Vol. 151, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 29, 1988 Pages 70-77

^{31}P NMR MEASUREMENTS OF MYOCARDIAL pH IN VIVO

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Received January 4, 1988

A ^{31}P NMR magnetization transfer method for measuring myocardial pH in vivo is demonstrated in the lamb, dog and cat. The method involves measuring the difference in chemical shift between the resonances of phosphocreatine and inorganic phosphate in magnetization transfer difference spectra in which the γ-phosphate resonance of ATP has been saturated. The method has been verified by measuring the chemical shift difference between the resonances of 2-deoxyglucose 6-phosphate and phosphocreatine following infusion of the animals with 2-deoxyglucose. The measured pH values are significantly lower than those obtained in previous studies on the heart in vivo.

^{31}P NMR has been used extensively to monitor changes in the concentrations of phosphorus metabolites and the intracellular pH in the perfused heart in response to various interventions such as changes in substrate (1,2) and ischemia (3). The myocardial pH can be determined from the chemical shift difference between the phosphocreatine (PCr) and inorganic phosphate (P_i) resonances. Similar measurements can also be made on the heart in vivo using open chested animals (4,5,6,7,8), implanted coils (9,10), catheter coils inserted through a peripheral blood vessel (1,12) and non-invasive spatial localisation techniques in dogs (13) and humans (14,15). The intracellular myocardial pH in vivo, however, can only be measured under ischemic conditions, when the concentration of P_i is relatively high. Under normoxic conditions the intracellular P_i resonance is of low intensity and overlap with resonances from the blood in the heart make the assignment of the myocardial P_i resonance and the determination of intracellular pH impossible (16). The interfering ^{31}P resonances from blood include those from 2 3-diphosphoglycerate (2,3-DPG) in the erythrocyte and plasma P_i. Spectral editing techniques, which exploit the proton coupling in the 2,3-DPG resonances, can be used to suppress these resonances (16). However application of this technique to the rabbit heart in

0006-291X/88 $1.50

vivo left only a single resolved P_i resonance, which was assigned to P_i in the blood on the basis of its chemical shift and P_i infusion experiments. There was no evidence of a separate myocardial P_i resonance at the signal-to-noise ratios obtained in these spectra.

In this study we have adopted two approaches in determining the myocardial pH in the normoxic heart in vivo. The first exploited the fact that the myocardial P_i resonance is unique among the ^{31}P resonances in this region of the heart spectrum, in that it is in rapid chemical exchange with the γ-phosphate resonance of ATP (17). In a saturation transfer experiment, saturation of the γ-phosphate resonance of ATP results in a reduction in the intensity of the exchanging P_i resonance, which can be readily observed in a difference spectrum in which the spectrum obtained with saturation of the γ-phosphate resonance of ATP is subtracted from a control spectrum. The P_i peaks observed in saturation transfer difference spectra obtained in surface coil experiments in the open-chested lamb, dog and cat have been used here to determine the myocardial pH in these species. The second approach that we have used is to infuse the animals with 2-deoxyglucose, which is taken up by the heart and phosphorylated to give 2-deoxyglucose 6-phosphate (18). This molecule gives an observable resonance in the heart spectrum which is sensitive to pH (18). The myocardial pH estimated using this probe has been compared with that determined from the magnetisation transfer measurements.

These measurements have allowed the first determination of intracellular pH in the normoxic heart in vivo. The results obtained support the results of a previous study (16) which showed that the pH values quoted for the normoxic heart in vivo are incorrect (7,13), since the blood P_i resonance had been wrongly assigned to the myocardial P_i. The determination of myocardial pH in vivo will allow calculation of the free ADP concentration in the heart muscle (1,19), which is an important indicator of cellular energy status, being sensitive to changes in the rate of ATP supply (3,20) and utilisation (21,22,23). Measurements of both pH and ADP concentration should be useful in assessing the pathology of certain disease states (24).

