Renal Corticomedullary Metabolite Gradients During Graded Arterial Occlusion: A Localized \(^{31}\)P Magnetic Resonance Spectroscopy Study\(^1\)

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ABSTRACT

In order to investigate the role of the outer medulla in acute ischemic renal failure (Epstein FH, Balaban RS, Ross BD: Redox state of cytochrome aa3 in isolated perfused rat kidney. Am J Physiol 1982;243: F356–F363), the distribution of ATP in the in vivo porcine kidney and its relationship to Na transport and to ischemia was examined by using localized \(^{31}\)P magnetic resonance spectroscopy. Renal cortex (ATP) was higher than medulla. Reduction in Na transport produced by partial renal arterial occlusion ("hypofiltration"), resulted in a 13% increase in the ATP/P ratio of the whole kidney (from 2.61 ± 0.26 to 2.96 ± 0.27; \(P < 0.03\)). This increase was accounted for by a statistically significant increase in (ATP) in the cortex, with medulla contributing to an insignificant extent. Further occlusion of the renal artery to reduce GFR to zero ("hypoperfusion") resulted in a 70% fall in ATP/P ratio. (ATP) was reduced most in the cortex, but pH fell equally in cortex and medulla. After release of arterial occlusion, cortical ATP recovered less completely than medulla ATP. Intracellular pH and P were restored in both cortex and medulla. It was concluded that cortex and medulla contribute equally to the pattern of disordered energy metabolism in acute renal failure. Sparing of ATP during hypofiltration may reflect the reduced energy requirements of active Na transport.

Key Words: Acute renal failure, phase encode, NMR, hypofiltration, ischemia, pH

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Acute renal failure (ARF) can follow hypoxia and partial or total ischemia of the kidney. The vulnerability of renal cells to hypoxia in part reflects their high resting oxygen consumption. The high oxygen consumption of the functioning kidney has been attributed to the energy requirements of tubular sodium reabsorption (1,2). Since the discovery of the role of sodium potassium ATP'ase (3), it has been recognized that ATP is consumed in active Na transport. Thus, the steady-state concentration of ATP in tubular cells represents the balance between ATP synthesis, by oxidative phosphorylation and glycolysis, and ATP hydrolysis by Na-K-ATP'ase and a number of other, quantitatively minor ATP'ases of the kidney. It can therefore be assumed that ATP is "spared" when active Na reabsorption is reduced, either by the specific inhibition of transport (diuretics) or by reduction in the filtered load. This in turn might result either in an increased use of ATP for other metabolic processes in the kidney (4,5) or in an increase in the steady-state [ATP] concentration in the relevant tubular cells. The intracellular concentration of ATP may therefore not simply be the reflection of ischemia. In an earlier study (6), this combination of factors, taken together with evidence of relative hypoxia in the outer medulla of the intact rat kidney, was developed as a theory for the etiology of ARF. In this model, the outer medulla, in particular, cells of the thick ascending limb, was proposed to be the most sensitive target in some forms of acute renal failure. Several subsequent studies from the laboratory of Epstein et al. have developed and substantially extended this view (7).

Experiments designed to examine the relationship between sodium transport and energy supply have been performed in kidney cortex slices (8) or in isolated renal tubules. The rate of gluconeogenesis, an ATP-requiring biosynthetic process of kidney cortex (9), increased in the response to diuretics (8), and to inhibition of Na-K-ATP'ase with ouabain. Confirming this interdependence, a reduction in renal \(O_2\) consumption has been noted in the intact kidney during progressive reduction in filtered load of sodium (10). The effect on [ATP] was not investigated.

On the other hand, it has long been recognized that an intrarenal ATP gradient exists between cortex and medulla (11). This could obscure any change in [ATP] which may occur in response to altered tubular sodium transport in selected nephron segments.
The advent of $^{31}$P nuclear magnetic resonance (NMR) resulted in a number of studies in which intrarenal [ATP] was monitored in the intact kidney during progressive hypoperfusion or complete ischemia (12–17). [ATP] has been reported to be reduced in "severe" hypoperfusion and absent in ischemia. [ATP] changes of intact kidney due to alteration of tubular Na$^+$ reabsorption has not been investigated. Because an increase in [ATP] due to reduction of Na transport is predicted from the discussions above, we designed studies to reexamine the question by using localized $^{31}$P MRS spectroscopy (MRS) of intact kidney in which only mild to moderate hypoperfusion was produced. Because cortex and medulla have very different requirements for oxygen and ATP and different [ATP], as well as different mechanisms, active or passive, or both (18) for sodium reabsorption, localized $^{31}$P MRS was used to separately assay these kidney regions. Thus, this study differs extensively from previous $^{31}$P MRS methods, in continuously monitoring both regions of the kidney. Further, MRS achieves what more destructive techniques cannot: the assay of metabolism in the intact organ. The disadvantages of MRS, its insensitivity and semi-quantitative results, are recognized.

To address the question of regional [ATP] in hypoperfusion, moderate ischemia, and ARF, we have monitored the metabolic response to progressive partial arterial occlusion. We confirmed the existence of renal corticomedullary gradients for ATP and, for the first time, present evidence of the expected ATP sparing. The effects of ischemia on [ATP] were found to be heterogeneously distributed within both cortex and medulla. Preliminary results have been briefly reported previously (19,20).

