A MODEL KIDNEY TRANSPLANT STUDIED BY PHOSPHORUS NUCLEAR MAGNETIC RESONANCE

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SUMMARY

Two types of measurements have been made on small rat kidneys using phosphorus nuclear magnetic resonance at 129 MHz. In the spectra of non-perfused kidney in ice-cold buffer, well resolved resonances are seen from the three phosphate groups of ATP, phosphocreatine, inorganic phosphate, AMP and a 'new' metabolite corresponding to a phosphodiester. The gradual depletion of the energy pool is followed during cold ischaemia. When such a kidney is perfused by blood derived from a live 'assist' rat (simulating a transplant operation) the recovery of ATP (and initially of ADP) is followed.

One major problem in human kidney transplant surgery is tissue preservation. The two major factors determining graft viability after transplantation are the condition of the donor immediately prior to cadaveric nephrectomy and the time which the kidney spends at body temperature without circulation before its removal (1,2). The kidney is then cooled and placed on ice and may be transplanted within the next 24 hours. The assessment of the viability of the tissue and ways of prolonging preservation are important goals in renal research.

Here we describe an experimental animal model in which we combine a new operative procedure with ^{31}P nuclear magnetic resonance (^{31}P NMR), a method that provides a continuous monitor for tissue energy metabolism. The recognition that ^{31}P NMR spectra can be recorded from intact skeletal muscle (3,4) led to rapid developments in the observation of phosphorus containing metabolites by the NMR method in muscle (5,6,7), heart tissue (8,9,10), adrenal glands (11) and other cellular (12) and subcellular (13) systems.

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The physiological function of the ischaemic kidney is conventionally measured by inducing injury in one kidney by renal artery clamping, followed by contralateral nephrectomy, and observing the survival of the experimental animal. Although such experiments represent the ultimate tests of organ viability after ischaemia, our method described here aims at assessing the resistance to ischaemia and the ability of the tissue to recover by measuring phosphorus-containing compounds in the renal tissue during and after ischaemic injury.

MATERIALS AND METHODS

Male Wistar rats weighing about 90g were anaesthetized by the intraperitoneal injection of Nembutal (0.1ml of sodium phenobarbital 60mg/ml,B Vet.C). After the abdomen was opened, one kidney was cooled by pouring ice-cold buffer on it and was rapidly excised and dropped into ice-cold buffer. (Krebs-Henseleit buffer containing 11mM glucose was used in all experiments). The kidney was then immediately placed in an 8mm NMR tube filled with cold buffer.

The operative procedures for the blood perfused kidney experiments are as follows. A 60g rat is similarly anaesthetized and its abdomen opened. The infra renal aorta is isolated and cannulated with a nylon cannula (external diameter 0.75mm). The inferior mesenteric artery and right renal pedicle are divided between ligatures. The left kidney is mobilized and the supra renal aorta is isolated and tied. Now the kidney is cooled by flooding the abdomen with ice cold buffer and rinsed by injection of buffer through the cannulated aorta. The kidney is excised with the aortic cannula in situ. It is kept in ice-cold buffer until the 'assist' rat is prepared and the kidney placed in the NMR tube can be linked to its blood circuit.

An arterial cannula from an anaesthetized adult (400g) 'assist' rat is linked via a peristaltic pump and a 37°C water jacket to the arterial Portex cannula, supplying the left kidney removed from a small rat. The kidney is then placed in an 8mm NMR tube, into which the venous effluent drains and blood is sucked out by a venous cannula and pump. The venous line runs from the NMR tube (again kept at 37°) via a second pump and bubble trap back to the vena cava of the 'assist' rat. Most technical problems in this preparation arise from keeping the 'assist' animal alive for a sufficiently long period, and the limitations on the size of the kidney dictated by the dimensions of the NMR tube.

NMR spectra of kidneys were recorded at 129MHz on a spectrometer constructed in Oxford (14). The spectrometer was operated in the Fourier Transform mode and was interfaced with a Nicolet B-NC 12 computer. Spectra were collected without the use of a field-frequency lock. (Field drift was 10 Hz/hour maximum). Phosphorus NMR spectra of extracts were also recorded on the same instrument. The spectra shown are all proton-coupled.

