Reactivity of Rat Basilar Artery to Serotonin after Short-Term Ischemia of Hindbrain and during Chronic Vertebrobasilar Insufficiency

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Contractile responses of the basilar artery to serotonin were examined *in vitro* on two models of circulation disturbances in the vertebrobasilar region of the brain. Two days after 30-min reversible occlusion of vertebral arteries, the sensitivity of the basilar artery to serotonin decreased, while chronic vertebrobasilar insufficiency had no effect on serotonin-induced contraction.

Key Words: *vertebrobasilar ischemia; basilar artery; serotonin*

Pathological changes in the reactivity of blood vessels in the brain after stroke can contribute to the development of repetitive disturbances of brain circulation even after resumption of cerebral blood flow. Some data corroborate the presence of such disturbances in respect to dilator and constrictor agents [5,7]. These abnormalities could be provoked by changes of the regulatory influences to blood vessels. For example, the content of some vasoactive substances sharply increases in cerebral tissues during ischemia [9].

Most researches use models of forebrain ischemia, since ischemic disturbances in cerebral circulation most frequently occur in the basin of the medial cerebral artery. The basin of the basilar artery (BA) ranks second by the incidence of ischemic cerebral infarction (24%) [2]. The hindbrain significantly differs from forebrain structures by phylogenesis, structure, vascular function, and innervation, [4]. It can be hypothesized that the data on reactivity of the vessels of the carotid basin obtained during brain ischemia and reperfusion cannot be unambiguously extrapolated to vessels of the BA basin. Moreover, there are few data on changes in cerebral vessels provoked by hindbrain ischemia.

Our aim was to study reactivity of rat BA to serotonin (5-HT) after short-term vertebrobasilar ischemia and during chronic vertebrobasilar insufficiency (CVBI).

MATERIALS AND METHODS

Experiments were carried out on male (n=29) Wistar rats weighing 378±12 g. Circulation deficiency in the brain stem was modeled by chronic bilateral occlusion of vertebral arteries in their prevertebral part or by short-term ischemia followed by reperfusion [8].

The rats were narcotized intraperitoneally with chloral hydrate (350 mg/kg). A longitudinal incision was made on the ventral side of the neck, the carotid arteries were displaced laterally, and *m. sternocleidomastoideus* was partially cut. The vertebral artery was found using brachial plexus nerves as a guide. CVBI was induced by complete ligation of one vertebral artery and partial ligation of the other by decreasing its external diameter to 150 μ. Chronic complete ligation of both vertebral arteries always led to fatal outcome. To model hindbrain ischemia and reperfusion, we softly clamped both vessels for 30 min, thereafter the arterial blood flow was resumed. In both experimental series, the control rats were sham-operated.

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On the next day after surgery, neurological deficit was scored [3].

The vasomotor reactions of isolated BA were studied 2 days after induction of cerebral circulation disturbances *i. e.* after complete formation of the infarction zone [1]. The rats were decapitated, the brain was removed, and 4-6 mm segment of BA was isolated. Under a binocular microscope, a steel cannula was inserted into proximal end of BA bathed in Krebs—Henseleit solution at 4-8°C. The artery was kept in this solution for 20 min (stabilizing period). Then it was placed into a 15-ml temperature-controlled flow camber (37°C, 4 ml/min) and perfused at the rate of 1.1-1.2 ml/min with a Rabbit peristaltic pump. The outflow from the distal end of the vessel and its lateral branches was free.

Under these conditions, the deviations in the perfusion pressure reflected changes in the hydraulic resistance of the vessel. The recorded perfusion pressure depended on the constant resistance of the cannula and variable resistance of the vessel. The perfusion pressure was recorded with a piezoelectric transducer at the vessel inlet. Both vessel and chamber were perfused with Krebs—Henseleit solution containing (in mM): 119.0 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 5.5 glucose, 1.5 $CaCl_2$ saturated with 95% O_2 and 5% CO_2 (37°C, pH 7.4). The high-potassium solutions with 60 or 100 mM [K^+] were prepared by substitution of sodium with potassium ions in Krebs—Henseleit solution.

Experiments began with a 30-min stabilizing perfusion with the control solution, short-term activation with 5-HT (10^{-6} M), and washout. Then 5-HT was applied in increasing concentrations (from 10^{-8} to 10^{-6} M). The vessel was not washed between each application, so the resulting dose—dependent curve was cumulative. Then the vessel was washed to the initial perfusion pressure, and its reaction to high-potassium solution was tested.

The data were processed statistically using nonparametric Mann—Whitney tests.

RESULTS

Two days after reversible 30-min occlusion of vertebral arteries, the symptoms of neurological deficiency were documented. This deficiency manifested in tachypnea, active involvement of accessory muscles in respiratory movements, respiratory arrhythmia, ptosis, bristling, untidy appearance of the animals, and in general decrease in locomotor activity. In experimental group, the neurological deficit scored 7.5±1.5 (p<0.05) [3]. In this case, the state of the rats was grave or moderate. In 83% rats of this group, respiratory arrhythmia was accompanied by activation of accessory respiratory muscles, which are indicative of circulatory disturbances in the brainstem involving the respiratory center. In the sham-operated group, the neurological deficit scored 1.0±0.8, which corresponded to virtually normal state. The rats with CVBI also demonstrated respiratory arrhythmia and activation of the accessory musculature.

