forms of mesenteric vessels. This is further confirmation that a disturbance of the venous outflow from the intestine represents a greater risk to the animal than disturbance of the inflow within the same time interval.

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A DIFFERENTIAL INDICATOR METHOD OF IDENTIFYING ZONES OF ISCHEMIA AND NECROSIS IN RATS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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KEY WORDS: size of a myocardial infarct; method of determination

Success in the search for therapeutic substances capable of reducing a zone of necrosis or infarction of the myocardium is largely dependent on the development of objective methods of assessment of damaged zones. Morphometric methods, most widely used at the present time, yield information on the size of zones of ischemia and necrosis [1, 6]. However, their use is limited by the complexity of the planimetric method of calculating the area of a lesion on the basis of individual sections, as well as its somewhat conventional nature.

A considerable step forward in improvement of the accuracy and simplification of the technique of mass investigations was the development of a method whereby the zone of ischemia and zone of necrosis can be estimated simultaneously as percentages of the total weight of the myocardium [4]. The basis for calculation of the zones of damage in this case is the difference in the quantity of dye contained in the hearts of rats with a myocardial infarct and intact animals. This last stage of affairs, however, is responsible for the main disadvantage of the method, namely the absence of an "internal" control, which makes the results obtained by this method much less comparable.

This paper describes a modification of the technique of determining zones of damage in rats with myocardial infarction, and which can yield original data for their calculation simultaneously in the same heart.

The principle of the method consists of using an "internal" control to identify zones of damage in each experiment on the basis of the difference between the quantity of dye in the damaged part of the myocardium and in its intact part. In this way the accuracy of the results can be greatly increased and the negative effect of possible deviations in the course of each experiment can be abolished.

As the indicator of the size of the zone of ischemia we used Evans' blue, perfusion of the isolated heart with which enables the size of the unperfused region to be estimated [3]. The indicator of the zone of necrosis was triphenyltetrazolium bromide which, like other soluble tetrazolium salts, on interaction with areas of myocardium preserving their dehydrogenase activity, is reduced into formazan [2, 5, 7].

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Description of the Method. A myocardial infarct was induced in noninbred male rats weighing 250-350 g by ligation of the descending branch of the left coronary artery at the level of the lower border of the auricle of the atrium, by the usual method. Animals were killed at various times after the operation, after receiving a preliminary intravenous injection of heparin (500 U/kg). The heart was quickly removed, washed in isotonic sodium chloride solution, after which the ascending part of the arch of the aorta was cannulated and the coronary vessels were irrigated with the same solution (37°C) under a pressure of 135 cm water, to remove traces of blood from the vessels. The myocardium was then perfused under the same pressure with a 0.025% solution of Evans' blue (37°C) until its undamaged parts were stained dark blue (the duration of staining in this case was 5-6 min).

After the end of perfusion with Evans' blue the myocardium was thoroughly washed with isotonic sodium chloride solution and the unstained region was excised, including tissue as far as 2-3 mm outside the clearly visible boundary of staining at the periphery.

Damaged and undamaged parts of the myocardium were weighed, cut into three or four approximately equal parts, and transferred into separate bottles, to which was added phosphatase buffer (37°C) containing triphenyltetrazolium bromide (1 mg/ml) at pH 7.4. The ratio of the area of tissue and buffer was 1:9 by weight. The bottles were then incubated for 1 h at 37°C. Under these conditions the myocardium, except the zone of necrosis, stained bright red due to the formation of formazan.

The dyes were isolated from the tissue and their concentrations determined by extracting them with chloroform [4], for which purpose the areas of damaged and undamaged myocardium were homogenized separately in the corresponding incubation buffer.

Determination of the Zone of Ischemia. To 0.5 ml of each homogenate 3 ml of 32% hydrochloric acid was added and the mixture incubated for 1 h at 37°C to destroy the formazan, after which 2 ml of a 12.8% solution of alkyldimethylbenzylammonium chloride was added to each. The mixture was shaken for 1 min until a colored complex of Evans' blue and alkyldimethylbenzylammonium chloride was formed. The complex was extracted with chloroform (2.5 ml) while the mixture was shaken for 2 min. The stained chloroform was separated by centrifugation and the concentration of Evans' blue determined on a spectrophotometer at 578 nm against pure chloroform. The concentration of Evans' blue in 1 g of tissue was found from a calibration graph. Subsequent calculation of the size of the zone of ischemia was done by means of the formula given below.

