Changes in the Steady-State Potential in Rats with Focal Cerebral Ischemia Receiving Cyclopentyladenosine

G. Z. Sufianova, S. E. Murik, L. A. Usov, A. A. Sufianov*, A. G. Shapkin, and M. V. Taborov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 12, pp. 653-656, December, 2003
Original article submitted February 18, 2002

Brain function and neuroprotective activity of cyclopentyladenosine in rats with focal cerebral ischemia were evaluated by recording the steady-state potential. Cerebral ischemia was modeled by intravasal occlusion of the left internal carotid and middle cerebral arteries and bilateral occlusion of the common carotid arteries. Recording of the steady-state brain potential during experimental ischemia allowed identifying the development of ischemic depolarization by a negative potential shift. Changes in the steady-state potential after cyclopentyladenosine administration reflected delayed development of ischemic depolarization in the nervous tissue. Cyclopentyladenosine holds much promise for the protection of nerve cells from ischemic injury.

Key Words: *A1 receptor agonist cyclopentyladenosine; ischemia; brain; rats; steady-state potential*

Study of brain function by recording spontaneous bioelectric activity is extensively used in experimental neurophysiology and medicine. Slow continuous potentials of the millivolt range attracted much attention in recent years. The steady-state potential (SSP) of the brain is a slowly fluctuating potential of the millivolt range that reflects membrane potentials of neurons, glia, and brain-blood barrier. Changes in SSP characterize the functional and metabolic state of brain structures.

In the present work brain function and neuroprotective activity of cyclopentyladenosine (CPA) during focal cerebral ischemia were evaluated by recording SSP.

MATERIALS AND METHODS

Experiments were performed on 27 male and female rats weighing 150-200 g. The animals were narcotized by intraperitoneal injection of sodium ethaminal in a dose of 50 mg/kg. The rats were divided into 2 groups. Physiological saline (0.9% NaCl) was administered into the right cerebral ventricle in control animals (n=12). Experimental rats (n=15) received 25 μg/kg CPA. CPA and physiological saline (10 μl) were introduced 60 min before modeling of ischemia.

Cerebral ischemia was modeled by intravasal occlusion of the left internal carotid and middle cerebral arteries and bilateral occlusion of the common carotid arteries [4].

SSP were recorded on a 4-channel DC-amplifier (input resistance 1 mΩ). AgCl electrodes were implanted epidurally above the right and left frontal and parietal regions of the cerebral cortex. Experiments were performed 3 days after implantation of electrodes. Recording of SSP started after falling asleep and continued for 1 min at 1-2-min intervals. The duration of cerebral ischemia was 1 h. SSP were recorded for 15-20 min after removal of the occluder.

The results were analyzed by Mann—Whitney U-test and Student's t test.

RESULTS

The introduction of the occluder into the middle cerebral artery induced a large negative shift of SSP in the

Department of Pharmacology, Irkutsk State Medical University; *Department of Childhood Neurosurgery, Irkutsk Regional Children's Hospital.
Address for correspondence: neuro@medk.baikal.ru. Sufianova G. Z.

0007-4888/03/1366-0576$25.00

left frontal and parietal cortex of control rats (Fig. 1). SSP in the frontal and parietal cortex decreased by 34 ($p<0.001$) and 40% ($p<0.001$), respectively. Small negative shift of SSP in the right parietal cortex (by 10%, $p<0.01$) was accompanied by its considerable increase in the frontal cortex (by 40%, $p<0.001$). Opposite changes in SSP were revealed after removal of the occluder and onset of reperfusion. SSP in the frontal and parietal cortex of the left hemisphere increased by 43 ($p<0.05$) and 40% ($p<0.05$), respectively, over 16 min. SSP in the right frontal cortex increased by 24% ($p<0.05$) after removal of the occluder. In the parietal cortex SSP increased by 17% (statistically insignificant).

Changes in SSP induced by CPA before modeling of ischemia were similar to those in control rats. SSP decreased by 9% compared to the level observed before administration of CPA and physiological saline ($p<0.01$). Introduction of the occluder to experimental rats induced changes in SSP that were similar to those in control animals (negative shift in the left hemisphere and positive shift in the right hemisphere). The mean decrease in SSP in the left hemisphere of control and CPA-receiving rats over 60 min was 37 and 33%, respectively ($p<0.05$). Therefore, the animals treated with CPA demonstrated less pronounced decrease in SSP in the left hemisphere and delayed development of ischemic injury.

