
Recent ^{31}P n.m.r. Studies of Myocardium [Abstract Only and Discussion]

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Recent ^{31}P n.m.r. studies of myocardium

[Abstract only]

BY D. P. HOLLIS AND R. L. NUNNALLY

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Earlier work from this laboratory has concerned the possible use of phosphorus n.m.r. as a method to monitor, in a non-invasive manner, the biochemical state of the perfused heart as a function of its mechanical performance. We showed that a simulated coronary infarction could be detected by ^{31}P n.m.r. (Hollis *et al.* 1978*a*) and that hypothermia and KCl arrest could preserve the pH and the ATP levels at more nearly normal values than in a non-arrested heart during long periods (40 min) of ischaemia (Hollis *et al.* 1978*b*). More recently it was shown that multiple doses of KCl, given at intervals, were more effective in this respect than was a single dose (Flaherty *et al.* 1979). These studies essentially followed the kinetics of transitions of the heart between two or more distinct physiological states (i.e. normoxic and ischaemic, with or without KCl arrest) by observation of the ^{31}P n.m.r. spectra at various time intervals over periods of up to 1 h. As described in detail and demonstrated in Dr Truman Brown's contribution to these discussions, the rates of chemical exchange reactions occurring in a steady state can be measured by the techniques of saturation transfer in various biological systems, including perfused hearts. We have applied a Fourier transform double irradiation technique to the study of the creatine kinase reaction in normal perfused rabbit hearts that are performing isometric contractions against a fluid-filled balloon, and in hearts, prepared in an identical manner, that have been subjected to 35 min of ischaemia and then reperfused to a state of stable performance before rate measurements are made (Hollis *et al.* 1978). The results support the hypothesis of cellular compartmentation of ATP and also demonstrate altered steady-state creatine kinase kinetics for altered physiological states of the heart. We base these conclusions on the fact that, in control experiments, the creatine kinase reaction appears not to be in a steady state as determined by comparison of the forward and reverse rates, which should be equal in the steady state. Since the heart is, in fact, in a steady state as judged by the long-term maintenance of ^{31}P -containing substrates and stable performance, the clear implication is that the creatine kinase systems are in a steady state. The saturation transfer results appear to contradict this simply because the n.m.r. measured concentrations of ATP and PCr needed to calculate these rates do not have appropriate values. One possible explanation for this, which we tentatively favour, is that two distinct pools of ATP exist within the heart cell. The observed indication of near steady-state kinetics in reperfused ischaemic hearts may result from the selective loss of ATP from one compartment during the ischaemic period. An observed decrease in T_1 of the γ -phosphate of the remaining ATP is consistent with this interpretation (Nunnally & Hollis 1979).

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Discussion

DR J. M. SALHANY (*Cardiovascular Center, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68105, U.S.A.*). We have studied cardiac pH in Langendorff-perfused guinea pig hearts. Hearts were perfused in both the absence and the presence of phosphate, with the use of Krebs–Henseleit bicarbonate buffer. Buffer pH was usually 7.4, but some perfusions were with buffer containing phosphate at a slightly higher pH. Hearts were suspended in a bath containing the given perfusion buffer. Measurements on over 23 control hearts yielded a value for cardiac pH of 7.0 ± 0.1 , not 7.4 as reported in the paper being discussed. When phosphate is in the buffer, a strong line from the external bath is observed, which obscures the very small line from internal phosphate. Raising the pH of the perfusing buffer causes the external (bath) line to move downfield from the pH 7.4 position while the internal line remains at 7.0. Perfusion with phosphate-free buffer causes the external line to disappear but does not effect the position of the internal 7.0 line, thus indicating that cardiac cellular pH is 7.0. Our n.m.r. value for cardiac pH is in excellent agreement with values determined by non-n.m.r. methods. Since one of the main uses of n.m.r. is to determine cardiac cellular pH in ischaemia, it was obviously essential to establish the n.m.r. value of cardiac pH in control hearts.

