Bilateral Occlusion of Carotid Artery in Awake Hypertensive Rats (SHR-SP) as a Model of Global Cerebral Ischemia

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Bilateral occlusion of carotid arteries in awake hypertensive rats (SHR-SP) was used as a model of global brain ischemia (duration of occlusion — until appearance of seizures). In normotensive rats (WKY), no seizures developed over 60 min. We revealed swelling of mitochondria in dendrites of hippocampal CA1 pyramidal cells, which was more pronounced in SHP-SP than in WKY rats. Blood pressure and heart rate in SHR-SP rats increased starting from the first minutes of occlusion, while in WKY rats these parameters remained unchanged. We proved that bilateral occlusion of the carotid arteries in awake SHR-SP rats can be used as an adequate model of global cerebral ischemia.

Key Words: *bilateral occlusion of carotid arteries; hypertensive rats (SHR-SP); mitochondria of neuronal dendrites; hippocampal CA1 area*

Brain ischemia is a condition when oxygen and glucose deficiency develops in the nervous tissue due to blood flow reduction. The decrease in blood flow leading to ischemia is the most common cause of brain dysfunction. Brain ischemia is divided into global cerebral ischemia which is a consequence of acute heart failure and focal ischemia resulting from disturbances in a local vascular basin due to ischemic stroke or transient ischemic attack [2,4]. The study of ischemic damage to the brain is important not only from medical, but also from social point of view [1,2].

The following experimental model of cerebral global ischemia is now most extensively used: bilateral occlusion of carotid arteries is conducted in rats in combination with controlled hypotension (to 50 mm Hg). Hypotension is necessary for modeling brain ischemia, because bilateral ligation of the carotid arteries does not reduce cerebral blood flow to a level required for the development of ischemic damage. Bilateral ligation of the carotid arteries and BP drop induce morphological changes in hippocampal CA1 neurons and impair energy metabolism in brain cells [3].

Specific defects of cerebral blood vessel leading to spontaneous strokes are typical of adult SHR-SP rats [9,10,12]. Similar processes develop against the background of arterial hypertension in humans [5,7]. Considering these peculiarities of SHR-SP rats, we hypothesized that global cerebral ischemia can be modeled by occlusion of carotid arteries without dosed blood pressure (BP) lowering.

MATERIALS AND METHODS

Experiments were performed on awake 4-5-month-old male hypertensive SHR-SP rats and normotensive WKY rats. The animals were kept in standard vivarium conditions with free access to food and water. All manipulations were done in accordance

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with the protocol approved by the Laboratory Animal Keeping and Usage Control Commission of the Branch of the Institute of Bioorganic Chemistry of Russian Academy of Sciences.

One day before the experiment, special occluders for the left and right carotid arteries and polyethylene catheter into the femoral artery (for BP recording with electromanometer) were implanted to the experimental animals. BP curves were processed using a computer-controlled device, mean BP and heart rate were calculated using an original software.

BP and heart rate were recorded over 15 min before and during carotid artery occlusion. In SHR-SP rats, the occlusion was continued until the appearance of seizures (40-50 min), in WKY rats for 60 min. After the experiment, ultrastructure of dendrite fragments of hippocampal CA1 pyramid neurons was analyzed.

Brain tissue was prepared for neuronal ultrastructure analysis. The animals were anesthetized, their chest was opened, a cannula was inserted into the left ventricle apex, and the brain was perfused with phosphate buffer (pH 7.4) and heparin (250 U/liter) for 4-5 min using a peristaltic pump in order to wash the blood out of the brain tissue. Then, the buffer was replaced with a fixative containing 3% formaldehyde and 0.5% glutaraldehyde, and the tissue was perfused for 4-5 min. After the end of fixation, the hippocampus was isolated and trans-

