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A Paracorporeal Rat Heart Model for Ischemic and Reperfusion Studies

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ABSTRACT

Paracorporeal rat hearts were supplied with blood derived from the abdominal aorta of a supporting rat. The circulatory stability and working capacity of the supporting animal was analyzed in the experimental situation in terms of PO_2, SO_2, PCO_2, HCO_3, pH and electrolytes, all of which were within the normal range before and during a 60 min period of paracorporeal perfusion. For evaluation of ischemic damage in this model studies were made on three groups of excised hearts. They were subjected to 10, 15 or 20 min of complete global ischemia at 37°C (ambient temperature) and reperfused for 30 min, including ECG, observations of contractility and an analysis of creatine kinase efflux in the coronary effluent. The results showed good reproducibility and the data were in accordance with reports from similar studies on Langendorff preparations. The model, which is easily set up, inexpensive and based upon pulsatile blood perfusion, should be more physiologic than the conventional Langendorff preparation.

INTRODUCTION

Improved myocardial protection is probably the most important contribution to recent advances in open-heart surgery. The clinical improvements are greatly attributable to extensive experimental laboratory investigations (1, 2, 13, 16). The isolated rat heart model has proved to be very useful in studies of myocardial ischemia, and experimental experiences have led to clinical innovations (10). An often used rat heart model is the Langendorff preparation (15) and its modifications (18), in which an isolated heart is perfused with a crystalloid solution in a non pulsatile way, usually a Krebs-Henseleit solution. In the clinical situation, however, the conditions are different. After the ischemic period the aortic cross-clamp is released and a pulsatile coronary blood flow is reinstituted.

The paracorporeal perfusion technique is known from earlier experiments (6, 17, 20). Gamble et al. (6) modified the paracorporeal heart model in the rat

but did not use a direct pulsatile retrograde perfusion technique in the aorta of the excised heart. Aiming at a situation closer to the clinic we have modified the paracorporeal rat heart model. Its reliability was evaluated and data on enzyme efflux correlated to ischemic heart damage are presented.

MATERIAL AND METHODS

Rats

Non-starved, 300 g male Sprague-Dawley rats were used.

The experimental model

The paracorporeal rat heart model consists of three main parts (Fig. 1): 1. The supporting rat. 2. The excised heart. 3. The retransfusion system. The ascending aorta of the excised heart was connected to the abdominal aorta of the supporting rat by a tube ("pump tube"). The coronary effluent from the excised heart was collected in a temperated water-jacketed funnel and retransfused to the supporting rat. After the ischemic period pulsatile blood flow was established from the supporting rat to the excised heart. The temperature of the supporting rat and the excised rat heart was kept at 37°C ± 1°C.

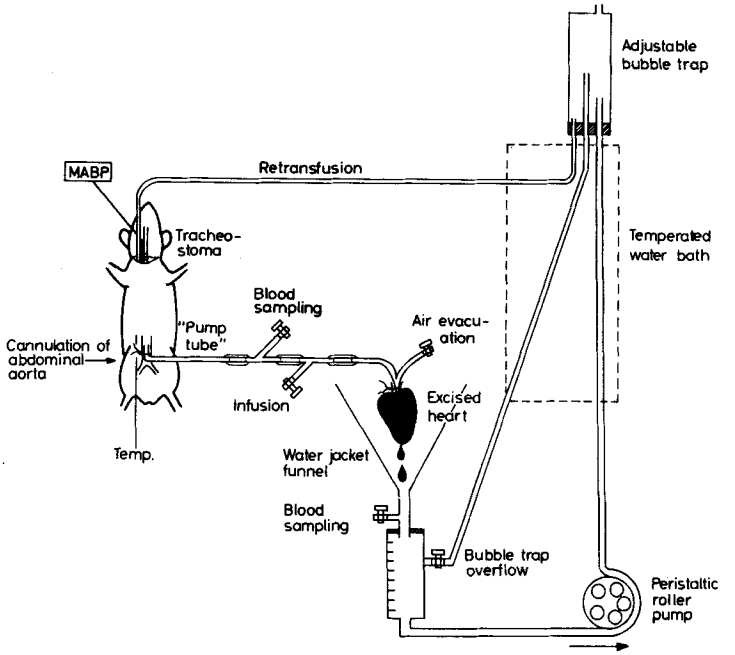

Fig. 1. The experimental model
The experimental model is composed of three main parts:
1. The supporting rat
2. The excised heart
3. The retransfusion system

