EFFECT OF GABA AND PIRACETAM ON DEVELOPMENT OF AN EXPERIMENTAL BRAIN INFARCT IN RATS AND ON PLATELET AGGREGATION IN PATIENTS WITH CEREBROVASCULAR PATHOLOGY

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Nootropic drugs are used in the treatment of acute disturbances of the cerebral circulation and chronic cerebrovascular insufficiency [3, 6]. Appreciable improvement of the parameters of the cerebral circulation has been demonstrated experimentally under the influence of GABA and its cyclic derivative, piracetam [4, 5]. An important role in the action of nootropic drugs on the cerebral circulation may be played by positive hemorheologic shifts. In the modern view an important role in the mechanism of the general regulatory function of the blood—vessel system may be played by metabolites of arachidonic acid (AA), synthesized in the blood cells and vessel walls [2].

The aim of this investigation was to study the action of GABA and piracetam on the development of an experimental brain infarct in rats and on platelet aggregation (PA) in patients with cerebrovascular pathology.

EXPERIMENTAL METHOD

PA was studied in platelet-enriched plasma (PEP) obtained from healthy blood donors (10) and patients with cerebrovascular disturbances (CVD), admitted to the Neurologic Department of the Lenin Regional Hospital (20 patients with a cerebrovascular accident of ischemic nature), by a turbidimetric method [8]. ADP (10^{-6} M), collagen (10^{-4} g/ml), and adrenalin (10^{-5} M) served as inducers of PA. Piracetam and GABA (each 10^{-5} M) were incubated in PEP for 15 min at 37°C. The results were subjected to statistical analysis by Student's t test.

A cerebral infarct was induced in anesthetized albino rats (2 groups, 6 rats in each group) by the method in [12]. A solution of AA (0.25 mg/ml in 0.25% ethyl alcohol with 0.025% sodium carbonate solution) was injected into the internal carotid artery (0.5 mg/2 ml/kg body weight) at the rate of 0.2 ml/min, after ligation of the lingual and occipital arteries on the same side. The test substances were injected through a tube into the stomach 3 h after the operation: GABA 5 mg/kg, piracetam 60 mg/kg. The control group (6 rats) received a 0.5% solution of methylcellulose. The rats were decapitated on the 3rd day after the operation, the brain fixed in 10% formalin, and 5 coronal sections were cut and embedded in paraffin wax, two sections from the frontal part were stained with hematoxylin and eosin. The zone of the infarct was studied microscopically.

EXPERIMENTAL RESULTS

In the group of normal blood donors (Fig. 1a) GABA inhibited collagen-induced aggregation by 38.77% ($p < 0.05$), and adrenalin-induced aggregation by 22.46% ($p < 0.05$) compared with the control (without inducers), but ADP-induced aggregation was unchanged. Piracetam caused similar but more marked changes: collagen aggregation was inhibited by 50.56%, adrenalin aggregation by 39.3% (in both cases $p < 0.05$), but ADP aggregation was unchanged. In patients with CVD (Fig. 1b) there was a marked increase in the control level of PA, on induction by ADP and adrenalin. At the same time, the antiaggregation effect

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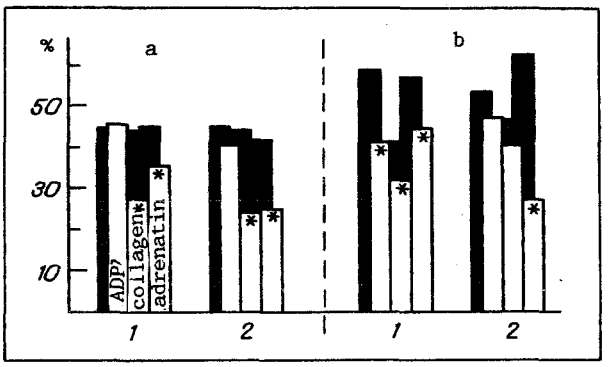

Fig. 1. Changes in platelet aggregation in plasma from patients with acute cerebrovascular disturbances (b) and normal blood donors (a) under the influence of GABA and piracetam, with induction of aggregation of ADP, collagen, and adrenalin. Black columns – control (without preparations); unshaded columns – action of GABA (1) and piracetam (2). Asterisks indicate statistically significant change ($p < 0.05$).

of GABA was strengthened in the patients by comparison with the group of normal donors: besides inhibition of collagen and adrenalin aggregation (22.52 and 21.38%, respectively) $p < 0.05$), inhibition of ADP-induced aggregation was found (28.6%, $p < 0.05$). Weakening of the ability of piracetam to inhibit PA in the patients was observed concurrently: its antiaggregation effect was preserved only against adrenalin-induced aggregation (55.9%, $p < 0.05$).