**METHODS**

**Animals**

Pigs were used for this study, because pig kidneys were large enough (2.5 to 3 cm thickness) to permit anatomical "separation" between cortex and medulla in a localizing one-dimensional (1-D) phase-encode experiment of approximately 30-min duration. With the nominal slice thickness of 0.5 cm, spectra could be assigned to a single anatomical region. Eleven mature male pigs (Great White strain; mean wt, 15 kg) were used and were allowed free access to water before surgery. Two animals served as physiological controls. In these, blood pressure (BP), blood gases, and renal function were monitored over a 6-h period under anesthesia but no renal artery occlusion was applied. In one of the controls, and nine experimental studies, $^{31}$P MRS was performed.

**Surgery**

Anesthesia was induced with ketamine hydrochloride (Ketaset; Bristol Laboratories, Syracuse, NY) (22 mg/kg) and xylazine (Rompum; Haver) (2 mg/kg) injected i.m. The pigs were intubated with a size 6 Portex endotracheal tube and ventilated on a Harvard ventilator (model 613: Harvard Apparatus, Natick, MA) at a respiratory rate of 16 to 20/min and a tidal volume of 200 mL. Anesthesia was maintained with halothane (0.5 to 1%) and oxygen (2 L/min). A left groin incision was made. An arterial cannula was placed in the femoral artery to monitor BP and from which to take samples for analysis of blood gases at 30-min intervals. A second cannula was placed in the femoral vein for infusion of fluids and venous sampling. With the catheters in place, the groin incision was closed. The left kidney was exposed through a subcostal incision, and the left ureter was cannulated with an 8 Fr Argyle catheter. An inflatable cuff was placed around the renal artery for future occlusion. The cuff consisted of an inflatable balloon placed inside a hook of 25 by 15 mm in diameter made of acrylic. With the hook around the artery, it was possible to remotely inflate the balloon with up to 2 mL of water via an 80-cm extension tubing, producing graded occlusion of the vessel. The kidney was wrapped in a plastic bag, to avoid desiccation and heat loss, and positioned inside the radiofrequency (RF) coil so that it lay in its natural anatomical position. The kidney was then placed in a 10-cm-diameter tray with a notch on the medial aspect to accommodate the renal vessels and the ureter. A 9.5-cm-diameter acrylic cylinder (Plexiglass) of 6 cm in height was lowered over the kidney so that the kidney lay within the cylinder, with the tray occluding one end and the renal pedicle protruding out through the notch. The cylinder and the dish were covered with copper foil, acting as shield, to exclude MR signals from the adjacent tissues. The muscles of the abdominal wall, the s.c. fat, the skin, and other extraperitoneal tissues were excluded from the sensitive volume of the coil by this device. The pig was placed in a cradle designed to fit the bore of the magnet. The RF coil and the acrylic cylinder were firmly fixed to the cradle to eliminate respiratory movements of the kidney. Finally, the cradle with the animal in it was placed in the magnet. Magnet bore temperature was maintained at 32°C by blowing warm air. BP was monitored by a Honeywell Simulator Recorder (model AR-6; Pleasantville, NY). To maintain fluid balance and continuous urine flow, physiologic saline was infused i.v. at a mean rate of 1.3 mL/min. Arterial blood gases were analyzed every 30 min on an ABL-2 blood gas analyzer (Radiometer, Copenhagen, Denmark). $P_{CO_2}$ was maintained around 30 mm Hg, and blood pH was maintained around 7.4 by artificial ventilation. The temperature of the kidney remained normal, because the whole organ and its retaining RF coil were enclosed within the abdomen throughout the 8 to 9 h of study.
RF Coil

An oval-shaped, two-turn Helmholtz type coil, 6.5 by 4 cm in diameter, was made of 2-mm-thick copper wire. The two turns were 3 cm apart to accommodate the kidney. The kidney extended outside the coil to avoid contamination of medullary "slices" by the cortex in the localizing experiment (coil size smaller than kidney size). The effective sensitive volume of the coil was 61 cm$^3$ ($6^{1/2} \times \frac{1}{2} \times \pi \times 3$). The coil was doubly tuned to 81 MHz for $^{31}$P and 200 MHz for proton by using the tuning circuit described by Schnall et al. (21) and was used both to transmit and receive.

Spectroscopic Methods

All experiments were performed on a CSl-II spectrometer from General Electric (GE) NMR Instruments (Fremont, CA), equipped with a 4.7-tesla, 33-cm bore magnet manufactured by Oxford Instruments (Oxford, United Kingdom). After shimming to a proton line width of 50 Hz or better, the coil was switched to $^{31}$P and spectra were recorded at 5-min intervals by using the following spectral parameters: pulse width of 100 $\mu$s (which corresponded to $\sim 36$ degrees), spectral width of 2,500 Hz, 2,048 complex datum points, 2-s interpulse delays, and 128 acquisitions. A digital exponential filter corresponding to a line broadening of 15 Hz was applied before Fourier transformation, followed by a frequency domain baseline deconvolution and baseline correction (IC routine, Nicolet).

Localized Spectroscopy Technique

The localized spectroscopy technique (also called "phase-encoded spectroscopy"; 1-D chemical shift imaging [CSI]) was used as described previously (22,23). The 1-D method provided the spectra to be acquired simultaneously from six to seven slices parallel to the coil and approximately 0.5 cm in thickness. Spectra were acquired by using the same pulse length and repetition rate as for the nonlocalized spectra. The amplitude of the phase-encode gradient was modulated according to a sine bell function. The duration of the gradient was 1.5 ms and was applied along the vertical axis (y), perpendicular to the plane of the coil. Sixteen gradient values were used (eight positive and eight negative), with 64 averages each; the field of view was set at 80 mm, giving a nominal slice thickness of 5 mm. The data set was processed by using 2-D Fourier transformation, and was corrected for phase variation as described previously (24). Because of the 1.5-ms phase-encode time, a first-order phase correction of approximately 3,000 degrees was required in the frequency dimension (in addition to the zero and first-order phase corrections applied in a pulse and collect experiment). The baseline artifacts induced by this large first-order phase correction were removed by a frequency domain baseline correction routine (the "IC" routine of CSI-Nicolet spectrometer). No phase correction (other than inverting alternate traces) or digital filtering was used in the distance dimension.

