GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Kinin Formation in the Plasma and Cerebral Blood Flow under Conditions of Cerebral Ischemia/Reperfusion in Rats during Modulation of Kinin System Activity

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Suppression of kininogenesis is an adaptive phenomenon in cerebral ischemia/reperfusion in rats aimed at elimination of the no-reflow phenomenon. Hyperkininogenesis and suppression of kinin destruction are pathogenetically significant, because they augment the manifestations of no-reflow phenomenon during reperfusion following ischemia.

Key Words: *ischemia; reperfusion; brain; cerebral bloodflow; kallikrein*

Cerebral ischemia/reperfusion is associated with activation of the kallikrein-kinin system in the blood [5,7,9]. It is assumed that kinins are involved in hemodynamic restructuring of the cerebral circulation during ischemia/reperfusion [4,5,7]. However, the significance of activation of the kinin system is not completely formulated. Some scientists report pathogenetic significance of kinin formation during cerebral ischemia [8,11], while others emphasize the adaptive effect of hyperkininogenesis [10,13]. The problem can be cleared out by studying ischemia/reperfusion of the brain under conditions of modulation of kinin system activity.

We studied the proportion between blood kininogenesis and cerebral hemodynamics in ischemia/reperfusion of the brain under conditions of modulation of kinin system activity.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=40; 280±30 g). The animals were narcotized with sodium thiopental (50 mg/kg intraperitoneally).

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The study was approved by the local Ethical Committee of Altai State Medical University.

The animals were divided into 4 groups. Drugs modulating kinin system activity were injected intraperitoneally 20 min before the experiment. Group 1 rats (control) were injected with saline. Group 2 animals received protease inhibitor contrycal (Arzneimittelwerke) in a dose of 5000 U/kg for kininogenesis suppression. In group 3, kinin degradation was suppressed by enap (KRKA), angiotensin-converting enzyme inhibitor, in a dose of 0.25 mg/kg, and in group 4 kininogenesis was activated by trypsin (Spofa) in a dose of 0.8 mg/kg. Blood pressure of rats in all groups was virtually the same at the start of the experiment.

Severe partial cerebral ischemia was modeled in all rats by ligation of both common carotid arteries for 30-min followed by 60-min reperfusion.

Local cerebral bloodflow (LCB) was measured in the brain cortex initially, during ischemia, and on minutes 20, 40, and 60 of reperfusion [4] by the hydrogen clearance method. The animals were sacrificed by decapitation.

Blood for measuring the kinin system components was collected from the saggittal cerebral sinus into polystyrene syringes containing 3.8% sodium citrate (1:9).

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The plasma was separated by 15-min centrifugation at 3000 rpm and subsequent freezing. Prekallikrein (PK) and kallikrein-like activities (KLA) in the plasma were measured using S-2302 chromogenic substrate (Chromogenix) by C. Kluft's method with some modifications [3].

In groups 1, 3, and 4, the blood was collected during hypoperfusion, which was determined by the hydrogen clearance curve. In group 2, no hypoperfusion period was observed, and hence, the blood was collected on minute 60 of reperfusion.

The data were processed statistically using Statistica 6.0 software. The significance of differences in the studied parameters was evaluated selectively using Student's *t* test and probability value (*p*). The relationships between blood flow level and kinin system activity in ischemia/reperfusion were evaluated using Spearman's coefficient of correlation.

