In vivo ^{31}P-NMR studies of myocardial high energy phosphate metabolism during anoxia and recovery

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^{31}P NMR spectra of heart in-situ in live guinea pigs were obtained continuously in 20.5 s time blocks during 3 min of anoxia, during subsequent reoxygenation and, in separate animals, during terminal anoxia. Reversible anoxia resulted in rapid degradation of phosphocreatine ($t_{1/2}$ = 54.5 ± 2.5 s) which recovered fully during reoxygenation. Heart P_i increased during anoxia and returned to basal levels after oxygen was restored. During 3 min of anoxia, no significant changes in ATP levels or pH were detected. The results demonstrate that it is feasible to measure rapid fluxes of high energy phosphates by ^{31}P NMR in intact animals during and after anoxic stress to the myocardium.

^{31}P-NMR in vivo *Heart metabolism* *High energy phosphate* *Anoxia* *Recovery*

1. INTRODUCTION

^{31}P-NMR spectroscopy offers the unique capability to non-destructively monitor high energy phosphates, P_i and pH in vitro and in vivo [1,2]. This technique has been employed in several studies of high energy phosphate metabolism in isolated, perfused hearts subjected to ischemia and hypoxia [3–8]. However, the relevance of these studies to the situation in the intact animal, where respiratory, vascular, neural and hormonal factors are known to play an important part in the control of myocardial function and physiology [9], is uncertain. Only two ^{31}P-NMR studies of heart in situ in the intact animal have been reported. ^{31}P-NMR spectra of rat heart were obtained in vivo [10], while we had studied the ^{31}P-NMR spectrum of guinea pig heart in vivo [11], combined with ^{13}C-NMR studies of myocardial glycogen metabolism. In [10], high energy phosphate metabolism was studied prior to and during respiratory arrest. However, 6.8 min were required for spectral accumulation. This time resolution does not permit analysis of the time course of decline of phosphocreatine. Here, we were able to obtain good quality ^{31}P-NMR spectra of heart in situ in live guinea pigs in 20.5 s. This allowed rapid measurement of myocardial levels of phosphocreatine and ATP during anoxia induced by respiratory arrest and during recovery from brief periods of anoxia.

2. MATERIALS AND METHODS

Experiments were carried out with Hartley strain guinea pigs, which were surgically prepared as in [11]. The animals were placed on a platform warmed to 40°C and ventilated on room air through a tracheal cannula. The common carotid artery was cannulated and arterial pressure and heart rate were monitored throughout each experiment. The thorax was opened through a mid-sternal incision and the pericardium removed. An incision was made between ribs on each side and the ribs were tied back, away from the heart. The heart was positioned within a 5-turn solenoidal receiver coil made of 1.5 mm diam. high purity copper wire. Only ^{31}P-NMR resonances from the heart were detected by the coil. The magnetic field was

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shimmed by using the ^{1}H-NMR resonance from the H_2O in the heart. ^{31}P-NMR spectra were obtained at 32.5 MHz on an Oxford Research Systems TMR-32/200 spectrometer.

3. RESULTS AND DISCUSSION

After obtaining control spectra (fig. 1A,B), anoxia was induced by turning off the respirator. ^{31}P-NMR spectra of the heart were accumulated in blocks of 20.5 s during 3 min of anoxia and during subsequent re-ventilation (fig. 1C, representative spectrum). Fig. 2 shows the time course of change in the intensity of the phosphocreatine signal during anoxia and recovery, averaged from data obtained in 5 animals. After the onset of anoxia, phosphocreatine levels showed a rapid decline. The data points could be fitted to a single exponential (correlation coefficient 0.96) with a $t_{1/2}$ of 54.5 ± 2.5 s ($\pm$SEM, $n = 5$). After 170 s, phosphocreatine had declined to 20% of the original level. At the onset of re-ventilation, at 3 min, rapid recovery of the phosphocreatine pool was observed (fig. 2). Regeneration to 50% of the original level was complete in 38.2 ± 3.3 s ($\pm$SEM, $n = 5$), thus the rate of recovery during re-ventilation was similar to that of phosphocreatine decline during the period of anoxia. Within 2.5 min, complete recovery of the phosphocreatine pool was observed (fig. 2). About 1–2 min after re-ventilation, arterial pressure and heart rate had returned to basal levels. In separate experiments, four animals were subjected to terminal anoxia. In these experiments, phosphocreatine declined to undetectable levels within about 5 min.