SSP in control and experimental rats decreased after introduction of the occluder into the frontal and parietal cerebral cortex (compared to the previous period, Fig. 2). SSP in the left frontal cortex of control and experimental animals decreased by 28 and 19%, respectively, over 20 min after introduction of the occluder ($p<0.05$, Fig. 2). Over the next 20 min SSP in control and experimental rats decreased by 35 and 26%, respectively, compared to the level observed before modeling of ischemia ($p<0.001$). Changes of SSP in control and experimental animals were similar over the last 20 min of observations (39 and 37%, respectively).

The negative shift of SSP in the parietal cortex of control rats developed more rapidly than in experimental animals. However, the degree of the negative shift in SSP in the parietal cortex became similar in the earlier period (second 20-min period, Fig. 2, *b*).

After reperfusion the opposite changes of SSP in experimental rats were less pronounced than in animals receiving physiological saline. No changes in SSP were observed over a 16-min period.

As differentiated from control rats, SSP in the right parietal cortex of experimental animals underwent a large positive shift during ischemia (26%, $p<0.001$). However, no changes were revealed in the right frontal cortex. Therefore, intracerebroventricular administration of CPA delayed the negative shift in SSP in the ischemic hemisphere.

Published data show that SSP reflects polarization in the nervous tissue [10,11]. The negative shift is related to depolarization of nerve cells. The positive shift in SSP probably reflects opposite changes (repolarization and hyperpolarization).

Negative shifts in SSP during cerebral ischemia were studied previously [5,6,9]. These changes were considered to be associated with ischemic depolarization in the nervous tissue [7]. We revealed negative shifts of SSP in the left hemisphere during ischemia, which reflected the development of ischemic depolarization. The observed changes were most pronounced in the left parietal cortex. Pretreatment with CPA before modeling of ischemia delayed the negative shift in SSP. It reflects suppression of depolarization processes in the brain.

Previous studies showed that adenosine and its analogues (*e.g.*, CPA) have specific A_1 and A_2 receptors in nervous tissue [2] and possess neuroprotective properties [3]. N^6-derivatives of adenosine display

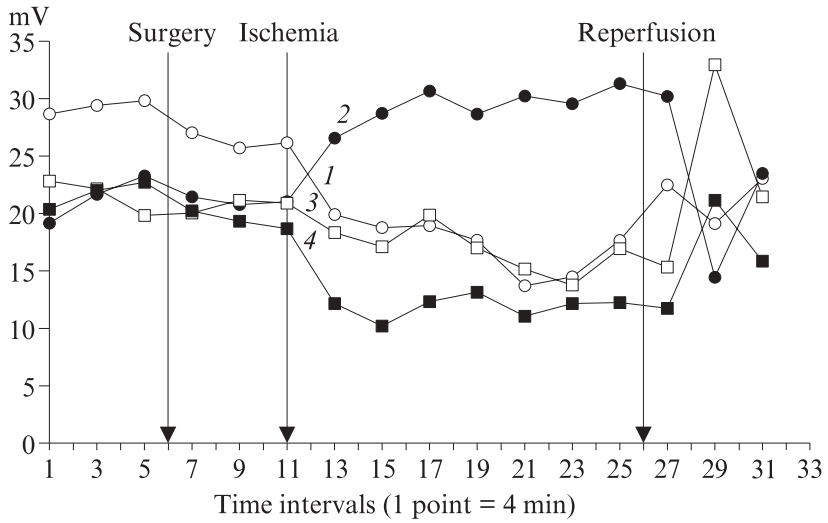

Fig. 1. Changes of the steady-state potential in control rats with ischemia: left frontal cortex (*1*), right frontal cortex (*2*), right parietal cortex (*3*), and left parietal cortex (*4*).