D. P. HOLLIS. We did *not* report a value of 7.4 for the cardiac pH in this paper, contrary to Dr Salhany's statement. The value that I did report is 7.18 ± 0.07 . It was brought out very clearly in the discussion following my paper that there is very little disagreement between our results and those given by Dr Salhany at this meeting. While we, as well as the Oxford group, initially reported a value of 7.4, this has been corrected and, indeed, the most recent result of 7.18 ± 0.07 has already been published (D. P. Hollis, *Bull. magn. Reson.* **1**, 27–37). In my opinion Dr Salhany's comment does not accurately represent the discussion that occurred following our paper, in that it suggests disagreement where none exists and it attributes to us a statement that we did not make. I should also like to point out that our earlier reports concerned rats, our current reports concern rabbits, and Dr Salhany's comments concern guinea pigs. It is not necessarily true that hearts from these three species will have the same pH under the experimental conditions used in measuring pH by n.m.r.

DR A. GANSSEN (*Siemens AG Medical, Erlangen, Germany*). In considering the effects of hypoxia on the pH value of the blood perfused myocard (and kidney), a very important additional effect should probably not be neglected; this is the effect on myocardial blood rheology.

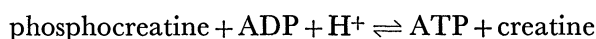
According to H. Schmid-Schönbein (Aachen) and others, the decrease in the pH of the blood caused by deficiency of oxygen gives rise also to an increase of the internal viscosity of the red cells. A simultaneous vaso-dilatatory effect is probably less effective within the capillary vessels since the diameter of the red cells is usually considerably larger than the inner diameter of the capillaries, so that the capillary blood flow works only as long as the red cells are soft and flexible. The stiffening erythrocytes produce an increased blood flow resistance within the capillary vessels, thus reducing the oxygen transport into the myocardium even further. In extreme cases, the pH values below 6.5, the red cells stiffen completely, thus plugging the capillaries. It can be seen that there is a negative feed back mechanism at work causing further deoxygenation and decrease of the overall pH.

This could be one of the effects connected with myocardial infarction.

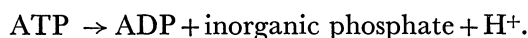
A paper on our work together with Schmid-Schönbein, concerning application and first clinical testing of n.m.r. applied to the measurement of blood plasma rheologic properties will appear in *Biorheology* this year.

D. G. GADIAN (*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU U.K.*). Following on from the collaborative studies with Dr T. Brown, Dr Joan Dawson and I have now measured by saturation transfer n.m.r. the activity of creatine kinase both in resting and in contracting muscle. We find that, in resting frog gastrocnemii at 4 °C, the rate of inter-conversion of ATP and phosphocreatine is $1.6 \text{ mmol kg}^{-1} \text{ s}^{-1}$. This is sufficiently rapid to ensure that the creatine kinase reaction is very close to equilibrium in resting muscle.

We have also made saturation transfer measurements during 3 s contractions of anaerobic frog gastrocnemii, repeated every 3 min over a period of 24 min. The only reactions that we need consider during contraction are:



and



We find by direct n.m.r. measurement that inorganic phosphate is formed during the contractions, at the rate of $0.8 \text{ mmol kg}^{-1} \text{ s}^{-1}$. We also find, from saturation transfer, that the forward rate (i.e. phosphocreatine \rightarrow ATP) remains approximately $1.6 \text{ mmol kg}^{-1} \text{ s}^{-1}$. We need to perform T_1 measurements during contraction to confirm this rate, and to establish the back rate. However, since the ATP level does not progressively decline during contraction, the reverse rate (i.e. ATP \rightarrow phosphocreatine) should be $0.8 \text{ mmol kg}^{-1} \text{ s}^{-1}$, for self-consistency.

The question arises as to whether the creatine kinase activity that we measure is high enough to account for the observation that there is no net ATP breakdown during contraction. Preliminary theoretical kinetic studies performed by Dr T. Chance suggest that our results are indeed consistent with this observation.