

Kinetics of Metabolism in Human Kidney Transplants

Measured by Dynamic ^{31}P NMR Spectroscopy

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Z. Naturforsch. **50 c**, 439–450(1995); received December 28, 1994/February 15, 1995

Dedicated to Professor Werner Müller-Warmuth on the occasion of his 65th birthday

Energy Metabolism, Intracellular pH, Kidney Transplantation, ^{31}P NMR Spectroscopy, Velocity Constants

The technique of *ex vivo* ^{31}P NMR spectroscopy has been used for the investigation of metabolic turnover in 15 cadaveric human kidneys obtained from brain-dead donors for transplantation. Measurements were carried out at 1.5 T time-dependently during regular hypothermic storage in histidine-tryptophan- α -ketoglutarate solution. The minimum delay between explantation and spectroscopy was 2 h 41 min. In one case of a discarded organ the measurements could be extended over a period of 161 h 35 min. A detailed kinetic model describing monoexponential ischemic phosphate turnover, which accounts for various interrelations of the NMR visible metabolites, has been derived. Averaged velocity constants of the decays of nucleotides and phosphomono- and -diesters ranged from 0.0047 to 0.294 h⁻¹ at approximately 4° C. Intracellular pH decreased monoexponentially with an averaged velocity constant of 0.31 h⁻¹.

Introduction

During the past two decades localized nuclear magnetic resonance (NMR) spectroscopy has become an important technique to provide non-destructively biochemical and physiological data on intact biological tissues. Among the widespread applications of NMR in biomedical research, investigation of the metabolic function of

the kidney *in vitro* and *in vivo* has found substantial interest (Balaban, 1989; Ross *et al.*, 1986). Since the first studies on isolated rat kidneys (Sehr *et al.*, 1977, 1979) renal viability during perfusion or ischemia was one of the major issues being addressed. Transplantation surgeons can be expected to be the clinical beneficiaries of such methods since an assessment of the metabolic state of excised donor organs is of important relevance, especially in relation to monitoring viability of organ storage and optimization of tissue preservation strategies. During transplantation renal ischemia is commonly associated with cadaver donor nephrectomy and subsequent storage of the kidney in a cooled flushing solution. When cadaveric kidneys are used, the recovery of renal function after transplantation is inversely related to the degree of ischemic injury. However, due to the complexity of the mechanisms leading to ischemic damage quantitative viability parameters are difficult to obtain by conventional techniques or cannot be used with human transplants due to their invasive character.

Abbreviations: AMP, adenosine 5'-monophosphate; ATP, adenosine 5'-triphosphate; GP, α -glycerolphosphate; GPC, glycerolphosphocholine; GPE, glycerolphosphoethanolamine; HTK, histidine-tryptophan- α -ketoglutarate; NDP, nucleoside 5'-diphosphates; NDP-Hex, nucleoside 5'-diphospho 1-hexoses; NDPN, nucleoside 5'-diphospho 5'-nucleosides; NDPX, sum of NDP-Hex plus NDPN; NMP, nucleoside 5'-monophosphate; NMR, nuclear magnetic resonance; NTP, nucleoside 5'-triphosphates; P_i , inorganic orthophosphate; PC, phosphocholine; PCr, creatine phosphate; PDE, phosphodiester; PE, phosphoethanolamine; PME, phosphomonoesters; rf, radio frequency.

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Therefore, simple estimates of total operative ischemia times and duration of cold storage of the organ are routinely used as predictors of the early outcome post-transplantation at the present. Similarly, the efficacy of different preservation strategies has largely been assessed by long-term survival studies.

The ability to detect relevant cytosolic metabolites like adenosine 5'-triphosphate (ATP), creatine phosphate (PCr), sugar phosphates and other phosphorylated glycolytic intermediates as well as inorganic orthophosphate (P_i) in living tissue by localized ^{31}P NMR spectroscopy provides an elegant non-invasive monitor of the energy status of the cell. Different animal models utilizing *in vitro* perfusion of isolated kidneys enabled the investigation of both the depletion of high-energy metabolites after arresting blood supply and the recovery of metabolic activity during reperfusion (Bretan *et al.*, 1987; Sehr *et al.*, 1977). Studies on isolated kidneys in a transplant setting without maintaining oxygen delivery demonstrated a correlation between relative metabolite ratios determined by ^{31}P NMR and the subsequent graft function or e. g. electron microscopy viability parameters (Bretan *et al.*, 1986a, 1986b; Kunikata *et al.*, 1989; Pomer *et al.*, 1988). The availability of large-bore magnets suited for whole-body investigations made it possible to extend such NMR techniques to cadaveric human kidneys removed for transplantation (Bretan *et al.*, 1989; Chan *et al.*, 1981; Möller *et al.*, 1993; van der Grond *et al.*, 1991). Spectroscopic parameters obtained on animal kidneys have also been used for an evaluation of different preservation solutions (Bore *et al.*, 1981; Bretan *et al.*, 1991).

Besides the acquisition of single spectra and correlation with the early graft function, an investigation of the time course of metabolic changes during ischemia leads to more profound insight into the underlying biochemistry. Turnover rates of individual metabolites have been obtained on rat kidneys applying ^{31}P NMR spectroscopy *ex vivo* (Pomer and Hull, 1993; Sehr *et al.*, 1979). However, for the further improvement of the reliability of spectroscopic parameters used as prognostic indices of post-transplantation function additional dynamic data derived from human kidneys are necessary. In the study presented here time-dependent changes in metabolite concentrations and tissue

pH during hypothermic storage of cadaveric human kidneys are analyzed. Quantitative turnover velocity constants are estimated based on a comprehensive kinetic model comprising all NMR visible phosphorus metabolites.

