Changes of Renal ATPase Enzymes in Different Types of Kidney Preservation

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Summary
After 24-hr storage of canine kidneys with extracellular or intracellular (Ursol) solutions, the cortical and medullary renal ATPase enzymes (total Na⁺ + K⁺ and Mg-ATPase, Na⁺ + K⁺ -ATPase, and Mg-ATPase) were examined. It was found that storage with extracellular solution decreased all cortical enzymes. This was not the case with intracellular solution or in kidneys cooled and stored without any solution. A decrease in the potassium concentration of the Ursol solution decreased cortical (Na⁺ + K⁺)-ATPase enzymatic activity. The medullary enzymatic changes were similar in the different groups, and lower than in unstored controls.

It appears that the changes on the level of the ATPase enzyme system which is related to the cation transport system might play a significant role in explaining the different results seen in clinical or experimental renal preservation systems. These changes can be related to the injury of preservation due to environmental effects of the cation concentrations and to a lesser degree to the damage of the enzyme system which provides the energy for cation transport.

Introduction
Electrolyte and water disturbances occurring during hypothermic preservation seem to play important roles in kidney function after preservation (Collins et al, 1969a; Keeler et al, 1966). The minor to severe cellular derangements seen with kidney preservation could be related to the function of the sodium pump system of the cell membrane which, in normal conditions, is responsible for accumulating potassium (K) and expelling sodium (Na) from the cells (Bonting, 1970; Collins et al, 1969a; Keller et al 1966). The function of the sodium pump is closely related to the enzymatic (Na⁺ + K⁺)-ATPase system which is an energy-dependent cation transport system (Bonting, 1970). However, the changes in that system in
different types of kidney storage are not understood. Indirect evidence of the function of the enzymatic system is shown by the loss of potassium in the kidneys preserved with extracellular-type solution as compared with that of intracellular solution (Collins et al, 1969a; Keeler et al, 1966). In the present study the effect of renal storage with extracellular- or intracellular-type solutions of the ATPase enzymatic system is examined. The changes of cortical or medullary or total (Na⁺ + K⁺) and Mg-ATPases will be discussed further in connection with the renal metabolic changes.

MATERIALS AND METHODS

Forty-nine kidneys from healthy mongrel dogs weighing between 15–20 kg, were used in the study. Solutions used for renal storage were: (1) extracellular solution (Ringer's lactate); (2) Ursol solution, which resembles Collins's solution — with the difference that the magnesium sulphate concentration has been reduced by half and the procaine concentration has been doubled (Varkarakis, et al., 1973); and (3) Ursol solution with a 50% reduction in potassium concentration.

For anaesthesia, pentobarbital 30 mg/kg was used intravenously. After nephrectomy the kidneys were perfused with 150 ml of the storage solution, then placed in an enamelled metal container with enough solution to cover the organ. The container was placed in a refrigeration unit and maintained at 4–8°C for 24 hr. After storage for 24 hr enzymatic studies for ATPase were conducted using cortical and medullary renal tissue. The same enzymes were also measured in cortical and medullary renal tissue in 12 unstored control kidneys (Group A). The stored kidneys used in the study were: Group B; eight kidneys perfused with 150 ml of Ringer's lactate and stored without solution: Group C; seven kidneys perfused and stored in Ringer's lactate: Group D; ten kidneys perfused and stored in Ursol solution: Group E; twelve kidneys perfused and stored in Ursol solution with decreased potassium concentration. Kidney slices were homogenised in a ‘Polytron’ homogeniser.

The method used for estimation of the different ATPase enzymatic activities has been described previously (Kimelberg and Papahadjopoulos, 1974). The results were expressed as μmoles phosphate produced per gramme of wet tissue per hour.

RESULTS

Cortical total ATPase was decreased in kidneys stored in extracellular solution (Group C) (p < 0.005) but not in kidneys stored in intracellular solutions (Groups D and E) or in kidneys perfused with extracellular solution and stored
in containers without solution (Group B) (p < 0.5) (Figure 1). The renal medullary total ATPase tissue levels were decreased in all stored kidneys (p < 0.05).

Figure 1. Changes of total ATPase in kidneys. Total cortical ATPase was decreased in group C, while medullary ATPase was decreased in all stored groups. Details of the various groups are given in the text.

Figure 2. Changes of (Na\(^+\) + K\(^+\)) ATPase in kidneys. Sufficient decrease of cortical (Na\(^+\) + K\(^+\)) ATPase was noted in groups C and E (p < 0.025). In all groups medullary (Na\(^+\) + K\(^+\)) ATPase enzymes were unchanged. Details of the various groups are given in the text.

Kidneys cooled and stored without any solution (Group B) had relatively unchanged cortical (Na\(^+\) + K\(^+\)) ATPase levels (p > 0.4). In contrast, storage of kidneys in intracellular solution with decreased initial potassium concentration
(Group E) and kidneys stored in extracellular solution (Group C) had a significant decrease in their cortical \((\text{Na}^+ + \text{K}^+)-\text{ATPase}\) levels \((p < 0.025)\) (Figure 2). Kidneys stored in Ursol solution (Group D) showed some decrease in these levels \((p > 0.5)\). On the other hand, medullary \((\text{Na}^+ + \text{K}^+)-\text{ATPase}\) levels were not significantly changed in any of the stored kidneys as compared to unstored kidneys \((p > 0.4)\).

