POSSIBLE ROLE OF CALCIUM IN REGULATION OF RNA SYNTHESIS BY BRAIN TISSUE CELL NUCLEI

N. I. Razumovskaya, S. A. Dambinova, M. N. Demina, and L. V. Govorova

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The effect of impulse generation on activity of the genetic apparatus of nerve and muscle cells is now a firmly established fact. Data obtained in recent years show that Ca^{++} ions participate in the coupling of electrical and metabolic processes in cells with excitable membranes [4, 7]. Ca^{++} ions are known to play an important role in the cyclic nucleotide system which transmits hormonal and nervous signals from the outer cell membrane to the internal structures of the cell, including the nucleus [9, 12]. Hypotheses on the role of Ca^{++} in the regulation of transcription during functional activity of nerve cells were expressed previously [2]. The basis for these hypotheses was the selective effect of Ca^{++} on RNA synthesis observed after microinjections of K^+, Na^+ and Ca^{++} inside giant neurons of Limnea stagnalis [2] and the presence of a definite optimum of Ca^{++} concentration added to isolated brain cell nuclei for the level of their RNA-synthesizing activity [6]. In this connection it was interesting to determine whether changes in the state of the excitable membrane of the cell are reflected in the endogenous Ca^{++} distribution in the intracellular structures and, in particular, in its concentration in the cell nuclei. It was also important to establish how changes in the Ca^{++} concentration affect RNA synthesis. These data would allow Ca^{++} to be more confidently identified as an intracellular agent which acts as mediator between the cell membrane and the genetic structures of the cells.

The writers found previously that a few hours after denervation of the rabbit gastrocnemius muscle there is a sharp fall in both RNA-synthesizing activity and in Ca^{++} concentration in the nuclei of the muscle fibers. Electrical stimulation of the denervated muscle restored both values. Addition of exogenous Ca^{++} to a suspension of nuclei activated RNA synthesis in the nuclei of the denervated muscle and inhibited it in nuclei of intact and stimulated muscles [5]. It was concluded that in order to maintain the normal level of transcription in cell nuclei, a certain optimal Ca^{++} concentration must be maintained; this concentration evidently depends on the state of the outer excitable membrane of the muscle fiber.

Data on the existence of similar correlation in brain cells will be found in this communication. Cell nuclei from the cerebral cortex and hippocampus of Wistar rats and of rats with genetically determined predisposition to audiogenic convulsions (the Krushinskii–Molodkina line) were used as the model.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar and Krushinskii–Molodkina rats weighing 200-250 g. The Krushinskii–Molodkina rats were investigated both during the tonic phase of epileptiform activity and in a state of quiet wakefulness. An epileptiform fit was evoked by the ringing of a bell with intensity 60 dB for 5 sec. Isolated cell nuclei were obtained by the method of McEwen et al. [11]. The purity of the preparations was verified in the light microscope after staining with toluidine blue. The DNA concentration was determined by Burton's method [8] and protein by Lowry's method [10]. The DNA/protein ratio in the nuclear preparations was 1:10.

RNA synthesis was judged from the quantity of ^3H-UTP incorporated into the acid-insoluble residue after incubation of the nuclei for 10 min at 37°C in medium of the following composition (in mM): Tris-HCl,

Laboratory of Biochemistry of Memory, Department of Pharmacology of Memory and Behavior, Biochemical Group, Department of Human Neurophysiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. Laboratory of Physiology of the Cerebral Circulation, A. L. Polonov Institute of Neurosurgery, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 677-679, June, 1981. Original article submitted January 14, 1981.

TABLE 1. Effect of Ca^{++} on Incorporation of ^3H-UTP (in cpm/mg DNA) into Rat Brain Cell Nuclei (M ± m)

Test object	Without additions	0.1 mM Ca^{2+} added	Activation or inhibition (-) under the influence of Ca^{2+}, %
Wistar rats (n = 6):			
Cortex	599 598	407 463	—33*
Hippocampus	299 597	408 039	+38*
Krushinskii - Molodkina rats:			
in a resting state (n=5)			
Cortex	736 350	485 583	—33*
Hippocampus	362 533	694 609	+91*
In a convulsive state (n=5)			
Cortex	341 922	539 859	+43*
Hippocampus	300 664	463 731	+37*

*P < 0.05.