Typically, spectra with readily interpretable signal-to-noise ratios were observed in 6 or 7 of the 16 total traces corresponding to an approximate renal thickness of 2.5 to 3 cm. Spectra from the medulla of the kidney were readily identified from the presence of a peak at $\sim 3.0$ ppm due to glycerophosphoryl choline (GPC) (22). pH was determined from the chemical shift difference between P$_1$ and [in the absence of phosphocreatine [PCr]] the $\gamma$-phosphate ATP as described before (22). Because the B1 field necessarily crosses both regions of the kidney, some contamination of slices is expected by this method but it is likely to be small.

Quantitation

A sealed glass capillary containing 35 $\mu$mol of methylene diphosphonate acid (MDP) was placed in a fixed position within the coil in the immediate vicinity of the kidney to serve as standard. To calibrate the standard, four solutions of 100, 200, 300, and 500 $\mu$mol of P, were prepared in bottles that occupied the sensitive volume of the coil. By using a predelay of 15 s, the 90-degree pulse was determined (200 $\mu$s), and, by using this pulse length, the P$_1$ solutions were each calibrated against the MDP standard. The areas under MDP and P$_1$ peaks were integrated, and, by comparing the ratio of the integrals, the P$_1$ content of the bottles was calculated. There was good correlation between the actual and the calculated P$_1$ content of the bottles ($r = 0.97$). These calibration data were used to quantitate [ATP] of the in vivo pig kidney in one experiment by comparison of the areas under MDP and $\beta$-ATP peaks. The concentration of other metabolites were similarly obtained by reference to MDP in this experiment. In each phase-encoding experiment (four to five per study), one slice was chosen as being representative of the medulla (and papilla) and two slices as representatives of the two cortices. After calibration of the appropriate volume of each slice ($6^{1/2} \times \frac{1}{2} \times \pi \times 0.5 = 10.2$ cm$^3$) [ATP] was quantified per gram wet weight of that slice assuming 1 g $= 1$ cm$^3$. Chemical shift artefacts inherent in the 1-D CSI procedure may introduce errors in pH determination in localized spectra (25). No correction has been applied; errors established in phantom studies did not exceed 0.2 ppm = 0.2 pH units for P$_1$ (data not presented in detail).

Calculation of Results

All of the experimental spectra were directly transferred to a Macintosh PC, and, by using the program...
Image, version 1.03 (National Institutes of Health, Bethesda, MD), the area under each peak from the whole volume and localized spectra was measured in pixels. All results were expressed as mean ± SE of absolute areas under the peaks or as relative ratios of the peaks. In only one experiment was absolute quantitation as described above carried out, and results were calculated without correction for tissue water.

**Experimental Protocol**

Experiments were carried out in four stages: Normoperfusion period (Stage 1), during which baseline measurements of renal metabolites and physiological function were obtained. Hypofiltration stage (Stage 2): partial occlusion of the artery to produce an approximately 50% reduction in urine flow, thereby markedly reducing total Na transport without evident ischemia (as judged by the spectra). Hypoperfusion stage (Stage 3): further inflation of the cuff to completely arrest urine flow and sufficient to just produce $^{31}$P MRS evidence of renal ischemia (decreased but detectable ATP, increased P). This phase is more closely defined in Results. "Recovery" (Stage 4): the arterial cuff was completely deflated and urine flow resumed (ATP/P, increased towards normal). Each stage lasted approximately 1 h, allowing acquisition of 5 to 10 1-pulse and 1 or 2 sets of 1-D localized spectra. Venous blood and urine samples were taken at 10- to 30-min intervals for measurement of electrolytes and glomerular filtration rate (GFR) from endogenous creatinine clearance. Total sodium reabsorption (TNa) was calculated per minute by using the relationship: filtered Na – excreted Na.

**RESULTS**

**Physiological Function**

Although experiments took 5.5 to 9.5 h to complete (mean duration, 7.0 ± 0.3 h, including anesthesia, surgery, and preparation time), pigs maintained constant arterial blood gases on artificial ventilation during this period (mean PCO$_2$, 31 ± 7 mm Hg and pH 7.42 ± 0.01). Mean initial BP of pigs was 102 ± 4.5 mm Hg ($N = 11$). There was a fall of 15% ± 3.1 in mean BP during the course of the experiments to 87 ± 5.2 mm Hg; $P < 0.001$). Figure 1 shows the time course of changes in GFR, urine output, TNa, percent Na reabsorption, and BP in control animals observed over 330 min. Expt. 1 (C): surgical preparation as described in Methods but without implantation of the coil or arterial cuff. Expt. 2 (*): the coil and cuff were implanted but no arterial occlusion was carried out.

