Moderate Hypobaric Hypoxia Modifies Ca^{2+}-Mediated Glutamatergic Signal Transduction in Rat Cerebral Cortex

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Translated from *Byulleten' Experimental'noi Biologii i Meditsiny*, Vol. 147, No 2, pp. 144-147, February, 2009
Original article submitted September 4, 2007

Examination of fluorescent Ca^{2+}-responses to stimulation by ionotropic glutamate receptor agonists in rat cerebral cortex slices, preconditioned with hypobaric hypoxia, has demonstrated different time course for up-regulation of their Ca^{2+}-conductivity and for stimulation of the consequent binding of the Ca^{2+} which entered the cell. The evident result is the development of AMPA-mediated moderate increase of intracellular Ca^{2+}.

Key Words: *hypobaric hypoxia, glutamate, calcium, preconditioning*

The phenomenon of "ischemic/hypoxic" brain tolerance consists in an enhanced resistance of neurons in the brain to consequent effects of stronger forms of hypoxia or ischemia [1,2,4,6-9] after an exposure to moderate hypoxia, and is being intensively studied during the last decade. The methodological procedure which initiates such a tolerance is known as "hypoxic preconditioning". It was proved that there exist an early and a late mechanism of induced hypoxic tolerance which are expressed respectively during the first tens of minutes or first hours after the preconditioning stimulus [1]. We previously demonstrated that the early tolerance in rat piriform cortex slices, induced by a 2-minute anoxia, is stipulated by a stable moderate activation of NMDA-receptors [9].

In this study we aimed to find out whether the ionotropic glutamate receptors are involved in mechanisms of expression of long-term hypoxic brain tolerance caused by an acute moderate hypobaric hypoxia. For this purpose we studied the time course of intracellular concentration of free and bound calcium, induced by application of ionotropic glutamate receptor agonists (AMPA and NMDA) on brain slices from intact and preconditioned rats.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 190-210 g which were kept in a flow altitude chamber at 360 mm Hg for 2 h (corresponds to an altitude of 5000 m above sea level). This procedure was repeated 3 times with 24-h intervals. The conditions of hypobaric preconditioning were chosen according to previous findings which demonstrated its high neuroprotective efficacy [3,7]. 24 h after the completion of preconditioning animals were decapitated and tangential survival slices of piriform cortex were prepared. Brain slices from animals not subjected to preconditioning were used as control. Brain slices (400 μm) were prepared on an EMS-4000 vibrotome (Electron Microscopy Sciences) and were placed into a flow incubation medium at 37.5°C. Changes in the content of intracellular bound calcium (Ca-c) were studied using chlortetracycline fluorescence probe (Sigma) and a LYuMAM-K contact fluorescent microscope (LOMO) equipped by photodiode illuminator L2523UVC (Kingbright) and a fiber-optic spectrometer AvaSpec-2048 (Avantes B.V.Eerbeek). The fluorescence quantum yield was measured in a spectral region with a maximum of 522 nm, excitation peak wave length 396 nm. Changes in free intracellular calcium (Ca'') content were detected using fura-2AM fluorochrome (Mo-

0007-4888/09/1472-0185

lecular Probes) and a spectrofluorimeter Hitachi F-2000 (Hitachi). The ratio between fluorescence signals with maximum at 510 nm during excitation at 340 nm and 380 nm was used as calculated characteristic of Ca'' level changes. This technique was described in detail previously [9]. Sodium L-glutamate (L-Glu) application in concentration 50 μM was used for non-specific adequate stimulation of the whole pool of glutamate receptors on the slice. 2-minute application was repeated twice with a 40-min interval. Stimulation of two types of ionotropic glutamate receptors (NMDA and AMPA) was performed using paired application of corresponding specific agonists: N-methyl-D-aspartate in final concentration 100 μM, in magnesium-free solution containing glycine for 15 min and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) in final concentration 50 μM for 2 min. Obtained results are shown on the graphs as series of mean percent of fluorescent signal±SEM and in the table in the form of integral calcium load during the fixed post-stimulus interval [5]. The results were analyzed by analysis of variance (ANOVA) using Dunnett test.

RESULTS

L-Glu and specific ionotropic glutamate receptor agonists application produced positive shifts of free calcium as well as fixed Ca^{2+} level on slices from control animals. The first index was characterized by restitution of Ca" level in the course of agonist washing off. The second index demonstrated a stable shift in the Ca-c level even upon washing off. Repeat exposure to the same doses of L-Glu and AMPA induced Ca"- and Ca-c responses of lower amplitude (Figs. 1, 2). At the same time the amplitude of repeat responses to NMDA application did not practically differ from the primary responses (Fig. 3). Comparative analysis of both types of calcium responses demonstrates the time course of extracellular Ca^{2+}entrance into neuronal cells and its accumulation in the intracellular depot.

