MICRODIALYSIS STUDY OF RELEASE OF ADENINE NUCLEOTIDE BREAKDOWN PRODUCTS INTO THE MYOCARDIAL INTERCELLULAR SPACE DURING ISCHEMIA AND REPERFUSION

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Adenosine (A) is one of the most important regulators of coronary vascular tone [4, 8]. At the present time changes in the A concentration in the intercellular space of the myocardium are judged on the basis of its concentration in the coronary venous outflow or in the transudate collected from the epicardial surface of the heart [5, 6]. In this communication we suggest a more direct method of determining extracellular A, based on perfusion of the rat heart by means of a microdialysis apparatus, implanted into the wall of the left ventricle, followed by determination of levels of adenosine nucleotide breakdown products (ANBP), namely A, inosine (I), and hypoxanthine (HX) in the dialysate. The microdialysis technique has already found wide application in neurophysiological and neuropharmacological research for quantitative analysis of neurotransmitters and other products in the extracellular space in various parts of the brain [9, 10]. It enables local changes in the concentration of ANBP, including A, to be assessed during ischemia and reperfusion of the heart.

EXPERIMENTAL METHOD

Experiments (n = 12) were carried out on isolated hearts of male Wistar rats weighing 300-350 g. The hearts were perfused in the retrograde direction with Krebs' solution at a constant rate of about 12 ml · min^{-1} · g^{-1}. The indicator of their contractile function was the pressure in a constant-volume balloon, introduced into the chamber of the left ventricle. Fuller details of the technique were given previously [1, 2]. After perfusion for 10 min a microdialysis apparatus, made from a dialysis tube (Cordis Dow, USA) with external diameter of 0.25 mm, and permeable for substances with mol. wt. of up to 5 kD, was introduced into the anterior wall of the left ventricle. For this purpose, a monofilament Prolene thread (Prolene 7.0, Ethicom, USA) was introduced inside the working region, after which one of its ends was glued into a silicone tube 12 cm long and with an internal diameter of 0.28 mm. A stainless steel needle was glued into the other end, and by means of it the dialysis apparatus was guided between the lateral branches of the descending part of the left coronary artery to a depth of about 1 mm. The needle was then disconnected and the free end of the dialysis fiber glues into a Silicone tube with external diameter of 0.4 mm and a length of 8 cm, intended for connecting with the other end to a perfusion pump (Braun, West Germany). Adequate fixation of the dialysis apparatus in the heart ensured firm contact between the ends of the silicone tubes and the epicardial surface. The effective length of the dialysis apparatus was about 8 mm. The operation to implant it took 8-12 min, after which the dialysis apparatus began perfusing at the rate of 2 μl/min with Krebs' solution not containing glucose. The beginning of the experiment was preceded by a 20-min period of control preperfusion of the dialysis apparatus. The results of one experiment are given in Figs. 1 and 2. Total ischemia lasting 40 min was created by complete arrest of the flow through the myocardium. Samples of dialysate were collected in microtubes as successive 10-min or 20-min fractions. Samples of perfusion fluid which had passed through the coronary vessels were collected at discrete (in time) fractions [2]. Concentrations of A, I, and HX in samples of dialysate and perfusion fluid were determined by high-performance liquid chromatography [1]. Calibration of the dialysis

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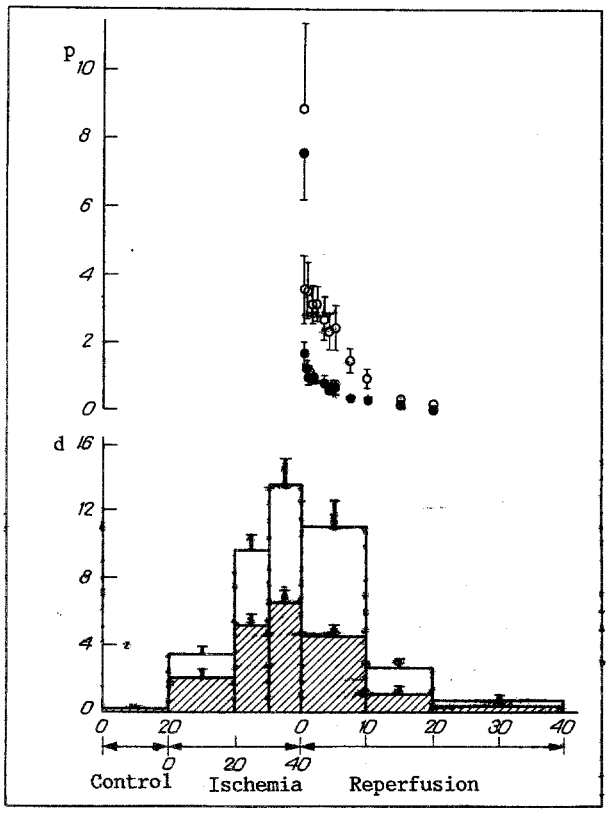

