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Preservation of Renal Function by Adenosine-Stimulated ATP Synthesis in Hypothermically Perfused Dog Kidneys[1]

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Dog kidneys were hypothermically perfused for 1 to 5 days in the presence or absence of adenosine (5 m*M*). Following 1, 3, and 5 days, kidneys were reperfused at normothermia in an isolated perfusion system using a bovine serum albumin containing perfusate and renal function was determined. At the end of normothermic perfusion, kidney cortical slices were removed for biochemical analysis. Kidneys preserved in the presence of adenosine generated much higher concentrations of ATP during normothermic perfusion than kidneys preserved in the absence of adenosine at all time periods studied. In kidneys reperfused (37°C) after 3 days of preservation, the ATP concentration averaged 9.15 μmol/g dry wt (+adenosine) vs 4.75 μmol/g dry wt (−adenosine). After 5 days, the average was 12.65 μmol/g dry wt (+adenosine) vs. 4.00 μmol/g dry wt (−adenosine). The tissue concentration of K^+ was higher in kidneys perfused in the presence of adenosine for all time periods studied. The presence of adenosine had little effect on the GFR (creatinine clearance) which was reduced by about 90% from control values at both 3 and 5 days of preservation. The primary effect of adenosine on renal function was a greater preservation of the capability of the isolated perfused kidney to reabsorb Na^+ from the glomerular filtrate. In the absence of adenosine Na^+ reabsorption was reduced from 97 to 50% whereas in the presence of adenosine was reduced to only 80% after 3 days of preservation. After 5 days of perfusion Na^+ reabsorption was unaffected by the presence of adenosine and the amount resorbed was only 25–30% of the amount filtered. These results suggest that the adenosine stimulated synthesis of ATP during perfusion preservation of kidneys preserved renal function for up to 3 days. After 5 days of preservation, even in the presence of high tissue concentrations of ATP, renal function is reduced. Thus, long-term kidney preservation requires not only the maintenance of energy metabolism but also preservation of other factors necessary for cell viability that have yet to be described.

The isolated perfused kidney (IPK) has become a popular method to study the effects of hypothermic preservation conditions on renal function (2–5, 7). The method involves reperfusion of the preserved kidney at normothermia with an albumin-containing electrolyte solution and the measurement of various renal functions. Although transplantation remains the ultimate test of the efficacy of organ preservation, the amount of data generated by this method is limited primarily to serum creatinine values and survival. Consequently, improvements in organ function resulting from a particular preservation maneuver may be obscured by this technique. The IPK model, however, provides the investigator with an opportunity to determine the effects of preservation conditions on specific renal functions including GFR, Na^+ reabsorption, protein in the urine, etc., as well as a biochemical analysis (adenosine nucleotides, tissue electrolytes, tissue edema) of the tissue following normothermic reperfusion. With this technique, many preservation conditions can be rapidly analyzed in terms of effects on the function of the kidney, resulting in the gen-

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eration of data which may be relevant to obtaining improved organ preservation.

In this study, we have used the IPK to study the effects of adenosine on renal function following 1 to 5 days of hypothermic perfusion of dog kidneys. Previous reports from this laboratory have shown that the addition of adenosine to our perfusate (17) stimulates ATP synthesis in hypothermically perfused kidneys. An analysis of the functional capabilities of cortical slices prepared from kidneys preserved for 5 days in the presence of adenosine indicated better K^+ transport, ATP synthesis, and reversal of tissue edema than in cortical slices from kidneys preserved in the absence of adenosine (16). Although controversy continues regarding the beneficial effect of ATP generating systems in organ preservation (13), these studies have relied on the transplant model; thus, the beneficial effects of ATP may be obscured when only survival or serum creatinine levels are determined following transplantation.

The results obtained in this study suggest that the adenosine stimulation of ATP synthesis during hypothermic perfusion of kidneys has a beneficial effect on renal function following reperfusion at normothermia including Na^+ reabsorption, ATP synthesis, and maintenance of near normal concentrations of tissue K^+.