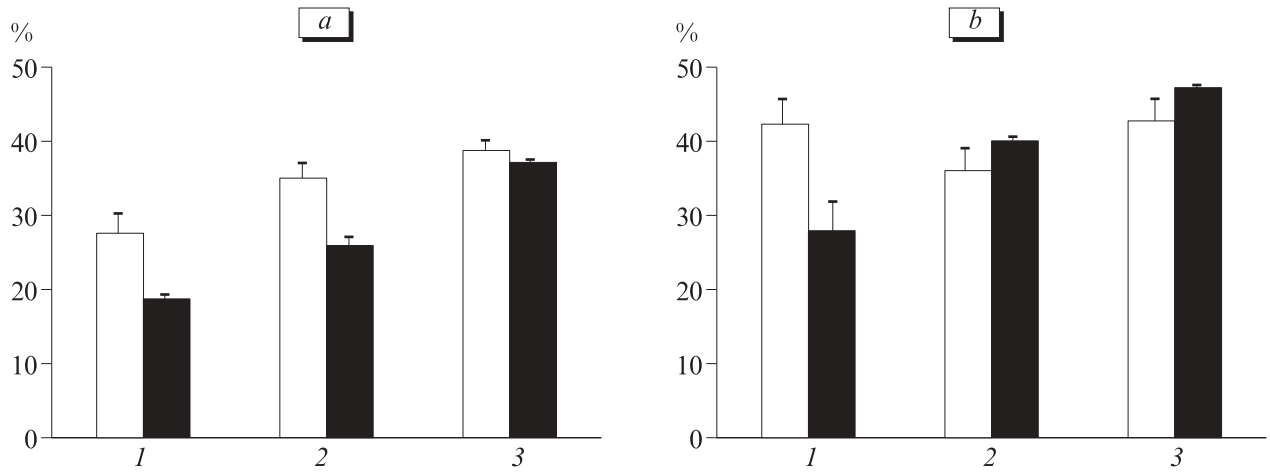

Fig. 2. Decrease in the steady-state potential (%) after the introduction of the occluder into the left carotid artery in the frontal (*a*) and parietal cortex of the left hemisphere (*b*) over the first (*1*), second (*2*), and third 20-min interval of ischemia (*3*). Light bars: control. Dark bars: cyclopentyladenosine.

high affinity for A_1 adenosine receptors. Activation of these receptors is followed by a decrease in cAMP content in tissues. Adenosine increases permeability of the cell membrane for K^+, blocks Ca^{2+} channels, and decreases intracellular calmodulin content [8]. Activation of transmembrane Ca^{2+} transport and accumulation of these ions in neurons play a role in the pathogenesis of various disorders in the central nervous system. CPA produces a protective effect and delays depolarization in neurons, which is probably related to deceleration of Ca^{2+} accumulation in cells and increase in permeability for K^+. The delayed development of ischemic depolarization can decrease mortality rate and/or severity of ischemic injury [7,9]. It is possible to affect the penumbra area and widen the "therapeutic window" (period of successful therapeutic treatment) [1].

During ischemia SSP underwent a positive shift in the right hemisphere (*i.e.*, zone opposite to site of occlusion), which was probably related to the redistribution of blood flow and better blood supply to this brain region. Previous experiments demonstrated that SSP reflects the strain of energy processes. The increase in SSP is associated with accumulation of acid products of energy metabolism at the blood-brain boundary. It results from intensification of energy metabolism in the brain. Changes in SSP suggest that cerebral ischemia is accompanied by activation of energy metabolism in the right hemisphere.

Thus, recording of brain SSP during focal cerebral ischemia allows identifying the development of ischemic depolarization in the left hemisphere by the negative shift in this potential. Changes in SSP induced by CPA indicate that this preparation delays the development of pathological processes in the nervous tissue.

REFERENCES

1. E. I. Gusev, V. I. Skvortsova, A. V. Kovalenko, and M. A. Sokolov, *Zh. Nevrol. Psikhiatr*., No. 2, 65-70 (1999).
2. V. V. Eliseev and G. M. Poltavchenko, *Role of Adenosine in the Regulation of Physiological Functions in the Organism* [in Russian], St. Petersburg (1991).
3. V. I. Kulinskii, G. Z. Sufianova, L. A. Usov, and A. A. Sufianov, *Byull. Eksp. Biol. Med*., **117**, No. 6, 622-624 (1994).
4. G. Z. Sufianova, L. A. Usov, A. A. Sufianov, *et al*., *Eksper. Klin. Farmakol*., **64**, No. 4, 63-67 (2001).
5. Q. Chen, M. Chopp, G. Bodzin, and H. Chen, *J. Cerebr. Blood Flow Metab*., **13**, No. 3, 389-394 (1993).
6. A. J. de Crespigny, J. Rother, C. Beaulieu, and M. E. Moseley, *Stroke*, **30**, No. 10, 2212-2222 (1999).
7. J. Li, Y. Takeda, and M. Hirakawa, *J. Neurosurg. Anesthesiol*., **12**, No. 3, 247-254 (2000).
8. M. L. Michaelis and F. F. Vincenzi, *Trends Pharm. Sci*., **2,** No. 9, 999-1005 (1981).
9. G. Mies, T. Iijima, and K. A. Hossmann, *Neuroreport*, **4**, No. 6, 709-711 (1993).
10. H. Rogers, P. J. Birch, and A. G. Hayes, *Naunyn Schmiedebergs Arch. Pharmacol.*, **342**, No. 5, 547-553 (1990).
11. G. B. Watson, R. K. Rader, and T. H. Lanthorn, *Brain Res*., **25**, No. 1, 81-88 (1989).