DTNB Inhibits Calcium Response of Rat Brain Cortical Slices to Anoxia of Various Duration

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The effects of DTNB on changes in intracellular Ca^{2+} content in slices of rat cortex induced by 2- or 10-min anoxia were studied fluorometrically. DTNB (200 μM) prevented excessive intracellular Ca^{2+} accumulation during and after 10-min anoxia and moderate increase in Ca^{2+} content induced by 2-min anoxia. Our results suggest that redox sites of NMDA receptors participates in both pathogenic and adaptive Ca^{2+}-mediated processes activated by anoxia.

Key Words: *hypoxia; calcium; NMDA receptor; DTNB*

According to modern neuropathological concept, hyperactivation of glutamate NMDA receptors plays a key role in the development of acute brain injuries (ischemia, trauma, and epilepsy) and chronic neurodegenerative diseases. These conditions are accompanied by calcium overload of brain cells resulting in disorganization of intracellular regulatory processes. In view of this, the search for new pharmacological preparations preventing hyperactivation of NMDA receptors is an important clinical problem. Ten-year experimental and clinical experience of using highly specific NMDA receptor antagonists as neuroprotectors during epilepsy and hypoxia showed that their positive effect (prevention of calcium overload) is often accompanied by impairment of physiological mechanisms underlying neuronal synaptic plasticity, which is manifested in various neurological disorders. Elaboration of mild antagonists blocking only pathological hyperactivation of NMDA receptors is therefore of considerable importance. The preparations oxidizing sulfhydryl groups of NMDA channel subunits forming the so-called redox site of NMDA receptors hold much promise in this respect. Reducing compounds affecting this modulatory complex promote opening of channels and entry of extracellular Ca^{2+}. Oxidizing compounds, including 5-5'-dithio-bis(2-nitrobenzoic acid) (DTNB), produce opposite effects [6,7,12]. DTNB prevents injuries during kainate-induced epilepsy in experimental animals [8-10]. Previous experiments showed that DTNB blocks long-term potentiation of synaptic transmission in hippocampal slices induced by short-term ischemia [6].

Here we evaluated the role of redox sites of NMDA receptor in 2 mechanisms underlying accumulation of intracellular Ca^{2+}: pathological calcium overload and moderate adaptive increase in cytoplasmic Ca^{2+} concentration (Ca^{2+}_{cyt}) induced by long-term and short-term anoxia, respectively [2]. These mechanisms were previously studied during measurements of Ca^{2+} content (Ca^{2+}_{cyt} and Ca^{2+} bound to hydrophobic intracellular domains, Ca-b) [3,5,11].

MATERIALS AND METHODS

Experiments were performed on 400-μ slices of the visual cortex from Kyoto-Wistar rats prepared on a 752 Vibroslice microtome (Campden Instruments). The slices were incubated in a standard oxygenated solution at 37.5°C [1]. The concentrations of Ca-b and Ca^{2+}_{cyt} were measured spectrofluorometrically using a LYuMAM KF contact fluorescent microscope and Hitachi F-2000 spectrofluorometer, respectively, in a 2-chamber incubation system [4]. Chlortetracycline flu-

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orescence probe (CTC, Sigma) was used to produce fluorescence signals characterizing the dynamics of Ca-b content. We measured the quantum yield of fluorescence at 520 nm after excitation with 410-nm light. The relative changes in Ca^{2+}_{cyt} content were recorded using a Fura-2/AM fluorochrome (Molecular Probes). The ratio between fluorescence signals with maximum of 510 nm after irradiation with 340 and 380 nm. Both methods were described previously [11]. Control measurements showed that DTNB solutions (200 μM) are characterized by intensive light absorption at 300-400 nm. Therefore, the presence of DTNB in the incubation solution markedly hinders fluorometry under these regimens. However, modification of redox sites in NMDA receptors produced by oxidizers and reducers persists for a long time after washout [7,10,12]. In our experiments brain slices were perfused with DTNB for 30 min, without DTNB for 60 min, and then subjected to 2- or10-min anoxia followed by 70-min reoxygenation. The data are presented as *M*±*SEM*. The results were analyzed by ANOVA using the Dunnett test.

