

Effect of Acute Ischaemia on Myocardial Function

Proceedings of the Seventh Pfizer International
Symposium held in Edinburgh



EFFECT OF ACUTE ISCHAEMIA ON MYOCARDIAL FUNCTION

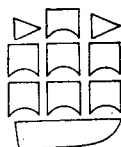
Proceedings of the
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Edited by

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PREFACE

OUR purpose in selecting the theme of acute myocardial ischaemia for the Seventh International Pfizer Symposium was twofold. First, knowledge of the effects of acute ischaemia on myocardial metabolism, electrophysiology and contractility has been accumulating rapidly in the last five years but there has been little opportunity for investigators working in different disciplines to meet together and discuss this subject in depth. Second, and no less important, there is a need to integrate and report as a whole much of the work which has recently and will shortly be published in specialized journals. It is hoped that this book will achieve this purpose.

It may be a surprise to the clinical cardiologist to realize how ill understood are so many of the basic mechanisms related to, for example, angina or ventricular fibrillation. But, on reflection, this will seem less unexpected if it is remembered that it has only recently been possible to measure changes in myocardial blood flow and action potential in the intact contracting heart, to estimate myocardial enzyme and amine concentration, to record the events leading to the onset of ventricular fibrillations, to measure substrate exchange across the myocardium, to determine what influences the requirements for myocardial nutrients and to analyse myocardial contractility. Many questions, currently unanswerable, are raised throughout this book and so a further aim should be achieved. This is the catalysis, provocation and reassembly of ideas so that more work on myocardial ischaemia will result.

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ACKNOWLEDGMENTS

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The rapporteurs for the sessions—Dr B. J. Kirby, Dr I. Nimmo, Dr S. R. Reuben, Dr R. Riemersma, Dr R. A. Strange and Dr C. W. Vellani—were assiduous in their help with the preparation of the manuscripts and we are most grateful to them. Mrs Eva Riemersma gave much valuable time to the references and index and Miss S. Bucher, Miss R. Dodd, Miss I. Johnston, Mrs J. Rodger, Miss M. Sharp, Mrs C. Winney were greatly appreciated for their secretarial assistance.

Finally, we wish to thank all those, including the publishers, who have made it possible to produce this book in such a short time.

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ISOLATED HEART PREPARATIONS

J. A. O'BRIEN

THIS paper is concerned with the problem of the measurement of the metabolic activity of the isolated rat heart and with the properties of the preparation in controlled hypoxia.

Changes occur in the properties of the isolated rat heart during the initial period of perfusion. Oxygen uptake,¹ the permeability to non-metabolized sugar in the absence of insulin,^{2,3} lactate output and, in some circumstances, glucose uptake⁴ decrease to constant rates. The period of change may be as short as 15 minutes in the case of oxygen consumption but as long as one hour for glucose uptake by hearts taken from fed rats. It is the practice in many laboratories to perfuse hearts for between 5 and 20 minutes before making any experimental measurements but change in properties may still occur subsequently. Estimates of the average metabolic rates over 30 minutes may therefore be misleading. Over short periods when a closed circuit system of recirculating perfusate is used a compromise must be struck between the difficulties of precisely measuring small changes in the amount of substrate and those of the interpretation of estimates made when the composition of the perfusate changes rapidly. The compromise favours the use of substrate concentrations high enough to make the rate of metabolism independent of changes in concentration. Such concentrations may be unphysiological.

Many of these problems can be overcome in a system of perfusion in which a substrate is infused steadily into recirculating perfusate whose volume is kept constant by withdrawal at a rate equal to infusion. An apparatus for this purpose has been described in detail by Fisher and O'Brien.¹ In this system the instantaneous rate of metabolism is given by the equation:

$$v = i(a - x) - P \frac{dx}{dt}$$

where v is the rate of substrate utilization, i is the rate of infusion, a is the concentration of the infused substrate, x is the concentration of substrate in the perfusate, P is the volume of recirculating perfusate and t is the time.