METHODS

Two male lambs, aged 55 and 68 days respectively, two female dogs and two female cats were anaesthetized with intravenous Nembutal. They were ventilated through a tracheostomy with 50% N_2O/O_2 and 1-2% Halothane, with a Harvard ventilator. Lines were inserted into the carotid artery for monitoring pH and blood gases and into the jugular vein for administration of fluids or drugs. The chest was opened through a mid-sternal incision and the pericardium opened sufficiently to allow a surface coil to be placed over the lateral surface of the left ventricle. Ventilation was adjusted to give an arterial pCO_2 of between 40-45 mm Hg and a pO_2 > 100mm Hg. Arterial pH was maintained at between 7.35 and 7.45. A 3cm diameter surface coil was used in the dogs and lambs and a 2cm diameter coil in the cats. The animals were placed in a plexiglass cradle,

the surface coil placed over the heart and tuned to 80 MHz and a hot water pad (at 37°C) placed over the animal. The cradle was inserted in a 4.7 T 40 cm horizontal bore magnet interfaced to a Bruker BIOSPEC II NMR spectrometer. Magnetic field homogeneity was optimized using the proton signal, which was gated to respiration.

^{31}P NMR spectra were obtained with a nominal 60° pulse (60 μsec for the 3cm coil and 20μsec for the 2cm coil) with the frequency of the pulse set between the γ and α-phosphate resonances of ATP. Spectra were acquired with a sweep width of 6000 Hz in 1K data points and were zero-filled to 4K prior to Fourier transformation. Between 15 and 20Hz line broadening was applied. For the saturation transfer experiments, the phosphate resonance of ATP was irradiated for 5s with a low power pulse. The amplitude of the pulse was set to the lowest value compatible with complete saturation of the ATP resonance. Control spectra were acquired with the irradiation set an equal frequency downfield from the P_i peak. Control and ATP irradiated spectra were acquired in alternate blocks of 128 scans. Blood pressure and pulse were monitored continuously with a Gould 4 channel recorder (Model 3400) and arterial blood gases were measured with an Instrumentation Laboratory System 1302 pH Blood Gas Analyzer every 30-45 min. The lungs were periodically sighed to prevent atelectasis. Metabolic acidosis was corrected with intravenous bicarbonate and hypotension was corrected with intravenous saline or lactated Ringer's solution. In some of the experiments, 0.4-0.8g of 2-deoxyglucose (Sigma)/Kg of body weight were infused over a period of 30-60 min.

RESULTS AND DISCUSSION

Magnetisation transfer difference spectra (fig.1a) i-ii, b) i-ii and c) i-ii) clearly show the effects of chemical exchange between the γ-phosphate of ATP and P_i and PCr. Exchange between ATP and P_i has been shown to be catalysed by the mitochondrial F_1F_0 ATP synthase and by the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase (17,25). Exchange between PCr and ATP is catalysed by creatine kinase (26,27). The difference in chemical shift between the P_i and PCr resonances in the difference spectra can be used to estimate the myocardial pH (see Table 1). Magnetisation transfer measurements on blood samples taken from these animals showed no detectable exchange between P_i and ATP (data not shown). The difference spectra also show that both the α- and β-phosphate peaks of ATP are decreased in intensity following saturation of the γ-phosphate peak. The decrease in the β-phosphate is due to exchange between the β-phosphates of ATP and ADP (28), an exchange which is catalysed by all enzymes that interconvert ATP and ADP. The exchange is observed since the β-phosphate of ADP is less than 1ppm upfield of the γ-phosphate of ATP and is saturated when the ATP resonance is saturated. The decrease in the ATP α-phosphate peak when the γ-phosphate is saturated has been observed in many previous saturation transfer studies in a variety of tissues. However the origin of this effect is obscure.

The heart spectra from the lamb, dog and cat clearly show signals from the blood in the heart. The lack of 2 3-DPG and the relatively high phosphate concentration in the blood of the cat and the lamb (see fig. 3) (29) are reflected in the appearance of the peaks between 5 and 6 ppm downfield of the

