DETECTION OF REGIONAL ISCHEMIA IN PERFUSED BEATING HEARTS BY PHOSPHORUS NUCLEAR MAGNETIC RESONANCE

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Summary: Phosphorus-31 NMR spectra of a perfused, beating rabbit heart regionally ischemic following ligation of the left anterior descending coronary artery revealed two peaks arising from inorganic phosphate. During the pre-ligation period there was only a single orthophosphate signal at a position corresponding to one of the two peaks noted during regional ischemia. Since the resonance frequency of the orthophosphate peak is determined by environmental pH and since ischemia results in intracellular acidosis, the two signals in the regionally ischemic heart result from tissue orthophosphate residing at different intracellular pH values in the normal (pH 7.4) and ischemic zones (pH 6.6). To document that one signal was derived from normal tissue and the other from the ischemic area, the pH dependent shift in the inorganic phosphate resonance was followed during global ischemia following the period of regional ischemia. After 20 minutes of total ischemia, only a single peak was observed at the more acidic region, pH 6.4. We conclude that it is possible to identify regional myocardial ischemia by this entirely non-invasive and non-destructive technique. In the future it is likely that rapid and repeated ischemic zone sizing and localization will be possible using existing NMR imaging methods.

We have initially reported that nuclear magnetic resonance can be used to monitor certain phosphorus containing metabolites and intracellular pH in perfused working rat hearts in normoxic and ischemic states (1), and similar observations were subsequently reported by others (2). This work followed naturally from earlier studies of other laboratories on the ^{31}P NMR analysis of oxygenated but non-perfused skeletal muscle and cellular preparations (3-5). Much of this previous research was recently reviewed by Burt et al.(6). More recently we have estimated that the intracellular pH as monitored by the chemical shift of inorganic phosphate decreased from about 7.45 to 6.41 when the heart was made globally ischemic by cross-clamping the aorta (7). This suggested to us and others (8) that normoxic and ischemic regions of the heart might be resolved and possibly quantitated

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by measuring the relative areas of the high and low pH inorganic phosphate signals. Recently, we have reported that high resolution ^{31}P spectra could be collected in only 5 minutes by increasing spectrometer frequency to 72.9 MHz and heart size to 6 grams (9). The purpose of this communication is to report the observation of resolved inorganic phosphate resonance in a rabbit heart in which regional myocardial ischemia was induced by ligation of the left anterior descending coronary artery.

MATERIALS AND METHODS

Male New Zealand rabbits weighing about 2 kg were anesthetized with sodium pentobarbital (250 mg) and injected with 2,000 units of Heparin intraperitoneally. Hearts weighing 6-8 grams were removed, placed in cold perfusate and perfused within 60 seconds by a cannula inserted into the aorta above the aortic valve. Perfusion pressure was 110 cm H_2O. A fluid filled latex balloon placed in the left ventricle measured isovolumic pressure as described previously (1). Perfusate composition, the method of monitoring function and other experimental details were as described previously (1). The hearts began beating spontaneously upon perfusion with warm (36°) perfusate. Substrate was 100 mg% glucose, and the Krebs buffer was bubbled with 95% O_2 + 5% CO_2.

Phosphorus NMR spectra were recorded at 72.9 MHz using a Bruker 180 Spectrometer operating in the Fourier transform mode and employed a Bruker BNC 1180 computer. Field frequency lock was not required over the 5 minute periods required to obtain high quality spectra. All spectra were obtained without proton decoupling. Hearts were contained in a 25 mm NMR tube. Samples were not spun.

RESULTS

Figure 1A shows the initial ^{31}P NMR control spectrum of a 6 gram working rabbit heart. The left ventricular pressure was initially 146 mm Hg systolic and 15 mm Hg at end diastole, at a spontaneous heart rate of 160 beats per minute. The spectrum shows the expected peaks previously assigned (1, 3, 4) to ATP, phosphocreatine, and inorganic phosphate. This spectrum was obtained with only five minutes of time averaging. Figure 1B was obtained under the same conditions and on the same heart as Figure 1A but immediately after ligation of the left anterior descending coronary artery proximal to the septal branch. Under these conditions, peak systolic left ventricular pressure dropped to 38 mm Hg and coronary flow decreased by 25%. Immediately following ligation a dark area of ischemia was observed over about 30% of the left ventricular anterior wall. The remainder of the heart

