Permeability of the Blood-Brain Barrier for Superoxide Dismutase after Unilateral Intracarotid Hyperperfusion of the Brain Vessels

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A rise of arterial pressure above the level of autoregulation of the brain blood flow causes blood-brain barrier (BBB) failure and vasogenic brain edema [1, 6]. It has been shown that damage to endotheliocytes caused by high blood perfusion pressure is accompanied by activation of arachidonic acid metabolism and free-radical processes in these cells [7,10]. These processes may be an important pathogenic event in severe hypertensive crises and strokes in patients with arterial hypertension. Superoxide dismutase (SOD), a key enzyme of the antioxidant defense system, protects the cell membrane structures from damage and inhibits the processes of lipid peroxidation through the uptake of free radicals. Injection of exogenous SOD reduces ischemia during experimental vasogenic brain edema [4,7]. It is not clear, however, whether this effect is restricted to the action of SOD on the vessel endothelium or whether the enzyme crosses the blood-brain barrier as well.

In the present study the activity of SOD in the brain parenchyma was determined using a model of vasogenic brain edema induced by an experimental "hypertensive crisis".

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MATERIALS AND METHODS

Thirty experiments were carried out on noninbred rats weighing 240-280 g. Unilateral hyperperfusion of the brain through internal carotid artery with autologous blood was performed under ketamine narcosis (150-200 mg/kg, i.p.), as described at length previously [2]. In brief, the autologous blood was infused (0.25 ml/0.5 sec three times at 3-min intervals) through a catheter introduced in the external carotid artery up to the bifurcation of the internal carotid artery, the common carotid artery being clamped at the moment of infusion. Such a mode of hyperperfusion damages the BBB primary in a certain zone of the subcortical structures and hypothalamus of the ipsilateral hemisphere, which is accompanied by a release of plasma albumins, water, and sodium ions into the intercellular space. Four minutes after the "crisis", 450 μg/kg SOD in a volume of 0.5 ml physiological saline were injected through the catheter during 5 min. After another 5 min the ipsilateral common carotid artery was ligated, after which the animal was decapitated and the brain vessels were immediately perfused through the catheter in the external common carotid artery with physiological saline under a pressure of 100 mm Hg. The brain was promptly removed, symmetrical samples (75-

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TABLE 1. Variation of SOD Activity (Ratio of SOD Activity in Right Hemisphere to Activity in Left Hemisphere in %) for Administration of Exogenous SOD.

Part of brain	1st group	2st group	3st group
Subcortex	109±7.2	95±6.2	265±3.4*,**
	$n=6$	$n=8$	$n=10$
Cortex	95±7.7	98±1.8	110±10.0
	$n=6$	$n=8$	$n=10$

Note. * $p<0.001$ in comparison with cortex; ** $p<0.001$ in comparison with control.

100 mg) were excised from the parieto-occipital cortex and subcortical structures (the thalamic nuclei) of each hemisphere, and a 10% homogenate in 0.1 M Na-phosphate buffer (pH 7.8) was prepared. The homogenates were centrifuged at 10,000 g for 30 min and the supernatants were subjected to chloroform-methanol extraction as described elsewhere [9]. A water-ethanol phase containing SOD activity was obtained after the extracts were centrifuged at 6,000 g for 30 min. The successive manipulations were performed at 4°C. The SOD activity was measured by the reduction of tetranitro blue tetrazolium into formazan by oxygen anion radicals generated in the $NADH_2$-phenazine methosulfate system [5] and estimated per mg protein of the supernatant. The protein concentration was measured after Lowry [8]. The statistical significance of the differences was determined using Student's t test. As has been found in the preliminary experiments, the intercarotid injection of SOD to animals without brain hyperperfusion does not increase the concentration of the enzyme in comparison with that in the contralateral hemisphere or in the brain of control animals. Thus, in the following experiments the brains from the control rats (1st group) and the rats after brain hyperperfusion without (2nd group) and with (3rd group) SOD injection were studied using the above methods.

RESULTS

As is seen from Table 1, 15 min after hyperperfusion the ratio of SOD activity in the right "damaged" hemisphere to that in the left "control" hemisphere in the second group does not differ from that observed in the control group either in the cortex or in the subcortical area. On the other hand, in the third group a reliable increase of this ratio in comparison with the 1st and 2nd groups was found due to an increase of the enzyme activity in the right "damaged" hemisphere and only in the subcortical nuclei.

Thus, when injected during the first few minutes after hyperperfusion of the brain vessels, SOD crosses the BBB and appears in the brain tissue, the area of its primary accumulation being the same as that of plasma albumin release by this mode of hyperperfusion with a constant blood volume [2]. These results confirmed quantitatively our previous data obtained with the same experimental model using the SOD immunochemical assay [3]. SOD injected after hyperperfusion occurred mainly in the subcortical structures of the damaged hemisphere and either was localized along the vessels or spread diffusely in both the intercellular space and cell cytoplasm.

It seems likely that in the cases of disturbed brain blood circulation in humans accompanied by an increased permeability of the BBB and vasogenic brain edema, SOD injection may boost the antiradical defense system by decreasing the processes of lipid peroxidation in both the endothelium of the brain vessels and directly in the brain parenchyma.

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