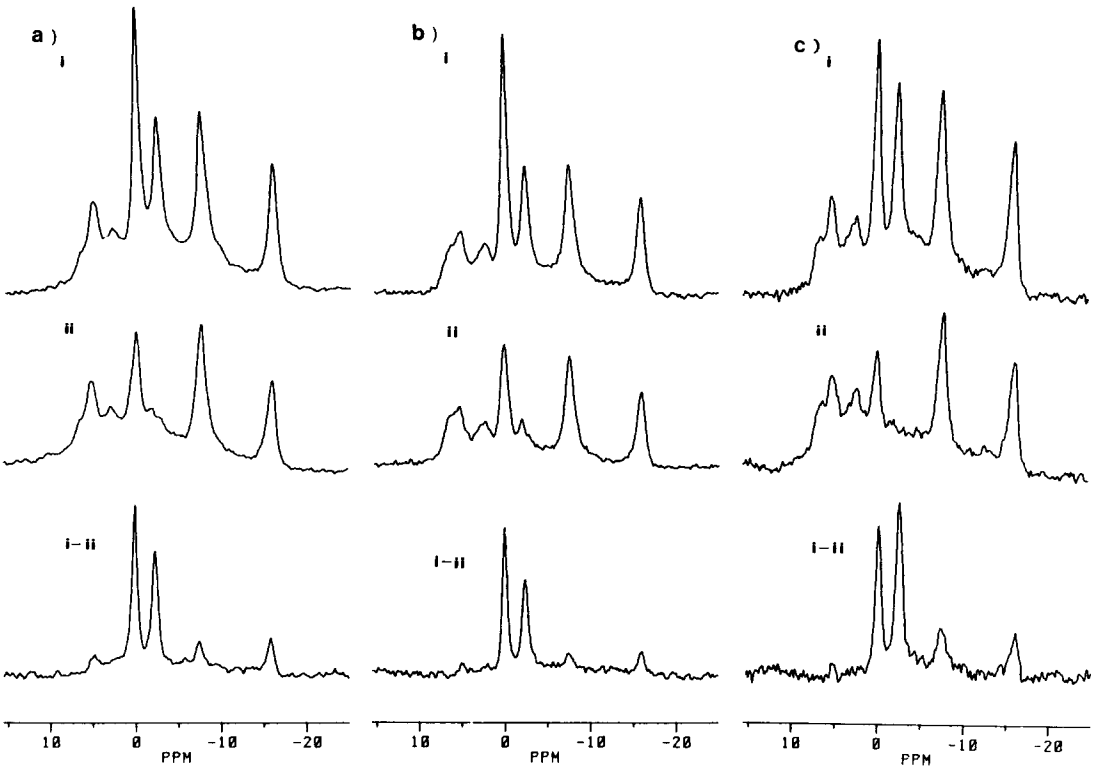


Figure 1. ^{31}P NMR magnetization transfer spectra from lamb, dog and cat heart in vivo.

The spectra labelled a), b) and c) were from lamb, dog and cat respectively. The chemical shift scale is referenced to the phosphocreatine resonance at 0.0 ppm. The lamb spectra are the sum of 512 scans, the dog spectra are the sum of 1024 scans and the cat spectra are the sum of 896 scans. The peak assignments in the difference spectra are, from the downfield end of the spectrum, P_i, PCr, γ-phosphate of ATP, α-phosphate of ATP and β-phosphate of ATP.

phosphocreatine resonance. Although the spectra were not collected under fully relaxed conditions, the spectra also show that the cat differs from either the lamb or the dog in that it has a significantly lower PCr/ATP ratio.

Infusion of 2-deoxyglucose in both the cat and the dog produced a readily observable 2-deoxyglucose 6-phosphate resonance in the heart spectrum. Spectra from the cat are shown in figure 2. Although some 2-deoxyglucose 6-phosphate appeared in the blood (fig.3), this was insufficient to cause the increase in intensity observed in the heart spectra. The assignment of the 2-deoxyglucose 6 phosphate resonance to the myocardium is further supported by the reciprocal changes in intensity between the 2-deoxyglucose 6-phosphate resonance and the ATP and PCr resonances. The phosphate group in 2-deoxyglucose 6-phosphate has a pKa of 6.3 and its resonance shifts by more than 3 ppm between pH 4 and pH 8.