P_i levels increased during anoxia (fig. 1), simultaneously with the decline in phosphocreatine. During re-ventilation, P_i returned to basal levels on a time scale similar to the recovery of phosphocreatine. However, the incremental changes in the P_i resonance (in % of control) seen during either reversible or terminal anoxia did not correspond in a uniform manner to the decrements in phosphocreatine seen in the same spectrum. Several factors are likely to contribute to this discordance, including:

(i) The phosphocreatine signal arises only from heart muscle, while both myocardium and blood within the ventricular chambers contribute to the P_i signal;

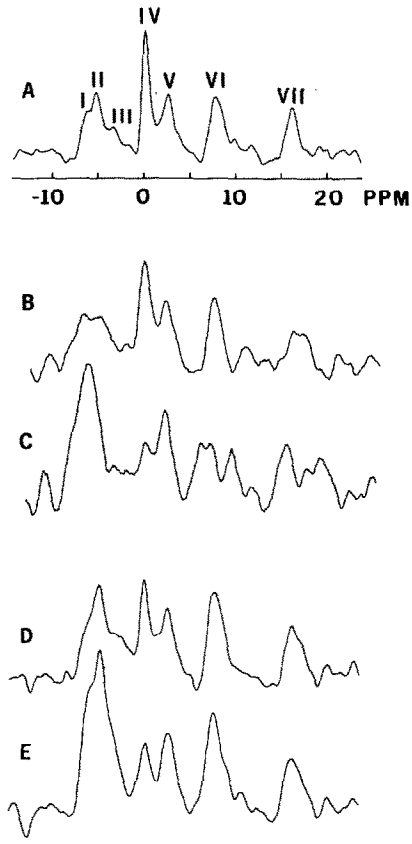

Fig. 1. (A) ^{31}P-NMR spectrum of guinea pig heart in vivo. The spectrum was recorded with 4 kHz spectral width, 65° pulse width, 10 s recycle time and 32 transients. Assignments: (I) 3-phosphate of 2,3-diphosphoglycerate and sugar phosphates; (II) 2-phosphate of 2,3-diphosphoglycerate and P_i; (III) phosphodiesters; (IV) phosphocreatine; (V) γ-ATP and β-ADP; (VI) α-ATP and α-ADP; (VII) β-ATP. (B) ^{31}P-NMR spectrum of guinea pig heart in vivo obtained prior to the induction of anoxia in 20.5 s with 3 kHz spectral width, 33° pulse width, 0.64 s recycle time and 32 transients. These parameters were chosen to yield optimal signal-to-noise per unit time for the phosphocreatine resonance ($T_1 \sim 3$–4 s) [10]. (C) ^{31}P-NMR spectrum obtained within 20.5 s at 78 s (midpoint of accumulation) after the induction of anoxia. (D) ^{31}P-NMR spectrum obtained prior to anoxia. The spectrum is the sum of 3 sequential spectra each collected in 48 s with 3 kHz spectral width, 65° pulse width, 0.54 s recycle time and 88 transients. These parameters were chosen to obtain near optimal signal-to-noise for the β-ATP signal ($T_1 \sim 0.5$–1 s). (E) Sum of 3 sequential spectra acquired (as in (D)) over 144 s immediately following induction of anoxia.

