GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Reactivity of the Basilar Artery in Krushinsky-Molodkina Rats 24 Hours After an Audiogenic Epileptic Seizure

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Examination of the reactivity exhibited by the basilar artery of the brain in Krushinsky-Molodkina rats before and after an induced epileptiform seizure accompanied by subarachnoid hemorrhages showed increased contractility of this artery in response to endothelin-1 and its reduced sensitivity to ATP 24 h after the seizure. Such changes in the reactivity of brain vessels may contribute to the development of vasospasm and secondary ischemia of the brain after an epileptic seizure.

Key Words: *epilepsy; subarachnoid hemorrhage; brain vessels; reactivity; endothelin*

During an epileptic seizure, especially if it is accompanied by generalized convulsions, the blood-brain barrier becomes more permeable and intracranial hemorrhages frequently occur. The resulting hematoma releases vasoactive factors that compromise brain hemodynamics and potentiate the vasospasm [6]. In addition, the reactivity of smooth-muscle cells in brain arteries is altered as a result of damage to neurons and changes in capillary filtration and in the composition of the cerebrospinal fluid. Since the nature of these changes has not been fully elucidated, we undertook the present study to evaluate the contraction and dilation of the basilar artery in response to vasoactive substances in rats that had sustained acute disturbances of cerebral circulation.

MATERIALS AND METHODS

Wistar rats and rats of the Krushinsky-Molodkina (KM) strain genetically predisposed to respond to strong acoustic stimulation by generalized convulsions [1,2] were used. Alternate exposure of KM rats, at 15-min intervals, to strong and weak acoustic stimuli according to a specially devised scheme resulted not only in convulsive seizures but also in subarachnoid hemorrhage (SAH) [1,2]. All tests in this study were performed with isolated basilar arteries from Wistar rats (group 1), intact KM rats (group 2), and KM rats that had developed, 24 h before the tests, an epileptiform seizure and an acute impairment of blood-brain barrier permeability accompanied by SAH (group 3).

The rats of all three groups were killed under ether anesthesia 24 h after the audiogenic stress produced in group 3, and a basilar artery segment about 4 mm in length was dissected out above the junction of the vertebral arteries. The proximal end of the isolated segment was connected to a polyethylene cannula and then immediately placed in a thermostated flow-through (3 ml/min) chamber, where it was perfused at a constant rate of 1 ml/min by means of a Rabbit peristaltic pump. Both the perfusate and the solution fed to the chamber had the following composition (mM): 130 NaCl, 4.7 KCl, 14.9 $NaHCO_3$, 1.5 $CaCl_2$, 1.2 $MgSO_4$,

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1.2 KH_2PO_4, and 6 D-glucose. In some tests, a calcium-free solution containing 0.1 mM EGTA and NaCl instead of $CaCl_2$ was used. The perfusate could flow freely into the chamber from the distal end and lateral branches of the vessel. The level of perfusion pressure, which was taken as an indicator of vessel contraction at the constant perfusion rate used, was recorded with Statham P23Db sensors (Gould Inc.) at the entry to the vessel.

After a 20-min perfusion with the solution of the indicated composition (control solution), the vessels were each tested with 100 mM KCl and then exposed to the following vasoactive substances in ascending concentrations added to the perfusate with a Secura perfusor: 5-hydroxytryptamine (5-HT; Sigma), adenosine triphosphate (ATP) or adenosine (Serva), and endothelin-1 (ET-1). These substances were introduced into the vessel lumen, with the exception of adenosine, which was added to the chamber. After the exposure to each agonist, the vessel was thoroughly washed with the control solution, in an attempt to bring the perfusion pressure as close to the baseline level as possible. In tests using the calcium-free solution, the vessel was preincubated in this solution for 8 min.

RESULTS

There were no significant intergroup differences in the reaction of the basilar artery to KCl, the perfusion pressures being 128±7, 133±8, and 137±12 mm Hg in groups 1, 2, and 3, respectively. In group 3 (KM rats that had sustained an epileptiform seizure), the contractile response of the basilar artery to 5-HT was significantly lower than in the other two groups (Fig. 1). In group 2 (intact KM rats), the artery was also less responsive to 5-HT than in group 1 (Wistar rats), though only at high concentrations of this substance. In some tests, 1 μM propranolol was added together with 5-HT in order to evaluate the possible contribution of β-receptors to the contractile response, but no increase in this response was noted. In the calcium-free medium, vasoconstriction failed to occur in any group in response to 5-HT (10^{-6} M).