Substantially increased Ca-c responses to the same agonists doses were observed in the slices from preconditioned animals. Upon that it was noted that while both L-Glu and NMDA exposures produced increased Ca-c responses, in case of AMPA application only the repeat response was increased as compared to control. Data analysis has shown that non-selective receptor stimulation by L-Glu of AMPA application invoke increase of both types of responses (Figs. 1, 2). At the same time NMDA produced only the increased Ca-c response, and the Ca"-response was not altered as compared to control (Fig. 3). Data translation into integral format and corresponding statistical analysis makes it possible to assess the significance of differences in implementation of ionotropic receptor Ca^{2+}-conductive function and also to estimate the capacity of systems which bind the entered Ca^{2+} (Table 1).

Data analysis suggests that hypobaric preconditioning enhances Ca-conductive properties of ionotropic glutamate receptors stimulated by agonists. Whereas Ca^{2+} entrance mediated by NMDA receptors is fully compensated by Ca^{2+}-binding systems, then the AMPA-mediated Ca^{2+} influx evidently produces a stable moderate increase of the intracellular

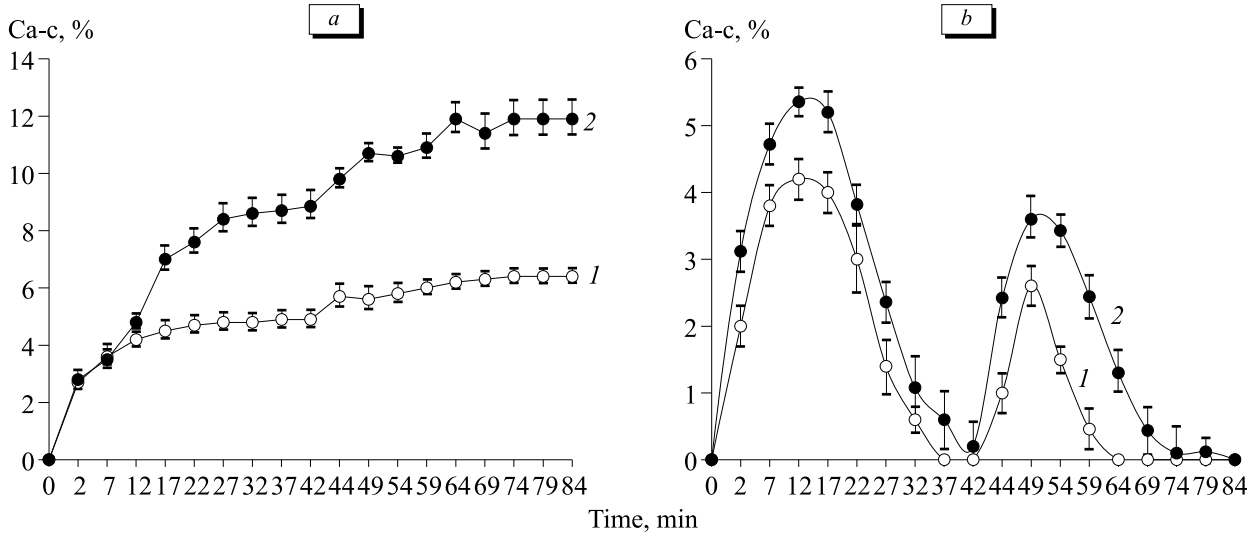

Fig. 1. Change of Ca-c (*a*) and free Ca^{2+} (*b*) levels in response to repeated (0-2 and 42-44 min) L-glutamate application on slices from control (*1*) and preconditioned (*2*) animals.