Fig. 1. Concentrations (in nmoles/ml) of I (empty circles and unshaded columns) and HX (filled circles and shaded columns) in perfusate (p) and dialysate (d) in control period of perfusion of rat hearts, during ischemia, and during reperfusion, in min (n = 6).

apparatus 8 mm long, with a flow fate of perfusion of 2 μl/min showed that when the concentrations of I and HX in the external medium were 50 nmoles/ml and that of A 15 nmoles/ml, their concentrations in the dialysate were 5.3, 6.0, and 9.3% of these values respectively.

Statistical analysis of the results was undertaken by Student's test.

EXPERIMENTAL RESULTS

In the period of control perfusion of the heart the concentration of ANBP in the dialysate was very low. Thus implantation of the dialysis apparatus into the myocardium, by contrast with its implantation into brain tissue [11], is evidently not a traumatic operation. The creation of total ischemia led to a significant rise of the ANBP level in the extracellular space and, correspondingly, in the dialysate (Figs. 1 and 2). Moreover, whereas accumulation of I and HX took place proportionally to the duration of ischemia, the rise of A during the first 20 min was comparatively small, probably reflecting the preferential nature of the AMP – IMP – I degradation pathway compared with dephosphorylation of AMP into A in the presence of mild ischemic damage of the rat heart [12]. An increase in the A concentration among ANBP indicates the increased importance of the second of these pathways of catabolism with increasing duration of ischemia (Fig. 3).

During reperfusion of the ischemic rat hearts the kinetics of elution of ANBP by the perfusion fluid was similar in type to that discovered previously for guinea pig hearts [2]. For I and HX it also was biphasic and could be described by the equation [2] $C(t) = A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t)$, where A, B, α, β are constants, and $\alpha >> \beta$. The fast phase of elution, characterized by the velocity constant α was correlated in [2] with elution of I (and HX), which had accumulated before the beginning of reperfusion in the extracellular space, endothelium, and vessels. The slow phase (with velocity constant β) ought evidently to characterize the inertia of the process of adenine nucleotide degradation after termination of ischemia [7] and elution of the excess of ANBP from the cardiomyocytes [2]. For rat hearts values of α_I, β_I, α_{HX}, and β_{HX} were 9.24 ± 1.20, 0.186 ± 0.021,

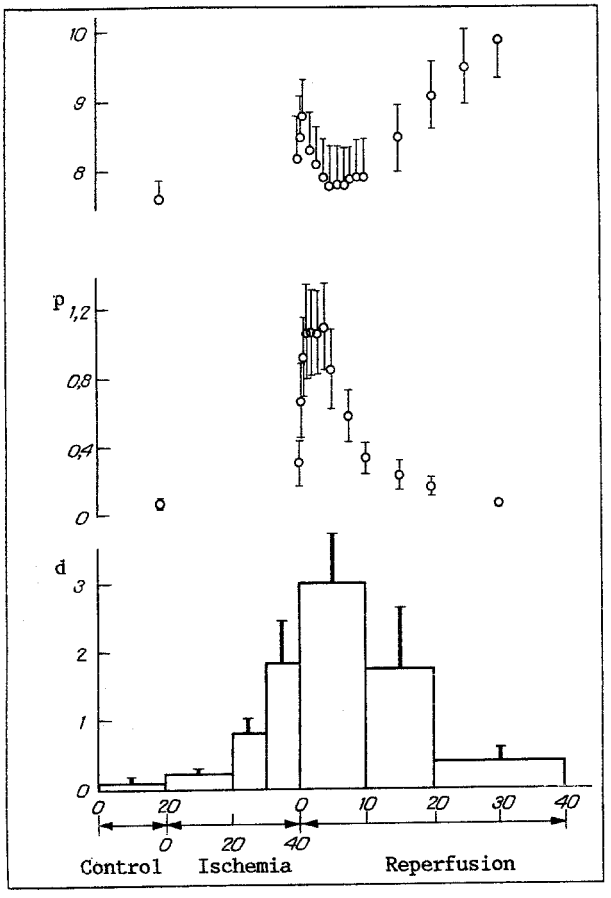

Fig. 2. Coronary resistance (in mm Hg · min · g · ml^{-1}; n = 12) and concentration of A (in nmoles/ml, n = 6) in perfusion fluid (p) and dialysate (d) in control period of perfusion of rat hearts, during ischemia, and during reperfusion (in min).