RESULTS AND DISCUSSION

The non-perfused hypothermic kidney. In the first series of experiments we studied the ^{31}P NMR spectra of small, non-perfused rat kidneys placed in

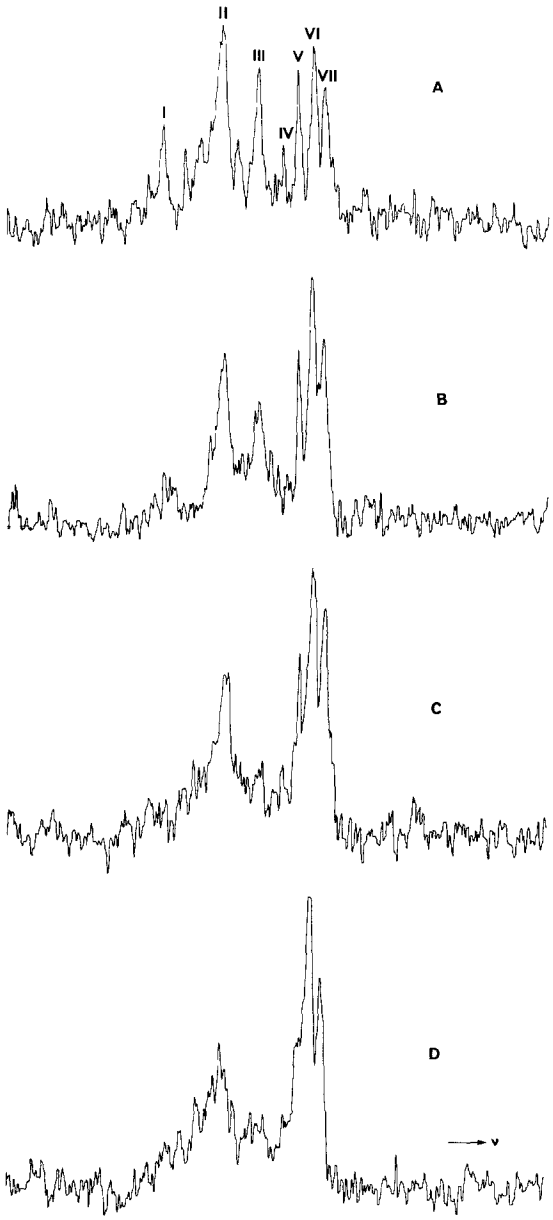

Fig. 1. 129 MHz phosphorus NMR spectra of a non-perfused rat kidney at 4°C. The kidney was excised, cooled for ten seconds in ice-cold Krebs buffer and then transferred to the thermostatted NMR tube. Sweep width 10 KHz. 200 70° radio-frequency pulses were applied at 2 s intervals. Spectral accumulations were started 1 min (A), 15 min (B), 41 min (C), and 57 min (D) after the onset of ischaemia.
Peak assignments: I = β-ATP; II = α-ATP + α-ADP + NAD;
III = γ-ATP + β-ADP; IV = phosphocreatine; V = unknown;
VI = inorganic phosphate; VII = AMP.
(Arrows in spectra indicate the direction of increasing frequency).

ice-cold (~4°C) buffer medium immediately after excision. Fig. 1 shows a typical series of spectra as a function of time. In common with other tissues, the spectra show well resolved resonances from the three phosphate groups of ATP, phosphocreatine, and inorganic phosphate. Notable differences from heart and skeletal muscle are the low level of creatine phosphate and the two intense signals closest to the inorganic phosphate resonance. Since the high frequency signal may arise either from nucleotide monophosphate (AMP and IMP) or sugar phosphate, its identification required freezing the tissue followed by perchloric acid extraction. Examination of the ^{31}P NMR spectrum of such an extract (c.f. ref. 9) showed that the highest frequency peak in the tissue spectrum was from AMP. The signal on the low frequency side of the inorganic phosphate resonance corresponds, in position, to the new metabolite observed in some skeletal muscle preparation, (5,15). Several phosphodiester compounds have been detected (15) and one of these has been identified as glycerophosphorylcholine. The concentration of this component in renal tissue is unusually high and its biological role remains to be elucidated.

Fig. 1 demonstrates that even at low temperatures the energy pool (ATP) of renal tissue is relatively rapidly depleted, probably partly as a result of the low phosphocreatine pool. In both heart and skeletal muscle lack of oxygen supply invariably leads to the acidification of the tissue cytoplasm that can be followed by measuring the shift in the position of the inorganic phosphate resonance (3,8). This tissue "acidosis" is a result of increased glycogen break-down under anaerobic conditions producing lactic acid as the final product of glycolysis. The apparent lack of any significant acidification of renal tissue during the 180min cold ischaemia must result from the low rates of glycolysis in this tissue at 4°C (the tissue pH remains at ~7 as measured from the position of the inorganic phosphate resonance). We have preliminary indications that the time of tissue acidosis may relate to the time of irreversible tissue damage.