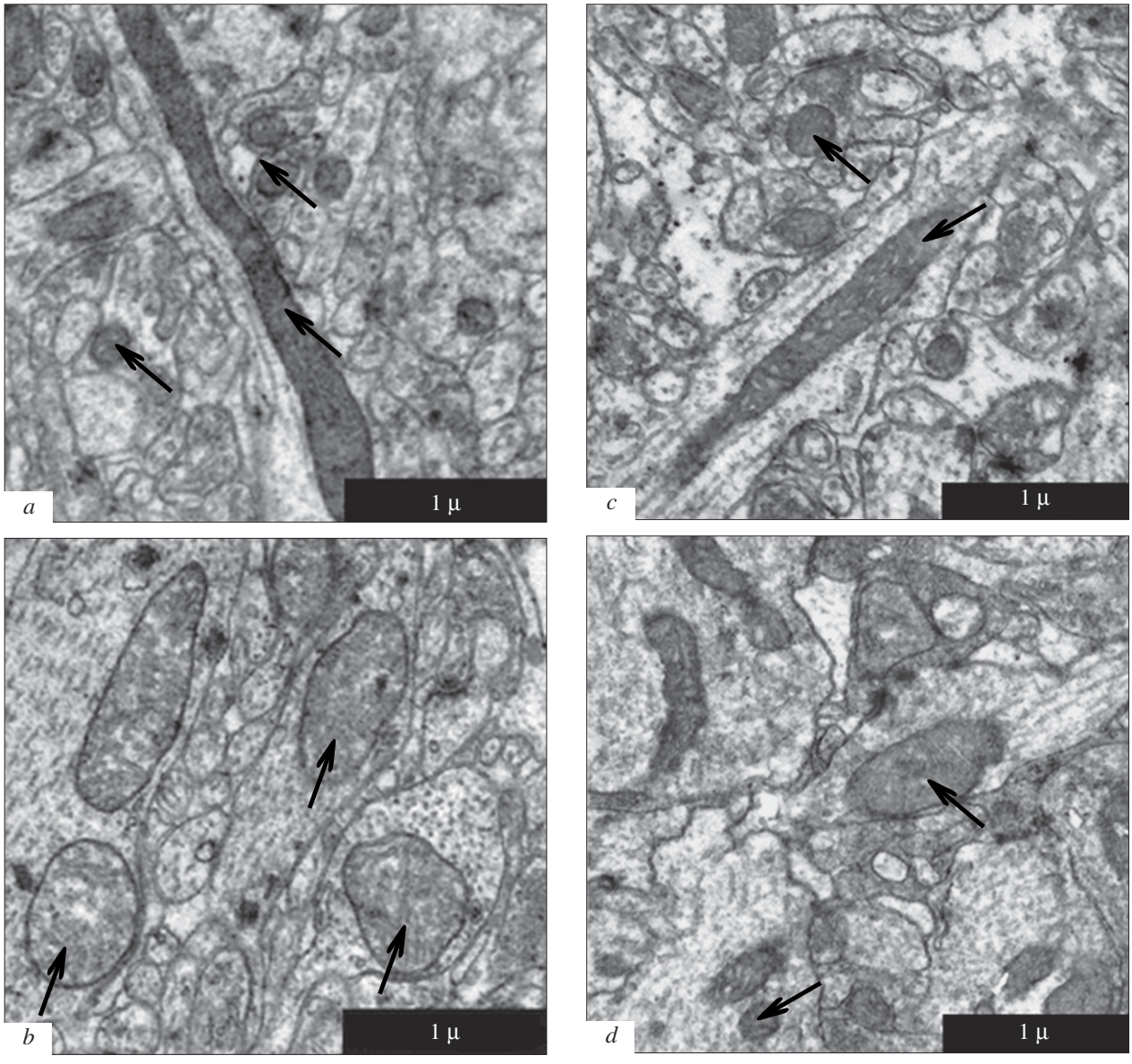

Fig. 1. Ultrastructure of hippocampal CA1 pyramidal neurons in SHR-SP (*a*, *b*) and WKY (*c*, *d*) rats before (*a*, *c*) and after (*b*, *d*) bilateral carotid artery occlusion. Arrows show mitochondria.

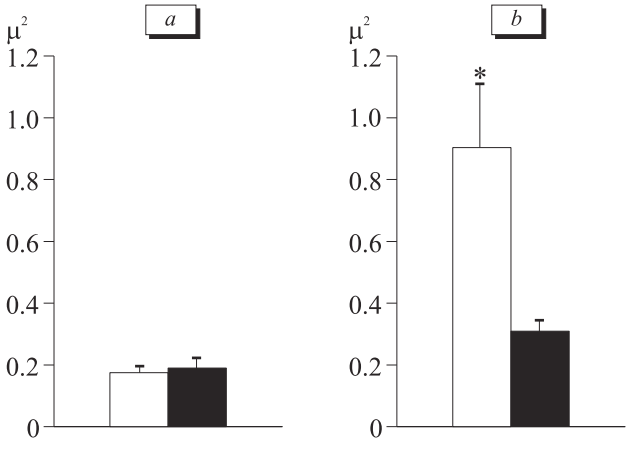

Fig. 2. Cross-section areas of mitochondria in dendrites of hippocampal CA1 pyramidal neurons of SHR-SP (light bars) and WKY (dark bars) rats before (*a*) and after (*b*) bilateral carotid artery occlusion. Here and on Fig. 3: **p*<0.05 compared to WKY rats.

verse segments of 1.5-2 mm width were prepared. The segments were postfixed and after special pretreatment ultrathin (60-70 nm) sections were prepared. The ultrathin sections were analyzed using a JEOL 1200-EX electron microscope. Statistical analysis was performed using Statistica software. Students' *t* test for independent samples was used for statistical evaluation of the results, differences were significant at $p<0.05$. The data are presented as means and standard errors of the means.

RESULTS

In SHR-SP rats, bilateral occlusion of the carotid arteries sharply disturbed of eyeball blood supply (it became pale), the pupil was dilated and did not respond to light; paresis, atonia, adynamia, complete disappearance of reflexes and sensitivity, deep infrequent periodic respiration, and intensive permanent tremor at rest were observed. After 40-50 min of bilateral carotid occlusion, SHR-SP rats developed seizures. In WKY rats, short-term disturbances in eyeball blood supply followed by its recovery, hypodynamia, and gait disorders were noted.