MATERIALS AND METHODS

Adult mongrel dogs (15–25 kg) were used in this study. The methods for hypothermic (8–10°C) pulsatile perfusion (9) and reperfusion (14, 18) at normothermia (37°C) have been described. Hypothermic perfusion utilized a synthetic perfusate containing dydroxyethyl starch (American Critical Care, McGaw Park, Ill.) for colloid osmotic pressure (8). Two kidneys were perfused simultaneously in the presence or absence of adenosine (5 m*M*). To obtain high tissue concentrations of ATP after 5 days of perfusion, the perfusate (800 ml) was exchanged (after 3 days) with fresh perfusate (800 ml) to resupply adenosine to the kidneys. Perfusate exchange was also done in the 5-day studies without adenosine.

Normothermic isolated perfusion utilized a bovine serum albumin containing perfusate (7, 14) oxygenated with O_2/CO_2 (95/5%): pO_2 = 450 ± 50 mm Hg. Perfusion pressure was maintained at 100 mm Hg. Urine was collected in calibrated tubes after a 30-min equilibration period. Urine was collected after 40, 50, and 60 min of reperfusion and the results were combined and averaged over these three time periods.

Creatinine was determined in both the urine and perfusate by the picric acid method (Sigma Kit No. 555), Na^+ by flame photometry, cortical tissue adenosine nucleotides by high performance liquid chromatography (19), and tissue water and electrolytes as described previously (10).

Results reported are mean values ± SEM obtained from at least five kidneys in each group. Statistical analysis was performed using Student's *t* test.

RESULTS

The effects of 1–5 days of hypothermic perfusion in the presence or absence of adenosine on renal functions tested in the isolated perfused kidney are shown in Figs. 1A–C. The ability of the isolated perfused kidney to clear creatinine was decreased by 72% in kidneys perfused for only 1 day and the presence of adenosine had no effect on this function (Fig. 1A). After 3 days of hypothermic perfusion the GFR was further reduced (95% of control) under both conditions. After 5 days of perfusion, kidneys perfused in the presence of adenosine. This difference was, however, small and the GFR after 5 days of preservation is still about 90% reduced.

The capability of the hypothermically perfused kidney to form urine was decreased from about 16 to 12 μl/min,g at 1 day and 10 μl/min,g at 5 days when preserved in the absence of adenosine (Fig. 1B). The presence of adenosine had no ef-

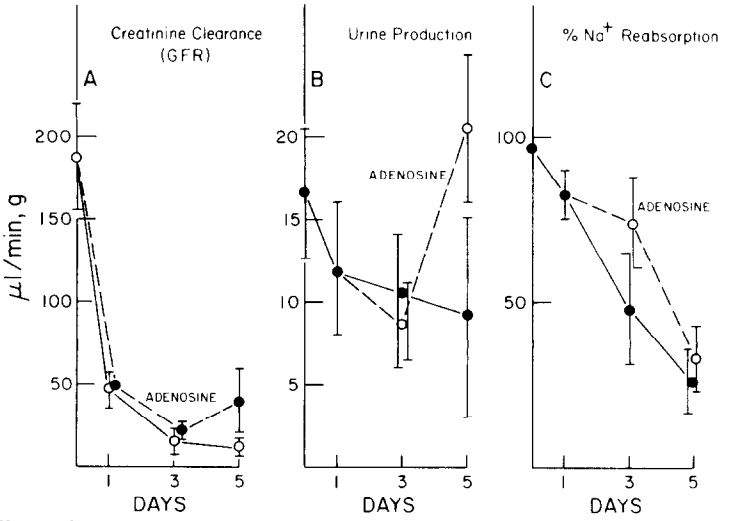

FIG. 1. Effects of hypothermic perfusion of dog kidneys on renal function. Methods are described under Materials and Methods. Dog kidneys were perfused for 1 to 5 days and reperfused at normothermia. Results shown are means ± SEM for GFR. Percentage Na^+ reabsorption at 3 days (+adenosine) was statistically different from 3 days (−adenosine) ($P < 0.05$).

fect on urine formation until 5 days of preservation. In the presence of adenosine the 5-day perfused kidney becomes slightly polyuric and urine formation averaged 20 μl/min,g. This adenosine stimulated urine formation may be the cause of the slight increase in GFR (Fig. 1A).