Experimental Details

Cadaveric kidneys

Fifteen human kidneys were obtained from different brain-dead donors by en bloc excision after *in situ* flush with cold ($\approx 4^\circ\text{C}$) histidine-tryptophan- α -ketoglutarate (HTK) solution. This standard procedure of nephrectomy for subsequent transplantation (Colberg 1980) achieves a minimal warm ischemia period of about 30 s. Triple-bagged transplants preserved with cold HTK solution were transferred into ice-containing styropor boxes and maintained in simple hypothermic storage until implantation. One of the kidneys, which could not be used for transplantation due to pronounced arteriosclerosis, served for long-term studies.

NMR measurements

A 1.5 T clinical whole-body tomograph (Siemens MAGNETOM 63 SP) operating at frequencies of 63.60 MHz (^1H) and 25.68 MHz (^{31}P) was used for all NMR investigations. Kidneys were studied without interruption of sterile hypothermic storage inside ordinary transplantation containers of slightly reduced height which fit within an external pair of rf coils. Because the method is non-invasive and non-destructive, it can be applied without prejudicing the subsequent transplantation of the donor organs. ^1H NMR imaging (scout views) to ensure an optimal coil position relative to the kidney and magnetic field homogeneity shimming were performed using a 23 cm Helmholtz transmit/receive coil placed directly below and above the container. A 17 cm Helmholtz transmit/receive coil arranged perpendicularly was employed for ^{31}P spectroscopy. Because phosphate-free HTK solution was used for preservation, all observed ^{31}P NMR signals entirely originate from the kidney. So an acquisition of the free induction decay following a single rectangular pulse of 500 μs duration ($\approx 72\text{ W}$ rf pulse power) without further volume localization could be applied (3 s repetition

time, spectral width ± 1 kHz, 1 K complex data points). The transmitter amplitude was adjusted to achieve maximum sensitivity, i. e. approximately 90° excitation. However, rf inhomogeneities of the Helmholtz coil produce a flip angle distribution across the kidney. Assuming complete absorption of the rf power in the kidney, HTK solution, and ice (≈ 7.5 kg total mass) the maximum specific absorption rate corresponds to ≈ 0.012 W kg $^{-1}$. Typically 200 averages (600 s total acquisition time) were sufficient to achieve satisfactory signal-to-noise ratios. Due to the short delay time of 500 μ s preceding data acquisition, differences in spin-spin relaxation times do not influence the spectra. In addition, no significant changes in relative peak intensities were observed between measurements at various repetition intervals ranging from 3 to 20 s, i. e. fully relaxed conditions are approximately fulfilled. Spectroscopy started between 2 h 41 min and 11 h 1 min (mean 5 h 52 min $\pm$ 2 h 7 min) after onset of cold ischemia and was repeated during 2 h 6 min and 11 h 44 min (mean 7 h 19 min $\pm$ 3 h 6 min) in the case of subsequent transplantation, while the long-term investigation of the discarded organ started 18 h 50 min after explantation and extended over a period of 161 h 35 min. Data processing included exponential multiplication (≈ 5 Hz line broadening), Fourier transformation (1.95 Hz digital resolution), and Lorentzian line fitting for the determination of peak positions, amplitudes, widths, and phases. Fitted peak areas were used to analyze metabolic changes.

Results and Discussion

Ex vivo kidney spectra

Representative spectra recorded from one human kidney *ex vivo* at different times of ischemic cold storage and corresponding peak assignments based on chemical shifts are presented in figure 1. The chemical shift scale is referenced to the position of PCr which is the usual internal standard for *in vivo* ^{31}P NMR. In living tissue PCr chemical shift varies between -2.3 ppm and -3.2 ppm with reference to external 85 % H_3PO_4 depending on sample geometry (Beall *et al.*, 1984). The results can be compared to high-field spectra of rat kidneys obtained at 11.7 T (Pomer and Hull, 1993) or rabbit kidneys collected at 4.7 T (Balaban, 1989) to provide a more detailed insight into the individual components contributing to the different peaks.

The signals labeled α -, β -, and γ -NTP arise from the three corresponding phosphorus nuclei of nucleoside 5'-triphosphates, predominantly ATP. Due to homonuclear J-coupling they split into two doublets (α - and γ -NTP) and a triplet (β -NTP), which are partly resolved in the case of the α - and γ -resonances and were fitted assuming coupling constants of 16 Hz. High-field spectra can resolve additional metabolite signals which are buried within the NTP signal envelopes at our field strength of 1.5 T. (i) α - and β -phosphorus of nucleoside 5'-diphosphate (NDP; -7 and -3 ppm) contribute