In the initial state, in mitochondrial structure and size were similar in SHR-SP (Fig. 1, *a*) and WKY rats (Fig. 1, *c*). After bilateral occlusion of the carotid arteries, enlargement of mitochondria was observed in both SHR-SP (Fig. 1, *b*) and WKY rats (Fig. 1, *d*). However, normal mitochondria were found after occlusion in only WKY rats.

Initial cross-section areas of mitochondria in dendrites of hippocampal CA1 pyramid neurons were similar in SHR-SP and WKY rats (Fig.2), while after occlusion this parameter in SHR-SP rats significantly surpassed that in WKY rats.

Thus, we revealed more pronounced changes in mitochondria in dendrites of hippocampal CA1 pyramid neurons of SHR-SP rats compared to WKY rats after bilateral occlusion of the carotid arteries. This conclusion is based both on qualitative evaluation of microphotographs, which allowed to reveal the absence of normal mitochondria in SHR-SP rats, and on quantitative analysis of mitochondria cross-section areas.

In SHR-SP rats, the mean BP and heart rate significantly increased after bilateral carotid occlusion compared to baseline values, while in WKY rats no statistically significant changes in these parameters were observed.

In SHR-SP rats, seizures developed 40-50 min after occlusion. This response of awake hypertensive rats to clamping of the common carotid trunks

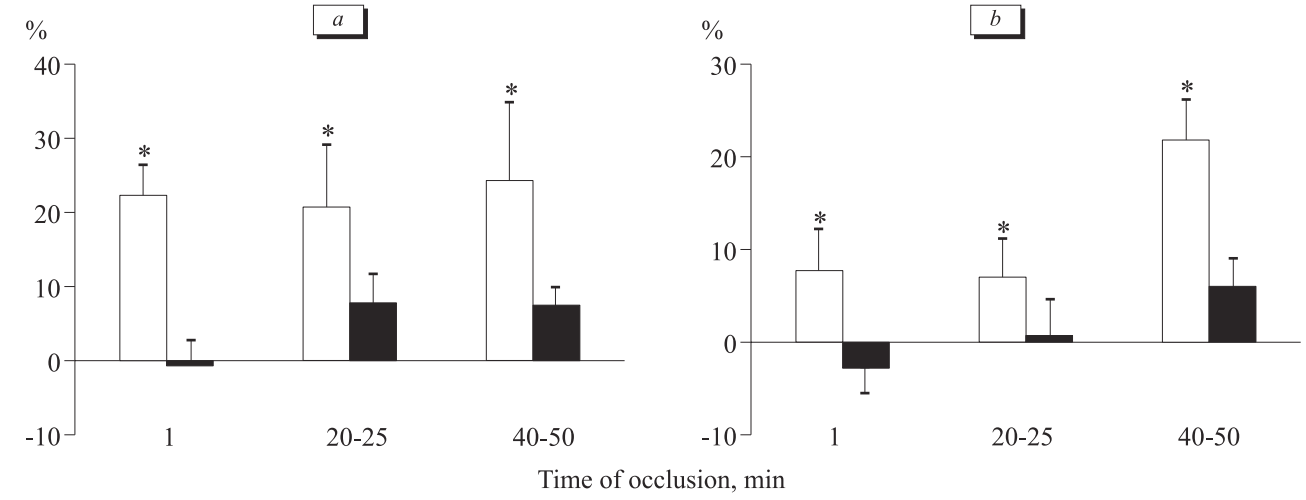

Fig. 3. Dynamics of mean BP (*a*) and heart rate (*b*) in SHR-SP (light bars, *n*=9) and WKY (dark bars, *n*=7) rats during bilateral carotid artery occlusion.

is in line with clinical data [8]. The absence of seizures in normotensive rats (WKY) can be explained by normally developed symmetric and closed circle of Willis.

It is well known that mitochondria swelling and vacuolization are the first signs of cell damage [11]. Nerve tissue resistance to ischemia is determined by the bioenergetic state of mitochondria [6]. We found that bilateral carotid artery occlusion in rats leads to mitochondria swelling. It is evident that these abnormalities of mitochondria were caused by brain hypoxia. These disturbances were more pronounced in SHR-SP rats than in WKY rats.

Elevated BP and heart rate under conditions of bilateral carotid artery occlusion in hypertensive rats are most likely associated with the development of compensatory responses aimed at increasing blood supply to the brain. This fact can also indicate that bilateral occlusion of the common trunks of the carotid artery in awake SHR-SP rats causes cerebral ischemia.

Thus, our findings prove the hypothesis that bilateral occlusion of the common trunks of carotid arteries without additional controlled hypotension causes cerebral ischemia in awake SHR-SP rats. Controlled hypotension is necessary for brain ischemia modeling only in normotensive rats, because bilateral common carotid artery ligation in these animals does not provide adequate reduction of cerebral blood flow for the development of ischemic damage. Thus, bilateral occlusion of the common trunks of carotid artery in awake SHR-SP rats can be used as an adequate model of global cerebral ischemia.

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