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PHOSPHORUS NUCLEAR MAGNETIC RESONANCE MEASUREMENTS OF INTRACELLULAR pH IN ISOLATED RABBIT HEART DURING THE CALCIUM PARADOX

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INTRODUCTION

In 1883 Ringer [1] reported that contraction of an isolated frog heart rapidly ceased when calcium was removed from the extracellular fluid. Similar experiments with isolated rat hearts were performed by Zimmerman and Hülsmann [2]. During perfusion with a calcium-free solution contraction of the heart ceased while electrical activity was maintained. They reported: "Returning to a normal perfusate after more than 2 min resulted in the disappearance of the electrocardiogram, and, after the heart had contracted a few times, these contractions passed off completely and contractile activity was not restored. The heart lost its colour and acquired a pale and mottled appearance. This phenomenon, whereby the heart maintained its red colour and electrical activity when the perfusion medium was changed from normal to calcium-free, but lost these properties on return to normal, we called the calcium paradox." The calcium paradox has been demonstrated in rat, mouse, guinea-pig, rabbit and dog heart [3,4], in superfused strips of the human heart [5], and also in the frog heart [6] although the amphibian heart is more resistant to the calcium paradox than the mammalian heart.

The calcium paradox is characterized by an excessive influx of calcium into the cells [7], which may be divided into an early, relatively small gain in cytosolic calcium, and a subsequent, massive influx [8]. Evidence has been provided that the slow channels and the Na^{+}-Ca^{++} exchange mechanism are involved in the primary gain in tissue calcium once calcium is readmitted to the perfusion fluid. The protective effect of calcium antagonists on the calcium paradox has been the subject of controversy [9]. From recent publications, however, it is clear that in a submaximal or mild form of the calcium paradox calcium antagonists may be protective [10,11]. Thus it is reasonable to assume that one of the routes of calcium entry during the calcium paradox is through

the slow channels. That calcium entry also occurs through the Na^+-Ca^{++} exchange mechanism, can be concluded from studies in which the extent of the calcium paradox was modified by changing the extracellular sodium concentration during the calcium-free or reperfusion period [12,13]. It should be noted, however, that the specific activity of the Na^+-Ca^{++} exchange mechanism as such is not affected by calcium depletion of the heart [14]. Other factors that may be responsible for the primary gain in calcium are the sodium-potassium and calcium pumps of the sarcolemma, and the calcium pump of the sarcoplasmic reticulum, whose activities are decreased by calcium-free perfusion [14-16].

The raised cytosolic calcium may then trigger a number of events, including energy-dependent accumulation of calcium and phosphate by mitochondria [17] and activation of various ATPases [18], contracture-mediated disruption of intercalated disc junctions [19], vesiculation of the sarcolemma and severe aggregation of intramembrane particles [20], loss of cytosolic constituents [2], and a secondary uncontrolled entry of calcium [8]. The precise sequence of these events and their relative importance still have to be elucidated. Hydrolysis of ATP and accumulation of calcium by mitochondria with deposition of insoluble calcium phosphate, are proton-generating processes [21,22]. Hence, it has been suggested that cytosolic acidification with a consequent stimulation of cytosolic and lysosomal (phospho)lipases, is an important factor in the origin of the calcium paradox [17,18]. In the present study phosphorus nuclear magnetic resonance (^{31}P NMR) spectroscopy was used to investigate the course of intracellular pH during the calcium paradox in isolated perfused rabbit heart.

MATERIALS AND METHODS

Isolated rabbit hearts were perfused at 37°C by the method of Langendorff [23] at a constant pressure of 80 cm H_2O. The standard perfusion fluid had the following composition (mmol/l): NaCl, 124.0; KCl, 4.7; $MgCl_2$, 1.0; $NaHCO_3$, 24.0; Na_2HPO_4, 0.5; $CaCl_2$, 1.3; glucose, 11.0. During calcium-free perfusion, calcium was omitted from the standard perfusion fluid and no correction was made for the small change in osmolarity. The perfusion fluids were equilibrated with 95% O_2 and 5% CO_2, and the resulting pH was 7.4 at 37°C. The perfusion sequence was: 10 min control perfusion with standard perfusate, 10 min calcium-free perfusion, and 5 min reperfusion with standard perfusate.

