Effects of Leu-Enkephalin Analog on Cerebral Circulation in Cerebral Ischemia of Different Severity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 11, pp. 516-519, November, 1998
Original article submitted October 10, 1998

Leu-enkephalin analog reduces cerebral circulation in mild and has no effect in moderate ischemia, while in severe cerebral ischemia it causes periodic compensatory enhancement of cerebral circulation in experimental animals, instead of its monotonous reduction, thus ensuring 100% survival during a 6-h period, whereas in the control group 60% animals die within 3 h.

Key Words: *ischemia; brain; circulation; leu-enkephalin analog*

We have previously demonstrated that the opioid neuropeptide leu-enkephalin (LE) improves pial microcirculation and total cerebral blood flow (CBF) in moderate brain ischemia [7,8]. Leu-enkephalin restored CBF and vasomotor reaction of the pial microvessels, rapidly and intensively improved peripheral and central lymph circulation against the background of decreased cardio- and hemodynamic parameters. The reduced content of opioid peptides in brain tissue [10] and vessels [11] in cerebral ischemia argues for replacement therapy in this disorder. However, clinical use of peptides is hampered by ambiguity of their effects. For instance, dalargin and other peptides have different effects in animals that survived blood loss [5] and emotional stress [9]. We assume that among other factors individual organism's reaction to damage and the severity of brain ischemia are responsible for different effects of peptides. To verify this assumption we studied the effects of LE analog (LEA) on local CBF in brain ischemia of different severity.

MATERIALS AND METHODS

Experiments were carried out on 48 male albino rats weighing 250-250 g narcotized with chloral hydrate (0.6 g/kg, intramuscularly). Cerebral ischemia was modeled by bilateral common carotid artery occlusion (CCAO). Local CBF was measured by the hydrogen clearance method in the sensorimotor cortex using an ITK-2M device 1 h before and during 3 h of ischemia. To evaluate the effect of LEA under the most disadvantageous conditions, only the animals with initially decreased CBF (below 60 ml/100g/min) were used. Thyrosine LEA (Laboratory of Peptide Synthesis, Cardiology Research-and-Production Center) is a hexapeptide Tyr-Tyr-Gly-Gly-Phe-Leu with direct lymphotropic activity 2-4-fold surpassing that of LE [6]. LEA was injected intraperitoneally in a dose of 40 μg/kg in 1 ml isotonic NaCl 15 min after CCAO. Experimental data were processed statistically using the Student *t* test.

RESULTS

In control rats, local CBF in the cortex was 35.01± 2.52 ml/100 g/min. Deviation of this parameter during a 3.5-h observation period did not surpass ±15% compared with the mean initial level over the first 40 min (100%).

Intraperitoneal injection of 1 ml 0.14 NaCl had no significant effects on CBF.

Bilateral CCAO reduced CBF by about 40% in comparison with the initial level. Despite the standard experimental procedure of ischemia modeling, the initial decrease in CBF (during the first 15 min

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0007-4888/98/0011-1100$20.00 ©1999 Kluwer Academic/Plenum Publishers

after CCAO) varied in different animals: in 23% rats this parameter decreased by less than 30% (81.51± 2.58%), while in 30% it decreased by more than 50% (41.40±1.76%). A moderate decrease in CBF by 30-50% (60.16±1.35) was observed in 47% rats. Taking these finding into account, we divided the animals into 3 groups: animals with mild (CBF>70%, group 1), moderate (CBF=50-70%, group 2), and severe (CBF<50%, group 3) cerebral ischemia.

Lethality in these groups during 3-h CCAO was 0, 11, and 60%. The maximum initial (15 min after the start of CCAO) decrease was observed in group 3 rats with the highest initial CBF (42.77±5.25 ml/ 100 g/min), while the minimum decrease was noted in group 1 rats with the lowest initial CBF (22.57± 3.39 ml/100 g/min, $p<0.01$). Due to different sensitivity of the rats to standard modeled ischemia, 15 min after the onset of CCAO CBF became similar in all experimental groups (from 16.94±1.83 to 21.82± 1.88 ml/100 g/min, $p>0.05$).

The dynamics of CBF during 3-h CCAO was different in different groups. In animals with mild ischemia, compensatory enhancement of CBF was observed periodically, and after 3-h CCAO CBF returned to and even surpassed the initial level, the correlation coefficient between the initial and final CBF being $r=-0.44$ (Fig. 1, *a*). In animals with moderate ischemia (group 2) the periods of compensatory rise of CBF during CCAO were also observed; however, in 50% animals CBF did not return to the initial level. This group was characterized by greater variations of CBF level and low correlation coefficient ($r=-0.1$). It was difficult to predict the dynamics of CBF from the initial drop of CBF during the first 15 min of CCAO. In group 3 rats, CBF progressively decreased and never increased during CCAO, 40% rats died within 1-h of CCAO. The initial drop of CBF (within 15-mih after the start of CCAP) positively correlated with its consequent decrease. The dynamics of CBF was similar and predictable in all group 3 animals; the correlation coefficient being -0.62.

In animals with mild ischemia (group 1), LEA reduced CBF in comparison with the control ($p<0.01$, Fig. 1, *a*) without lethality in experimental animals. In moderate ischemia, LEA had practically no effect of the dynamics of CBF (except for the 3.5-h ischemia point. Fig. 1, *b*). Despite the absence of CBF recovery, all animals survived within 7 h after CCAO, whereas 11% control rats died. The effect of LEA on the dynamics of CBF was noted only in rats with severe ischemia (Fig. 1, *c*): the periods of compensatory CBF rise typical of mild and moderate ischemia appeared, implying transition from severe decompensated to compensated CBF disturbances.