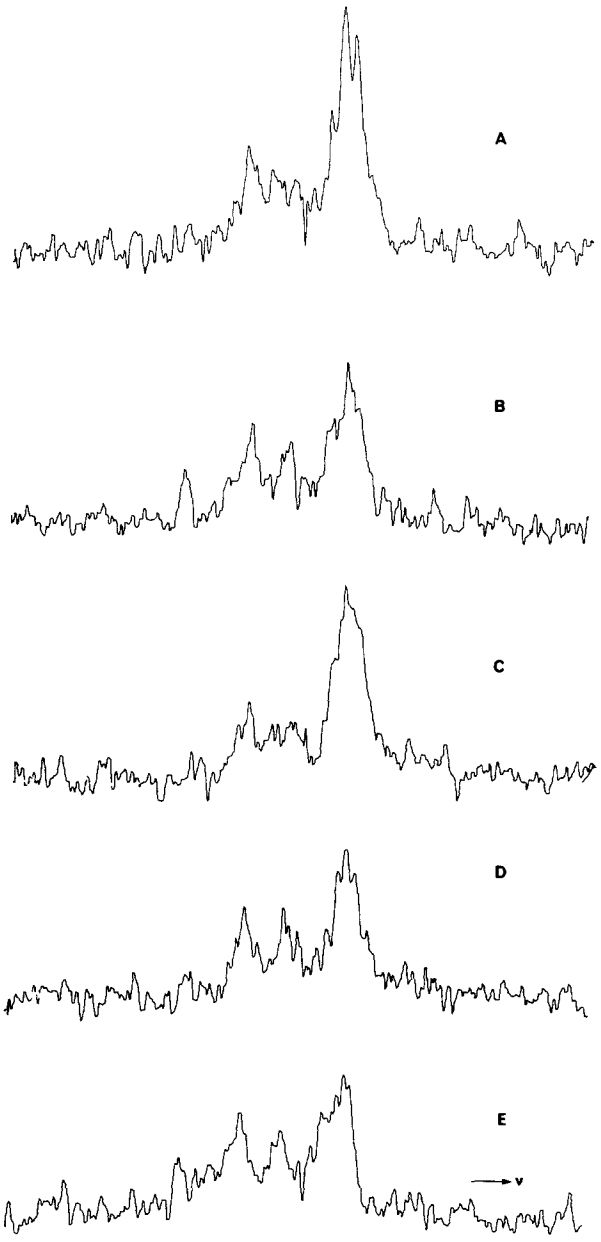

Fig. 2. 129 MHz phosphorus NMR spectra of a blood perfused kidney. Sweep width 10 KHz. 200 70° radio-frequency pulses were applied at 2 s intervals. The kidney was excised and rinsed with ice-cold buffer. There was then a 15 min period of cold ischaemia and spectrum A was started together with perfusion. Data accumulation for spectrum B was initiated after 51 min of perfusion. After a total of 133 min the pump was turned off. Spectrum C was started after 12 min of warm ischaemia. Reperfusion began as data collection for C came to an end. Spectra D and E were begun after 12 and 24 min of reperfusion respectively.