By the application of the above equation to perfusate collections made at regular short intervals (2 to 5 min) the uptake by hearts of glucose at physiological concentrations has been followed in detail and it has been shown that in the absence of insulin it decreases smoothly to an almost constant level and that, once this has happened, the perfusate concentration also moves in an exponential fashion to a constant level. In the presence of sufficient insulin, glucose uptake is constant from the beginning of perfusion, and perfusate glucose concentration reaches a steady state concentration in a relatively short time thereafter.

The decrease in glucose uptake rate in the absence of added insulin is probably due to the decrease in effective insulin in the perfusion system, since in the presence of adequate insulin there is no sign of any other cause of decrease. If this is right, then it takes 30 to 60 min after preliminary washout to bring insulin activity down to a negligible level.

With this system no arbitrary assumption has to be made about when the state of the heart becomes basal, and there is one other substantial advantage, namely, that there is no lower limit to the substrate concentration which can be used other than those imposed by analytical methods, so that the system can be used to study very rapidly turning over metabolites. Glucose metabolism has been studied over the range 0.02 mM to 10 mM (0.4 to 180 mg/100 ml). At the lowest concentration the rate of uptake by the heart each hour is equal to ten times the glucose content of recirculating perfusate.

The apparatus of Fisher and O'Brien has been adapted to allow controlled variation in the oxygen tension presented to the heart. Figure 1, 1 depicts the modification. Infusate is introduced into the lead carrying perfusate to the heart. Two perfusates are used, one equilibrated with 5 per cent CO_2 in oxygen, the other equilibrated with 5 per cent CO_2 in nitrogen. One of them is used for recirculation and the other for infusion. With variation of the infusion rate this allows any arterial oxygen tension to be established in the range 10 to 700 mmHg. The oxygen tension can be altered almost instantaneously by adjusting the infusion rate.

Flow-through oxygen electrodes are placed before and after the point of infusion. From the oxygen tensions determined at these points and the rate of infusion the coronary flow can be calculated.

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A third electrode measures the oxygen tension of the effluent from the heart so that the oxygen consumption can be estimated. The arrangements for gaseous exchange in the apparatus are sufficient to re-equilibrate the recirculating perfusate in one passage.

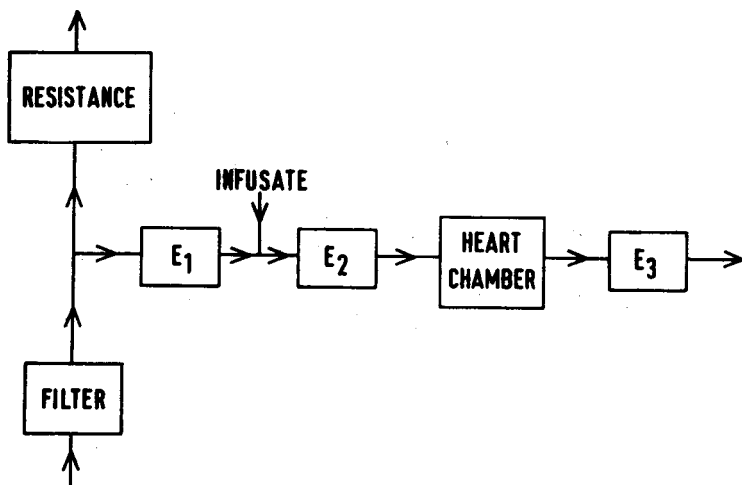


FIG. 1, 1. Diagram of the modification of the apparatus of Fisher and O'Brien⁴ for the study of the hypoxic heart. E₁, E₂ and E₃ are oxygen electrodes.

Preliminary experiments have been made with this system on the effect of insulin and hypoxia on oxygen consumption and glucose utilization. The infusate glucose concentration was 4 mM and the steady state concentration was never less than 3 mM at which level glucose utilization by the aerobic heart is maximal. Insulin, when used, was present at 100 mU/ml or 2 mU/ml and the perfusion pressure was always 40 mmHg. The stability of the rates of oxygen consumption, coronary flow and glucose uptake at any one level of hypoxia was indicated by the constancy of the outputs from the oxygen electrodes and the analysis of the time-course of the perfusate glucose concentration.