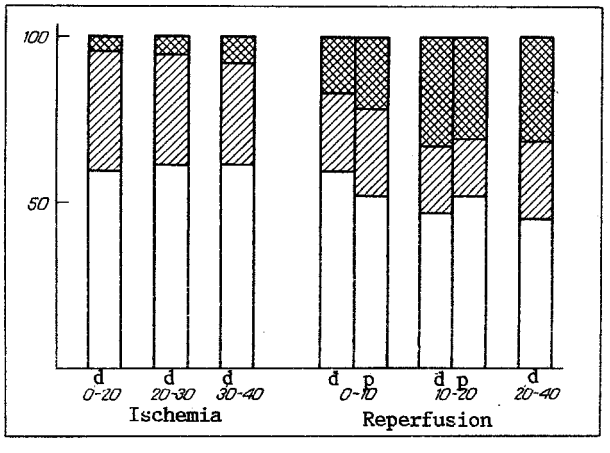

Fig. 3. Relative levels (in per cent) of I (unshaded part of column), HX (shaded part), and A (cross-hatched part) in dialysate (d) and perfusion fluid (p) during ischemia and reperfusion (in min).

8.36 ± 1.10, and 0.238 ± 0.070 min^{-1} respectively. Dependence of the A concentration in the perfusion fluid on the time of reperfusion of the rat hearts, just as for guinea pigs, had a maximum at 2.2 ± 0.4 min (Fig. 2). This course of the curve is evidently due to competitive inhibition of carrier-mediated A transport through the membranes of cardiomyocytes and endothelial cells by I (and, possibly, by HX also), as a result of which A accumulates during ischemia in the cardiomyocytes (where it is formed [8]) by a greater degree than in the interstitial tissues, and at the beginning of reperfusion, its elution from these pools is delayed until a sufficient amount of I and HX has been released into the perfusion fluid [2]. On the basis of these suggestions it must be expected that, unlike I and HX, the maximum of the A concentration in the intercellular space ought to occur, not at the end of ischemia, but shifted somewhat into the region of reperfusion, and the contribution of A to ANBP during reperfusion ought to be greater than in the period of ischemia. Results in this direction were in fact obtained by the use of microdialysis. It will be noted that during reperfusion the relative percentages of concentrations of ANBP in the dialysate and perfusion fluid were closely similar, indicating the unimportant role of catabolism of ANBP in passage across the endothelial barrier [the concentrations of ANBP in fractions of the perfusion fluid (Fig. 3) can be easily calculated from the kinetic data].

Thus whereas during total ischemia, unique results on the dynamics of release of ANBP into the intercellular space can be obtained by microdialysis, during reperfusion qualitative agreement is observed between changes in the ANBP levels in the coronary outflow and in the intercellular medium. On the basis of these changes it can be postulated for A, a metabolic vasodilator, that it is the character of its release into the intercellular space during reperfusion that determines the formation of the minimum in the change of coronary resistance (Fig. 2). The quantitative ratio of concentrations of ANBP in the dialysate and perfusion fluid (average values for the first 10 min of reperfusion) was 5.2 ± 1.4 for A, 4.5 ± 1.2 for I, and 5.0 ± 1.3 for HX. Allowing for the degree of extraction of ANBP during dialysis, it can be estimated that the ANBP level in the extracellular medium was about 70 times higher than its level in the venous outflow. If their level in the transudate is used as an index of the intercellular ANBP concentration, this ratio (under conditions of cardiac hypoxia) was significantly less (about 4-6) [5].

The study of the kinetics of elution of ANBP during reperfusion of the heart after total ischemia, besides determination of kinetic constants, can also be used to calculate integral characteristics of the process: loss of ANBP during reperfusion of infinite duration (R) [2] and by the use of the method of statistical moments [3] the mean retention time (MRT) of ANBP during reperfusion. For the rat heart, values of R (per gram wet weight of heart) and MRT were: 130.7 ± 38.0 nmoles/g and 4.87 ± 0.96 min for A, 154.4 ± 38.2 nmoles/g and 1.89 ± 0.56 min for Hx, and 348.0 ± 114.8 nmoles/g and 3.02 ± 0.35 min for I. Thus adenosine, released by the rat heart in a smaller amount than the other adenine nucleotide breakdown products, is the product of adenine nucleotide catabolism that is retained the most during reperfusion ($p < 0.05$ relative to MRT of HX).

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