Figure 2 shows the time course of changes taking place in GFR and ATP/P, ratio II a representative experiment, and Table 1 summarizes physiological function of the kidney in controls and experimental animals during control period, the two stages of occlusion of the renal artery, and recovery. Significantly reduced GFR and TNa, as well as a small and insignificant reduction in fractional Na reabsorption, were observed in animals in which the arterial cuff and RF coil were implanted ($N = 9$), but renal function was within physiological limits. When hypofiltration was established by partial occlusion of the renal artery, there was a disproportionate fall in GFR (59%) compared with urine flow (50%), together with a 2.4% reduction in percent Na reabsorption. Fractional K reabsorption was reduced by 25%.

Ischemia, indicated by a significant fall in renal [ATP], was achieved by further occlusion of the renal artery. During hypoperfusion, urine flow was zero in all but one of the animals so that GFR, Na transport,
and K reabsorption for this group were not significantly different from zero.

After deflation of the cuff, there was a lag before recovery of GFR occurred (Figure 3A). Urine flow recommenced after 2 to 17 min (mean, 8.0 ± 2) and mean GFR had reached its maximum (0.17 ± 0.02 mL/min/g) at 23 min. During the whole period of recovery, GFR was only 50% of control, indicating a degree of renal failure for the period of observation (maximum, 130 min). Fractional Na reabsorption and TNa were not significantly less than starting values (Table 1).

Light microscopy of a kidney in which GFR had recovered to 70% of normal did not show any evidence of structural damage to account for these abnormalities. In particular, thick ascending limb and glomerular morphology were normal.

TABLE 1. Physiological function of the pig kidney in vivo

<table>
<thead>
<tr>
<th></th>
<th>GFR (mL/min/g)</th>
<th>Urine (mL/min)</th>
<th>TNa (µmol/min)</th>
<th>% Na Reabsorption</th>
<th>% K Reabsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.37 ± 0.005 (2)</td>
<td>0.33 ± 0.11 (2)</td>
<td>27.9 ± 1.4 (2)</td>
<td>98.2 ± 0.4 (2)</td>
<td>53.9 ± 4.8 (2)</td>
</tr>
<tr>
<td>P &lt; 0.05</td>
<td>0.006*</td>
<td>0.18*</td>
<td>0.006*</td>
<td>0.14*</td>
<td>0.40*</td>
</tr>
<tr>
<td>Normoperfusion</td>
<td>0.24 ± 0.02 (9)</td>
<td>0.62 ± 0.20 (9)</td>
<td>18.9 ± 1.9 (9)</td>
<td>95.1 ± 1.8 (9)</td>
<td>59.1 ± 5.8 (9)</td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>0.001</td>
<td>0.08</td>
<td>0.001</td>
<td>NS</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypofiltration</td>
<td>0.10 ± 0.01 (9)</td>
<td>0.31 ± 0.08 (9)</td>
<td>10.0 ± 1.8 (9)</td>
<td>93.8 ± 1.9 (9)</td>
<td>44.7 ± 7.0 (9)</td>
</tr>
<tr>
<td>P &lt; 0.009</td>
<td>0.009</td>
<td>0.01</td>
<td>0.001</td>
<td>NS</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>0.008 ± 0.008 (9)</td>
<td>0.02 ± 0.02 (9)</td>
<td>0.49 ± 0.49 (9)</td>
<td>10.5 ± 10.5 (9)</td>
<td>5.1 ± 5.1 (9)</td>
</tr>
<tr>
<td>P &lt; 0.002</td>
<td>0.002</td>
<td>0.02</td>
<td>0.05</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.12 ± 0.01 (9)</td>
<td>0.35 ± 0.09 (9)</td>
<td>16.8 ± 7.2 (9)</td>
<td>94.6 ± 2.1 (9)</td>
<td>52.1 ± 6.0 (9)</td>
</tr>
<tr>
<td>P &lt; (versus normo-)</td>
<td>0.008</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Values are mean ± SE. Statistics refer to Student's paired t test except for * where a one-group t test was carried out comparing normoperfusion values with control means. P < 0.05 = significant. Values in parentheses are number of animals. NS, not significant.
Renal Energy Metabolism

Figure 4A shows the $^{31}$P spectrum of in vivo pig kidney, obtained during normoperfusion, with renal arterial cuff in position. Resonances from left to right are: MDP reference, phosphomonooester (PME), P, glycerophosphoryl ethanolamine (GPE), GPC (PCr was below the limits of detection in this example), and $\gamma$-, $\alpha$-, and $\beta$-ATP. ATP/P ratio in this spectrum was 2.46 and intracellular pH was 7.37. [ATP] was 2.5 $\mu$mol/g wet wt (weight of kidney, 54 g). Total [PME], [PDE], and [P] concentrations in this spectrum were 3.1, 1.5, and 1.0 $\mu$mol/g, respectively. The area of $\gamma$-ATP was 14% greater than that of $\beta$-ATP, possibly because of the small proportion of NMR-visible $\beta$-ADP. In contrast, peak area of $\alpha$-ATP exceeded $\beta$-ATP by 80%, equivalent to approximately 2 $\mu$mol of $^{31}$P/g wet wt of kidney. By reference to a high-resolution spectrum of a perchloric acid extract of porcine kidney (Figure 4B), the additional metabolites comprising the $\alpha$-ATP resonance were shown to include uridine diphosphoglucone (UDPG), pyridine dinucleotides (NAD), as well as a proportion of $\alpha$-ADP. The complex resonance of PME (resolved in some examples but not in the spectrum in Figure 4A), along with other components, comprised phosphoryl choline and phosphoryl ethanolamine in proportions ~1:1.7 as shown in the spectrum of the renal extract (Figure 4B). In the spectra of intact kidney, phosphodiester (PDE) was present as two peaks of chemical shift: GPE was 2.69 to 3.40 and GPC was 1.72 to 2.76 ppm, comprised of more GPE than GPC. However, the GPC-to-GPE ratio was reversed in the high-resolution spectra of aqueous extracts of the kidney (Figure 4B); this is discussed below.