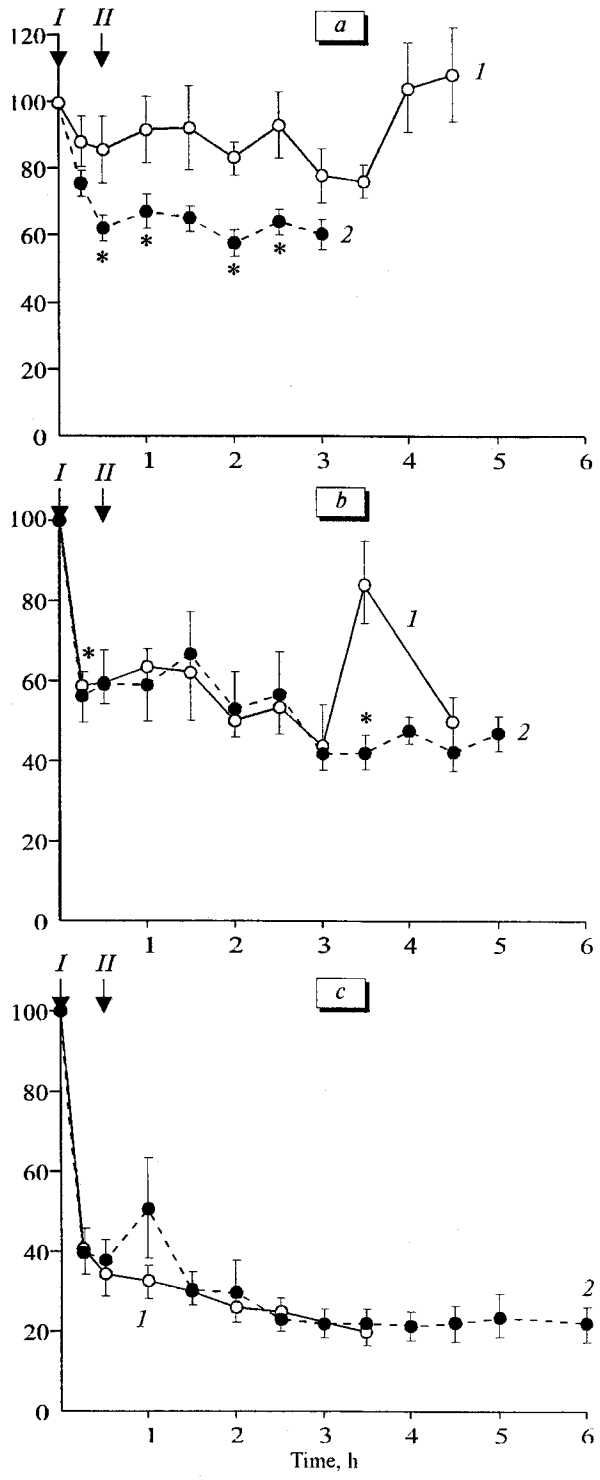

Fig. 1. Local cerebral blood flow (CBF) in rat cortex under conditions of bilateral common carotid artery occlusion (CCAO) in the control (*1*) and after injection of leu-enkephalin analog (40 μg/kg, *2*). Cerebral ischemia: mild (CBF>70%, *a*), moderate (CBF=50-70%, *b*), and severe (CBF<50%, *c*). Ordinate: CBF, %. Arrows indicate start of occlusion (*I*) and injection of leu-enkephalin analog (*II*). *$p<0.05$ compared with the control.

Quantitative LEA-induced changes in CBF were insignificant due to large scatter of experimental data; however, we observed important qualitative changes: repeated compensatory increase instead of progressive decrease in CBF accompanied by 100% survival of experimental animals (60% control rats died).

These findings suggest that LEA has different effects on CBF in cerebral ischemia of different severity. There is growing evidence that organism's resistance to disease depends on individual factors [1-5,9]. The animals were divided into low- and high-resistant to ischemia [2], stress [4,5], hypoxia [1], and blood loss [3,9].

In our experiments the initial decrease of CBF in response to CCAO had the maximum prognostic value. It should be emphasized that despite considerable variations in the initial CBF, after CCAO it was similar in all groups. This low level of CBF did not predict its subsequent dynamics and animal lethality. Another interesting finding is the dependence of CBF reduction during CCAO on its initial drop: the higher the initial CBF, the greater the decrease induced by CCAO. Similar phenomenon was described by others [4]. This reaction is probably determined by CBF reserve and the minimal level of CBF attainable under conditions of this experimental model. The considerable CBF drop in animals with high initial CBF was accompanied by decompensation and high lethality, while in rats with low initial CBF this decrease was less pronounced, which was a beneficial factor. These features of cerebral hemodynamics are probably responsible for grave ischemic strokes in young people with high initial CBF and relatively mild strokes in old patients with low CBF. Our findings suggest that LEA can be beneficially used in severe cerebral ischemia characterized by maximum CBF decrease and lethality, whereas in mild ischemia, when cerebral circulation can recover spontaneously, LEA should not be used, since it reduces CBF. These findings agree with the data [3,8,9] on inefficiency of the peptide preparation Semax and $ACTH_{1-24}$ fragment in animals with compensated blood pressure and liver blood flow under conditions of fatal blood loss and, on the contrary, prolongation of lifetime and transition from decompensated to compensated hemodynamic disturbances in animals with severe blood loss.

The study was supported by the Ministry of Science and Technology.

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