Figure 1, 2 shows that even at arterial oxygen tensions much higher than physiological, the oxygen uptake is dependent on oxygen tension, but that it tends to decrease less sharply in the presence of insulin. Figure 1, 3 shows that in the presence of insulin

oxygen uptake is sustained until the venous oxygen tension corresponds to a true anoxia, but that the decrease in oxygen uptake in the absence of insulin occurs long before the heart is truly anoxic.

The presumption is that the determining factor is the ability of the heart to oxidize glucose at a given oxygen tension. Preliminary experiments have shown that the oxygen uptake falls at least as much in the absence of glucose as in its presence and that insulin

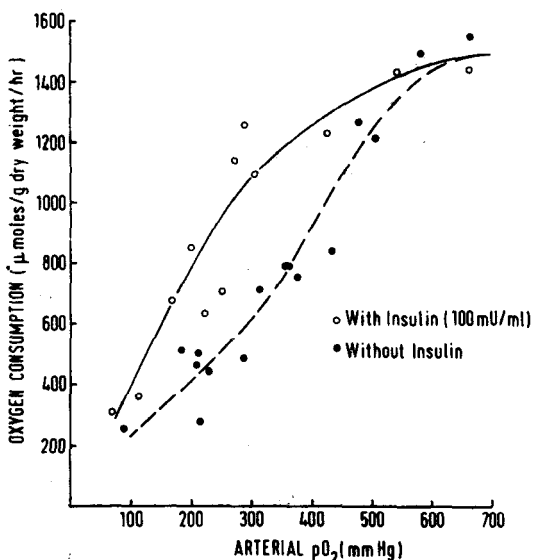


FIG. 1, 2. The relationship between arterial oxygen tension and oxygen consumption in the presence and absence of insulin. The data were obtained from 15 hearts.

does not then restore the oxygen uptake. The sustaining effect of insulin therefore seems to be due to an increase in the rate of oxidation of exogenous glucose. At the lower insulin concentration (2 mU/ml) the same sustaining effect is seen.

The effects of insulin and hypoxia on glucose utilization are shown in Figure 1, 4. Glucose uptake is stimulated by insulin at all degrees of hypoxia and is markedly increased when the oxygen tension of the effluent from the heart approaches zero. If the oxygen consumption equivalent to the complete oxidation of the glucose uptake of the

ISOLATED HEART PREPARATIONS

heart is calculated, the increase in the equivalent oxygen consumption due to insulin is of the same order as the maximum difference in oxygen consumption sustained by insulin (Fig. 1, 3). This is consistent with the notion that insulin increases the rate of oxidation of exogenous glucose. It is implicit that the oxidation of endogenous substrate must limit oxygen consumption by the hypoxic heart in the absence of insulin.

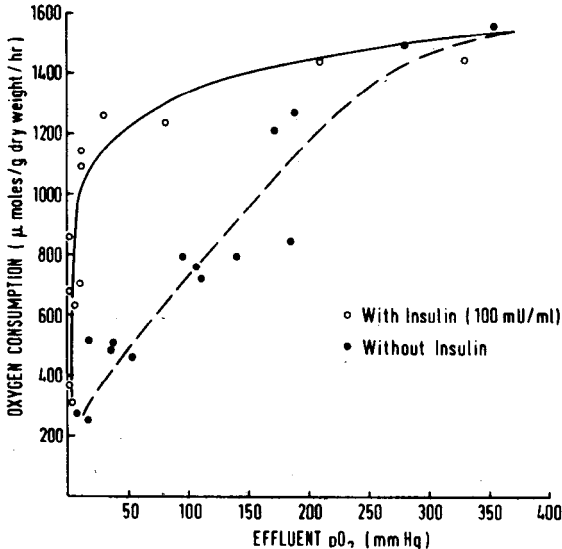


FIG. 1, 3. The relationship between the oxygen tension of the coronary effluent and oxygen consumption in the presence and absence of insulin. The data were obtained from 15 hearts.