Two days after 30-min hindbrain ischemia-reperfusion (n=6), the amplitude of the constrictor responses of BA to 5-HT in a dose range from 10^{-8} to 10^{-7} M significantly decreased by 14-52% compared to the control (n=9, p<0.05, Fig. 1, *a*). However, the constrictor responses of BA to greater doses of 5-HT (2×10^{-7} to 10^{-6} M) did not significantly differ from those in the control group. High-potassium solution

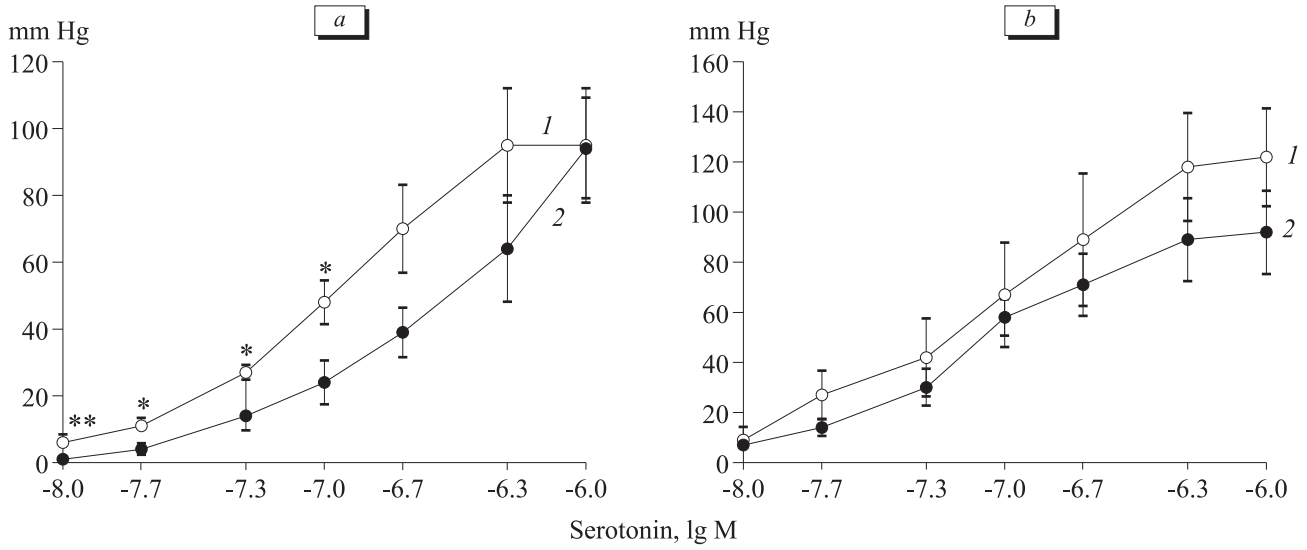

Fig. 1. Reactivity of basilar artery to serotonin in rats two days after 30-min complete occlusion of vertebral arteries (*a*) and after induction of incomplete chronic vertebrobasilar ischemia (*b*). *1*) control; *2*) experiment. *p<0.05, **p<0.01 compared to the control.

([K^+]=100 mM) induced similar contractions of BA in the experimental and sham-operated groups: 106±19 and 109±19 mm Hg, respectively.

In rats with CVBI (n=7), the constrictor responses of BA to 5-HT were identical to those in sham-operated rats (n=7) in the entire dose range from 10^{-8} to 10^{-6} M (Fig. 1, *b*). The same similarity in responses was observed for 60 mM KCl: 119±21 and 129±15 mm Hg in experimental and control groups, respectively.

There are data that the content of 5-HT, the most powerful vasoconstrictors involved in the regulation of cerebral circulation, increases in the ischemic brain [9]. This suggests that 5-HT participates in the development of postischemic disturbances of cerebral blood flow by promoting vascular spasm.

Two days after short-term hindbrain ischemia, the sensitivity BA artery to 5-HT (10^{-8} to 10^{-7} M) was markedly reduced. The controversial data are available about the effect of ischemia on the constrictor potency of 5-HT in the basin of internal carotid artery. Some papers report a decrease in serotonin-induced vasoconstrictor response after ischemia [5,7], while other revealed no changes [10] or even potentiation of this response in postischemic tests [11]. It was shown that the decrease in 5-HT reactivity of rat medial cerebral artery after infarction in the basin of this artery was accompanied by inhibition of myogenic reactions and by a decrease in the content of fibrillar actin in vascular smooth muscles [6]. This suggests that under these conditions, moderation of constrictor response to 5-HT resulted from weakening of the contractile potency of the artery.

Vasoconstriction induced by high-potassium solution results from depolarization of the membrane of smooth muscle cells and is not mediated by receptor mechanisms. In our experiments, high-potassium solution induced practically identical constrictions of experimental and control arteries, which confirmed preserved of contractile potency of BA in the ischemic region. Probably, changes in the reactivity to 5-HT demonstrated in our study resulted not from weakening of contractile potency of the artery, but from changes in the cell receptor moiety. Moderation of the response of cerebral arteries to the constrictor influences can impede the development of delayed vasospasm, but at the same time, this adaptive process can promote cerebral edema.

CVBI had no effect on BA reaction to 5-HT. Despite deficiency of blood supply to hindbrain and described neurological symptoms, the residual perfusion coupled with activation of collateral circulation probably compensated (to some degree) the subnormal blood supply in this cerebral region. Preserved reactivity of BA in CVBI rats and induction of these changes by short-term ischemia and reperfusion demonstrated that damages to the arteries caused by ischemia-reperfusion procedure involving activation of free-radical oxidation and LPO in cell membranes [1] were more essential than those caused by chronic pathological processes.

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