^{31}P NMR spectra were recorded on a Bruker MSL 200 spectrometer equipped with a wide bore (150 mm) 4.7 Tesla superconducting magnet, using a pulse

repetition rate of 2.32 s and a pulse angle of 90°. Accumulated free induction decays were obtained from 6 or 128 transients on submerged rabbit hearts in a total volume of 55 ml in a 30 mm tube and exponentially multiplied, resulting in a line broadening of 20 Hz and 10 Hz, respectively. Zero ppm was assigned to the resonance position of creatine phosphate at pH 7.0. Intracellular pH was measured from the chemical shift of the intracellular inorganic phosphate (P_i) peak, using a titration curve obtained from a solution containing 10 mmol/l ATP, 10 mmol/l creatine phosphate, 10 mmol/l P_i, 10 mmol/l NADPH, 10 mmol/l glucose-6P, and 10 mmol/l $MgCl_2$. Results are expressed as mean ± S.D. of six experiments.

RESULTS AND DISCUSSION

Fig. 1 shows a typical ^{31}P NMR spectrum during control perfusion. In all hearts intracellular pH, which was calculated from the position of the intra-

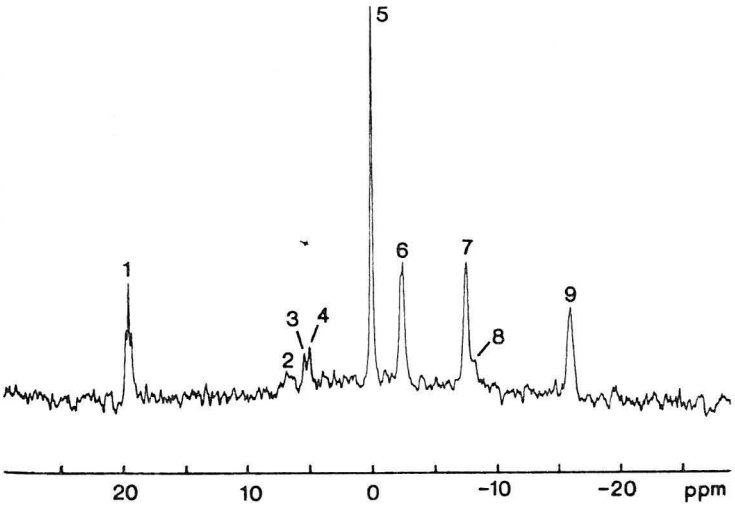

Fig. 1. ^{31}P NMR spectrum of a rabbit heart obtained from 128 scans, and taken between 5 and 10 min of control perfusion with standard perfusate. Resonances are assigned as follows:

1: methylene diphosphonate (external reference compound)
2: phospho-monoesters
3: extracellular P_i
4: intracellular P_i
5: creatine phosphate
6: γ phosphate of ATP
7: α phosphate of ATP
8: NAD(H)
9: β phosphate of ATP

cellular P_i peak, amounted to 7.1. In some hearts the intracellular P_i peak was absent in the spectrum that was taken during the subsequent calcium-free perfusion (Fig. 2). This is most likely a result of incorporation of P_i into high-energy phosphates, since myocardial creatine phosphate and ATP levels increase during calcium-free perfusion [24]. When the intracellular P_i peak was present, intracellular pH was calculated to be 7.1. Upon reperfusion with calcium-containing solution there was a sudden and severe decline of intracellular creatine phosphate and ATP levels. In the heart that was used for Fig. 3, intracellular pH varied from 7.1 to 6.9 between zero and 80 s of reperfusion. After approximately 1.5 min of reperfusion the intracellular P_i peak, and also the high-energy phosphate peaks, were no longer perceptible. Mean intracellular pH values of all hearts amounted to 7.1 ± 0.1 (0-20 s); 7.1 ± 0.1 (40-60 s); 7.0 ± 0.1 (80-100 s).

These results demonstrate that there was no appreciable fall of intracellular pH when the hearts were reperfused with calcium-containing solution. It is true that pH data were obtained only during the first 100 s of reperfusion, i.e. the period that the intracellular P_i peak was perceptible. It should be noted, however, that the calcium paradox damage develops so rapidly