Surgical and technical procedures

Rats intended for supporting function were anaesthetized by intraperitoneal administration of Inactin^{R} (Byk-Gulden, Germany), 120 mg·kg^{-1}. After tracheostomy the right carotid artery was cannulated for continuous blood pressure recording via an EMT 34 transducer (Elema-Schönander, Sweden) and a M 34 recorder (Elema-Schönander, Sweden). The right jugular vein was cannulated for transfusion. Heparin in a dose of 200 IU was given intravenously. The supporting rat was breathing air spontaneously.

The abdominal aorta was cannulated and the cannula was fixed to the aorta by silk ligatures. Close to the aorta, the cannula was cut and rejoined by a more flexible piece of silicone rubber tubing to permit easy clamping. Connections permitted blood sampling and infusion. The body temperature was recorded continuously in the abdominal cavity.

Heart donors were anaesthetized with ether. After 200 IU of heparin intravenously, the blood was rapidly collected from the abdominal aorta. Following thoracotomy and ligation of the aortic arch branches the heart-arch preparation was excised. The time required for this procedure was 3-5 min.

The ascending aorta was attached to the cannula from the supporting rat. Electrodes for bipolar ECG recording were placed at the apex, the right atrium and the aortic arch. During the ischemic period the heart was kept in a water-jacketed funnel filled with plain saline at 37°C-38°C.

The retransfusion system consisted of a peristaltic roller pump (Multiperpex 2115, LKB Products, Bromma, Sweden), a temperature controlled water bath and an air bubble trap. All connections were of silicon rubber tubing. The system was primed with 20 ml of heparinized blood collected from the heart donor and an additional rat. 10 ml of saline was added to the system during the first 7 min of reperfusion. Subsequently no extra volume was added. The retransfusion of blood was adjusted by regulating the height of the bubble trap. The mean arterial blood pressure (MABP) of the supporting rat was maintained at 95±10 mm Hg.

Experimental protocols

To test the endurance to ischemia, excised hearts were subjected to complete global ischemia at 37°C (ambient temperature) for 10, 15 or 20 min. The number of hearts examined in these three groups was 6, 8 and 7, respectively. Samples of arterial blood from the supporting rat were obtained before reperfusion was started and 10 and 30 min after the start. Sampling of the coronary effluent from the excised hearts was performed via the funnel after 10, 20 and 30 min of reperfusion. No buffer solutions were used. The following recordings were made:

1. Supporting rat: MABP and heart rate.
2. Arterial line (pump tube): MABP

$$PO_2,\ SO_2,\ PCO_2,\ pH$$

HCO_3^-, base excess (BE)

Electrolytes Na^+, K^+, Ca^{++}, and glucose

3. Excised heart: ECG (continuous recording)
4. Coronary effluent: Flow rate

Creatine kinase (CK) efflux

The CK activity was determined according to the recommendations given by the Scandinavian Committee on Enzymes. For background measurements samples were taken from the supporting animal and from the blood prime before the start of reperfusion.

For control six excised hearts were reperfused directly after cannulation for determination of CK efflux and to evaluate the influence of enzyme efflux from the supporting rat, another six rats were cannulated as supporting animals, but with no excised heart in the paracorporeal line. The flow through the cannula was adjusted to 8 $ml \cdot min^{-1}$. Samples taken from the blood prime and from the paracorporeal line after 10, 20 and 30 min were analysed for CK activity.

RESULTS

Supporting rat

Most rats showed a stable condition during the experiment. Their body temperature, blood gases, electrolytes and blood pressure had to be within normal range for acceptance as supporting animals.