TABLE 2. Calcium Concentration in Rat Brain Cell Nuclei (M ± m)

Test object	Calcium concentration, nmoles/mg protein	
Wistar rats (n=6):		
Cortex	2004±414	
Hippocampus	1286±140	P_1<0,05
Krushinskii - Molodkina rats:		
in a resting state (n=3)		
Cortex	3451±122	
Hippocampus	2414±194	P_1<0,05
In a convulsive state		
Cortex (n = 7)	1272±236	P_2<0,05
Hippocampus (n = 3)	1601±402	P_2<0,05

Legend. P_1) Differences between cortex and hippocampus, P_2) differences compared with resting state.

pH 8.0, 40, KCl 70, $MgCl_2$ 8, $MnCl_2$ 2, dithiothreitol 40, ATP 1, GTP 1, CTP 1, ^3H-UTP 0.15 (2 μCi), and a suspension of nuclei containing 20-30 μg DNA. At the end of the reaction the samples were placed on ice and treated with an equal volume of cold 10% TCA with 0.015 M sodium pyrophosphate and 0.2 ml of 1% serum albumin as carrier. After the samples had been washed three times with 5% TCA the residues were dissolved in 5% KOH.

Radioactivity was counted in a standard scintillation mixture containing Methylcellosolve on a Nuclear Chicago (USA) counter. Incorporation of label was expressed in cpm/mg DNA.

The content of endogenous Ca^{++} in the nuclei was determined as described previously [5]. Statistical analysis was carried out by the difference method [1].

EXPERIMENTAL RESULTS

Investigation of the RNA-synthesizing activity of rat brain cell nuclei showed that the level of incorporation of ^3H-UTP into nuclei of the cerebral cortex was significantly higher than into the hippocampal nuclei (Table 1). This relationship was found both in Wistar rats and in Krushinskii–Molodkina rats when studied in a state of quiet wakefulness; meanwhile in the latter both cortical and hippocampal cell nuclei had a rather higher level of activity than cell nuclei from the corresponding zones of the Wistar rat brain, in agreement with data obtained by other workers who found a higher RNA concentration in the brain of these animals [3].

Epileptiform fits lead to a decrease in RNA synthesis in the cell nuclei. This decrease was much greater in cell nuclei from the cerebral cortex than in those from the hippocampus. Unlike nuclei from the cortex and hippocampus in the waking state, nuclei from these parts of the rat brain during epileptiform activity responded to addition of 0.1 mM Ca^{++} by activation of RNA synthesis. Yet another interesting feature, regularly observed, may be noted. Ca^{++} ions inhibit nuclei with higher ability to incorporate ^3H-UTP and increase incorporation of label into nuclei with a low initial level of activity, i.e., they exhibit the same rule as was observed with skeletal muscle nuclei [5]. Direct determination of the endogenous calcium content in rat brain cell nuclei showed that fluctuations in RNA-synthesizing activity, just as in muscle, correlate with fluctuations in calcium content (Table 2).

The calcium concentration in hippocampal cell nuclei of Wistar rats was much lower than in the cerebral cortex. The same relationship also was found in Krushinskii–Molodkina rats in a quiet state, but the absolute calcium content was higher in their brain cell nuclei than in those of Wistar rats. Audiogenic convulsions, just as in the case of RNA-synthesizing activity, had a stronger influence on cortical than on hippocampal cell nuclei. A fall in the endogenous calcium content in cortical cell nuclei was accompanied by manifestation of their ability to increase incorporation of ^3H-UTP under the influence of exogenous Ca^{++}.

It is difficult at present to explain the cause of the decrease in calcium content in brain cell nuclei as a result of an epileptiform fit, for the mechanisms regulating the calcium concentration in the nucleus have not yet been explained. An essential factor in the development of convulsions is known to be destabilization of cell membranes and, in particular, a decrease in their ability to bind Ca^{++}. It may be that loss of Ca^{++} by the nuclei is associated with this membrane defect.

The results suggest that a change in the Ca^{++} concentration in the cell nuclei is one of the factors which regulate RNA synthesis in the cells of the brain in its various functional states.

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