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Ischaemic Contracture and Myocardial Perfusion in Isolated Rat Heart

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Summary. The development of left ventricular contracture and myocardial perfusion defect was studied in isolated rat hearts during global ischaemia of 90 min duration. The left ventricular pressure was measured by a balloon catheter inserted into the ventricle and filled with water. The pressure reached the maximum at 16 min of ischaemia. The left ventricular volume and compliance (passive distensibility) were measured by the same balloon, the former by connecting the balloon to an open catheter and the latter by applying a constant additional volume (0.020 ml) into the balloon. The left ventricular volume and compliance both decreased progressively for 20 min of ischaemia after which they remained low for the rest of the observation period (90 min). The myocardial perfusability was tested by infusing 0.1 per cent sodium fluorescein in isotonic saline into the cannulated aortic root of the isolated heart preparation. The percentage perfused with the fluorescent tracer in horizontal frozen myocardial sections was estimated by point counting from colour photographs taken under ultraviolet light. The proportion of the perfused area decreased gradually from 100% at 0 min of ischaemia to 93, 67, 43 and 37% at 15, 30, 60 and 90 min of ischaemia, respectively. It was concluded that ischaemic contracture of the left ventricle is followed by the development of a myocardial perfusion defect in isolated ischaemic rat heart.

Key words: Coronary vessels – Ischaemic cell injury – Myocardial infarct – No-reflow phenomenon – Rigor mortis.

Introduction

Contracture of the left ventricle occurs in isolated rat heart during global ischaemia. The mechanism of this ischaemic contracture is not known in detail, al-

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though its development seems to depend on ATP depletion which leads to myocardial rigor (Hearse et al., 1977; Katz and Tada, 1972, 1977). Rigor was recently implicated as the major determinant of the impaired myocardial perfusion observed in experimental canine myocardial infarcts (Gavin et al., 1978a) and in isolated ischaemic dog hearts (Nevalainen et al., 1978); the same authors suggest that rigor could be the main cause of the "no-reflow"phenomenon described in experimental myocardial infarcts by Kloner et al. (1974).

In the present investigation myocardial perfusion was studied by a fluorescent tracer in ischaemic isolated rat hearts. Ischaemic contracture of the left ventricle was followed by a myocardial perfusion defect in this experimental model.

Materials and Methods

A modified Langendorff (1895) method was used. Wistar rats weighing 200–400 g, maintained on a standard diet, were anesthetized by intraperitoneal (i.p.) Nembutal (0.1 ml/100 g), and given 0.5 ml heparin i.p. The abdominal cavity was opened, the diaphragm was transected, and lateral incisions were made along both sides of the rib cage. The anterior chest wall was folded back. The left lung was pulled away and the descending aorta was cannulated with a glass cannula. The right auricle was incized and retrograde coronary infusion was immediately started from an infusate reservoir at 37° C and at a pressure of 65 cm H_2O. The infusate consisted of Krebs-Henseleit bicarbonate buffer (Na 118.46 mM, KCl 4.73 mM, $CaCl_2$ 2.53 mM, $NaH_2PO_4 \cdot H_2O$ 1.18 mM, $MgCl_2 \cdot 6\,H_2O$ 1.18 mM, $NaHCO_3$ 24.87 mM, pH 7.40), glucose 10.09 mM, and 0.002 per cent phenol red. It was gassed continuously with 95% oxygen and 5% carbon dioxide. The great vessels arising from the aortic arch were ligated. The pericardium, the lungs and mediastinal tissues were trimmed away and the isolated heart was placed in a beaker at 37° C.

For the measurement of the left ventricular volume, pressure, and compliance a balloon catheter was inserted into the left ventricle through the left atrium. The balloon was made of thin latex (condom), approximately to the size of the left ventricular cavity, and secured to a teflon catheter by number 5-0 surgical silk. It was filled with water and either left open or connected to a recorder (Omni Scribe, Houston Instrument, Bellaire, Texas) through a pressure transducer (EMT 490 A, Elema-Schönander, Stockholm, Sweden) and amplifier (EMT 460, Elema-Schönander, Stockholm, Sweden).