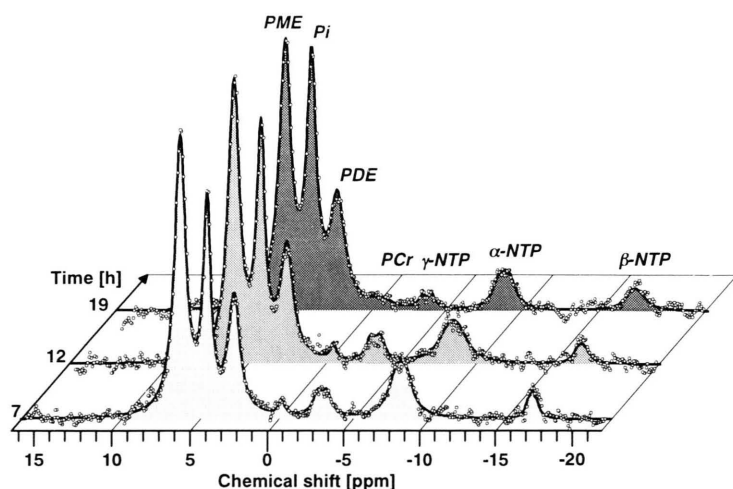


Fig. 1. 25.68 MHz ^{31}P NMR spectra of one human kidney *ex vivo* obtained at different times of cold ischemia. The spectra comprise resonances of the α - (-7.66 ppm), β - (-16.52 ppm), and γ -phosphorus (-2.67 ppm) of NTP, PCr (0.00 ppm, chemical shift reference), P_i (5.03 ppm, corresponding to a pH of 7.18), and phosphoesters PME (6.77 ppm) and PDE (3.20 ppm). As peak positions are slightly pH dependent they shift with progression of ischemia, and the numerical values given above refer to the measurement performed 7 hours after explantation.

to α - and γ -NTP, respectively. (ii) Both the α - and β -phosphorus of nucleoside 5'-diphospho 5'-nucleosides (NDPN, predominantly nicotinamide adenine dinucleotides; -8 ppm) and of nucleoside 5'-diphospho 1-hexoses (NDP-Hex, e. g. uridine 5'-diphospho 1-glucose; -8 and -9.5 ppm) contribute to α -NTP. Even at 11.7 T additional contributions to the NTP resonances from uridine- (UTP, UDP), cytidine- (CTP, CDP), and guanosine 5'-tri- and -diphosphates (GTP, GDP) cannot be separated from the adenine nucleotide signals. Chromatographic studies yielded a nucleotide distribution of 82 % ATP plus ADP, 11 % GTP plus GDP, 7 % UTP plus UDP, and only traces of CTP plus CDP in the normal rat kidney (Gerlach *et al.*, 1963). After 10 min of warm ischemia these authors observed a reduction of the ATP plus ADP fraction by 86 %, while GTP plus GDP and UTP plus UDP decreased by 73 % and 79 %, respectively, i. e. the relative contribution of the adenosine nucleotides was slightly reduced to 74 %. Similarly, our measurements showed typical reductions of the NTP peak areas by 50 % during 24 h of cold ischemia. Therefore, we expect that the dominance of the adenosine 5'-phosphate contributions to the NTP signals is maintained during regular cold storage periods.

Based on NMR results PCr concentrations of 0.7 mM (Pomer and Hull, 1993) or below 0.2 mM (Freeman *et al.*, 1983; Iles *et al.*, 1982) were estimated for the rat kidney. Consequently, only very weak lines were detectable in eight organs decaying to zero intensity with prolonged ischemia (Fig. 1). To the best knowledge of the authors,

this represents the first NMR observation of this metabolite in human transplants. Because the maximum integral was always below 5 % of the total spectral area, PCr depletion via the creatine kinase reaction was not included in the kinetic model.

Contrary to previous studies on kidneys preserved in EuroCollins or University of Wisconsin solution, which both contain large amounts of a phosphate buffer (Bretan *et al.*, 1987, 1989; van der Grond *et al.*, 1991), no additional buffer P_i fraction has to be considered in cases of HTK flush. Therefore, the P_i peak completely results from cytosolic inorganic phosphate. In ^{31}P NMR of muscle, heart, or brain its chemical shift versus PCr is routinely used for a determination of intracellular pH. As PCr was detectable in eight transplants this method could now be extended to human kidneys without need of an external reference. In these organs pH varied time-dependently between 7.3 and 7.0.

Since no proton decoupling was applied in this study, the resonance bands of phosphomono- and -diesters (PME and PDE) are broad due to unresolved ^1H - ^{31}P spin couplings and individual metabolites cannot be resolved. It is known, however, that the major component of the PDE signal results from glycerolphosphocholine (GPC) with an additional contribution of glycerolphosphoethanolamine (GPE) (Balaban, 1989; Pomer and Hull, 1993). The phosphomonoesters at relevant concentrations are phosphoethanolamine (PE, being the main fraction of the PME signal under *in vivo* conditions) and phosphocholine (PC), while glycolytic intermediates are only of minor importance

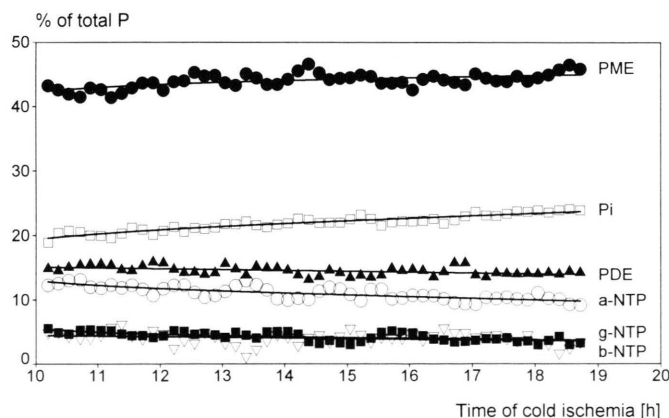


Fig. 2. Phosphate metabolite levels (% of total NMR detectable phosphorus) of a human kidney *ex vivo* versus time during the first 19 h of cold storage. ^{31}P NMR measurements started approximately 10 h after onset of ischemia. Results of the data analysis based on Eqns (20) – (25) are shown as solid lines. Qualitatively, similar patterns resulted in all short-term studies, independent of differences in the delay between nephrectomy and NMR measurements, which varied between 2 h 41 min and 11 h 1 min.

