HISTOCHEMICAL INVESTIGATION OF THE DISTRIBUTION OF PLASMINOGEN ACTIVATOR IN EXPERIMENTAL ISCHEMIA

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Experimental ischemia induced in rats by ligation of the coronary artery leads to a decrease in the concentration of plasminogen activator in the myocardial tissues, especially in the zone of necrosis 1 day after ligation of the vessel. After 3 days the concentration of plasminogen activator is restored. The results of the histochemical tests were confirmed by biochemical analysis.

KEY WORDS: fibrinolysis; plasminogen activator; myocardial ischemia.

The histochemical method of determination of the fibrinolytic activity of tissues by autography, suggested by Todd [12], has played an important role in the study of fibrinolysis. By means of this method it is possible to investigate the fine distribution of two of the most important components of the fibrinolytic system, namely plasmin and plasminogen activator (PA), under physiological conditions without any gross preparative interference (such as at times significantly alters the structure and, correspondingly, the activity of enzymes), not only in the organs or tissues of the animal, but also at the cellular and subcellular levels [12]. The topography of these two enzymes and changes in their activity have been studied under normal and pathological conditions under the influence of various neurohumoral factors [8].

The use of Todd's method [12] is particularly appropriate for the study of diseases in whose pathogenesis plasmin and PA play an active role: in thrombophlebitis, venous stasis, atherosclerosis, and myocardial infarction.

The object of this investigation was to study changes in PA activity by the fibrinolytic autography method in rats with experimental ischemia.

EXPERIMENTAL METHOD

Myocardial ischemia was induced in male albino rats weighing 200-250 g by ligation of a large branch of the coronary artery under ether anesthesia. The heart was removed and the myocardial tissue from seven animals was investigated histochemically on the day after ligation of the artery, and in another 12 animals 3 days after the operation. A mock operation was performed on the five animals of the control group. Histochemical analysis of the myocardial tissue was carried out by Todd's method [12] in the modification of Rejniak et al. [11]. Fibrinolytic activity of the tissue samples was estimated quantitatively by Pandolfi's method [9] from the time of lysis of a fibrin microfilm 60 μ in thickness above a tissue section during incubation at 37°C. Simultaneously with histochemical analysis of the heart, in some animal tissue activator was isolated and its activity was determined by the method of Andreenko and Migalina [2].

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TABLE 1. Time (in h) of Lysis of Fibrin Microfilm by Tissue Preparations

Group of animals		Right atrium	Left atrium	Right ventricle	Left ventricle	Region of ischemia
Ischemia for 1 day	$M \pm m$	14,5± 2,0 (7)	15,0± 1,4 (7)	17,0± 1,74 (5)	19,5± 0,72 (7)	24,5± 1,7 (6)
	P	<0,05	N.s.	<0,02	N.s.	<0,05<0,05
Control	$M \pm m$	10,0± 1,2 (5)	11,0± 1,2 (3)	12,5± 1,35 (5)	15,0± 1,2 (5)	17,0± 2,0 (5)
	P					
Ischemia for 3 days	$M \pm m$	14,0± 1,5 (12)	13,0± 0,8 (11)	15,0± 1,0 (12)	17,0± 2,0 (11)	21,0± 1,4 (9)
	P	<0,02	N.s.	N.s.	N.s.	<0,05 N.s.

Note. Numbers in parentheses show numbers of animals. During statistical analysis indices were compared from experimental and control groups and, in addition, indices were compared for the left ventricle and the region of ischemia in the experimental groups; N. S.) not statistically significant.