Sodium, potassium and calcium concentrations in samples from the arterial line determined in six cases before reperfusion and after 10, 20 and 30 min (ischemic periods 10, 15 and 20 min) were all within the normal range. Arterial blood gases analysed before and 10 and 30 min after the start of reperfusion showed only minor variations, indicating high stability in the system (Table I). For control, additional blood gas analyses performed on blood samples after 5, 15 and 20 min of reperfusion with the same results. Blood glucose values at repeated determinations were within the normal range.

Table I. Blood gas data for the supporting rats before and after 10 and 30 min of reperfusion of excised hearts subjected to 10, 15 and 20 min of complete global ischemia at 37°C. As there was no significant difference between the three groups, the means and SD were calculated from all supporting rats. Significant differences ($p<0.05$) during support compared with pre-supporting values are indicated with an asterisk. n = number of animals.

	pH	PCO_2 (kPa)	PO_2 (kPa)	BE	SO_2 (%)	HCO_3 ($mmol \cdot l^{-1}$)
Before reperfusion	7.40±0.03	5.3±0.6	12.4±1.0	-0.1±1.7	95±1	24±1
n	19	19	18	18	18	18
10 min of reperfusion	7.37±0.03	5.0±0.5	13.0±1.5	-3.1±2.0*	96±1	22±2*
n	20	20	20	19	19	19
30 min of reperfusion	7.38±0.03	5.1±0.5	12.2±1.2	-2.3±1.6	95±1.3	23±1.3
n	22	22	22	21	21	21

The hemodilutive effect of added saline resulted in a Hb concentration of 13.6 $g \cdot l^{-1}$ (SD±2.1) and 14.4 $g \cdot l^{-1}$ (SD±2.1) at 10 and 30 min of reperfusion, respectively, as compared with 17.5 $g \cdot l^{-1}$ (SD±2.0) before transfusion.

Excised hearts

All hearts subjected to 10 min of ischemia regained sinus rhythm and a rate of at least 140 beats/min after 10 min of reperfusion. After 15 min of ischemia only 1/3 of the hearts returned to normal rhythm, while the other hearts developed serious arrhythmias. After 20 min of ischemia no heart returned to normal rhythm (Table II).

Table II. Heart activity after 10 min of reperfusion of hearts subjected to 10, 15 and 20 min of complete global ischemia at 37°C, verified by ECG and observed contractility. n = number of examined hearts.

		Duration of pre-reperfusion ischemia		
		10 min n = 10	15 min n = 12	20 min n = 9
Heart activity	Sinus rhythm > 140 beats/min	10	4	-
	Non-reversible arrhythmias	-	8	9

The coronary flow rate in the 15-min ischemic group is given in Table III. It was already fairly high during the first minute and increased to a maximum of 9 ml/min after 4-5 min of reperfusion. After 20 min a constant flow rate of about 5 ml/min was established.

Table III. Coronary flow ($ml \cdot min^{-1}$) during reperfusion of 7 excised hearts subjected to 15 min of complete global ischemia at 37°C. Sampling periods one min. Mean±SD.

Duration of reperfusion (min)	0-1	2'	4'	10'	20'	30'
Flow $ml \cdot min^{-1}$	6.8±1.7	8.7±2.4	9.2±2.9	7.1±1.9	5.0±1.8	4.6±1.5

The CK background activity was found to be stable, with a mean of 8.1 (SD ± 2.0 $ukat \cdot l^{-1}$) in supporting animals (27 animals) and 4.8 (SD ± 1.8 $ukat \cdot l^{-1}$) in the blood prime (27 experiments). In the control group with no heart in the paracorporeal line the background activity was similar.

The CK activity in the coronary effluent after 10, 20 and 30 min of reperfusion showed similar patterns depending upon the period of ischemia (Table IV). In control samples after 40 and 50 min of reperfusion there was a greater increase in the accumulation of CK in the 20-min ischemic group compared to the other groups. A significant difference ($p<0.05$) between the CK activity in

samples from hearts subjected to 10 min of ischemia, compared to the 15- and 20-min groups, was observed. The difference between the two last groups, however, was insignificant. In the controls the efflux of CK was low (Table IV).