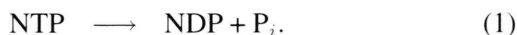
(Pomer and Hull, 1993). There are indications from combined NMR and high-performance liquid chromatography assays that adenosine 5'-monophosphate (AMP) can make a significant contribution to the PME signal in the ischemic kidney (Bretan *et al.*, 1989; Kunikata *et al.*, 1989).

Metabolic consequences of cold ischemia

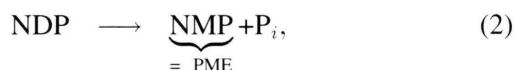
Qualitatively, time-dependent changes of the NMR visible metabolites in human kidney grafts are similar to results from animal models applying comparable storage conditions (Pomer and Hull, 1993; Sehr *et al.*, 1979). Examples, including the first period of ischemia as well as the long-term study are presented in Figs 2 and 3.

It has been pointed out that the kidney is a heterogeneous organ with different types of tissue. However, global metabolite concentrations detected by ^{31}P NMR without sufficient spatial resolution are weighted averages over the whole organ, including cortex, medulla, papilla, and tubular volume. Cellular heterogeneity is also apparent in the energy metabolism of the *in vivo* kidney; the cortex is almost completely dependent on fatty acids oxidation and oxidative phosphorylation, whereas the inner medulla is mostly glycolytic (Balaban, 1989). However, since energy conversion processes are limited to anaerobic glycolysis after the oxygen supply is arrested, this specific metabolic heterogeneity can be neglected in the present study. ATP utilization mainly arises from $\text{Na}^+\text{K}^+\text{ATPase}$ activity, which is essential for the maintenance of transmembraneous Na^+ and K^+

gradients (Belzer and Southard, 1988). During ischemia ATP synthesis cannot keep up with the energy demands, and there is net ATP hydrolysis. Consequently, a decrease in the cytosolic concentration of NTP with a rise in intracellular P_i can be observed as a function of cold storage time (Figs 2 and 3) and may be described by the comprehensive reaction



Because NDP contributes to the α - and γ -NTP resonances only the β -NTP peak should indicate NTP depletion directly. Both NDP and NMP are also hydrolyzed during hypothermic storage (Gerlach *et al.*, 1963; Southard *et al.*, 1977):



From chromatographic assays it is known that AMP catabolism proceeds via inosine 5'-monophosphate in the ischemic kidney (Gerlach *et al.*, 1963). Only the second step of this reaction, being expressed by Eqn. (3), leads to changes in the ^{31}P NMR spectrum. Frequently, NDP has been quantified by subtraction of the γ - and β -NTP peak areas. However, chemical extraction followed by enzymatic assay yields at least a fourfold higher NDP content (Freeman *et al.*, 1983, 1986; Stubbs *et al.*, 1984). The general explanation of this large discrepancy is that the biochemical methods measure the total content (bound to macromolecules

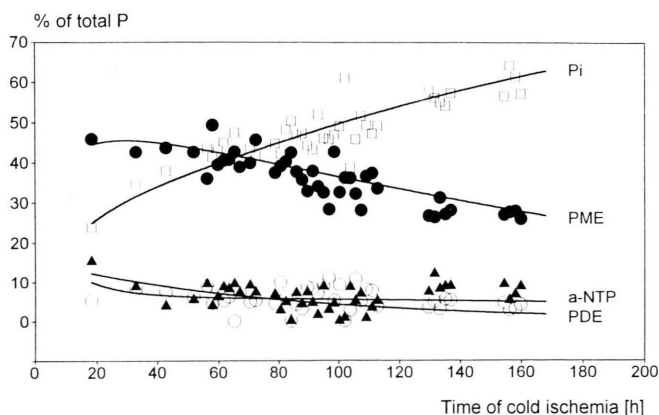
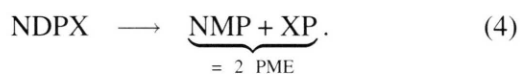


Fig. 3. Ischemic long-term changes of phosphate metabolites (% of total NMR detectable phosphorus) of the non-transplanted human kidney *ex vivo* during approximately 160 h of cold storage. For simplicity, only the α -resonance of NTP is shown besides PME, P_i , and PDE. Results of the data analysis based on Eqns (20) – (25) are shown as solid lines.