The antiaggregation effect of GABA and piracetam may be due to a combined influence, including potentiation of ATP synthesis from ADP, and also a peripheral (adrenolytic and antiserotonin) action [9, 13]. The absence of any effect of the preparations against direct ADP-induced aggregation in the blood donors will be noted. In patients with CVD the antiaggregation effect of GABA was potentiated and a new quality was exhibited, namely ability to inhibit ADP-induced aggregation, which can provisionally be considered to be the result of increased sensitivity of the GABA receptors in pathology, bearing in mind evidence obtained of the physiological role of GABA in adaptation and compensation processes when the cerebral circulation is disturbed [1]. The antiaggregation effect of piracetam, on the other hand, is weakened under CVD conditions, i.e., the drug loses its ability to improve the rheologic parameters of the patients' blood. The mechanism of action of piracetam is known to be dominated by metabolic factors, namely activation of synthesis of macromolecules, restoration of membrane phospholipids and of the neurotransmitter levels, etc., which evidently predetermines the secondary character of its hemorheologic effects [7, 15].

On microscopic investigation of the rat brain after injection of AA no appreciable changes could be found in the cortex. They were found, however, in the white matter, affecting the microcirculatory bed. Besides marked perivascular edema (Fig. 2a) and multiple hemorrhagic foci (Fig. 2b), aggregates of erythrocytes could be seen in most vessels. As a result of intravascular blood clotting, homogeneous, amorphous, slightly basophilic contents could be identified in the dilated venules, in the form of a "hyaline" thrombus, completely occluding the lumen (Figs. 2a and 3a). The capillaries were mainly constricted. Against the background of these vascular changes foci of destruction and softening with a marked decrease in the eosinophilic staining of the tissues, were observed in the white matter. Foci of necrosis were located mainly in regions with well-marked vascular changes. Confluent in places, they formed larger areas of softening of brain tissue. In two animals of this group, because of destruction and encephalomalacia of the white matter, cavities filled with softened tissue and erythrocytes could be identified (Fig. 3b).

Thus after injection of AA severe disturbances developed in the brain, affecting the microcirculatory bed, in the form of aggregation of erythrocytes, widespread intravascular blood coagulation, perivascular edema, and hemorrhages, accompanied by destruction and necrosis of individual areas of white matter of the brain.

In the group of rats receiving GABA, microscopic examination revealed dilatation and congestion of small arteries and arterioles both in the cortex, and, in particular, in the white matter of the brain. In some areas marked perivascular edema was present. Against this background dilated venules and small veins were found, with hyaline-like thrombi in their lumen. Marked changes were also observed in the microcirculatory bed and the vascular plexus. If dilated and congested vessels were present, extensive hemorrhages could be identified with damage to the underlying brain tissue.