The decrease of the left ventricular volume during the development of the contracture was measured with the open catheter, placed vertically on a millimeter scale, as an increase of the water surface. When the left ventricular pressure was measured the balloon was filled with water under hydrostatic pressure of 65 cm H_2O after which the catheter was connected to the pressure transducer and the system remained isovolumic during the experiments. Compliance (C) or the passive distensibility of the left ventricle measured in the system described above. At 2-min intervals, additional 0.020 ml water (ΔV) was applied into the balloon from a syringe for 30 s and the pressure change (ΔP) was recorded. $C = \Delta V / \Delta P$.

Myocardial perfusion was measured by infusing 0.1% sodium fluorescein dissolved in isotonic saline into the aortic cannula for 1 min at the pressure of 65 cm H_2O. After the infusion the hearts were placed into a freezer (– 18° C) for 1–7 days. The frozen hearts were sectioned horizontally in the middle and 50 µm thick cryostat sections were photographed under ultraviolet light (w.l. 350 nm) at magnification 1:1 on Kodak Ektachrome 200 color slide film. The proportion of the myocardium perfused with the fluorescent tracer was quantified by projecting the color slide on a grid mesh drawn on white paper with test points 0.7 cm apart. The number of test points falling on perfused and non-perfused areas in the pictures is directly proportional to the corresponding areas in the tissue sections (Chalkley, 1943). The myocardial perfusion is expressed as per cent of the section containing the fluorescein tracer. In pilot experiments, a number of hearts were sectioned semi-serially and it was found that the perfusion was even throughout the organ as measured by the method described above.

After a stabilizing period of 15 min the hearts were made globally ischaemic by closing the valve to the infusate reservoir. The perfusability was measured at 0, 15, 30, 60 and 90 min of ischaemia.

Results

Ischaemic Contracture

1. Left Ventricular Volume. The decrease of the left ventricular volume during the development of the ischaemic contracture was determined by a balloon filled with water and connected to an open catheter which allowed the expulsion of the water against minimal resistance. The contracture began at 8 min of ischaemia and was complete at 20 min (Fig. 1). The contracture, as measured in the decrease of the left ventricular volume, was maintained practically at its maximum until the termination of the experiment (90 min of ischaemia).

2. Left Ventricular Pressure. The left ventricular pressure was measured by the water filled balloon catheter filled with a constant volume during the experiment. During ischaemia the contractions ceased within a couple of minutes and the pressure remained low. After approximately 6 min of ischaemia the pressure began to increase relatively rapidly and reached a maximum somewhat above the systolic pressure at 16 ± 3 (mean $\pm$ SD, $n=5$) minutes of ischaemia.

3. Compliance Change. Compliance of the left ventricle was measured in order to characterize the passive distensibility of the myocardium during the development of the ischaemic contracture. The results are given in Fig. 2. There was a steady decline in the compliance levelling off at 20 min.

Myocardial Perfusion

The myocardial perfusion with the fluorescein solution declined in the ischaemic hearts as recorded by color photography of frozen horizontal sections through

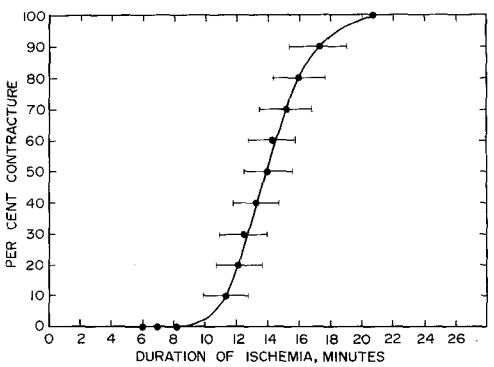

Fig. 1. The development of contracture as per cent of the final contracture during ischaemia. A balloon was inserted in the left ventricle, filled with water, connected to an open teflon catheter, and the increase of the water level in the catheter was recorded. Mean $\pm$ SEM, $n=7$