Dose-dependent rises of the perfusion pressure were also observed in response to added ET-1 (Fig. 2). In group 3, the basilar artery was significantly less sensitive to ET-1 than in group 1 (Wistar controls), although the maximal response to ET-1 was even higher (by 20±4.3%). In group 2 (intact KM rats), the sensitivity to ET-1 was similar to that in the control group (Fig. 2). In the calcium-free medium, the contractile responses in groups 1, 2, and 3 to the highest ET-1 dose (2.5×10^{-9} M) were, respectively, 16.2±3.4%, 15.1±2.1%, and 19.0±2.5% of the values recorded in the tests with 100 mM KCl. Although responses after

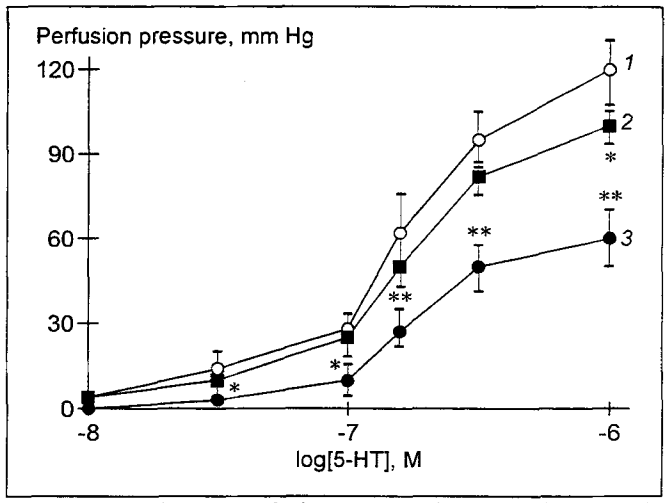

Fig. 1. Variations of perfusion pressure in the basilar artery in response to 5-hydroxytryptamine (5-HT). Here and in Figs. 2 and 3: curves *1*, *2*, and *3* were obtained for the corresponding three groups of rats. $*p<0.05$, $**p<0.01$ in comparison with group 1 (Wistar rats).

the epileptiform seizure tended to be higher, the increases were not significant.

The relaxation of basilar artery preparations in response to ATP and adenosine was examined after their contraction induced by 10^{-7} 5-HT, which amounted to 80% of the maximal contraction observed in response to this substance. ATP was found to cause relaxation of the artery in concentrations of 5×10^{-7} M to 5×10^{-5} M and its dilation in a concentration of 5×10^{-4} M (Fig. 3). The dilation was greatest at 5×10^{-5} M in both groups of KM rats and at 5×10^{-6} M in the Wistar group, which indicates reduced sensitivity of the basilar artery smooth muscle to ATP in KM rats irrespective of whether or not they developed SAH. Artery dilations in response to adenosine (used in

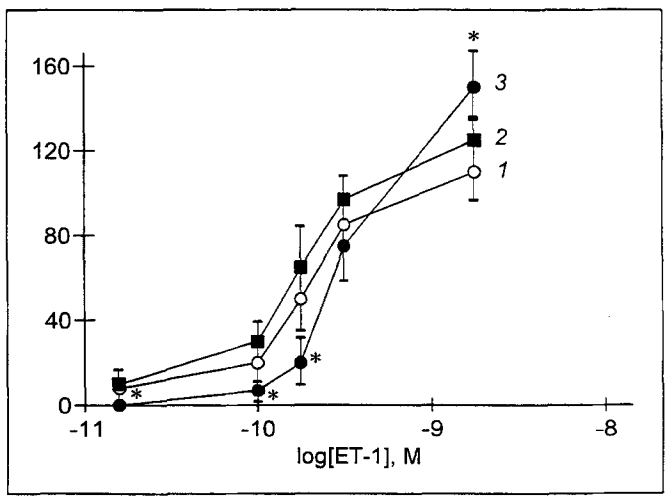

Fig. 2. Variations of perfusion pressure in the basilar artery in response to endothelin-1 (ET-1). Ordinate: % changes in perfusion pressure relative to the highest pressure developed in response to 100 mM KCl taken as 100%.

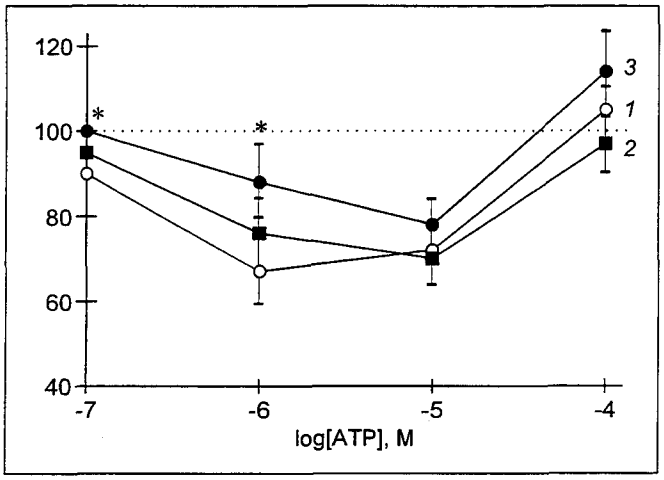

Fig. 3. Variations of perfusion pressure in the basilar artery in response to adenosine triphosphate (ATP). Ordinate: % changes in perfusion pressure relative to the baseline level taken as 100%.

concentrations of 10^{-7} M to 5×10^{-4} M) were similar in the three groups, the maximal response having a mean value of 53.2±1.6%.