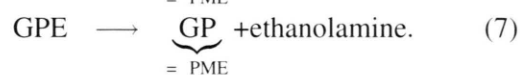
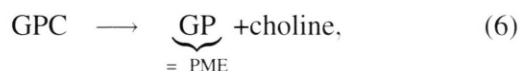
plus unbound), whereas localized NMR only detects mobile phosphorus compounds. For this reason Eqn. (2) only refers to the free and therefore probably cytosolic concentrations from NMR studies. In a similar manner, subtraction of the γ -NTP peak area from the area of the α -NTP signal provides a measure of total NDPX, i. e. the sum of NDPN plus NDP-Hex. Pyrophosphatase hydrolyzes the pyrophosphate bond producing two phosphomonoesters, namely NMP and XP, which may be nicotinamide mononucleotide or hexosemonophosphates:



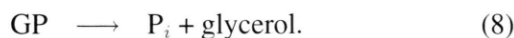
By analogy to NMP catabolism via the 5'-nucleotidase reaction of Eqn. (3), hydrolysis of the monoesters XP results in:



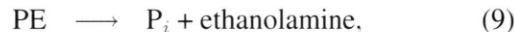
In many tissues the main constituents of the PME and PDE resonances are related to the synthesis and degradation of cellular membranes. However, diester concentrations found in renal medulla are by far too high to be attributed to these biochemical pathways. Rather, their principle function is to protect the cells from the hyperosmotic environment. In contrast to inorganic salts GPC and further NMR-invisible organic osmolytes do not significantly perturb enzymatic function over a wide range of concentrations (Garcia-Perez and Burg, 1990). Low temperatures inhibit $\text{Na}^+\text{K}^+\text{ATPase}$ leading to increased intracellular Na^+ concentrations in the hypothermic preserved kidney. Because levels of compatible solutes vary with tonicity a depletion of GPC can be expected, as it is demonstrated in Figs 2 and 3 by PDE reduction. In the kidney, both GPC and GPE are hydrolyzed by GPC diesterase (Garcia-Perez and Burg, 1990; Nakanishi and Burg, 1989; Philippon, 1964) producing α -glycerolphosphate (GP), which contributes to the PME peak:



At first approximation ^{31}P NMR of the whole kidney without further intrarenal localization should not be sensitive to changes in permeability or active transport, which also might influence intracellular GPC levels. Via α -glycerolphosphate dehydrogenase reaction and formation of dihydroxyacetone phosphate GP can be introduced into glycolysis for ATP regeneration. Finally, as far as spectroscopic changes are involved, this would result in a net production of P_i due to Eqns (1) – (3) and may be summarized by the apparent reaction



As Eqns (2), (4), (6), and (7) lead to a production of phosphomonoesters an increase of the PME peak area during the first 20 hours of ischemia is observed (Fig. 2). Little is known about the biochemical function of the major PME components, PE and PC, in renal metabolism. However, they have been proposed to play a similar role in the cortex to that of GPC in the medulla (Wolf *et al.*, 1989). By analogy to Eqn. (3), indicating NMP catabolism during ischemia, the following reactions may be assumed for these monoesters:



Due to educt depletion at long storage times PME formation now no longer keeps up with hydrolysis leading to a marked decrease in PME peak area after 20 hours of cold storage (Fig. 3).

Kinetic model

The technique of *ex vivo* ^{31}P NMR utilized in this study provides information about the overall phosphate turnover rather than individual biochemical reactions, which may be investigated e. g. by saturation transfer experiments if applicable (Freeman *et al.*, 1983, 1986). Previous animal studies only analyzed time-dependent variations of single metabolites, without regarding interrelations of the various fluxes. However, these properties of phosphate metabolism detected by NMR have to be introduced into a comprehensive model.

Due to the multicomponent nature of most signals the scenario of cold ischemia metabolic turnover as described in the preceding section is by

far too complicated to be analyzed in all details. Therefore, no attempts are made to quantify true Michaelis constants K_m and V_{max} . Instead, simple monoexponential behavior is assumed as it would be correct in the limit of low substrate concentrations. Further, a single velocity constant k_{PDE} is related to total PDE decomposition, Eqns (6) and (7). The same simplification is used for PME catabolism, Eqns (3), (5), and (8) – (10), being described by a single velocity constant k_{PME} .

The final model therefore comprises a set of six combined differential equations:

$$\frac{\partial c_{NTP}}{\partial t} = -k_{NTP} c_{NTP}, \quad (11)$$

$$\frac{\partial c_{NDP}}{\partial t} = k_{NTP} c_{NTP} - k_{NDP} c_{NDP}, \quad (12)$$

$$\frac{\partial c_{NDPX}}{\partial t} = -k_{NDPX} c_{NDPX}, \quad (13)$$

$$\frac{\partial c_{PDE}}{\partial t} = -k_{PDE} c_{PDE}, \quad (14)$$

$$\frac{\partial c_{PME}}{\partial t} = k_{PDE} c_{PDE} - k_{PME} c_{PME} + k_{NDP} c_{NDP} + 2 k_{NDPX} c_{NDPX}, \quad (15)$$

$$\frac{\partial c_{P_i}}{\partial t} = k_{PME} c_{PME} + k_{NTP} c_{NTP} + k_{NDP} c_{NDP}. \quad (16)$$