The absolute peak areas and relative ratios for $^{31}$P metabolites of nine kidneys are summarized in Table 2.

### Table 2. Effect of progressive renal artery occlusion on whole-kidney intrarenal phosphate metabolites

<table>
<thead>
<tr>
<th>Stage</th>
<th>PME (nmol/g)</th>
<th>P (nmol/g)</th>
<th>PDE (nmol/g)</th>
<th>PCr (nmol/g)</th>
<th>ATP (nmol/g)</th>
<th>ATP/P (nmol/g)</th>
<th>ATP/PME (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoperfusion</td>
<td>1459 ± 115 (9)</td>
<td>581 ± 81 (9)</td>
<td>616 ± 64 (9)</td>
<td>224 ± 69 (6)</td>
<td>1387 ± 115 (9)</td>
<td>2.61 ± 0.26 (9)</td>
<td>0.89 ± 0.08 (9)</td>
</tr>
<tr>
<td>Hypofiltration</td>
<td>1488 ± 104 (9)</td>
<td>555 ± 70 (9)</td>
<td>607 ± 65 (9)</td>
<td>158 ± 12 (6)</td>
<td>1465 ± 153 (9)</td>
<td>2.96 ± 0.27 (9)</td>
<td>0.97 ± 0.10 (9)</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>1640 ± 94 (9)</td>
<td>962 ± 82 (9)</td>
<td>283 ± 45 (9)</td>
<td>98 ± 22 (6)</td>
<td>550 ± 64 (9)</td>
<td>0.81 ± 0.09 (9)</td>
<td>0.48 ± 0.06 (9)</td>
</tr>
<tr>
<td>Recovery</td>
<td>1305 ± 73 (9)</td>
<td>699 ± 91 (9)</td>
<td>416 ± 52 (9)</td>
<td>178 ± 17 (6)</td>
<td>985 ± 71 (9)</td>
<td>1.70 ± 0.25 (9)</td>
<td>0.75 ± 0.05 (9)</td>
</tr>
<tr>
<td>P &lt; (versus control)</td>
<td>NS</td>
<td>NS</td>
<td>0.006</td>
<td>0.07</td>
<td>0.001</td>
<td>0.004</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^{a}$ Metabolite ratios and areas under individual peaks (in arbitrary units) were obtained from two to nine whole-volume spectra of the pig kidney during each stage of the experiment (~25 spectra per experiment). For definition of the stages, see Methods. Values are mean ± SE. Statistics refer to paired t-test. P < 0.05, significant; NS, not significant. Values in parentheses are the number of animals. In three kidneys, (PCr) was too low to be reliably quantitated.
TABLE 3. Concentration of phosphorus metabolites of the whole kidney, cortex, and medulla in a representative experiment

<table>
<thead>
<tr>
<th>Whole Volume</th>
<th>Cortex</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PME)</td>
<td>(P)</td>
<td>(PDE)</td>
</tr>
<tr>
<td>Control</td>
<td>3.3 ± 0.2 (9)</td>
<td>1.1 ± 0.1 (9)</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>3.4 ± 0.3 (7)</td>
<td>1.3 ± 0.1 (7)</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>4.4 ± 0.7 (2)</td>
<td>2.8 ± 0.9 (2)</td>
</tr>
<tr>
<td>Recovery</td>
<td>4.3 ± 0.5 (5)</td>
<td>2.8 ± 0.7 (5)</td>
</tr>
</tbody>
</table>

* The in situ porcine kidney was continuously monitored with 31P MRS and 1-D CSI localization as described in the text. A single 1-D CSI experiment was performed at each stage of the study with the exception of hypofiltration when two complete 1-D CSI assays were performed. Absolute quantitation of metabolites are derived from peak areas with reference to a standard of MDP and expressed as micromoles per gram wet weight of the kidney. Values in parentheses are the number of spectra acquired at each stage and used for determination.

2 (line 1). The absolute concentration of ATP, Pm, PME, and PDE during all four stages of the representative study are shown in Table 3. The value for [ATP], in particular, is close to that assayed enzymatically in perchloric acid extracts of quick-frozen kidney (27).

Localized MRS, by providing six to seven spectra in a single examination, yielded significantly more information than single whole volume spectra (Figure 5). Each spectrum represented a slice of approximately 0 .5 cm in thickness and 10 .2 cm1 in volume. The 35-μmol MDP (70-μmol 31P) resonance was almost completely confined, as expected, to one slice of the series. An additional but much-reduced MDP signal was observed in a single neighboring slice. Localized spectra differed from one another in the presence of PDE in only the central slices, corresponding to the papilla and medulla of the kidney. No PDE was observed in the cortical slices as PDE is present in the cortex at a much lower concentration. There was improved spectral resolution of PME into two components, identified as PC and PE (see above). The lineshape of the P1 resonance was generally broadened by the appearance of a shoulder, possibly attributed to 2:3 diphosphoglycerate. Therefore, assigning an accurate chemical shift to Pm in localized spectra was difficult (see Methods). However, localized intracellular pH determinations, obtained when mean blood (extracellular) pH was 7.42 ± 0.01, showed no difference between cortex and medulla (Table 4).