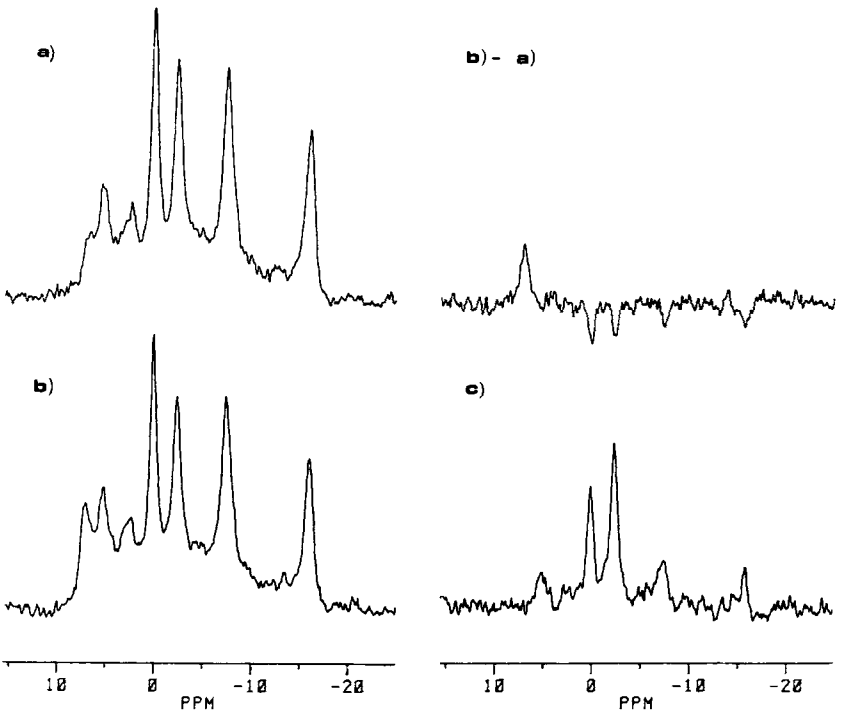
Table 1
Estimated pH in the heart from magnetisation transfer and 2-deoxyglucose infusion experiments

Animal	Measurements made before 2-deoxyglucose infusion	Measurements made following 2-deoxyglucose infusion	
	pH from P_i chemical shift	pH from P_i chemical shift	pH from 2-DOGP chemical shift
Lamb 1	6.93		
Lamb 2	6.97		
Dog 1	7.15		
Dog 2		6.92	6.81
Cat 1	7.02	7.12	7.02
Cat 2	7.27	7.15	7.07

The pH values were calculated from the chemical shift differences between the resonances of P_i and PCr and 2-deoxyglucose 6 phosphate (2-DOGP) and PCr, using the following algorithms.

$$\text{pH PCr-P}_i = 6.75 + \log([\sigma - 3.27]/[5.69 - \sigma])$$
$$\text{pH PCr-2-DOGP} = 6.30 + \log([\sigma - 4.35]/[7.59 - \sigma])$$

where σ is the chemical shift difference in ppm between PCr and P_i and between 2-deoxyglucose 6-phosphate and PCr. The algorithm for the PCr-P_i chemical shift difference was obtained from (22) and the algorithm for the PCr-2-deoxyglucose 6-phosphate chemical shift difference was obtained by constructing a titration curve for 2-deoxyglucose 6-phosphate. This was done by titrating a solution containing 10mM 2-deoxyglucose 6-phosphate, 10mM phosphocreatine, 2.5mM $MgCl_2$, 100mM KCl and 10mM P_i with 0.1N KOH and 0.1 N HCl at 37°C. ^{31}P NMR measurements were made at 121.49MHz.



