ROLE OF CALCIUM IONS IN THE DEVELOPMENT OF THE ANTICONVULSANT EFFECT OF TAURINE

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Much experimental material on the anticonvulsant action of taurine has now accumulated [3]. There have also been successful attempts to use this sulfoamino acid for the treatment of epilepsy in man, although the results are somewhat ambiguous [4]. However, the molecular mechanism of the action of taurine on excitable tissues has not yet been explained. It is likewise unknown whether taurine is an inhibitory mediator or a modulator of activity of nerve cells [11]. Meanwhile, there is indirect evidence that taurine participates in the redistribution of Ca^{++} in the intracellular compartments of heart muscle [5] as well as of an increase in the uptake of Ca^{++} by certain cell organelles in the presence of taurine [6].

Considering the important role of calcium in the generation and conduction of excitation and the importance of stabilization of intracellular membranes for elevation of the threshold of excitability of brain cells [12], the investigation described below was carried out to demonstrate correlation between the effect of taurine on the Ca-transporting system of nerve cells and its ability to induce an anticonvulsant effect.

EXPERIMENTAL METHOD

Microsomes and mitochondria were isolated from cerebral cortical cells of male Krushinskii–Molodkina and Wistar rats (weighing 120-140 g) by differential centrifugation. Equilibrium dialysis was carried out in a Teflon chamber with compartments with a volume of 0.5 cm^3, separated from the external volume by a dialysis membrane (from Sigma). Samples of 200 μl were taken every 10 min after the beginning of dialysis

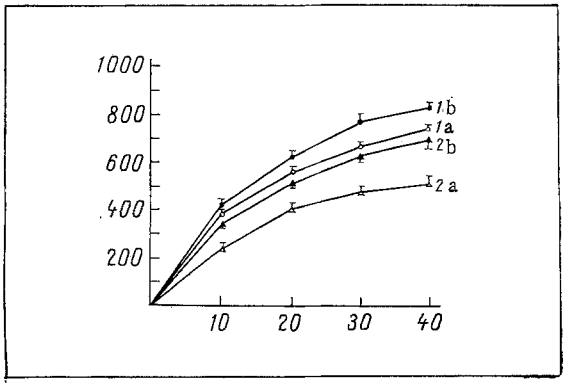

Fig. 1. Effect of taurine on uptake of $^{45}Ca^{++}$ by microsomes of cerebral cortical cells. 1) Wistar rats, a) control, b) incubation with 1 mM taurine. 2) Krushinskii–Molodkina rats; a) control, b) incubation with 1 mM taurine. Abscissa, time (in min); ordinate, radioactivity (in cpm/mg protein).

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TABLE 1. Effect of Taurine on Ca, Mg-ATPase Activity of Rat Cerebral Cortical Cells (M ± m)

Line of rats	Microsomes		Mitochondria	
	control	1 mM taurine	control	1 mM taurine
Wistar	2,1±0,4	3,6±0,3	7,4±1,1	7,6±0,3
Krushinskii–Molodkina	1,2±0,2	2,4±0,4	7,0±0,8	7,0±0,9

from the inner volumes. The initial radioactivity of the external solution was 0.1 μCi $^{45}Ca^{++}$/100 ml. Dialysis was carried out in 0.05 M Tris-HCl, pH 7.2. A control of passive diffusion was set up simultaneously. The quantity of calcium taken up was estimated from the difference between the radioactive samples containing and not containing microsomes. Radioactivity was measured on a "Nuclear Chicago" scintillation counter. The efficiency of counting was 80%. Activity of Ca-activated, Mg-dependent ATPase, expressed in μmoles P_i/mg protein/15 min of incubation, was determined by Eibl's method [7]. The composition of the incubation medium was: 23 mM Tris-HCl; 5 mM $MgCl_2$; 2.5 mM ATP; 0.15 mM ouabain; 1 mM EGTA. The experiments to study the effect of taurine on the development of convulsions were carried out on three male rabbits weighing 3-5.5 kg, with chronically implanted monopolar electrodes in the sensomotor and visual areas of the cortex and bipolar electrodes in the dorsal hippocampus, by the method described previously [2]. An epileptogenic focus was produced by injection of a solution of the sodium salt of benzylpenicillin in a dose of 500 units in a volume of 1 μl through a chemical electrode by the method of Borodkin et al. [1]. Solutions of the substances to be studied were injected in volumes of not more than 2 μl through the same chemical electrode. The EEG was recorded on an "Orion" electroencephalograph.