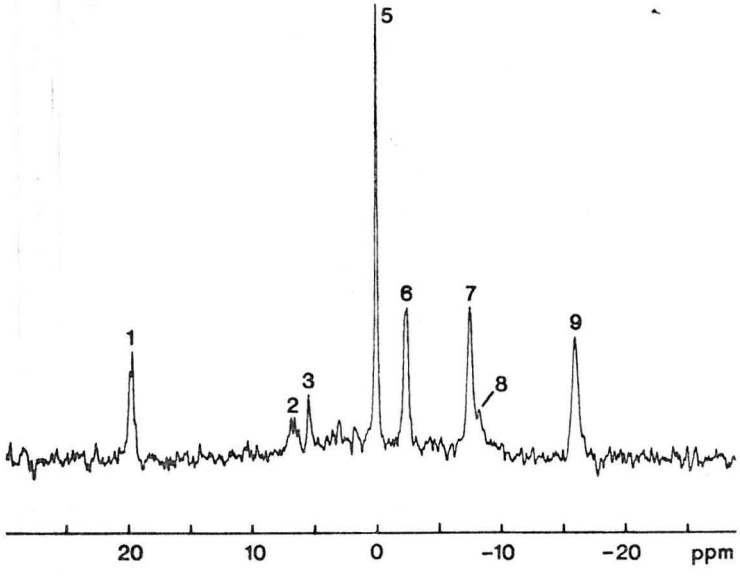

Fig. 2. ^{31}P NMR spectrum of a rabbit heart obtained from 128 scans, and taken between 5 and 10 min of calcium-free perfusion. Numbered peaks: see legend to Fig. 1.

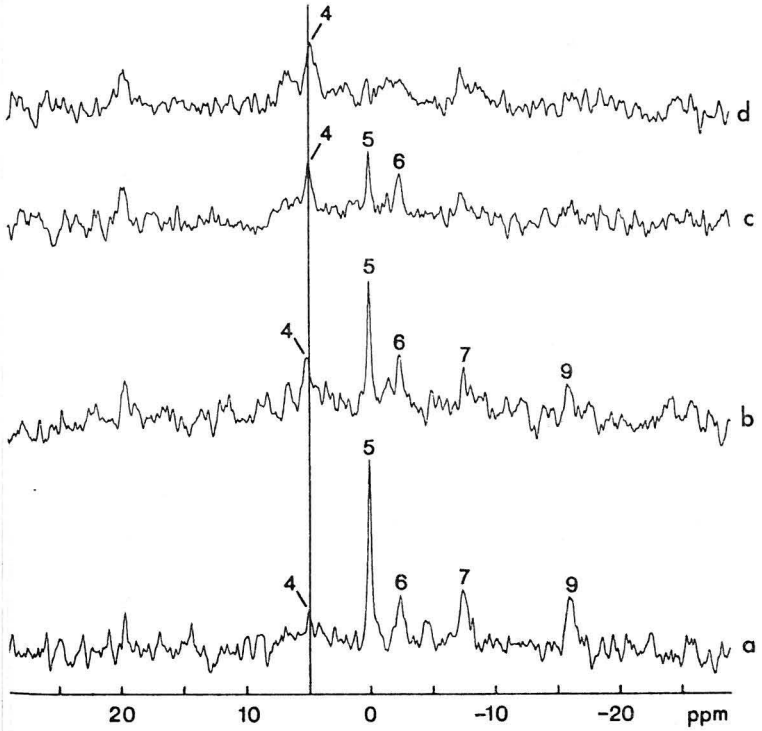

Fig. 3. ^{31}P NMR spectra of a rabbit heart obtained from 6 scans, and taken between 0-20 s (a), 20-40 s (b), 40-60 s (c) and 60-80 s (d) of reperfusion with calcium-containing solution. Numbered peaks: see legend to Fig. 1. Intracellular pH was measured from the chemical shift of the intracellular P_i peak, and amounted to 7.1 (a), 7.2 (b), 7.0 (c) and 6.9 (d). The vertical line indicates the position of the P_i peak at pH 7.0.

that the release of intracellular components such as enzymes, which is one of the characteristics of the calcium paradox, is maximal between 60 and 90 s of reperfusion [25]. Fig. 3 clearly shows that there is an almost simultaneous disappearance of the high-energy phosphate peaks and the P_i peak during the calcium paradox. This is in contrast with the situation during ischemia, where creatine phosphate and particularly ATP levels decline more gradually and the P_i peak shows a marked increase [26]. Leakage through the disrupted sarcolemma [20] into the extracellular space, and uptake by mitochondria together with calcium may be responsible for the rapid disappearance of P_i from the cytosol during the calcium paradox.

As mentioned in the introduction, both breakdown of ATP and accumulation of calcium by mitochondria are proton-generating processes. However, breakdown

of creatine phosphate is a proton-consuming reaction [18,22]. Apparently these opposite effects cancel during the calcium paradox and the result is little or no change in intracellular pH. Other factors that may contribute are the buffering capacity of the cell and leakage of protons into the extracellular space. It can be concluded that acidification of the cytosol does not play a causal role in the development of the calcium paradox.