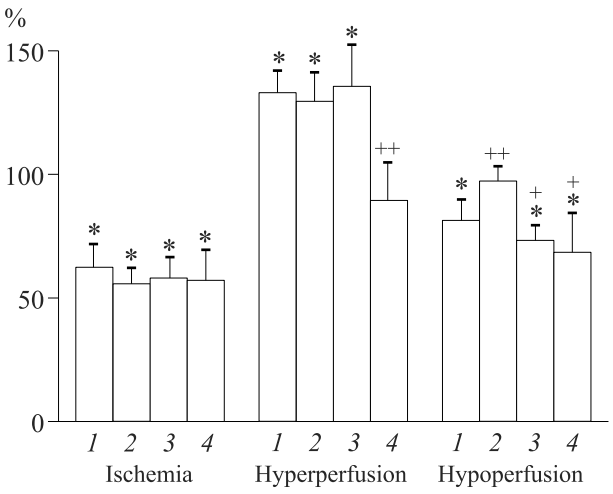

Fig. 1. Local cerebral bloodflow in rats during ischemia/reperfusion of the brain. *1*) control group; *2*) contrycal; *3*) enap; *4*) trypsin. **p*<0.01 compared to initial LCB; ++*p*<0.01, +*p*<0.05 compared to the control group. Initial LCB is taken as 100%.

RESULTS

Pharmacological modulation of the kinin system was associated with changes in activity of plasma kininogenesis during cerebral ischemia/reperfusion (Table 1). Injection of contrycal (a polyvalent inhibitor of proteases, including kallikrein [6]) inhibited kininogenesis, which manifested by an increase in PK level (1.8 times) and decrease in KLA in comparison with the control. By contrast, injection of trypsin led to kininogenesis stimulation, which was associated with KLA increase and reduction of PK level in the plasma. Injection of enap inhibiting bradykinin hydrolysis and leading to its accumulation in the blood [1] caused no changes in PK content and KLA level.

Cerebral hemodynamics of rats during the reperfusion period differed significantly depending on the kinin system status, LCB level initially and during ischemia did not differ from control values (Fig. 1).

Removal of ligatures in animals with kininogenesis suppressed by contrycal was followed (similarly as in the controls) by an increase in LCB and hyperperfusion period on minute 20. This was followed by LCB reduction, but in contrast to the control, no no-reflow phenomenon was observed until the end of the experiment.

Trypsin activation of kininogenesis had an opposite effect on the cerebral hemodynamics during reperfusion. Local cerebral blood flow increased after resumption of circulation, but there was no postischemic hyperperfusion period, and no-reflow phenomenon was recorded on minute 40, LCB was 1.5 times below the initial level.

Despite enap had no effect on kininogenesis, LCB level during hypoperfusion in this group was significantly lower than in the control. This effect of enap can be also explained by accumulation of bradykinin in the blood, but not as a result of kininogenesis activation, but because of inhibition of its destruction by angiotensin-converting enzyme [1].

A strong correlation (r=0.7; p<0.05) between LCB level during hypoperfusion and PK content in venous blood plasma and a negative correlation between LCB and KLA (r=-0.7; p<0.05) in the plasma were detected in animals injected with saline and contrycal. In rats treated with trypsin and enap, LCB level by the end of reperfusion period correlated (r=-0.7; p<0.05) with KLA level.

TABLE 1. Kininogenesis Parameters in Venous Blood Plasma in Ischemia/Reperfusion of the Brain in Rats during Modulation of Kinin System Activity (*M*±s)

Parameter	Control	Contrycal	Trypsin	Enap
PK, U/liter	428.9±109.6 (*n*=10)	773.3±197.7* (*n*=10)	155.9±39.0* (*n*=10)	419.9±74.6 (*n*=10)
KLA, U/liter	32.9±11.2 (*n*=10)	18.6±3.8* (*n*=10)	57.1±21.8* (*n*=10)	27.5±5.8 (*n*=10)

Note. *n*: number of studies; **p*<0.01 compared to the control.

Hence, the data attest to adaptive significance of kininogenesis suppression during the reperfusion period, this suppression eliminating the no-reflow phenomenon, thus maintaining perfusion of the brain. By contrast, activation of kininogenesis during cerebral ischemia/reperfusion is pathogenetically significant, because it augments the course of no-reflow period. It is known that hyperkininogenesis causes cerebral vasodilatation, leads to endothelium damage, and increases vascular wall permeability [12]. This can aggravate brain edema, disorders in cerebral hemodynamics, and augment no-reflow phenomenon during reperfusion period after ischemia.

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