While c_{NTP} , c_{NDPX} , and c_{PDE} decays are expressed by ordinary first-order reactions, c_{NDP} obeys the well-known expression of series first-order reactions and PME kinetics includes both parallel and series first-order reactions. A general discussion was given e. g. by Frost and Pearson (1953). Assuming that total NMR-visible phosphate is constant, c_{P_i} can be found most conveniently from the stoichiometric relation. Therefore, straightforward integration can be performed analytically by standard techniques, yielding:

$$c_{NTP} = c_{NTP}^0 E_{NTP}, \quad (17)$$

$$c_{NDP} = \frac{k_{NTP}}{k_{NDP} - k_{NTP}} c_{NTP}^0 (E_{NTP} - E_{NDP}) + c_{NDP}^0 E_{NDP}, \quad (18)$$

$$c_{NDPX} = c_{NDPX}^0 E_{NDPX}, \quad (19)$$

$$c_{PDE} = c_{PDE}^0 E_{PDE}, \quad (20)$$

$$\begin{aligned} c_{PME} = & \frac{k_{PDE}}{k_{PME} - k_{PDE}} c_{PDE}^0 (E_{PDE} - E_{PME}) \\ & + c_{PME}^0 E_{PME} \\ & + \frac{k_{NTP} k_{NDP}}{k_{NDP} - k_{NTP}} c_{NTP}^0 \\ & \times \left(\frac{E_{PME} - E_{NTP}}{k_{NTP} - k_{PME}} - \frac{E_{PME} - E_{NDP}}{k_{NDP} - k_{PME}} \right) \\ & + \frac{k_{NDP}}{k_{NDP} - k_{PME}} c_{NDP}^0 (E_{PME} - E_{NDP}) \\ & + \frac{2 k_{NDPX}}{k_{NDPX} - k_{PME}} c_{NDPX}^0 (E_{PME} - E_{NDPX}), \end{aligned} \quad (21)$$

$$\begin{aligned} c_{P_i} = & c_{P_i}^0 \\ & + c_{PDE}^0 \left(1 - \frac{k_{PME} E_{PDE} - k_{PDE} E_{PME}}{k_{PME} - k_{PDE}} \right) \\ & + c_{PME}^0 (1 - E_{PME}) \\ & + c_{NTP}^0 \left\{ 3 - E_{NTP} - \frac{1}{k_{NDP} - k_{NTP}} \right. \\ & \times \left[k_{NDP} E_{NTP} - k_{NTP} E_{NDP} \right. \\ & + \frac{k_{NDP}}{k_{NTP} - k_{PME}} (k_{NTP} E_{PME} - k_{PME} E_{NTP}) \\ & \left. \left. - \frac{k_{NTP}}{k_{NDP} - k_{PME}} (k_{NDP} E_{PME} - k_{PME} E_{NDP}) \right] \right\} \\ & + c_{NDP}^0 \left(2 - E_{NDP} - \frac{k_{NDP} E_{PME} - k_{PME} E_{NDP}}{k_{NDP} - k_{PME}} \right) \\ & + 2 c_{NDPX}^0 \left(1 - \frac{k_{NDPX} E_{PME} - k_{PME} E_{NDPX}}{k_{NDPX} - k_{PME}} \right), \end{aligned} \quad (22)$$

with $E_j = \exp(-k_j t)$ and $j = NTP, NDP, NDPX, PDE, PME$. Although quite complicated expressions result for c_{PME} and c_{P_i} their parameters are actually obtained from the simultaneous time course of all six concentrations, i. e. all PME production terms are totally determined by the simple

Eqns (17) – (20). Similarly, P_i production is completely included in Eqns (17) – (21).

If normalized concentrations are used, Eqns (20) – (22) also apply to the time course of the corresponding ^{31}P NMR peak areas. Fits to the α -, β -, and γ -NTP decays can easily be obtained by appropriate combination of Eqns (17) – (19):

$$A_{\alpha\text{-NTP}} \sim c_{\text{NTP}}^0 E_{\text{NTP}} \quad (23)$$

$$+ \frac{k_{\text{NTP}}}{k_{\text{NDP}} - k_{\text{NTP}}} c_{\text{NTP}}^0 (E_{\text{NTP}} - E_{\text{NDP}})$$

$$+ c_{\text{NDP}}^0 E_{\text{NDP}} + 2 c_{\text{NDPX}}^0 E_{\text{NDPX}},$$

$$A_{\beta\text{-NTP}} \sim c_{\text{NTP}}^0 E_{\text{NTP}}, \quad (24)$$

$$A_{\gamma\text{-NTP}} \sim c_{\text{NTP}}^0 E_{\text{NTP}} + c_{\text{NDP}}^0 E_{\text{NDP}} \quad (25)$$

$$+ \frac{k_{\text{NTP}}}{k_{\text{NDP}} - k_{\text{NTP}}} c_{\text{NTP}}^0 (E_{\text{NTP}} - E_{\text{NDP}}).$$

Metabolic turnover rates

The integral of the entire ^{31}P NMR spectrum provides a measure of the total cytosolic phosphate. The dynamic measurements showed no significant changes of total phosphate with time during cold ischemia for any kidney. Standard deviations varied between 2.4 % and 11.4 % depending on signal-to-noise ratio. The assumption of total NMR-visible phosphate being constant is therefore verified within these limits.