Determination of the Zone of Necrosis. To 2.5 ml of each homogenate 2.5 ml of chloroform was added and the mixture shaken for 2 min. The formazan was extracted with chloroform for 15 min at 4°C, the mixture being shaken every 5 min for 30 sec. The stained chloroform was separated by centrifugation, and to remove all traces of Evans' blue it was mixed with alumina (100 mg/ml), recentrifuged, and after preliminary separation of the chloroform in the ratio of 1:5, the formazan concentration was determined at a wavelength of 492 nm. The formazan content in 1 g of tissue was found from a calibration graph. Subsequent calculation of the size of the zone of necrosis was done by the formula given below.

Calculation of the Zone of Ischemia and Zone of Necrosis. The dimensions of the zone of ischemia and zone of necrosis were calculated as percentages of the total weight of the myocardium by the following formula:

$$X = 100 - \frac{K_1 M_1 + K_2 M_2}{K_1 (M_1 + M_2)} \cdot 100,$$

where X denotes the size of the zone of necrosis or the zone of ischemia; K_1 the concentration of dye in 1 g of the undamaged part, and K_2 its concentration in 1 g of the damaged part, M_1 the weight of the undamaged part, and M_2 the weight of the damaged part.

A value reflecting the real content of dye in the whole heart is formed in the numerator of the fraction after the procedures have been carried out, and the value of the "expected" content of the dye, i.e., the amount of it which could accumulate in the heart if there had been no unperfused area ("internal" control) is formed in the denominator. Under these circumstances it is possible to eliminate possible deviations in the degree of staining of the tissue with the dye, which may be connected both with the different functional state of the undamaged parts of the infarcted myocardium and with other factors.

After determination of the size of the zones of damage, the ratio of the zone of

necrosis to the zone of ischemia, i.e., the value which was most stable and least dependent on fluctuations in the size of the zone of ischemia, was calculated as a percentage. Investigations conducted by the method described above showed that the zone of necrosis, 1 h after ligation of the coronary artery, was 25 ± 3.7% of the zone of ischemia, and after 2 and 4 h it was 41 ± 4.5 and 68 ± 4.3% respectively. Intravenous injection of propranolol (1 mg/kg) 12 min and 2.5 h after creation of the infarct caused a decrease in size of the zone of necrosis, which was 52 ± 2.7% of the area of ischemia ($p < 0.05$) 4 h after occlusion of the coronary artery. These figures are close to the results obtained by other workers who conducted similar investigations [4].

The differential indicator method of determining the dimensions of the zones of ischemia and necrosis in myocardial infarction is easily reproducible, accurate, and sufficiently informative and has high throughput capacity, thus making it promising for use in experimental cardiac pharmacology.

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DEPENDENCE OF PLATELET AGGREGATION RESPONSE ON ARGINYL-GLYCYL-ASPARAGINE TRIPEPTIDE

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Fibronectin (FN) is the term given to a class of structurally and immunologically related polyfunctional high-molecular-weight glycoproteins, which are involved in various functions of cells, including adhesion of cells, formation of the extracellular matrix, and changes in the cytoskeleton, cell mobility, phagocytosis, differentiation, and neoplastic transformation [6]. The role of the blood plasma FN in functions of the reticuloendothelial system, in regenerative processes, and in the mechanism of hemostasis, of which the key components are platelet aggregation and the formation of the primary platelet plug, has frequently been described [2, 3, 5, 9]. For the platelet aggregation response (PAR) to develop, not only is an inducer (aggregant) necessary [8], but so also are bivalent cations (Ca^{++}) and protein cofactors, which may include fibrinogen, FN, and Willebrandt's factor [1].

This paper describes a study of the action of exogenously added FN on PAR and also the effect of binding of endogenous FN with specific antibodies (AB), and the action of the synthetic tripeptide arginyl-glycyl-asparagine (AGA), which is an amino-acid sequence found in the region (domain) of the FN molecule which interacts with the cell receptors of platelets and other cells [7], on PAR.

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