ATP/Pm ratio in the cortex, 1.32 ± 0.09 (N = 11), was significantly higher than that in the medulla, 1.00 ± 0.07 (P < 0.001) (Table 5). [ATP] concentration in the cortex in the representative study was 2.8 μmol/g and in the medulla was 2.3 μmol/g (Table 3), confirming the findings of Burch et al. (11). [PDE], observed only in the medullary slices was 1.2 μmol/g, considerably less than that calculated for the whole kidney. PDE invariably appeared in localized spectra as a single narrow peak, whereas that in the whole volume spectra was more than 40 Hz wide. PDE observed in the whole volume spectra may therefore be attributed in part to an unresolved broad resonance, lost from localized spectra, acquired after a 1.5-ms delay.

The ratios of metabolites from localized spectra were not equal to those of whole-volume spectra. This discrepancy, we believe, was due to one or a combination of the following factor(s): (1) The 1.5-ms delay in acquisition which is inherent in phase-encoding-affected quantitation by eliminating any rapidly decaying, that is, relatively immobile, metabolites from the spectrum. (2) Baseline correction of the spectra in the frequency domain (IC routine of GE-CSI spectrometer) especially in the spectra with the highest
Figure 6. Localized spectra during hypoperfusion. Spectral are oriented as shown in Figure 5. A kidney in which a detectable concentration of ATP (A) and one in which (ATP) was below the limit of NMR detection (B) are shown for comparison. Note unequal elevation of P and PME in cortex (C) and medulla (M) and absence of PDE peak in medullary slices of hypoxic kidney.

TABLE 4. Intracellular pH of kidney obtained from $^{31}$P MRS

<table>
<thead>
<tr>
<th></th>
<th>Intracellular pH</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Whole Volume</td>
<td>Cortex</td>
<td>Medulla</td>
</tr>
<tr>
<td>Normoperfusion</td>
<td>7.46 ± 0.02 (9)</td>
<td>7.41 ± 0.02 (9)</td>
<td>7.40 ± 0.03 (9)</td>
</tr>
<tr>
<td>$P&lt;$</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Hypofiltration</td>
<td>7.49 ± 0.02 (9)</td>
<td>7.35 ± 0.01 (7)</td>
<td>7.34 ± 0.02 (7)</td>
</tr>
<tr>
<td>$P&lt;$</td>
<td>0.02</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypoperfusion (a)</td>
<td>7.15 ± 0.04 (2)</td>
<td>6.94 ± 0.06 (2)</td>
<td>7.09 ± 0.26 (2)</td>
</tr>
<tr>
<td>Hypoperfusion (b)</td>
<td>7.25 ± 0.10 (5)</td>
<td>6.77 ± 0.06 (5)</td>
<td>6.79 ± 0.08 (5)</td>
</tr>
<tr>
<td>$P&lt;$</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Recovery</td>
<td>7.47 ± 0.01 (9)</td>
<td>7.35 ± 0.02 (7)</td>
<td>7.36 ± 0.03 (7)</td>
</tr>
<tr>
<td>$P&lt;$ (versus control)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Chemical shifts of P were determined as described in Methods. Results are mean ± SE of pH values obtained from two to nine spectra at each stage of the experiment. Values in parentheses are the number of animals. Group a during hypoperfusion are the two experiments in which ATP was detectable and group b are the five experiments in which ATP was unmeasurable. Localized pH determinations may be subject to errors of ±0.2 pH units, as described in Methods.

“roll”-affected quantitation. (3) Reduced signal-to-noise due to a lower number of spins in the 0.5-cm-thick slice of localized spectra compared with the whole volume. (4) Varying magnetic field gradients and B1 field inhomogeneities in different slices.

Hypofiltration

Metabolic changes of whole kidney and its major regions during progressive occlusion of the renal artery (Figure 2) are presented in Tables 2, 3, and 5.
Renal Metabolism in Acute Renal Failure

During hypofiltration, there was significant (13%; \(P < 0.003\)) increase in ATP/Pm ratio of the whole kidney. Intrarenal PCR, originating, we believe, in vascular smooth muscle, fell by 30\% (\(P < 0.01\)). Localized spectroscopy indicated that the increase in ATP/Pm was significant in the cortex (+25\%; \(P < 0.005\)), whereas that in the medulla was not (\(P > 0.2\)) (Table 5). In the representative study, the [ATP] determined in the whole kidney increased 10\%, to 3.1 \(\mu\)mol/g. This was accounted for by a higher increase in the [ATP] of the cortex than medulla (Table 3).

**Hyoperfusion**

As has been repeatedly demonstrated for the whole kidney, during hypoperfusion, the phosphorus spectrum changed abruptly. ATP was markedly reduced in concentration, and P, and PME increased. It was difficult to maintain a steady metabolic state throughout this period (Figure 2). Accordingly, [ATP] at the end of this period was generally lower than at the outset. There was, as expected, a significant reduction of ATP/Pm ratio and [ATP] (from 3.1 to 1.1 \(\mu\)mol/g) (Tables 2 and 3). Reduction of ATP exceeded elevation of Pm. Because one or more moles of Pm is released per mole of ATP hydrolyzed, this result indicates the presence of an invisible pool of Pm in the kidney similar to that developed during complete ischemia (28). [PME] increased, and [PDE] and [PCR] were reduced, most probably because of direct effects of localized ischemia. Whereas ATP was always present in whole volume spectra \([\text{ATP}] \sim 1.1 \pm 0.5 \text{ mM;} \ P < 0.01\), significantly greater than zero), in the localized spectra ATP was detectable in only two experiments at significantly reduced concentration. In the remainder, the ATP signal was below the limit of detection by localized MRS. This was attributed to the drop in signal from reduced total ATP in the smaller volume of each slice compared with the whole kidney volume. The results in Tables 4 and 5 have been presented separately for those kidneys in which ATP was relatively well conserved (upper line), and those in which ATP was undetectable (lower line). The results obtained are sufficient to indicate that no kidney was completely ischemic during the hypoperfusion period.