SUMMARY

^{31}P NMR spectroscopy was used to investigate the course of intracellular pH during the calcium paradox in isolated rabbit heart at 37°C. Intracellular pH was measured from the chemical shift of the intracellular P_i peak. During control perfusion and the subsequent calcium-free period intracellular pH amounted to 7.1. After induction of the calcium paradox by readmitting calcium to the perfusion fluid, intracellular pH amounted to 7.0. It is concluded that acidification of the cytosol does not play a causal role in the development of the calcium paradox.

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REFERENCES

1. Ringer, S. J. Physiol. 4: 29-42, 1883.
2. Zimmerman, A.N.E. and Hülsmann, W.C. Nature 211: 646-647, 1966.
3. Hearse, D.J., Humphrey, S.M., Boink, A.B.T.J. and Ruigrok, T.J.C. Eur. J. Cardiol. 7: 241-256, 1978.
4. Zimmerman, A.N.E., Daems, W., Hülsmann, W.C., Snijder, J., Wisse, E. and Durrer, D. Cardiovasc. Res. 1: 201-209, 1967.
5. Lomský, M., Ekroth, R. and Poupa, O. Eur. Heart J. 4 (Suppl. H): 139-142, 1983.
6. Ruigrok, T.J.C., Slade, A.M. and Poole-Wilson, P.A. Eur. Heart J. 4 (Suppl. H): 89-96, 1983.
7. Alto, L.E. and Dhalla, N.S. Am. J. Physiol. 237: H713-H719, 1979.
8. Nayler, W.G., Elz, J.S., Perry, S.E. and Daly, M.J. Eur. Heart J. 4 (Suppl. H): 29-41, 1983.
9. Ruigrok, T.J.C. In: Control and Manipulation of Calcium Movement (Ed. J.R. Parratt), Raven Press, New York, 1985, pp. 341-365.
10. Meno, H., Kanaide, H. and Nakamura, M. J. Pharmacol. Exp. Ther. 228: 220-224, 1984.
11. Ohhara, H., Kanaide, H. and Nakamura, M. J. Mol. Cell. Cardiol. 14: 13-20, 1982.
12. Dhalla, N.S., Alto, L.E. and Singal, P.K. Eur. Heart J. 4 (Suppl. H): 51-56, 1983.
13. Nayler, W.G., Perry, S.E., Elz, J.S. and Daly, M.J. Circ. Res. 55: 227-237, 1984.

14. Lamers, J.M.J., Stinis, J.T. and Ruigrok, T.J.C. Circ. Res. 54: 217-226, 1984.
15. Lamers, J.M.J. and Ruigrok, T.J.C. Eur. Heart J. 4 (Suppl. H): 73-79, 1983.
16. Preuner, J. Basic Res. Cardiol. 80 (Suppl. 2), 19-24, 1985.
17. Ruigrok, T.J.C., Boink, A.B.T.J., Spies, F., Blok, F.J., Maas, A.H.J. and Zimmerman, A.N.E. J. Mol. Cell. Cardiol. 10: 991-1002, 1978.
18. Grinwald, P.M. and Nayler, W.G. J. Mol. Cell. Cardiol. 13: 867-880, 1981.
19. Ganote, C.E. and Nayler, W.G. J. Mol. Cell. Cardiol. 17: 733-745, 1985.
20. Post, J.A., Nievelstein, P.F.E.M., Leunissen-Bijvelt, J., Verkleij, A.J. and Ruigrok, T.J.C. J. Mol. Cell. Cardiol. 17: 265-273, 1985.
21. Brierley, G.P., Murer, E. and Bachmann, E. Arch. Biochem. Biophys. 105: 89-102, 1964.
22. Gevers, W. J. Mol. Cell. Cardiol. 9: 867-874, 1977.
23. Langendorff, O. Pfluegers Arch. 61: 291-332, 1895.
24. Boink, A.B.T.J., Ruigrok, T.J.C., Maas, A.H.J. and Zimmerman, A.N.E. J. Mol. Cell. Cardiol. 8: 973-979, 1976.
25. Ruigrok, T.J.C., Boink, A.B.T.J., Slade, A., Zimmerman, A.N.E., Meijler, F.L. and Nayler, W.G. Am. J. Pathol. 98: 769-790, 1980.
26. Ruigrok, T.J.C., Van Echteld, C.J.A., De Kruijff, B., Borst, C. and Meijler, F.L. Eur. Heart J. 4 (Suppl. C): 109-113, 1983.