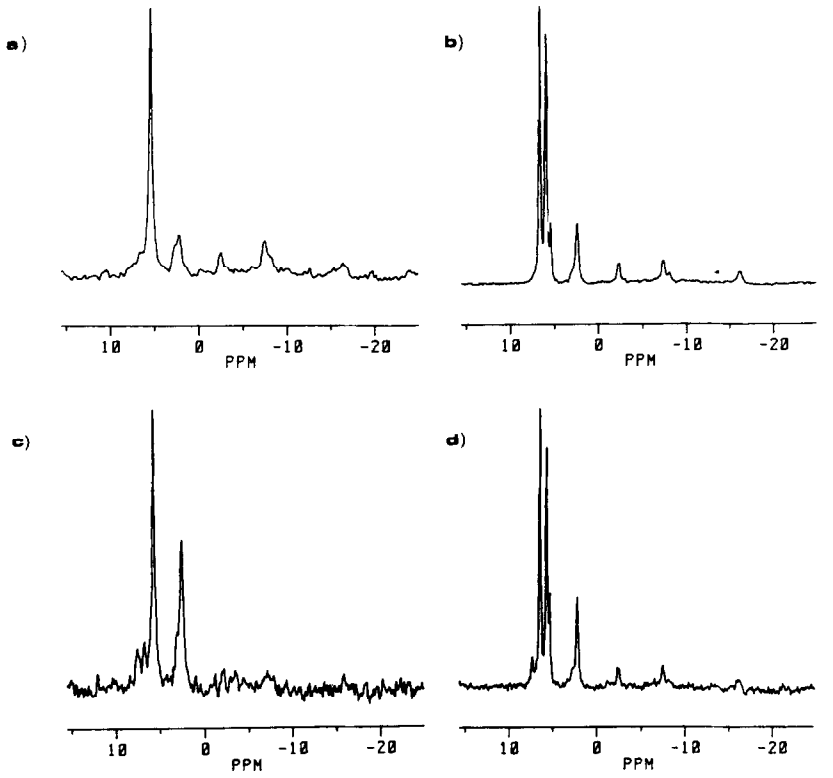


Figure 3. ^{31}P NMR spectra of arterial blood samples from the lamb, dog and cat.

Spectrum a) is from lamb's blood. Spectrum b) is from dog blood prior to 2-deoxyglucose infusion and spectrum d) is from dog blood after 2-deoxyglucose infusion. Spectrum c) is from cat blood following 2-deoxyglucose infusion. The chemical shift scale is referenced to the resonance frequency of phosphocreatine at 0.0 ppm. The assignments are, from the downfield end of spectrum b); 3-phosphate of 2,3-DPG; 2-phosphate of 2,3-DPG; plasma phosphodiesters; γ-phosphate of ATP; α-phosphate of ATP; NAD^+; β-phosphate of ATP. The low field resonances in spectra d) and c) are assigned to 2-deoxyglucose 6-phosphate on the basis of their chemical shift and appearance after 2-deoxyglucose infusion.

The molecule can, therefore, be used as a pH probe for the myocardium (18) (see table 1). The pH in the myocardium following 2-deoxyglucose infusion was also determined using the magnetisation transfer experiment described above. The saturation transfer difference spectrum obtained following 2-deoxyglucose infusion in the cat is shown in fig.2c. The pHs determined using these two

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Figure 2. Effects of 2-deoxyglucose infusion on the spectrum from cat heart.

The spectra are the sum of 640 scans. Spectra a) and b) were acquired before and after 2-deoxyglucose infusion respectively. The resonance at 7.07 ppm in the difference spectrum (b-a) was assigned to 2-deoxyglucose 6-phosphate. A magnetisation transfer difference spectrum, acquired after 2-deoxyglucose infusion, is shown in c).

techniques show reasonable agreement (table 1). However the pH values determined here of between 7.0 and 7.2 are significantly lower than previously reported values of 7.4 (7.13) and support the conclusions of a previous study which indicated that the high pH was due to assignment of the blood P_i resonance to the myocardium (16).

A non-invasive ^{31}P NMR magnetisation transfer method for determining myocardial pH has been described and the results obtained using the technique verified using 2-deoxyglucose 6-phosphate as a pH probe. The technique could be combined with spatial localisation techniques such as the rotating frame imaging experiment (15), which would allow regional determination of pH. Ultimately this technique, in combination with the rotating frame localization experiment may be used to determine regional pH of the human heart in vivo.

ACKNOWLEDGEMENTS

We wish to thank Mr Virgil Simplaceanu and Mrs Nancy T. Ho for their assistance. The Pittsburgh NMR Center for Biomedical Research was established through funding by the Richard King Mellon Foundation, the Ben Franklin Partnership of the Commonwealth of Pennsylvania, the University of Pittsburgh and Carnegie Mellon University. This research is supported, in part, by a grant from the National Institutes of Health (HL-24525 awarded to CH.). J.A.D. is supported by a postdoctoral fellowship from the National Institutes of Health (NS-08280). K.M.B., B.R. and G.K.R. wish to thank the Medical Research Council of Great Britain, the British Heart Foundation and NIH (HL-18708) for support. K.M.B. thanks the Royal Society for a University Research Fellowship.

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