The reabsorption of Na^+ was practically normal in control, isolated perfused dog kidneys (97% reabsorption of the filtered Na^+) (Fig. 1C). After 1 day of preservation the reabsorption was decreased to about 80% (±adenosine). A difference in Na^+ reabsorption, however, occurred after 3 days of preservation. Kidneys preserved for 3 days in the presence of adenosine reabsorb Na^+ to an extent approximately equal to that observed after 1 day of preservation (80%). However, in the absence of adenosine the reabsorption of Na^+ was decreased to 50%. The increased capability of the kidney to reabsorb Na^+ when preserved in the presence of adenosine, seen at 3 days, was not observed after 5 days of preservation and Na^+ reabsorption was reduced to about 20–30% in kidneys preserved both in the presence and absence of adenosine.

Kidneys preserved in the presence of adenosine contain a high concentration of ATP at the end of preservation (17), and also regenerate high concentrations of ATP during isolated reperfusion (Fig. 2A). Kidneys preserved in the presence of adenosine for 5 days regenerate three times the concentration of ATP than in kidneys preserved for 5 days in the absence of adenosine. After 3 days of preservation, the difference was twofold.

The degree of tissue edema (total tissue water) was not affected by the presence of adenosine (Fig. 2C) and all kidneys remained slightly swollen following 1 hr of normothermic perfusion.

The concentration of tissue K^+ in the reperfused (IPK) kidney was consistently greater when kidneys were hypothermically perfused in the presence of adenosine (Fig. 2B) at all preservation periods studied. Normothermic perfusion of kidneys, following preservation for 1 day, resulted in a loss of tissue K^+ (280 mEq/kg dry wt for controls to 230 mEq/kg dry wt at 1 day). However, kidneys preserved in the presence of adenosine maintained near normal tissue concentrations of K^+ fol-

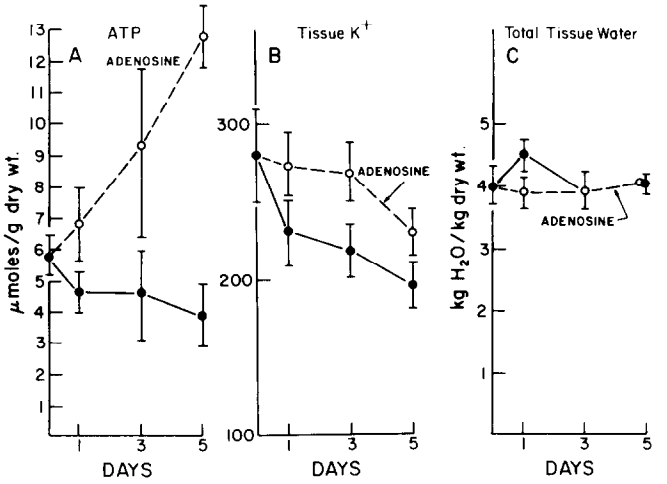

FIG. 2. Effects of hypothermic perfusion of dog kidneys on renal cell cortical tissue ATP (A), total tissue water (C), and K+ (B). Methods are described under Materials and Methods and Fig. 1. Results shown are means ± SEM. ATP and K^- concentrations = μmol/g dry wt and total tissue water = kg H_2O/kg dry wt. K^- content (+ adenosine) was statistically different from K^+ content (− adenosine) at all time periods ($P < 0.05$).

lowing both 1 and 3 days of preservation and 1 hr of reperfusion. After 5 days of preservation, kidneys perfused with adenosine lose K^+ (230 mEq/kg dry wt) but not to the same extent as those preserved in the absence of adenosine (200 mEq/kg dry wt).