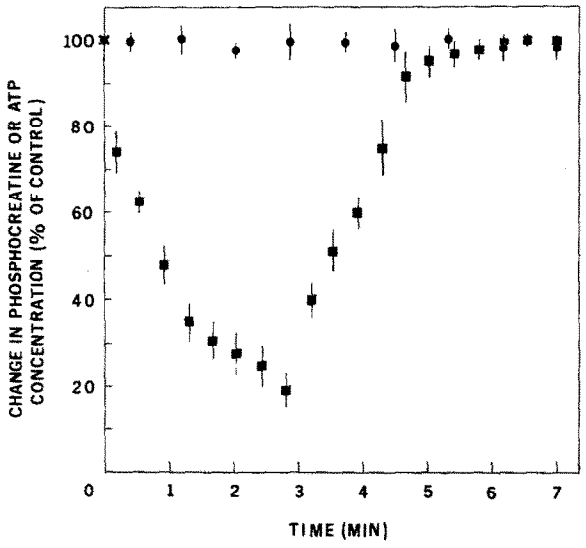

Fig. 2. (■) Time dependence of the intensity of the phosphocreatine resonance during 3 min of anoxia and subsequent re-ventilation. Data points are midpoints of 20.5 s accumulations and represent the average of 5 animals (±SEM). (●) Time dependence of the β-ATP signal during terminal anoxia. Data points are midpoints of 48 s accumulations (±SEM, $n=4$). There is a 2 s delay between subsequent spectra due to filing of individual spectra on floppy disk in an automated sequence.

(ii) P_i liberated from high energy phosphates may diffuse out of the myocardium.

The pH of guinea pig heart in vivo, as determined from the chemical shift of the P_i resonance, was 7.35 ± 0.05 (±SEM, $n=9$). This value is essentially the same as the pH for rat heart in vivo and reflects an average of the cardiac intracellular and the whole blood pH [10]. During 3 min of anoxia no significant change in pH was detected, while at later times during terminal anoxia progressive acidosis was observed.

In order to monitor the ATP pool during 3 min anoxia followed by re-ventilation as well as during terminal anoxia, ^{31}P-NMR spectra were collected in blocks of 48 s with different parameters (see legend to fig. 1) to yield near optimal signal-to-noise per unit time for the β-ATP signal [12]. Fig. 1D and E each show sums of 3 such spectra, representing about 2.5 min of data accumulation, before and immediately following the induction of anoxia. While these spectra again clearly demonstrate the decline in phosphocreatine and the increase in P_i, no change in the intensity of the β-ATP signal is detected. Fig. 2 demonstrates that ATP levels did not change significantly during the first 7 min of terminal anoxia. Between 20–30 min after the induction of terminal anoxia, ATP had declined to undetectable levels; heart rate and blood pressure could no longer be detected at 12–14 min.

In [10], no change in ATP concentration was found during the first 10 min of respiratory arrest in their ^{31}P-NMR study of rat heart in vivo. In addition, in the ^{31}P-NMR study of hypoxia in isolated, perfused rat heart [7], ATP decreased significantly only after 4 min. The present results obtained during anoxia in vivo by ^{31}P-NMR show that the ATP levels are constant during the first 7 min of this insult. However, they are in contrast to those obtained with isolated, perfused hearts, using the freeze-clamping technique, where a rapid decline of ATP after the onset of anoxia was found [13–16].

The time resolution achieved in the present ^{31}P-NMR experiments allows measurements of the rates of decline and recovery of high energy phosphates and of changes in P_i levels and pH during and after periods of anoxia in vivo.

The result of these ^{31}P-NMR studies, combined with our ^{13}C-NMR studies of myocardial glycogen metabolism in vivo [11], demonstrate that it is now feasible to monitor rapid fluxes in myocardial high energy phosphate and glycogen pools non-destructively, in intact animals. Such studies should provide a more comprehensive picture of the utilization of myocardial high energy compounds during anoxia, or other types of stress, and an opportunity to correlate observed biochemical changes with changes in myocardial performance.

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