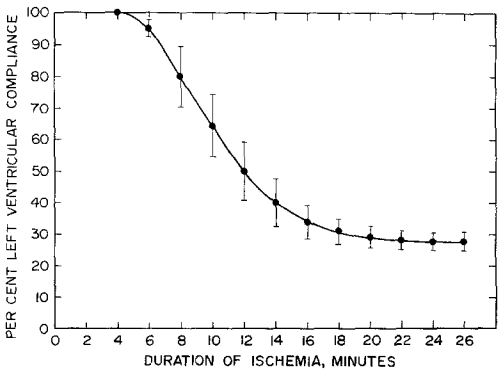

Fig. 2. The compliance (passive distensibility) of the left ventricle during the development of ischaemic contracture. The decline of compliance levels off at 20 min of ischaemia. The results are expressed as per cent of the maximal compliance which was obtained immediately after the cessation of the heart beat at the beginning of global ischaemia. Mean ± SEM, $n=5$

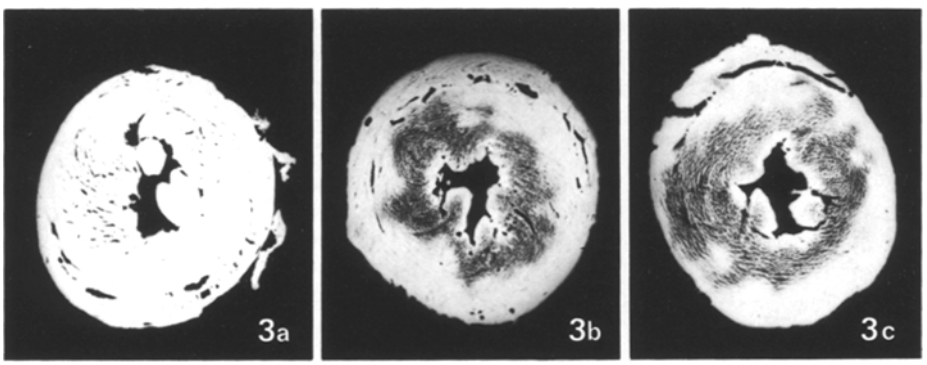

Fig. 3a–c. The development of the perfusion defect is illustrated in these photographs of frozen sections through fluorescein-perfused ischaemic hearts. **a** Ischaemia 0 min, fully perfused. **b** Ischaemia 30 min, subendocardial myocardium not perfused. **c** Ischaemia 60 min, subendocardial and mid-myocardium not perfused, subepicardial myocardium perfused

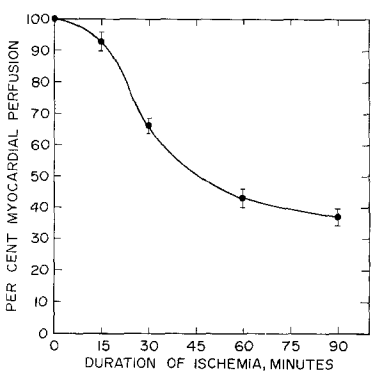

Fig. 4. The myocardial perfusion as quantified by measuring the fluorescent areas by point counting on color photographs of frozen sections through the ischaemic hearts perfused with the fluorescein tracer. Mean ± SEM (0 min, $n=5$; 15 min, $n=7$; 30 min, $n=8$; 60 min, $n=5$; 90 min, $n=6$)

the heart (Fig. 3). The perfusion defect developed first in the subendocardial myocardium, whereas the subepicardial myocardium was nearly always fully perfused. When the perfused areas in the sections were quantified (Fig. 4) it was found that at 15 min of ischaemia, 93% of the left ventricular wall was

perfused. At 30 min this value decreased to 67% and at 60 and 90 min it was 43 and 37, respectively.