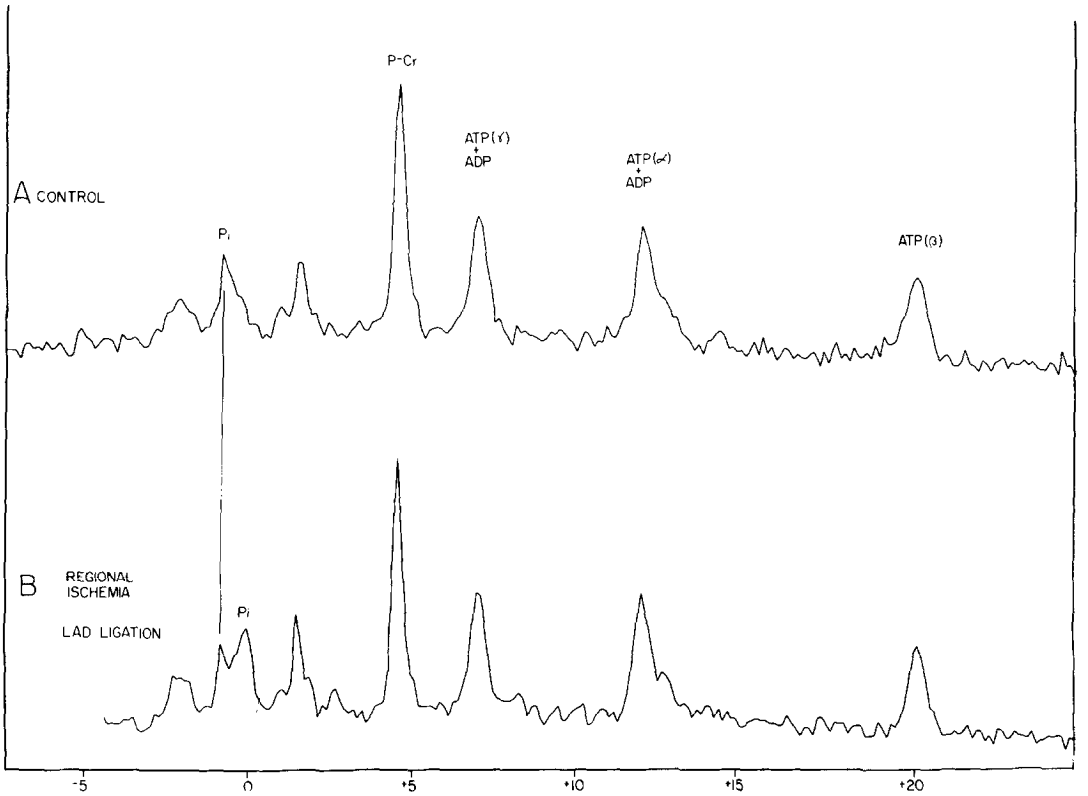

Fig. 1. ^{31}P NMR spectrum (72.9 MHz) of a perfused rabbit heart. Each trace represents the Fourier transformed average of 150 transients requiring 5 minutes total accumulation time. Chemical shifts are expressed in parts per million relative to a solution of .2 M H_3PO_4 in 15% $HClO_4$ contained in a 1 mm diameter capillary tube. Positive values indicate shifts to a higher field than the reference. (A) fully perfused heart; (B) same heart and same condition as in (A) but spectrum obtained immediately after ligation of the left anterior descending coronary artery. The bar line calibrates the location of the control phosphate peak.

appeared to be normally perfused. The crucial point is that the orthophosphate resonance split into two components, Figure 1B. One component remained in the position of the control phosphate, pH 7.4, while the other appeared at a higher field indicating a decrease in tissue pH to 6.6. The lower field resonance was assigned to phosphate in the well perfused portion of the heart since it is in the same position as the phosphate peak prior to ligation. The higher field resonance indicates phosphate in the occluded ischemic region of the heart which has become more acidic. Evidence supporting this latter assignment is presented in Figure 2.