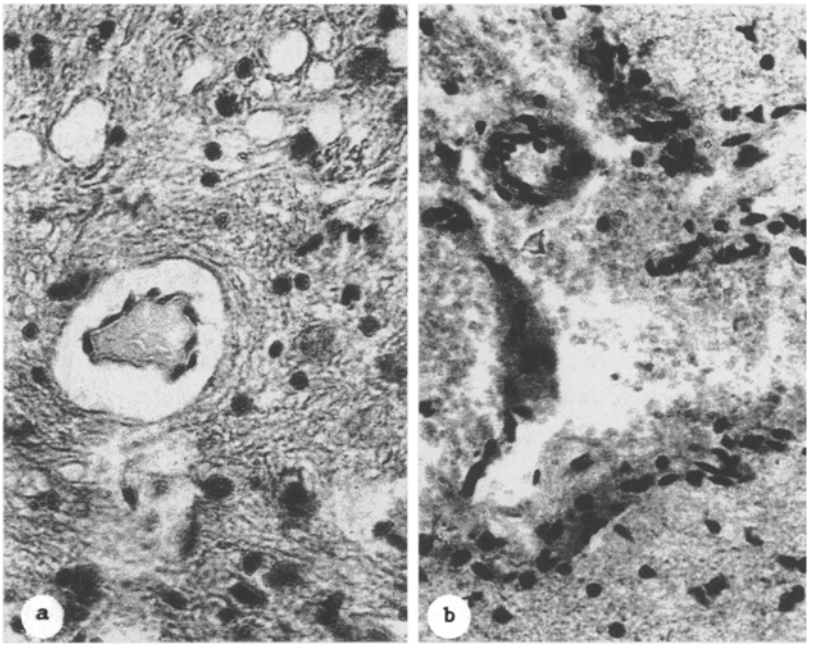

Fig. 2. Changes in rat brain on 3rd day after injection of AA: a) marked perivascular edema with intravascular blood clotting, b) focus of hemorrhage in the brain. Hematoxylin and eosin. 400×.

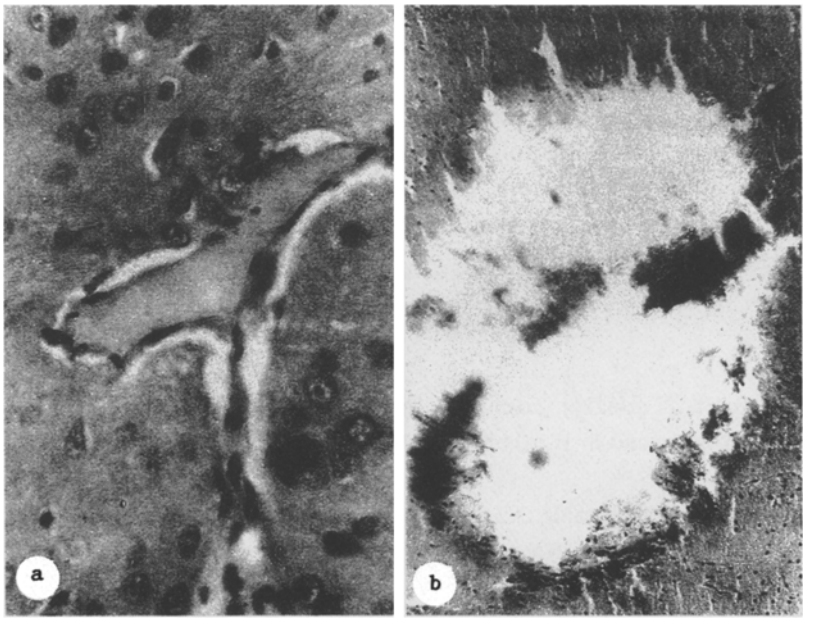

Fig. 3. Brain 3 days after intra-arterial injection of AA. a) "Hyaline" thrombus in a venule against the background of constriction of the capillary lumen and perivascular edema. 400×; b) focus of necrosis with cavity formation in white matter of brain. 100×. Hematoxylin and eosin.

Thus in the animals of this group, unlike in the control group, dilatation and congestion of vessels of the microcirculatory bed was observed, with some increase in vascular permeability and with the appearance of perivascular edema, mainly around the small veins and venules. Destructive changes in the brain tissue were mild in degree, and were manifested only as widespread but less frequent areas of micronecrosis. Unlike the other groups, in this group damage to vessels of the meninges and of the vascular plexus was particularly well marked, and led to the appearance of severe hemorrhages.

Microscopically marked perivascular, and in some cases pericellular, edema was observed in all parts of the brain of the rats receiving piracetam. Separate small foci of necrosis and softening, with the presence of multiple extravascular erythrocytes, were found in the white matter. In some areas, especially around the small arteries and veins, perivascular concentrations of erythrocytes were large. As regards the microcirculatory bed, besides the changes already described, general constriction of the lumen was found, with multiple venules containing hyaline thrombi. More severe changes affecting the blood vessels were discovered in the vascular plexuses of the meninges, where large, confluent hemorrhages were discovered around the small arteries and arterioles.

Thus in the animals of this group severe changes were found mainly in the microcirculatory bed, where foci of destruction developed in the white matter against the background of diffuse vasoconstriction, increased permeability, and perivascular edema. By contrast with the control group, more severe spasm of the vessels was observed with increased permeability of their walls and with foci of hemorrhage. If signs of intravascular blood clotting were present, aggregation of erythrocytes was not observed in the lumen of the small venules and veins. Under these circumstances necrosis and softening of the tissues were microfocal but diffuse in character.