Discussion

Kloner et al. (1974) described the "no-reflow" phenomenon in experimental myocardial infarcts. They observed that blood failed to flow into the affected myocardium when an occlusion of the circumflex branch of the left coronary artery of dog was removed after 90 min of ischaemia. A reduction of myocardial reflow and an increase in coronary vascular resistance was observed after temporary ligation of the anterior descending branch of the left coronary artery in dog by Willerson et al. (1975). Apstein et al. (1977) reported increased coronary vascular resistance during reperfusion of the ischaemic isolated rat heart. Several explanations have been offered as to the decreased reflow of ischaemic myocardium (Gavin et al., 1978b; Kloner et al., 1974; Fabiani, 1976). Although various factors such as endothelial cell swelling or injury, myocardial cell swelling or tissue oedema, alterations in blood viscosity or thrombosis, and collapse or compression of capillaries might be involved to varying degrees under various experimental conditions, myocardial rigor seemed the most feasable explanation of the "no-reflow" phenomenon in canine experimental infarcts (Gavin et al., 1978a) and in isolated ischaemic dog hearts (Nevalainen et al., 1978).

In the present experimental model myocardial perfusibility with fluorescein solution decreased gradually during 60 min of ischaemia in isolated rat hearts. Although various mechanisms might be involved in this experimental model, ischaemic contracture or myocardial rigor most probably plays a significant role in the development of the observed perfusion defect. Several authors (Camilleri et al., 1976; Baghirzade et al., 1970; Hauschild et al., 1970) have suggested that perfusion defects in ischaemic hearts might be associated with the contractile state of myocardium. It is interesting to notice that in the present experiment the contracture was fully developed by 20 min of ischaemia as measured in the left ventricular volume, pressure and compliance, whereas the perfusion defect developed slower, still advancing at 30 and 60 min of ischaemia. Thus there seem to be other late mechanisms in addition to ischaemic contracture or rigor, restricting the myocardial perfusability of ischaemic isolated rat hearts. The development of ischaemic contracture was studied by Hearse et al. (1977) in an isolated rat heart preparation. The contracture was complete within 17 min of ischaemia, similar to the observations in the present experiment.

The decrease of the compliance (passive distensibility) of the left ventricle characterizes the stiffening of the ischaemic myocardium in the same way as the change in the extensibility under a standard load characterizes the development of rigor in skeletal muscle (Bendall, 1973). The compliance of left ventricle decreased within 20 min of ischaemia. There was no further change in this variable during the rest of the experiment, i.e., up to 90 min of ischaemia.

The present results indicate that, during the ischaemic contracture, the compliance of the left ventricle decreases rapidly and permanently and that there is a rapidly developing increase in the intraventricular pressure. In the isolated

rat heart preparation there seem to be at least two chronologically overlapping components in ischaemic contracture: tension development in the left ventricular wall similar to the systolic contraction of the myocardium and stiffening or loss of extensibility which can be interpreted either as the rigor-change of the muscle or as a continuous tetanic myocardial contraction.

The mechanisms of ischaemic contracture are not fully understood. ATP-depletion in the myocardium under hypoxic conditions appears the cause of the rigor-change (Hearse et al., 1977). Contracture might be caused by an abnormal increase in intracellular calcium accumulation which activates the contractile process (Katz and Tada, 1972). Such calcium imbalance might result from hypoxic cell injury, which leads to disturbances in the permeability properties of the plasma and other cellular membranes.

It can be concluded from the present results that in the isolated ischaemic rat heart ischaemic contracture of the left ventricle is followed by the development of a perfusion defect, first in the subendocardial myocardium and later also in the mid-myocardial zone. Other mechanisms in addition to ischaemic contracture seem to be involved in the development of the myocardial perfusion defect in this experimental model.

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