Table IV. The efflux of creatine kinase (ukat·l^{-1}) in the coronary effluent after 10, 20 and 30 min of reperfusion of hearts subjected to 10, 15 or 20 min of complete global ischemia at 37°C. To compare, values are given from a group of excised hearts reperfused directly after cannulation and another group with no excised heart in the paracorporeal line, i.e. CK efflux from the supporting rat. Mean ± SD. n = number of experiments.

		Reperfusion of excised hearts		
		10 min	20 min	30 min
Duration of ischemia	10 min	12.3±4.1	15.9±4.3	18.0±4.2
	n	6	6	6
	15 min	19.8±5.7	30.0±9.6	34.7±8.7
	n	8	8	8
	20 min	25.1±10.7	37.0±13.9	46.2±17.8
	n	7	7	7
	Control group (reperfused directly)	7.9±0.7	11.3±1.1	13.8±1.6
	n	6	6	6
	Control group (no excised heart)	6.1±0.9	7.4±1.1	10.1±1.1
	n	6	6	6

DISCUSSION

Investigations using different models have increased the knowledge concerning heart metabolism. In many ways the Langendorff model and its modifications (9, 18) are superior to in situ models. Any perfusion medium may be administered and any heart temperature, perfusion pressure and degree of oxygenation can be maintained. The perfusate is easily collected, measured and examined.

The Langendorff preparation, however, constitutes a denervated heart which is devoid of normally occurring humoral factors, and most often a non-corpuscular perfusion medium is used. This may influence not only the physiological distribution of oxygen and substrate to the different parts of the myocardium but also the removal of metabolites (6, 21).

These disadvantages can partly be overcome by the use of a mechanical pump and with donor blood as perfusion medium. However, the best "pump" qualities in terms of pulse rate and pressure are obtained by the most superior pump available - another heart. Thus, the paracorporeal heart model (17, 20) is a middle course optimized in terms of pump function and pump flow. The paracorporeal heart can be influenced by hormones and substrates from the supporting rat, which is important for clinical applications. Variations in acid-base and electrolytes can also be corrected by the pump rat.

To stimulate the clinical reperfusion situation we used a retransfusion system. The model was stable for at least 60 min of paracorporeal circulation but 30 min was enough to cover our interest in early reperfusion after ischemia.

A prerequisite for good function of this system was that the supporting animal was able to stand the surgical trauma and could increase its cardiac output to compensate for the high demands due to the high flow through the excised heart. These requirements were fulfilled. Thus their acid-base status, blood-gas balance and electrolytes were stable both before and during perfusion of the excised hearts. Quickly retransfused volumes neither caused deficient reoxygenation nor signs of "pump" insufficiency in terms of pulse pressure failure in the aortic arch of the experimental heart. Concerning the reliability of the model, our findings corroborate the results of other investigators using Langendorff preparations. Thus, the critical period of complete global ischemia at 37°C was between 15 and 20 min (12). The efflux of CK after different periods of ischemia was also in accordance with other reports (12, 22). The model showed a high degree of reproducibility. Heart function in terms of electrical activity and observed contractility correlated well to the ischemic time. The efflux of CK was also proportional to the ischemic time. This model can easily be modified to allow the use of a left ventricular latex balloon for isovolumetric contraction studies (4, 14, 19).

The mechanisms of ischemic heart damage associated with heart surgery have been extensively discussed. Increased attention has been paid to the early period of recirculation (7, 11). Significant damage to heart muscle seems to occur within a few minutes of recirculation. The role of calcium and eventually further energy content depletion during the reperfusion period has been stressed. Furthermore, an adverse effect of molecular oxygen, "the oxygen paradox", has been proposed (3, 12). This may be based upon a possible toxic effect of oxygen-derived free radicals, such as the superoxide (O_2^-) and hydroxyl (OH$^\cdot$) radicals (5).

We believe that the presented model, which is simple and inexpensive, is suitable for studies of myocardial ischemia and the reperfusion phenomena. Although the present experimental arrangement is still far from the clinical situation, it is closer than the Langendorff preparation.