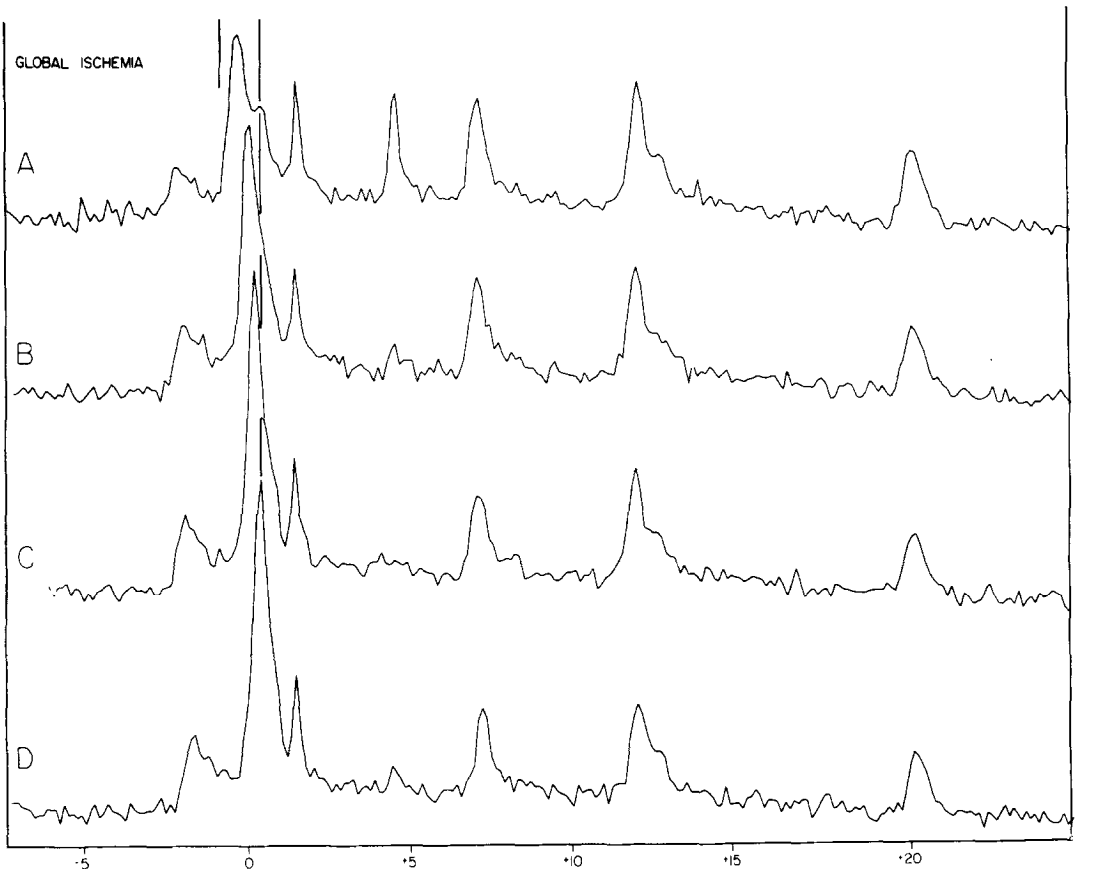

Fig. 2. Time course of pH changes as measured by ^{31}P NMR in a regionally ischemic heart which was subsequently made globally ischemic by cross-clamping the aorta. This is the same heart as shown in Figure 1. (A) through (D) represents four successive 5 minute accumulations following the clamping the aorta. All instrumental conditions are the same as in Figure 1. The chemical shift scale is also identical to that of Figure 1. The left bar line calibrates the phosphate peak in the control heart; the right line is the phosphate position in the regionally ischemic preparation.

Figure 2 shows the effects of global ischemia on the same rabbit heart employed in Figure 1A and 1B. After the perfusion is stopped, Figure 2A, the low field signal intensity increased markedly, shifting to pH 6.8. The peak progressively moved toward higher field with time, Figures 2B and 2C, finally overlapping the original high field signal when the entire heart had become severely acidotic, Figure 2D. This required about 20 minutes. During this time pH dropped from pH 7.4 to pH 6.4.

DISCUSSION

While it is tempting to relate the relative area of the acidic phosphate peak directly to the volume of ischemic tissue (8), this assumption is an oversimplification. The net amount of tissue orthophosphate observed as an acidic NMR peak will be a function of the severity of tissue ischemia, the duration of ischemia, the volume of ischemic myocardium, and the extent of phosphate wash-out by coronary arterial collateral flow. In man, acute myocardial infarction is not necessarily an occlusive event. To induce ischemic myocardial damage, flow need only be restricted to a rate such that oxygen delivery is compromixed (10. Additionally, in an ischemic region there are graded zones of perfusion (11) and damage (12). We assume that cells in these distinct regions would contain different levels of orthophosphate. Furthermore, the effects of the duration of ischemia are noted in Figure 2. In the initial scan of global ischemia, the low field phosphate peak is small. During the 20 minutes of ischemia, the total amount of phosphate progressively increased. Therefore, the amount of orthophosphate phosphate present in the tissue is time dependent. And finally, it must be borne in mind that such an estimation would assume that the total phosphate content of both the normoxic and ischemic regions remained constant. However, we have repeatedly observed a progressive loss in total phosphate content in ischemic myocardium as determined by NMR (Pi). Phosphate loss from ischemic tissue has also been reported by others (13, 14). Phosphate wash-out by even minimal collateral flow thus complicates any calculation of the volume of ischemic tissue present.

However, our results demonstrate the feasibility of using ^{31}P NMR to identify the presence of regional myocardial ischemia. They also illustrate the substantial advantage of using large bore NMR magnets with large hearts to obtain satisfactory ^{31}P NMR spectra in physiologically relevant time blocks. The high sensitivity thus obtained and phosphate peak resolution suggest the possibility of using ^{31}P NMR to obtain a three dimensional image of the heart in vivo based either on inorganic phosphate concentration or on tissue

pH, using for example the method of Lauterbur (15). This would permit the rapid localization and sizing of ischemic regions of the intact heart by entirely non-invasive, non-destructive and repeatable methods. Additionally, previous areas of infarction containing frank scar tissue might appear as metabolic voids in the spatial image. This technique therefore holds much promise for the detailed evaluation and differentiation of both past and current ischemic myocardial tissue damage.

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