EXPERIMENTAL RESULTS

The course of uptake of labeled Ca^{++} by cortical microsomes was investigated by equilibrium dialysis in Krushinskii–Molodkina rats, genetically predisposed to audiogenic convulsions (AC), and in Wistar rats, in which AC practically never arise. As a first step, observations by other workers that taurine has no effect on passive transport of $^{45}Ca^{++}$ through an artificial membrane [6] were confirmed. It will be clear from Fig. 1 that uptake of $^{45}Ca^{++}$ by microsomes in rats with AC was less than in normal animals. Taurine intensifies binding of $^{45}Ca^{++}$ by cortical microsomes of animals of both groups. It was shown previously [13] that Ca-ATPase activity of membrane fractions of brain cells from DBA/2N mice with AC is reduced. Our own observations on Krushinskii–Molodkina rats (Table 1) show that a disturbance of the Ca-transporting system may be common to different lines and species of animals with AC. It is interesting to note that taurine stimulates Ca, Mg-ATPase only in microsomes and does not affect the activity of this enzyme in mitochondria (Table 1).

The results, on the one hand, confirm yet again the role of the endoplasmic reticulum in regulating the intracellular calcium concentration [10] and, on the other hand, they demand a search for confirmation of the role of Ca^{++} in the manifestation of the inhibitory action of taurine on the CNS in experiments in vivo. One such attempt was undertaken by Izumi et al., who showed that a general fall in the concentration of bivalent ions in experimental animals is accompanied by weakening of the anticonvulsant action of taurine, but has hardly any effect on the anticonvulsant effect of GABA [8]. In the present experiments rabbits with implanted electrodes and chemical electrodes were used, by means of which microinjections of penicillin into the region of the hippocampus evoked epileptiform discharges in deep and superficial structures of the brain, accompanied by convulsions (Fig. 2a). Injection of 2 μl 1 mM taurine through the chemical electrode 10 min before injection of penicillin completely prevented the development of fits (Fig. 2b). When a convulsant dose of penicillin was injected after taurine the EEG was indistinguishable from normal (Fig. 2b). However, if taurine was injected together with 1 mM EGTA, a specific Ca^{++} chelating agent, no anticonvulsant effect was observed (Fig. 2c). This is evidence that free Ca^{++} ions are necessary for the inhibitory function of taurine. EGTA itself, incidentally, does not have the property of inducing convulsions. Furthermore, neither taurine nor EGTA affect the normal EEG. Since convulsant activity largely depends on the calcium concentration in nerve cells [9], it can be postulated that taurine, a natural regulator of the intracellular distribution of this ion, determines its own anticonvulsant effect by facilitating mobilization of additional amounts of Ca^{++} in the appropriate parts of membrane structures, thereby raising the threshold of excitability of the nerve cells. A search for taurine receptors, the possible existence of which is being intensively discussed [11], and the study of the likely agonists and antagonists of taurine may lead in the future both to a more detailed understanding of the

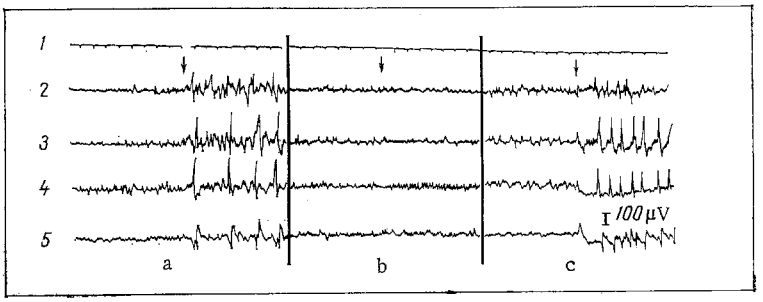

Fig. 2. Effect of taurine on EEG of rabbit during development of fits provoked by microinjection (arrow) of penicillin solution into dorsal hippocampus of left hemisphere. a) 10 min after injection of 500 units penicillin, b) after microinjection of 500 units penicillin preceded by injection of 1 mM taurine 10 min beforehand, c) after injection of 500 units penicillin preceded by injection of a mixture of 1 mM taurine and 1 mM EGTA 10 min beforehand. 1) Time marker (each interval 1 sec), 2) sensomotor cortex of right and left hemispheres, 3) visual cortex of right and left hemispheres, 4) dorsal hippocampus of right hemisphere, 5) dorsal hippocampus of left hemisphere.

mechanism of its action and to the optimization of the use of this natural compound as a therapeutic preparation.

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