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REFERENCES

1. Bretschneider, J.H., Hubner, G., Knoll, D., Lohr, B., Nordbeck, H. & Spieckermann, P.G.: Myocardial resistance and tolerance to ischemia. Physiological and biochemical basis. J Cardiovasc Surg 16:241-260, 1975.
2. Buckberg, G.D., Brazier, J.R., Nelson, R.L., Goldstein, S.M., McConnell, D.H. & Cooper, N.: Studies on the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass. I. The adequately perfused beating, bifrillating and arrested heart. J Thorac Cardiovasc Surg 73:87-94, 1977.
3. Demopoulous, H.B.: Free radicals in medicine and biology. Acta Phys Scand Suppl 492 Symposia, 1979.
4. Fallen, E.L., Elliott, W.C. & Gorlin, R.: Apparatus for study of ventricular function and metabolism in the isolated perfused rat heart. J Appl Physiol 22:836-839, 1967.
5. Fridovich, I.: Quantitative aspects of the production of superoxide anon radical by milk xanthine oxidase. J Biol Chem 245:4053-4057, 1970.
6. Gamle, W.J., Conn, P.A., Edalju Kumar, A., Plenge, R. & Monroe, R.G.: Myocardial oxygen consumption of blood-perfused, isolated, supported, rat heart. Am J Physiol 219:604-612, 1970.
7. Ganote, C.E., Worstell, J. & Kaltenbach, J.P.: Oxygen-induced enzyme release after irreversible myocardial injury. Effects of cyanide in perfused rat hearts. Amer J Pathol 84:327-350, 1976.
8. Gordon, K.M. & Visscher, M.B.: The mechanism of failure in the completely isolated mammalian heart. Am J Physiol 125:461-473, 1939.
9. Hearse, D.J., Humphrey, S.M. & Chain, E.B.: Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: A study of myocardial enzyme release. J Molec Cell Cardiol 5:395-487, 1973.
10. Hearse, D.J., Humphrey, S.M. & Garlick, P.B.: Species variation in myocardial anoxic enzyme release, glucose protection and reoxygenation damage. J Molec Cell Cardiol 8:329-339, 1976.
11. Hearse, D.J.: Reperfusion of the ischemic myocardium. J Molec Cell Cardiol 9:606-616, 1977.
12. Hearse, D.J. & de Leiris, J. (Eds.) Enzymes in Cardiology. Diagnosis and research. D.J. Hearse and J. de Leiris, New York, John Wiley & Sons, Chichester, Brisbane, Toronto, 417-444, 1979.
13. Kirsch, U., Rodewald, G. & Kalmar, P.: Induced ischemic arrest. J Cardiovasc Surg 63:121-130, 1972.
14. Kligfield, P., Horner, H. & Brachfeld, N.: A model of graded ischemia in the isolated perfused rat heart. J Appl Physiol 6:1004-1008, 1976.
15. Langendorff, O.: Untersuchungen am überlebenden Säugetieren. Pflügers Archiv 61:291-332, 1895.
16. Melrose, D.G., Dreyer, B., Bentall, H.H. & Baker, J.B.E.: Elective cardiac arrest. Lancet ii:21, 1955.
17. Mulch, J., Schaper, J., Gottwik, M. & Hehrlein, F.W.: Recovery of the heart after normothermic ischemia. Thorac Cardiovasc Surg 27:145-152, 1979.
18. Neely, J.R., Liebermeister, H., Battersby, E.J. & Morgan, H.E.: Effect of pressure development on oxygen consumption by isolated rat heart. Amer J Physiol 212:804-814, 1967.
19. Opie, L.H.: Effect of extracellular pH on function and metabolism of isolated perfused rat heart. Amer J Physiol 209:1075-1080, 1965.
20. Sarnoff, S.J., Case, R.B., Welch, G.H.Jr., Braunwald, E. & Stainsby, W.N.: Performance characteristics and oxygen debt in nonfailing, metabolically supported isolated heart preparation. Amer J Physiol 192:141-147, 1958.
21. Taegtmeyer, H., Hems, R. & Krebs, H.A.: Utilization of energy-providing substrates in the isolated working rat heart. Biochem J 186:701-711, 1980.
22. Waldenström, A.P., Hjalmarsson, Å.C., Jodal, M. & Waldenström, J.: Significance of enzyme release from ischemic isolated rat heart. Acta Med Scand 201:525-532, 1977.