Thus injection of AA causes infarction of the brain, whose development is based on interaction between the AA metabolite thromboxane A_2 (TxA_2) with the specific TxA_2/PGH_2 (a peroxide metabolite of AA) receptor [11]. This accompanies the process of secretion of various substances from blood cells: proaggregants (ADP, collagen, thrombin), stimulating intravascular thrombus formation, in addition to the ability of TxA_2 and PGH_2 to induce spasm of cerebral vessels [14]. During AA metabolism, free oxygen radicals also are formed, and these can damage the tissues and cause lysis of erythrocytes [10]. The preparations studied differed in their effects on the course of the cerebral infarct. For instance, under the influence of GABA the destruction process in the brain tissue followed a milder course, there were no signs of aggregation of erythrocytes, and the blood vessels were dilated. Under the influence of piracetam destruction and necrosis were observed, but were microfocal in character, and intravascular clotting continued to be noted, although with no signs of aggregation of erythrocytes. Spasm of the vessels was more intensive than in the control.

Consequently, the morphological picture of the changes in cerebral infarction was a little improved under the influence of both preparations, more especially when GABA was used. The results are in agreement with those obtained by workers who noted the stronger action of piracetam on the cerebral blood flow than of GABA [1]. Also, unlike piracetam, GABA does not pass through the blood–brain barrier, and because of that, a definite role in the effects of GABA on the cerebral circulation is played by peripheral mechanisms, including an antiaggregation mechanism.

Thus GABA and piracetam have a certain beneficial action on the course of experimental brain infarct in rats; this action is stronger in the case of GABA, in whose mechanisms of action a definite role may be played by peripheral rheologic shifts, activated under conditions of cerebrovascular pathology.

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EFFECT OF A PEPTIDE PREPARATION FROM THE HEART ON THE ISCHEMIC MYOCARDIUM

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The writers showed previously that cordialin, a polypeptide preparation from myocardial tissue, can limit the size of an ischemic focus [4] and have a beneficial effect on the state of cells in the peri-infarct zone [5] in animals with experimental myocardial infarction. However, to determine whether treatment of a myocardial infarct (MI) with cordialin is indicated, a more detailed study of its effects on the formation of the zone of necrosis and on the various stages of the healing process is essential.

The aim of this investigation was to study the effects of cordialin on mortality of animals with experimental MI, the degree of heart damage, and the time course of healing of the pathological focus.

EXPERIMENTAL METHOD

The possibility of a direct effect of cordialin on physiological and biochemical parameters characterizing the state of the myocardium was studied on isolated hearts. Experiments were carried out on 150 noninbred albino rats and 20 guinea pigs. MI was produced by ligation of the left coronary artery. The size of the zone of necrosis (ZN) in the myocardium was determined by the method in [1]. The size of the unperfused zone (UPZ) was determined by filling the coronary arteries [2]. Cordialin was injected intraperitoneally in a dose of 0.5 mg/kg, 1, 2, and 6 h after coronary occlusion. Fragments of myocardium were fixed in 10% neutral formalin, dehydrated in alcohols of increasing concentration, and embedded in paraffin wax. Paraffix sections 5-7 μm thick, were stained with hematoxylin and eosin and by Van Gieson's method. The guinea pigs' hearts were perfused by Langendorff's method at 30°C and with saturation of the perfusion fluid with carbogen. Perfusion was carried out without stimulation of the heart muscle, at a constant flow rate of 10 ml/min, which was assigned by a peristaltic pump. One hour after the beginning of perfusion, during which the work of the heart became adapted and stabilized, total ischemia was created for a period of 30 min by compressing the tube introducing the solution. The change in the parameters of contraction (frequency and amplitude) was assessed and the malonic dialdehyde (MDA) concentration determined in biopsy material from the myocardium.

EXPERIMENTAL RESULTS

Table 1 shows that by the end of the first day the ZN in animals receiving cordialin was smaller than in the control, but on the second day the dimensions of ZN in the groups compared were identical and did not differ from the control values obtained 24 h after coronary occlusion. One hour after creation of MI total death of the cells was observed in the control group

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