-mentioned conditions of their use, did not completely prevent the development of kindling, despite a considerable fall of the free Ca^{2+} concentration in the synaptosomes. A change in calcium homeostasis is therefore not the only mechanism of plastic and long-term changes in neurons during kindling.

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