RESULTS

DTNB markedly changed the dynamics of Ca^{2+}_{cyt} content in slices subjected to 10-min hypoxia: it inhibited the increase in Ca^{2+}_{cyt} content during anoxia and prevented its secondary rise after 25-min reoxygenation compared to the control (Fig. 1, *a*). DTNB had no effect on the dynamics of Ca-b content during anoxia, but 2-fold attenuated the posthypoxic increase in this parameter (Fig. 1, *b*). These changes in Ca^{2+}_{cyt} and Ca-b contents suggest that DTNB-induced oxidation of NMDA receptor redox sites blocks enter of extracellular Ca^{2+} during 10-min anoxia and after 25-min reoxygenation. Therefore, the contribution of Ca^{2+}-binding intracellular sequesters into compensatory elimination of excessive Ca^{2+}_{cyt} decreases.

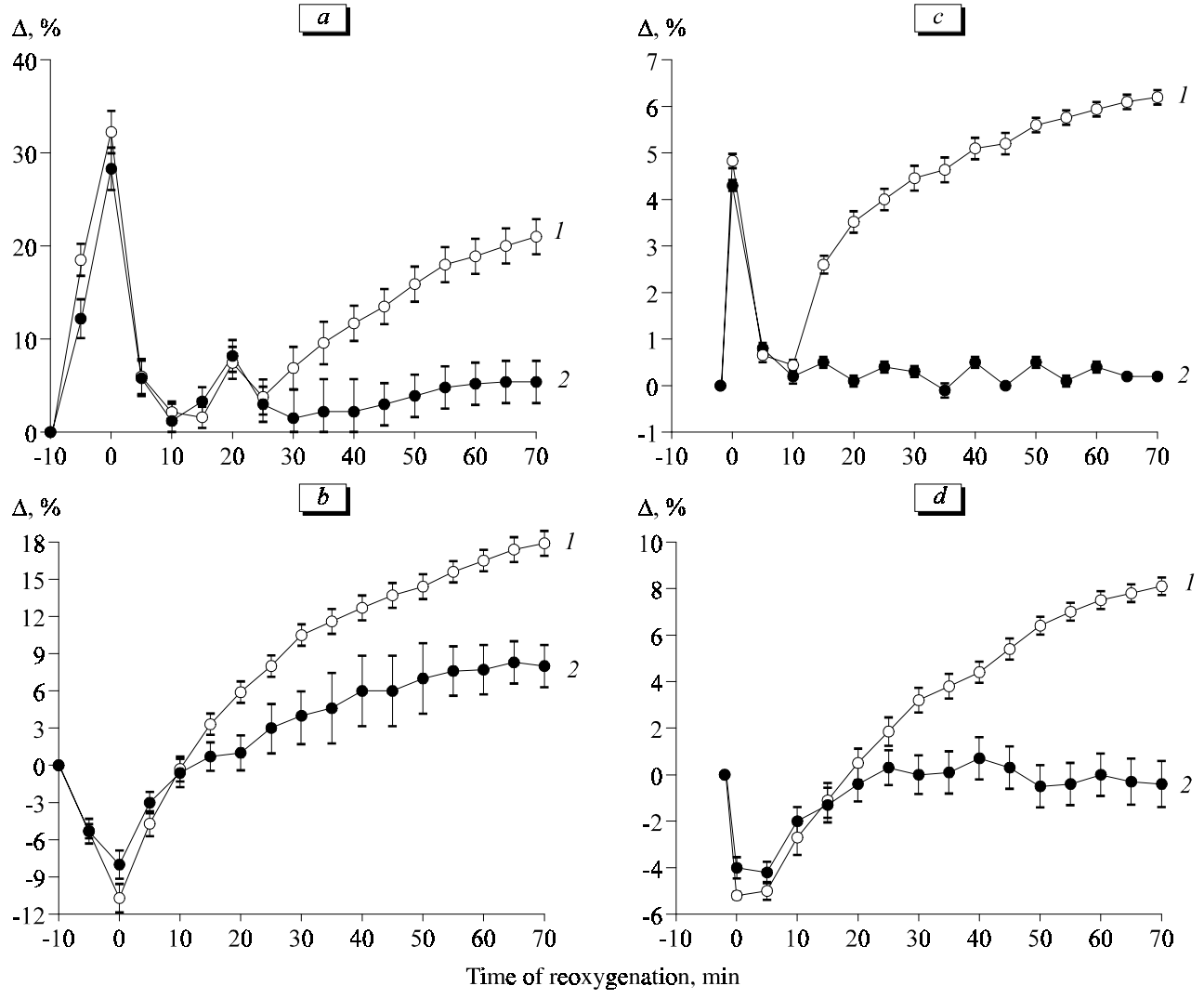

Fig. 1. Content of cytoplasmic Ca^{2+} (*a*, *c*) and calcium bound to hydrophobic intracellular domains (*b*, *d*) during 10- (*a*, *b*) and 2-min anoxia (*c*, *d*) in control (*1*) and DTNB-treated slices (*2*). Negative values on abscissa correspond to anoxia; zero point: start of reoxygenation.

Our previous studies showed that short-term anoxia (2 min) was accompanied by Ca-b release and that this process, but not Ca^{2+} entry was responsible for slight increase in Ca^{2+}_{cyt} content during short-term anoxia. During reoxygenation Ca^{2+} entry through NMDA channels is activated [3]. In the present study, treatment with DTNB before short-term anoxia had no effect on anoxia-induced changes in intracellular Ca^{2+} content, but completely blocked posthypoxic NMDA receptor-mediated Ca^{2+} entry (the contents of Ca^{2+}_{cyt} and Ca-b before and after anoxia were the same, Fig. 1, *d*).