In discussing the results presented above, it should be borne in mind that the sharp rise in arterial pressure occurring during an epileptic seizure accompanied by SAH [1] results in damage to the endothelium and directly to the smooth muscles of cerebral vessels [10]. Blood components gain entry into the adventitial layer and interfere with interendothelial junctions; the smooth muscles undergo partial necrosis and the mitochondria are lyzed [10]. The reduced basilar artery reactivity after hemorrhages may be associated not only with altered receptor-effector relationships but also with morphological injuries to the vessel. This might explain the diminished contractile responses to 5-HT and threshold ET-1 doses of basilar arteries isolated from KM rats after the seizure. In the literature there are reports of decreased contractions of the basilar artery (in dogs) [9] as well as of its dilatation (in rabbits) [7] in response to 5-HT after the development of SAH. In group 2 (intact KM rats) we also observed some decrease in the maximal reaction to 5-HT, which points to a genetically determined alteration in smooth muscle responsiveness to this agonist in the KM strain. In group 3 (rats after an epileptiform seizure), the maximal response of the basilar artery to ET-1 was significantly higher than in group 2, although its response to 5-HT and its sensitivity to ET-1 were reduced (Fig. 2). Other authors reported a more than twofold increase in the responsiveness of basilar artery smooth muscle to ET-1 after SAH [4]. These findings make ET-1 a more likely candidate for the role of trigger of the ischemia secondary to SAH. The reduced sensitivity to ET-1 may be due to receptor desensitization, since ET-1 levels in biological fluids have been shown to rise during the development of SAH [13]. The synthesis of this substance in vascular endothelium is enhanced and its effects are potentiated by the oxyhemoglobin produced in the presence of intracranial hemorrhages [12]. ET-1, unlike 5-HT, cannot be washed off a smooth muscle after its strong and prolonged contraction. Such strong contraction is apparently of the same type as the rigid contraction of skeletal muscle and requires very little ATP [5], whose stores in the brain after an epileptic seizure are drastically depleted [2]. That ET-1 is involved in the development of vasospasm is confirmed by the recorded elevation of protein kinase C activity in brain arteries after SAH [11]. Moreover, ET-1 is able to enhance the calcium sensitivity of the phosphorylation undergone by the light chains of myosin, and to elicit muscle contraction in a calcium-free medium even when the intracellular calcium concentration is not increased [8]. In the present study, some contraction of the basilar artery in the calcium-free medium was observed in response to ET-1, especially after the seizure, but not in response to 5-HT. The vasospasm resulting from SAH is very refractory to treatment with calcium blockers [14].

Judging by our tests with adenosine and ATP, the capacity of smooth muscle for relaxation did not change in KM rats after the SAH, but its sensitivity to ATP decreased (Fig. 3). The relaxation of brain vessels in response to ATP is known to be mediated by endothelial P_{2y} receptors [3]. Our results do not allow us to determine whether the reduced basilar artery sensitivity to ATP is due to endothelial damage, receptor desensitization, and diminished prostacyclin and nitric oxide synthesis in the endothelial cells or to a stress-induced fall in the activity of the ATP-dependent calcium pump in the smooth-muscle cells. This question requires further study.

In summary, the present study revealed some differences in basilar artery reactivity between Wistar rats and intact KM rats genetically predisposed to audiogenic epilepsy and marked differences in this respect between these two groups and KM rats that had sustained an epileptiform seizure. Increased contractility of brain vessels in response to ET-1 and their reduced sensitivity to ATP may be factors contributing to the development of vasospasm and secondary brain ischemia after an epileptic seizure.

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The Effect of Some Botanical Preparations on the Production of Lymphocyte-Activating Factor by Mouse Macrophages after Rotation Stress

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It is found that a short-term rotation stress triggers the production of lymphocyte-activating factors by peritoneal macrophages of (CBA×C57Bl/6) F_1 mice and raises blood levels of interleukin-1α and corticosterone. Botanical preparations administered to unstressed animals induce no secretion of lymphocyte-activating factors by macrophages and do not change blood levels of interleukin-1α and corticosterone. The herbals limit the stress-induced production of lymphocyte-activating factors by peritoneal macrophages.

Key Woords *interleukin-1α; lymphocyte-activating factor; botanical preparations, stress*

Botanical preparations (BP), complex naturally occurring compounds, have recently found wide application as adaptogens producing a complex effect on the organism's resistance to various damaging factors [1,2,11]. However, the mechanisms of their influence on resistance to stress are unclear. From the scant literature the modulation of the production of cytokines [13], including interleukin-1 (IL-1) [8], can be assumed to be one of the mechanisms responsible for the effects of BP.

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MATERIALS AND METHODS

Male (CBA×C57Bl/6) F_1 mice weighing 18-20 g were maintained at room temperature and on 12-h light-darkness cycles with free access to food and water. All experiments were started at 10 a.m. Stress was induced by rotation at 78 rpm for 1 h: 10-min rotations with 5-min breaks. The animals were decapitated 0, 0.5, 1, 2, 24, and 48 h after stress, and blood was collected to determine the hormone and IL-1α concentrations. Intact mice were not exposed to rotation stress.

Aqueous decoctions (1:20) of the underground parts of *Eleutherococcus senticosus*, *Aralia mandschurica*, and *Rhaponticum carthamoides* were used. The

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