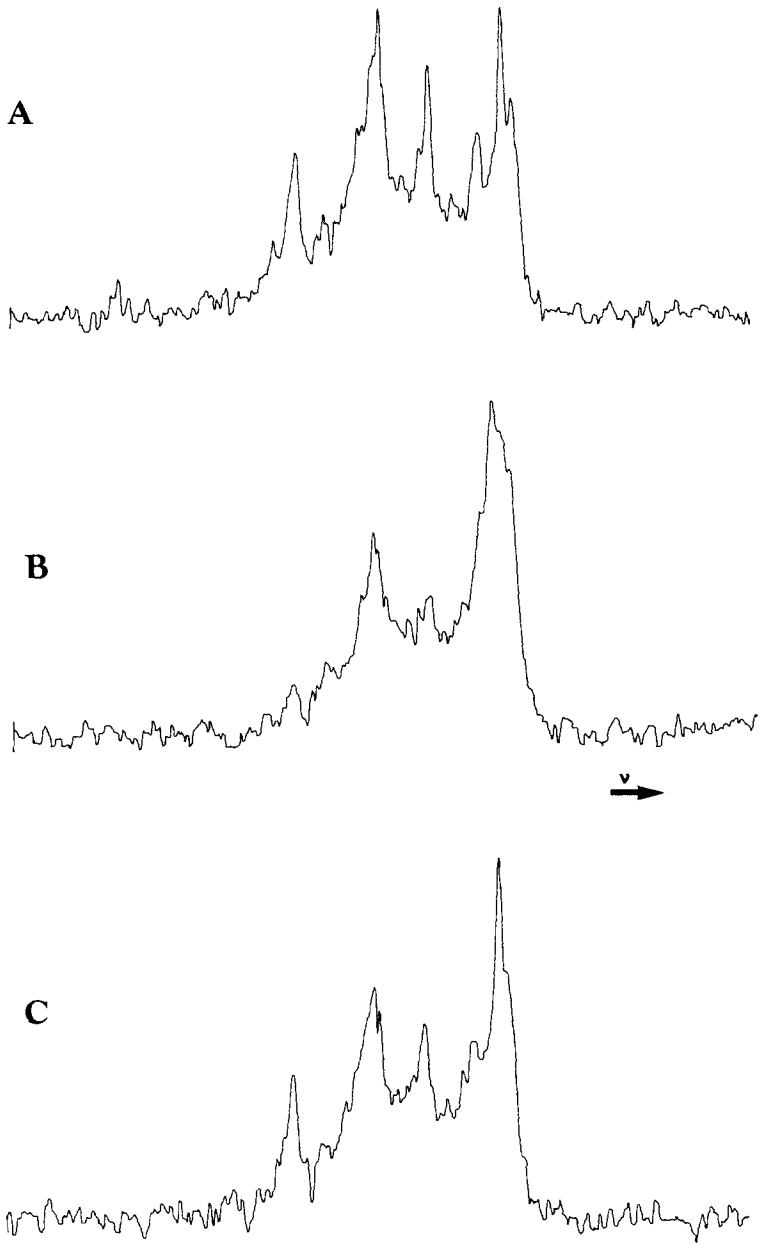

Fig. 3. 129 MHz phosphorus NMR spectra of blood perfused kidney using longer accumulations. The kidney was excised, rinsed with ice cold buffer and then kept in the same buffer for 58 min. Data for spectrum A (800 scans) were collected between 16 and 48 min after the start of perfusion. After 61 min of perfusion, the blood circuit was turned off. Data for the warm ischaemic kidney were collected for 23 min (spectrum B, 600 scans) and perfusion resumed immediately thereafter. Spectrum C shows data accumulated between 8 and 40 min after the onset of reperfusion (800 scans).

Kidney recovery. To establish a relation between the condition of an ischaemic non-perfused kidney and its ability to recover its metabolic functions, we have developed a model system in which the energy state of the tissue is monitored by ^{31}P NMR during normothermic whole blood perfusion.

Fig. 2 shows the ^{31}P NMR spectra of the small kidney at various times after commencing perfusion. It can be seen that ATP was resynthesised after about 1 hour of perfusion and remained relatively steady for several hours after this time. After 133 min, perfusion was stopped for a period of 12 min, the kidney being kept at 37°C in the spectrometer. The ischaemic spectrum was recorded (Fig. 2C). Reperfusion again resulted in the recovery of the original ATP levels (Fig. 2E) but interestingly through the initial synthesis of ADP (Fig.2D). (Control experiments with blood alone have shown that the ATP or ADP signal do not arise from the blood in the sample tube and that only a relatively small contribution to the rather complex signal in the inorganic phosphate region results from blood in the tissue).

The signal to noise ratio in the spectra shown in Fig. 2. is not particularly good as we were attempting to follow the time course of recovery. However once recovery was achieved we were able to keep the system in a steady state. We can then average the spectra over a longer period. Fig.3 shows the results of such an experiment on another preparation and demonstrates that reperfusion after the second ischaemic period results in a nearly identical spectrum to that observed prior to ischaemia.

In conclusion, we have demonstrated that ^{31}P NMR can be used as a continuous and non-destructive method for following the metabolic state of isolated and physiologically perfused rat kidneys. The experiments were designed to follow the metabolic state of a rat kidney under conditions that are closely analogous to those commonly used in renal transplantation surgery. The ability of the tissue to regenerate its ATP after an ischaemic period has been shown to be a valid index of tissue viability in animal survival experiments (16). NMR provides a way of directly following the course of ATP recov-

ery in the isolated perfused organ, while simultaneously detecting other metabolites and the intracellular pH. The conditions that affect tissue preservation and its metabolic recovery can thus be studied.

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