These findings suggest that redox-modulating sites of NMDA receptors are involved in hyperactivation and moderate activation of glutamatergic signal transduction induced by long-term and short-term anoxia, respectively. Thus, oxidizers of NMDA receptor redox sites produce a neuroprotective effect associated with prevention of calcium overload. On the other hand, these agents can suppress adaptive mechanisms triggered by moderate activation of intracellular calcium-dependent regulatory processes underlying hypoxic preconditioning.

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REFERENCES

1. M. I. Mityushov, N. A. Emel'yanov, A. A. Mokrushin, *et al*., *Brain Slices in Neurophysiological and Neurochemical Assays* [in Russian], Leningrad (1986).
2. M. O. Samoilov, *Brain and Adaptation* [in Russian], St. Petersburg (1999).
3. M. O. Samoilov, A. A. Mokrushin, D. G. Semenov, *et al*., *Vestn. Ros. Akad. Med. Neuk*, No. 9, 32-39 (2000).
4. D. G. Semenov, *Ros. Fiziol. Zh*., **85**, No. 8, 1121-1125 (1999).
5. D. G. Semenov, E. I. Tyul'kova, M. O. Samoilov, and E. V. Lazarevich, *Ibid*., **85**, No. 1, 139-148 (1999).
6. H. Gozlan, D. Diabira, P. Chinestra, and Y. Ben-Ari, *J. Neurophysiol*., **72**, No. 6, 3017-3022 (1994).
7. J. Lazarewicz, J. Wroblewski, M. Palmer, and E. Costa, *Neurosci. Res. Commun*., No. 4, 91-97 (1989).
8. O. Quesada, J. Hirsch, Y. Ben-Ari, and C. Bernard, *Neurosci. Lett*., **212**, No. 3, 171-174 (1996).
9. O. Quesada, J. Hirsch, H. Gozlan, *et al*., *Epilepsy Res*., **26**, No. 2, 2409-2417 (1997).
10. R. M. Sanchez, C. Wang, G. Gardner, *et al*., *Neuroscience*, **20**, No. 6, 2409-2417 (2000).
11. D. Semenov, M. Samoilov, P. Zielonka, and J. Lazarewicz, *Resuscitation*, **44**, 207-214 (2000).
12. T. Yamakura and K. Shimoji, *Progr. Neurobiol*., **59**, 279-298 (1999).