Velocity constants describing phosphate turnover in the ischemic kidney were calculated from simultaneous least-squares fits of the time-dependent variations of all six phosphate peak areas to the model functions applying a Marquardt algorithm. Examples are shown in Figs 2 and 3. Qualitatively the patterns of metabolic changes during the observed periods of cold ischemia were identical for all kidneys, independently of differences in the delay between nephrectomy and start of the NMR measurements and could be well described by the assumed model functions. A summary of the numerical data is presented in Table I. Considering the uncertainty of the fitting procedure the kinetic parameters of a single kidney are de-

Table I. Mean values and inter-individual ranges of the velocity constants k_j describing ischemic turnover of j = NTP, NDP, NDPX, PDE, and PME and changes in intracellular pH during hypothermic storage ($\approx 4^\circ\text{C}$) in cadaveric human kidneys.

Metabolite	$\langle k_j \rangle$	range of k_j
NTP	0.027 h^{-1}	0.010 – 0.047 h^{-1}
NDP	0.294 h^{-1}	0.140 – 0.347 h^{-1}
NDPX	0.0047 h^{-1}	0.0001 – 0.0094 h^{-1}
PDE	0.0197 h^{-1}	0.0132 – 0.0241 h^{-1}
PME	0.0068 h^{-1}	0.0037 – 0.010 h^{-1}
pH	0.31 h^{-1}	0.102 – 0.49 h^{-1}

termined with an accuracy of $\pm 30\%$ (exception NDPX).

Within these experimental errors no indications of multiphasic behavior were detectable in any of the transplants. Therefore, the approximation that the individual substances in significant quantities of each resonance line do not have widely different velocity constants, seem to be justified during the observed period of cold ischemia. Whereas animal studies can start almost directly after nephrectomy this is impossible when human kidneys are excised for subsequent transplantation (explantation is normally performed at a different hospital, thus transportation is necessary). Our minimum delay period between onset of cold ischemia and spectroscopy was therefore 2 h 41 min and no information about significant deviations from the observed kinetics at shorter storage times can be derived. Biphasic behavior in nucleotide loss (Pomer and Hull, 1993) and pH (Pomer and Hull, 1993; Sehr *et al.*, 1979) was found in rat studies, i. e. an initial rapid kinetic phase, being observable during the first two hours of ischemia, was followed by a slower one. However, the rapid short-term decay was missing in rat kidneys flushed with a preservation solution, probably due to a brief warm ischemia introduced by the flushing procedure (Pomer and Hull, 1993). Therefore, multiphasic behavior may further be levelled below detectability, since all of our transplants were flushed with cold HTK solution.

While fits of individual kidney data could be performed with typical standard errors of about 30 %, between two- and fourfold increased inter-individual variations of the kinetic parameters were obtained when the results from the collection of

all different transplants were compared (see Table I). These individual results did not simply correlate to the duration of ischemia preceding spectroscopy. Since all kidneys were obtained from different brain-dead donors such variations in the kinetic parameters may reflect differences of various factors influencing transplant quality like donor hydration, circulatory condition, age, etc.

The NDPX decay was found to be extremely slow even in the long-term study. Thus the velocity constant k_{NDPX} is attached with a large uncertainty and only its order of magnitude can be estimated. Consistently, NDPN remained stable over 18 h of cold storage in the rat kidney, while NDP-Hex decreased to about 50 % of its initial level (Pomer and Hull, 1993). Since both metabolites are present in similar quantities and cannot be separated at our field strength of 1.5 T biexponential behavior may thus significantly contribute to the large experimental error in this case.

Changes of the adenine nucleotides are of special interest as the energy charge of the adenylate system (half of the average number of anhydride-bound phosphate groups per adenine moiety) is an important regulatory parameter describing the energy balance of the cell (Atkinson, 1968). Pomer and Hull (1993) reported time constants of 5–10 h for the nucleotides in rat kidneys with hypothermic EuroCollins preservation. In contrast to these results NTP loss is by about one order of magnitude slower in human transplants (averaged time constant: 53 h, inter-individual range 21–96 h). Similar qualitative findings have already been discussed by Ross *et al.* (1986). According to these authors the rapid disappearance of NTP after arresting blood supply in small animals with high O_2 consumption and accelerated NTP metabolism may be regarded as a reflection of the inverse relationship between oxygen consumption and body size as pointed out by Krebs.

Based on previous animal studies (Gerlach *et al.*, 1963; Kallerhoff *et al.*, 1988; Kunikata *et al.*, 1989; Southard *et al.*, 1977) a sequence $k_{\text{NTP}} \geq k_{\text{NDP}} \gg k_{\text{NMP}}$ should be expected concerning depletion of the adenosine nucleotides. In accordance with these biochemical measurements (performed after freeze-clamping and acid extraction) our NMR results indicate that NMP catabolism (quantified by k_{PME} in Table I) proceeds signifi-

cantly slower than NTP hydrolysis. This is also consistent with NMR investigations of rat kidneys (Pomer and Hull, 1993; Sehr *et al.*, 1979). In contrast, NDP decays one or two orders of magnitude faster as compared to the turnover timescales of all other phosphates. However, this result has to be interpreted with caution. Stubbs *et al.* (1984) compared NMR and chemical extract measurements of adenine nucleotides in control and ischemic rat kidneys and concluded that NDP formed during ischemia is largely invisible to NMR. This implies that an additional exchange between the two NDP fractions (unbound and bound to macromolecules) has to be considered



and the apparent velocity constant k_{NDP} comprises both cytosolic NDP hydrolysis, Eqn. (2), and immobilization, Eqn. (26). NDP disappearance in the NMR spectrum may therefore be expected to be dominated by the NDP flux to the NMR invisible compartment. Due to the low concentrations of NMR detectable NDP (below 5 % of total detectable phosphate) this "phosphate loss" produces no change in total phosphate (determined from NMR peak areas) within the experimental error.