DISCUSSION

In previous publications (14, 18) we have utilized the IPK to compare the effects of various preservation maneuvers on renal function in dog kidneys cold stored for 24 hr or perfused for 24 hr. Also, we have shown that results obtained using the isolated perfused dog kidney to measure initial renal function after 72 hr of perfusion preservation are similar to results obtained using the transplant model in which similar renal functions were observed. In these reports, we indicated that the IPK is an effective method to differentiate between the effect of various preservation maneuvers on initial renal function in dog kidneys, a conclusion supported by other studies using small animal models (2–5, 7). The study reported here illustrates the use of the IPK model to compare: (i) the effects of duration of preservation (1–5 days) on renal function and (ii) the effect of adenosine on renal function in kidneys hypothermically perfused for up to 5 days.

Kidneys preserved for 3 days under these conditions are fully viable and preservation-induced renal damage is rapidly reversed upon transplantation (8). However, kidneys preserved for 5 days under these conditions are not viable. Thus, this study provides information concerning the differences in renal function between potentially viable and nonviable kidneys. The cause(s) for loss of kidney viability during hypothermic preservation are not known. In a previous study (16) we presented data suggesting that the difference between potentially viable (3 day perfused) and nonviable kidneys (5 day perfused) was an increased permeability of the cells to electrolytes. These data were obtained using tissue slices from preserved kidneys. The results reported here also show that kidneys preserved for 3 days were more capable of maintaining higher tissue concentrations of K^+ during reperfusion at normothermia, especially when adenosine was included in

the preservation fluid, than kidneys preserved for 5 days. Thus, long-term preservation appears to affect membrane-related functions.

Another difference between potentially viable and nonviable kidneys was the effect of long-term preservation on Na^+ reabsorption. After 3 days of preservation Na^+ reabsorption was about 50% of the filtered load whereas after 5 days of preservation it was reduced to about 25%. These results also suggest that electrolyte transport was more disturbed in nonviable than viable kidneys and that Na^+ reabsorption may be a good indication of renal viability. A similar conclusion has been suggested by Fuller and Pegg (6). However, without directly correlating Na^+ reabsorption with actual transplantation and survival studies such a conclusion is only suggestive and not yet definitive.

The results reported here also demonstrate a beneficial effect of including adenosine in the preservation medium during continuous hypothermic perfusion of dog kidneys. In kidneys preserved in the absence of adenosine, cortical tissue concentrations of ATP are slightly lower than control concentrations at the end of reperfusion (control = 6 vs 4–5 μmol/g dry wt). Also, kidneys preserved in the absence of adenosine are unable to restore tissue K^+ to control concentrations and the degree of loss of K^+ is dependent upon the length of preservation. The beneficial effect of adenosine on renal functions was observed in kidneys preserved for 3 days and included an increased capacity of the isolated perfused kidney to reaccumulate filtered Na^+, regenerate ATP, and maintain near normal concentrations of tissue K^+. Each of these determinants of renal function is related to energy metabolism and indicate that the adenosine stimulation of ATP synthesis during hypothermic perfusion is beneficial to conserving these energy requiring reactions. Following 5 days of preservation, there was no apparent beneficial effect of adenosine on physiological functions of the kidney. However, adenosine as the perfusate did stimulate ATP resynthesis and conservation of tissue K^+.

These results suggest that although ATP is beneficial to kidneys during hypothermic perfusion, the presence of ATP alone does not guarantee good quality function following long-term preservation (5 days). Thus, other changes in either renal metabolism or cell morphology occur during long-term preservation that compromise the ability of the kidney to survive. Some changes that may be induced during 5 days of preservation that may affect kidney survival include an activation of lysosomal enzymes (12) lipid peroxidation (11), membrane disruption (15), or other as yet unidentified change that affect viability.

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