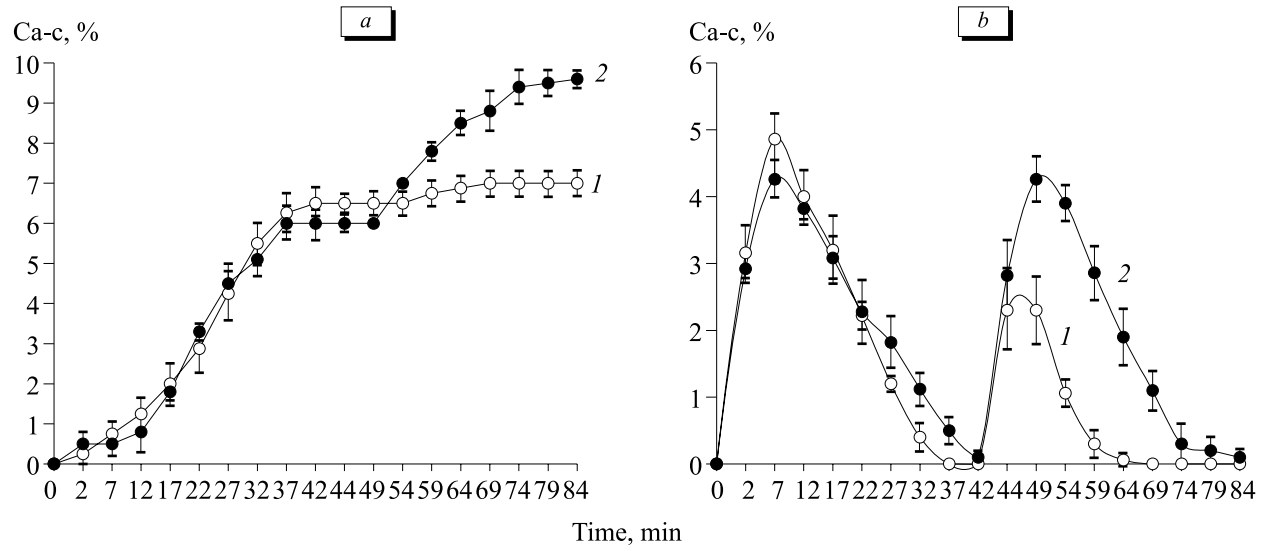

Fig. 2. Change of Ca-c (*a*) and free Ca^{2+} (*b*) levels in response to repeated (0-2 and 42-44 min) AMPA application on slices from control (*1*) and preconditioned (*2*) animals.

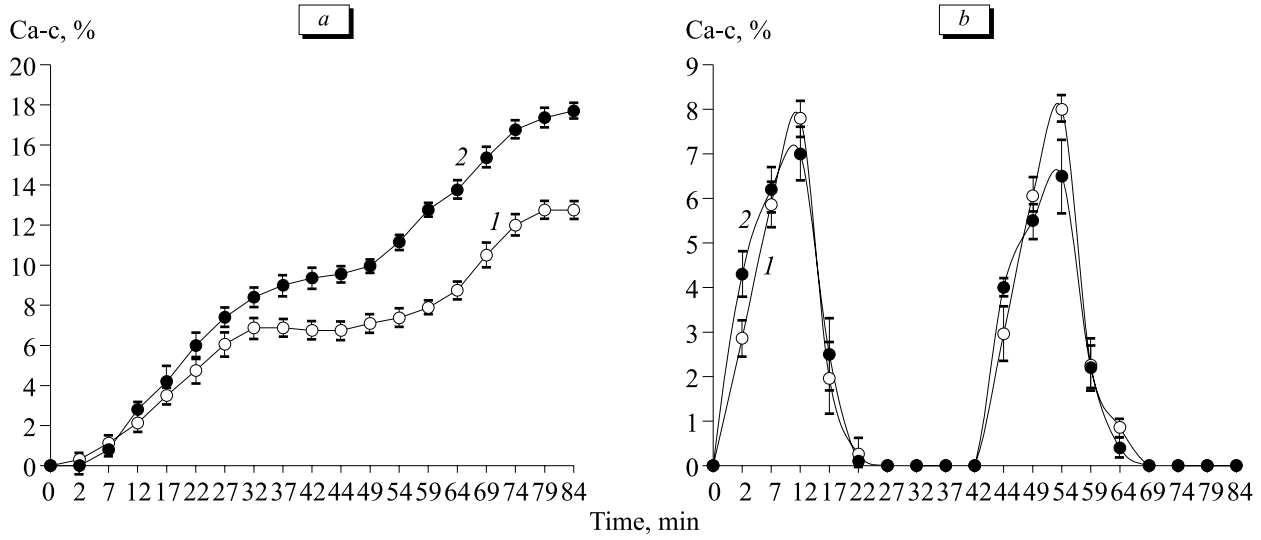

Fig. 3. Change of Ca-c (*a*) and free Ca^{2+} (*b*) levels in response to repeated (0-15 and 45-60 min) NMDA application on slices from control (*1*) and preconditioned (*2*) animals.

TABLE 1. Time course of integral Ca-c and Ca" load in response to repeated agonist application (rel. units)

Agonist, response		Ca-c		Ca''	
		control	PH	control	PH
L-glutamate	1st response (0-42 min)	35.8±1.1	52.4±1.7*	19.0±0.9	26.3±2.2*
	2nd response (42-84 min)	11.3±0.7	17.0±1.5*	5.6±0.2	14.5±1.9*
AMPA	1st response (0-42 min)	32.0±2.7	28.4±0.9	19.0±1.6	20.0±2.1
	2nd response (42-84 min)	5.9±0.8	20.0±1.6*	6.0±1.2	17.4±1.8*
NMDA	1st response (0-42 min)	37.0±2.5	49.8±1.8*	20.1±1.3	20.1±2.5
	2nd response (42-84 min)	26.1±1.8	42.0±2.0*	18.7±0.95	18.2±1.1

Note. PH — preconditioning. *$p<0.02$ compared to the control.

Ca^{2+} level. This effect can induce or maintain the development of long-term hypoxic tolerance; mechanisms of this tolerance are known to be coupled with Ca^{2+}-mediated gene expression and enhanced protein and peptide synthesis [1,2,8].

The work was supported by the Russian Foundation for Basic Research (grant No. 07-04-00664).

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