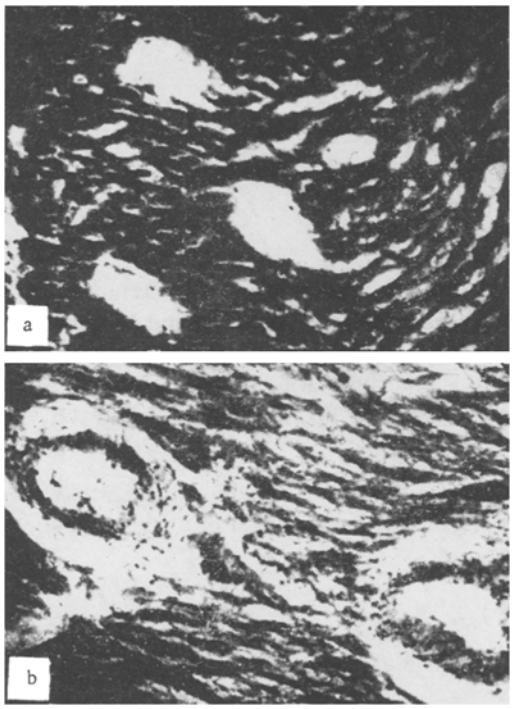

Fig. 1. Photomicrograph of myocardium of apex of left ventricle on rat with experimental ischemia (a) and control rat (b) 40 min after beginning of incubation of fibrin film with section. Hematoxylin, 70x.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that 24 h after ligation of the coronary artery the time taken for lysis of the fibrin film by tissues from various parts of the heart was increased, indicating a decrease in the PA content in the myocardial tissues of the atria and ventricles as a result of ischemia. The greatest decrease (statistically significant) in fibrinolytic activity was observed actually in the region of necrosis. A marked difference in the PA content in the necrotic zone of the left ventricle and in its apex, outside the zone of necrosis, was observed in the experimental animals. A photomicrograph of the myocardium from the ischemic apex of the left ventricle, taken 40 min after the beginning of incubation of the microslide (Fig. 1a), shows the absence of active PA in the vascular endothelium by contrast with the section through the control heart, in which distinct pale zones of lysis can be seen around the small vessels after incubation for 40 min with the fibrin film (Fig. 1b). The results agree with data in the literature indicating that fibrinolytic activity of the ischemic region falls in the period of organization of the infarct [6].

A decrease in the activity of other enzyme systems in the inital period of experimental ischemia has been observed by several workers [1, 3, 5]: lactate dehydrogenase, cytochrome oxidase, alkaline phosphatase, and the enzymes of glycolytic metabolism; in most cases this decrease has been attributed to the development of degenerative changes in the myocardium and to elution of enzymes from the tissues into the blood and lymph [4, 10]. Observations made by some workers who found an increase in the PA content in blood taken from the left ventricle of animals with ischemia lasting 24 h can be explained by the breakdown of cells in the necrotic zone and the liberation of PA into the blood, causing, on the one hand, a decrease in PA activity in the zone of necrosis and, on the other hand, an increase in its content in the blood.

The PA level 3 days after ligation of the artery was lower than in the tissues of the control heart, but less so than on the day after ligation of the vessel (Table 1). The time of lysis of the fibrin film above the section of the myocardium from the region of ischemia in the animals with ischemia for 3 days was 28% less than the time of lysis in an animal with ischemia lasting 1 day. This agrees with data showing increased fibrinolytic activity in the young vessels of the necrotic region of the heart in the period of restoration of the disturbed structures of that region [7].

Heating the microfilm in a moist chamber for 40 min at 85°C [5] rendered it completely incapable of lysis by tissue enzymes, showing that the tissue sections contained no active plasmin.

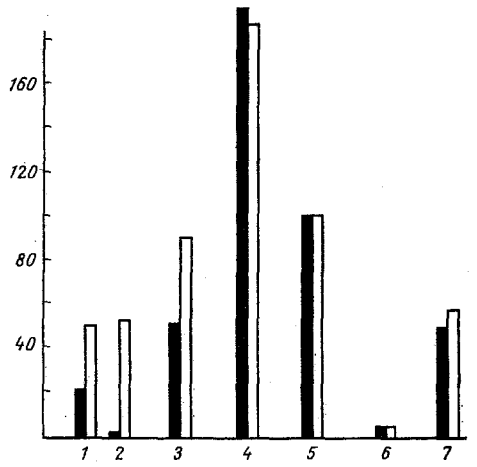

Fig. 2. PA activity in various organs of rats. Shaded columns) experimental animals, unshaded columns) control; 1) left ventricle, 2) region of necrosis, 3) right ventricle, 4) lungs, 5) kidneys, 6) liver, 7) spleen. Ordinate, PA activity (in mm^2/100 mg weight of dry powder).

The decrease in the PA content in the myocardial tissues of the ischemic heart was confirmed by biochemical tests after isolation and partial purification of PA by the method of Andreenko and Migalina [2]. Activity of the heart (various parts thereof) and other organs taken from three intact animals and three animals with myocardial ischemia is shown in Fig. 2. Clearly PA activity was sharply reduced in the heart tissues of the experimental animals, whereas in the other organs the differences in PA activity with the control animals were not significant.

The histochemical tests thus showed that experimental ischemia leads to a decrease in the PA content in the myocardium, especially in the zone of necrosis, followed by a gradual restoration of activity of the enzyme. Biochemical analysis confirmed the histological observations.

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