Bretan *et al.* (1986a, 1986b, 1987, 1989) used the PME/P_i ratio as a viability parameter to predict functional recovery after transplantation. However, Pomer and Hull (1993) pointed out that this ratio has no direct biochemical significance in a sense that NTP plus NDP would have. Our results clearly show that their argumentation derived from high-field rat studies can completely be transferred to human transplants. Since k_{NTP} , being a measure of NMP production exceeds k_{PME} , the relative PME signal area slightly increases during regular clinical storage periods (Figs 2 and 3). Therefore, PME/P_i does not simply decrease with prolonged ischemia due to PME depletion but because P_i increases faster than changes in PME occur.

Changes in intracellular pH

An example of the time course of the change in intracellular pH during hypothermic ischemia is presented in Fig. 4. Qualitatively identical patterns were obtained from all eight kidneys with detectable PCr, independent of differences in the

delay between nephrectomy and NMR measurements, which varied between 3 h 18 min and 11 h 1 min in these cases. As before, all experimental data could be fitted accurately to a single exponential decay according to

$$\text{pH} = \Delta\text{pH}_0 \exp(-k_{\text{pH}}t) + \text{pH}_\infty \quad (27)$$

with typical velocity constants $k_{\text{pH}} \approx 0.3 \text{ h}^{-1}$ (Table I). Again, the range of inter-individual variations exceeded the estimated fitting uncertainties of about $\pm 30 \%$ by a factor of four. Previous animal studies yielded rates of proton production, expressed by k_{pH} , being comparable to the rate of P_i production (Pomer and Hull, 1993; Sehr *et al.*, 1979). Accordingly, a linear correlation of P_i concentration and pH was obtained in the ischemic rat kidney. A similar linear relationship was also observed for the human transplants investigated here ($R = 0.88$; plot not shown). Since PCr could be detected only in a limited number of cases we currently evaluate alternative strategies for a pH estimation based on the chemical shifts of the PME and PDE signals.

Conclusions

Previously, insight into metabolic turnover in the hypothermic preserved kidney during ischemia was obtained from sophisticated animal studies but these data allow only limited quantitative

conclusions when transferred to human transplants. The present work shows that even at 1.5 T high-quality spectra of cadaveric human kidneys can be obtained by *ex vivo* ^{31}P NMR allowing the investigation of major phosphate metabolites. A precise and quantitative monitoring of the underlying kinetics is therefore possible without prejudicing the subsequent transplantation of the donor organs. For example, from our results NTP can be expected to decrease by about 50 % in human kidneys after 24 h of cold storage. However, further investigations during the first hours after nephrectomy are necessary because animal studies showed that the kinetic behavior may be different in the initial phase of ischemia.

Besides the biochemical relevance of detailed kinetic investigations simply obtainable parameters suited for a prediction of post-operative functional recovery are of interest in clinical practice. Based on the kinetic results metabolite ratios involving either NTP (e. g. $\beta\text{-NTP}/\text{P}_i$) or NTP plus NDP (e. g. $\gamma\text{-NTP}/\text{P}_i$) should be more sensitive than Bretan's viability parameter PME/P_i . In view of the high quality of the spectra presented in Fig. 1 reliable quantitation of the NTP signals is no longer limited to high-field animal studies but can also be obtained from human transplants in a clinical setting. Use of HTK solution for preservation allows precise quantitation of intrarenal P_i since it does not contain additional phosphate. However, additional investigations are necessary to achieve statistical significance. Such experiments comprising various correlations with

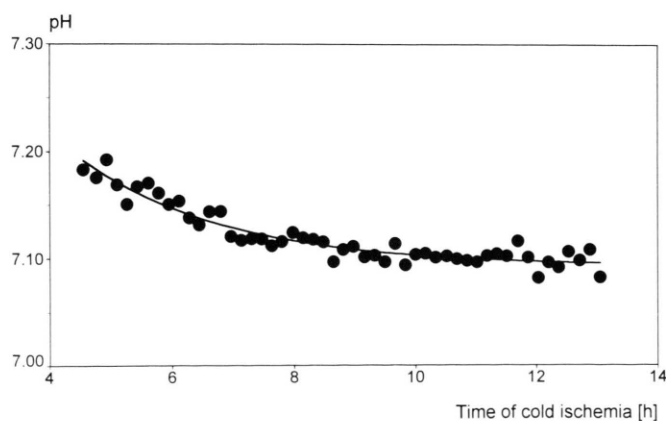


Fig. 4. Ischemic changes of intracellular pH in a human kidney during the first 13 h of cold storage. ^{31}P NMR measurements started 4 h 41 min after onset of ischemia. The result of a fit to Eqn. (27) is shown as a solid line.

post-operative functional parameters are currently being performed at our institution.

Knowledge of relevant velocity constants being determined under regular hypothermic storage conditions also provides a novel basis for a reliable assessment of further investigations on the improvement of preservation strategies. In addition to phosphate levels, intracellular pH may now be included in the analysis.

Acknowledgements

This work was supported by a grant to H. E. M. and T. V. (Bennigsen-Foerder-Preis) from the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen. We thank all members of the transplantation team and the magnetic resonance imaging facility of the University of Münster for their kind cooperation.

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