PME and P, were elevated in both cortex and medulla. Figure 6 shows localized spectra of two kidneys during hypoperfusion, one in which ATP was conserved (panel A), and the other in which ATP was below limits of detection of the localized assay (panel B).

As expected, pH fell significantly in the whole kidney during hypoperfusion to 7.21 \(\pm 0.06\) (Table 4). The pH of both cortex and medulla, measured independently, was also markedly reduced during hypoperfusion. Differences between these values and the corresponding whole-kidney measurements may be

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**Table 5. Effect of progressive renal artery occlusion on cortical and medullary phosphorus metabolites**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Cortex</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>294 ± 45 (11)</td>
<td>151 ± 15 (11)</td>
</tr>
<tr>
<td>ATP</td>
<td>257 ± 29 (7)</td>
<td>164 ± 28 (7)</td>
</tr>
<tr>
<td>ATP/Pm</td>
<td>1.32 ± 0.09 (11)</td>
<td>1.64 ± 0.09 (7)</td>
</tr>
<tr>
<td>PME</td>
<td>230 ± 127 (12)</td>
<td>311 ± 205 (2)</td>
</tr>
<tr>
<td>PCr</td>
<td>260 ± 58 (5)</td>
<td>248 ± 26 (5)</td>
</tr>
<tr>
<td>P</td>
<td>267 ± 46 (7)</td>
<td>165 ± 18 (7)</td>
</tr>
</tbody>
</table>

\*Values are the mean ± S.E. of absolute areas under individual peaks in milliamps. Values in parentheses are the number of 1-D phase-encoding experiments carried out during each stage. Statistics refer to paired t-test for a given renal artery occlusion pressure in each kidney. Significant values were labeled as follows: \(P < 0.05\), \(P < 0.01\), \(P < 0.005\). Differences between these values and the corresponding whole-kidney measurements may be

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**Table 4.**
partly attributed to methodological differences (see above).

The regional effects of ischemia on renal metabolism observed in this model may be summarized as follows. (1) P1 and PME were invariably elevated in both cortex and medulla but showed considerable heterogeneity of response. Thus, there was variation in the extent of ischemia within areas of the cortex and within the medulla (Figure 6). (2) Intracellular pH was reduced markedly and equally in both cortex and medulla (Table 4). (3) [ATP]/[Pi] ratio, measurable in two experiments, fell more drastically in the cortex than in the medulla (Table 5). More frequently, ATP was undetectable by this assay in either cortex or medulla, even though some renal blood flow continued (ATP present in whole-kidney spectra).

Recovery

After deflation of the cuff, metabolic recovery continued for up to 110 min. As has been shown previously (16), recovery of ATP was more rapid than that of GFR. ATP/Pi reached 50% of control after 7.4 ± 1.2 min when GFR was still unmeasurable (Figure 3A). ATP/Pi ratio measured as much as 2 h after release of partial renal arterial occlusion remained significantly lower than normoperfusion values (Table 2). Recovery of PME and PCr was complete, and only PDE remained significantly outside the normal range at the end of 110 min of observation (Table 2). ATP/Pi gradient between cortex and medulla was not restored (Table 5). In the representative study presented in Table 3, [ATP] after recovery was 2.1 ± 0.2 μmol/g for the whole kidney, with [ATP] in cortex and medulla of 2.8 and 1.8 μmol/g, respectively. No statistical correlation was found between final recovery of GFR and initial (r = 0.01) or final (r = 0.52) ATP/Pi ratio determined during the recovery period (Figure 3B). We attribute the failure to recover ATP to the additional work load of Na reabsorption imposed with restoration of glomerular filtration.

DISCUSSION

We have earlier applied localized 31P MRS to defining regional renal metabolic responses to the isolated perfused kidney (22). This study demonstrates the distribution of high-energy phosphate metabolites, particularly ATP, within the functioning kidney regions in vivo. It further highlights the importance of localization in any 31P MR study of the kidney.

If the hypothesis developed by Epstein et al. (6) is correct, we might expect higher concentrations of ATP in cortex than in medulla, greater sparing of ATP in medulla than in cortex during hypofiltration, and possibly more drastic loss of ATP, or accumulation of H+, in medulla, during incomplete ischemia.

These were not the findings of our study. ATP was indeed higher in cortex than in medulla, but sparing of ATP by hypofiltration was equally present in cortex and medulla, reaching statistical significance only in the cortex. Partial ischemia reduced ATP and pH throughout the kidney, and, in the limited number of studies where the method showed adequate sensitivity, medulla [ATP] was not more affected than cortex. pH was equally affected in cortex and medulla. Higher [ATP] of the cortex is compatible with higher blood flow in this region, and consistent with directly assayed ATP content of cortical nephron segments in the rat (11).