The effects of insulin on oxygen consumption can be interpreted as being secondary to an increase in the cell permeability to glucose. Anoxia has also been shown to increase permeability to non-metabolized sugars.⁵ The effects of hypoxia on the rates of glucose uptake are consistent with these observations, but they suggest that the effects of hypoxia and insulin are additive. Estimates of the parameters of glucose permeation from an analysis of the kinetics of the glucose utilization by the aerobic rat heart indicate that the maximum rate of permeation in the presence of insulin is approximately 0.45 mmoles/g. dry weight/hour (Fisher and O'Brien, un-

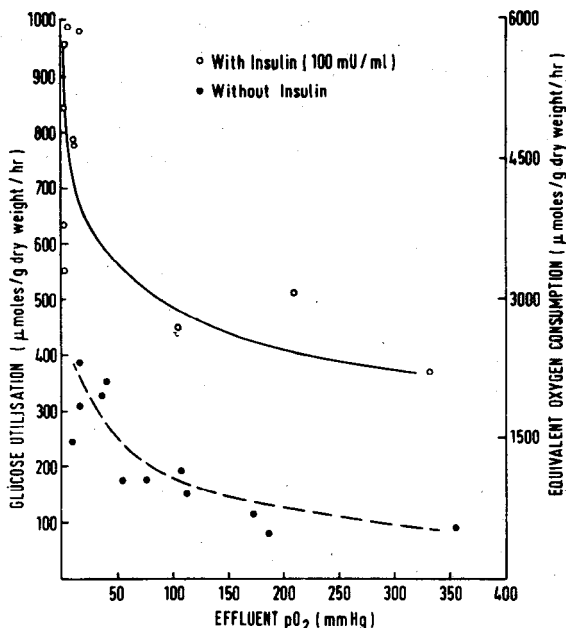


FIG. 1, 4. The relationship between the oxygen tension of the coronary effluent and glucose utilization and equivalent oxygen consumption in the presence and absence of insulin. The data were obtained from 15 hearts.

published observation). The rate of uptake of nearly 1 mmole/g dry weight/hour observed when the coronary effluent is almost totally depleted of oxygen can only be accounted for by an increase in permeability due to anoxia.

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DISCUSSION

Fisher. Your work suggests the effects of hypoxia are dependent upon the hormonal status of the heart which is difficult to determine in most systems. Would you agree with this?

O'Brien. Yes, I would.

Noble. Could you give us an idea of the mechanical performance of a heart set up like this?

O'Brien. Heart rate and oxygen consumption are constant at a particular oxygen tension.

Carlson. Does your perfusion medium contain fatty acids?

O'Brien. It does not.

Carlson. Two points of interest are the decrease in glucose uptake without insulin and the increased glucose uptake in the presence of hypoxia plus insulin. With regard to the first point, have you measured if there is release of insulin from your preparation, and could the decrease in glucose uptake be due to a decrease in the insulin content of the receptor, or would you rather think that it could be related to the rate of utilization of endogenous substrates?

O'Brien. The permeability of the perfused rat heart to non-metabolized sugars falls progressively over the period during which glucose uptake is falling. The basal level of permeability is achieved immediately in hearts from animals treated with anti-insulin serum. Both effects can be explained in terms of elution or destruction of insulin without involving endogenous substrates.

Carlson. Could the increased permeability for glucose during hypoxia be explained by an increase in binding of insulin under these conditions? Could the oxygen content influence the amount of insulin bound to membranes?

O'Brien. I do not think so, because hypoxia considerably increases glucose uptake by the insulin-free heart.

Randle. It is unlikely that the effects of anoxia are due to insulin because they are as marked in the heart from the alloxan diabetic