During partial renal arterial occlusion, when GFR and therefore Na transport were reduced, [ATP] increased. This, we propose, was due to sparing of ATP which is normally used for Na transport. The inevitable ischemia, however small, produced by this reduction in renal blood flow (RBF) might have resulted in reduced ATP synthesis which was more than offset by this ATP sparing, resulting in elevation of ATP. The proof of this may lie in an experiment in which Na transport is reduced without reduction of RBF by using the diuretics acetazolamide, bumetamide, or furosemide. Our preliminary results indicate that increases in GFR produced by these drugs further complicate interpretation and have not yet proved conclusive (Parivar F, Lee R, Narasimhan PT, Ross BD, unpublished observations). We believe that the present result is compatible with a model in which kidney functions under conditions of relative hypoxia; a significant reduction in energy demand for transport being reflected in net ATP synthesis (6).

There are three potential sources of error in 31P MRS assays of ATP in vivo. Partial occlusion of the renal artery produces inevitable changes in the T1 values of the metabolites that may affect quantitation. We believe that under these experimental conditions the quantitative changes are negligible. By using well-known MR theoretical principles (29), it can be calculated that with a flip angle of 36 degrees as used in these experiments (90-degree pulse, 250 μs; pulse width, 100 μs) and with a maximum T1 value of 2.0 s for Pi, a 15% increase in T1 of Pi would reduce its intensity by ~2% whereas a 15% decrease in T1 will increase its intensity by ~2%. On the other hand, with a maximum T1 of 0.7 s for β-ATP, flip angle of 36 degrees, and TR of 2.0 s, a 15% change in T1 value of β-ATP during partial occlusion will lead to only ~0.5% change in its intensity. Therefore, we can confidently conclude that under these experimental conditions the β-ATP signal intensity changes are true changes in the concentration and not due to T1 effects. An alternate possible explanation which we may also exclude is that renal volume was reduced when renal artery occlusion was applied. The resultant "compression" of renal cellular elements within the observed volume of the RF coil would produce a lower filling factor and lead to de-
creased signals, which could be interpreted as a coordinated apparent increase in concentration of all intracellular metabolites, including ATP and P, per gram weight. Finally, edema and changes in water distribution might be expected under ischemic or hypoxic conditions but probably play a minor role in these experiments in which some renal ATP was conserved throughout.

The second part of this study was concerned with the regional metabolic effects of more severe ischemia and is relevant to the proposed role of outer medulla in ARF. As we (26) and others (15) have noted, renal [ATP] falls precipitately as perfusion pressure is lowered over a narrow critical range. This was true, even though the animal respired ~100% O2. We were successful in reducing and maintaining the [ATP] content of whole kidney at approximately 30% of normal (about 1 mM) and observing cortex and medulla separately. However, the method of localized spectroscopy is relatively slow and there was a further small loss of ATP from the whole kidney during 30 min of apparently controlled hypoperfusion (Figure 2). Because 1-D CSI detects total ATP in the reduced volume of interest of individual “slices,” we could not measure [ATP] below ~1 mM. Nevertheless, in two experiments in which sufficient ATP was conserved to be detected in localized spectra, the ATP/P, gradient between cortex and medulla was lost (ratio ≈ 1), pH fell, and P, was increased equally in both cortex and medulla. An advantage of the 1-D phase-encode method is the acquisition of six or seven spectra simultaneously, each from a different kidney region. Considerable heterogeneity of the distribution of [ATP] was found during this stage. This implies that both cortex and medulla are affected by moderate hypoperfusion. In the more severely ischemic kidneys, a similar heterogeneity of distribution of P, and PME may be taken as indicating variable degrees of hypoxia in different regions within the cortex, as well as within the medulla. As Leichtweiss et al. (30) have previously demonstrated, regions of low oxygen tension exist in well-perfused rat kidney, despite a high P, tension in the renal vein blood. Evidence was obtained from cytochrome aa, determination (6) that renal oxidative metabolism was significantly impaired in the rat even in the normal state. These observations make it possible for heterogeneous distribution of ATP to occur in cortex, medulla, or both. The anatomical studies establish the existence of “fingers” of medullary tissue which extend out into the cortex. The simple localization procedure applied in the studies presented here most probably results in the inclusion of some islands of medulla in “cortical” spectra and vice versa. Thus, a pattern of heterogeneous distribution of both ATP and P, in renal hypoxia demonstrated by this work cannot conclusively distinguish cortex and medulla. The results are, however, consistent with present views of the great heterogeneity of renal structure, blood flow, metabolism, and function.

Recovery from this prolonged period of partial renal arterial occlusion was incomplete and may be regarded, even without histological proof, as ARF. Whether further recovery might occur if several hours elapsed, could only be examined in a nonsurgical, in vitro model. No simple relationship between ATP/P, ratio of whole kidney, cortex, or medulla, and GFR determined after recovery in individual kidneys, was obtained. Indeed, final GFR was independent of the ATP/P, ratio determined in the early (r = 0.01) or late phase of recovery (r = 0.52) (Figure 3B). The data presented here indicate that partial loss of renal ATP can contribute to the development of ischemic ARF. Taken together with the heterogeneous distribution of that ATP which remains, it is unlikely that single measurements of intrarenal [ATP] for the whole kidney will be predictive of ARF. Localized assays of intrarenal metabolism could be more informative. The methods of 2- and 3-D phase-encode spectroscopy (phosphorus imaging) would be appropriate but will inevitably encounter severe problems because of the loss of signal to noise when renal [ATP] is reduced.

The need for caution in interpreting renal [ATP] alone as a measure of ischemia is evident from this work. A balance exists between the demand of ATP for tubular Na+ transport and its provision by oxidative phosphorylation. Further understanding of the role of [ATP] in ARF may require assay of ATP turnover on a regional basis by using localized saturation transfer NMR (17,27,31). Techniques for such a study are now available.

REFERENCES


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