Cortical Mg-ATPase levels were unchanged in kidneys stored in intracellular solutions \((p > 0.5)\) (Groups D and E) or in kidneys stored without solution (Group B) but decreased significantly in stored kidneys with Ringer’s lactate solution (Group C) \((p < 0.005)\) (Figure 3). In contrast, medullary Mg-ATPase levels were decreased significantly in all stored kidneys \((p < 0.001)\).

![Renal Cortex and Renal Medulla](image)

Figure 3. Changes of Mg**ATPase in kidneys. Cortical Mg-ATPase was decreased in kidneys stored in extracellular solution \((p < 0.05)\). Medullary Mg-ATPase was decreased in all stored \((p < 0.01)\). See text for details of groups.

**DISCUSSION**

The maintenance of transport of relatively high potassium with low sodium into the cells seems to play an important role in renal preservation. Under normal conditions there is a passive leakage of both ions down their electrochemical gradients, while in addition, a pump system draws potassium ions into the cell and extrudes sodium ions out of the cell against these gradients (pump-leak concept) (Bonting, 1970). The first action is passive, not requiring metabolic energy, while the pump function requires the hydrolysis of ATP.

During hypothermia and preservation with extracellular solutions, potassium and magnesium leak out of cells (Keeler et al, 1966; Leonard and Scribner, 1971)
while during preservation with intracellular solution this is less evident. This feature was claimed as a reason for the success of the latter method in experimental or human preservation (Collins et al., 1969a; Collins et al., 1969b).

In the present studies, the changes in the levels of the enzyme system responsible for the active cation movement across the cell membrane have been followed during storage under different conditions. It was shown that all the cortical ATPase enzymes are decreased in kidneys stored for 24 h with hypothermia and extracellular solution. It seems that it is storage of kidneys in an extracellular environment, rather than cold preservation, which plays a role in the decreased activity of ATPase enzymes. Thus kidneys which were washed out with Ringer’s lactate and stored without any solution did not change in any of the examined cortical ATPase enzymes. In contrast, when Ursol, was used during storage, it was found that the ATPases were not different from those of the unstored kidneys of normal dogs.

The decreased activity of the cortical ATPases seen with kidneys stored in Ringer’s lactate solution (Group C) may be due to damage due to storage or poor availability of ATP, which is provided mainly by oxidative metabolism (Lee and Peter, 1969). We have shown previously that kidneys preserved for 24 h with extracellular solution have unchanged oxygen consumption (Varkarakis et al., 1973). It has been shown that kidneys stored in Ringer’s lactate have decreased total, cortical, and medullary blood flow (Burdick et al., 1973). It is possible that these derangements can be reversed with extracellular solution preservation of less than 12-hr duration (Pegg et al., 1964).

The unchanged cortical ATPase enzymes with the Ursol solution seen in this study are in agreement with the unchanged oxidative metabolism also seen in similarly stored kidneys (Varkarakis et al., 1973). The renal cortex is considered to be a site for aerobic metabolism (Lee and Peter, 1969). On the other hand cortical anaerobic metabolism, which is decreased in kidneys stored in intracellular-type solutions (Varkarakis et al., 1973), is not normally important as an energy source (Lee and Peter, 1969). The decreased cortical and medullary blood flow seen in similarly preserved kidneys in a previous study did not relate to the enzymatic system examined in this study (Burdick et al., 1973).

The decreased medullary total, or Mg-ATPase enzymes, seen in all stored kidneys, may result from disorders of the vascular system seen in kidneys stored in intracellular and extracellular solutions (Burdick et al., 1973) or to energy deficiency (Varkarakis et al., 1973). Anaerobic glycolysis is decreased in kidneys stored in intracellular solution and unchanged in kidneys stored in extracellular solution (Varkarakis et al., 1973).

A recent study on the effects of hypothermic storage on the levels of membrane ATPases in kidney slices indicated a significant decrease in the level of (Na⁺ + K⁺)-ATPase following 48-hr storage in Ringer’s buffer at 4°C (States et al., 1972). On the other hand, it was shown in the same study that the same slices were unimpaired in their ability to accumulate potassium and extrude sodium.
It is thus not clear at present whether the observed decrease in the ATPase following storage in extracellular solutions represents irreversible damage to the enzyme system. Further, the lack of a substantial difference between the enzyme levels in control kidneys as compared to those stored simply at 4°C indicates that the activity of these enzymes might not be the most sensitive indicator for optimal conditions of renal preservation. In any case, the observed differences in cortical enzyme levels between kidneys stored in intracellular solutions and those stored in extracellular solutions do suggest that the preservation solutions originally proposed by Collins et al., (1969b) are desirable in relation to the activity of the membrane transport enzymatic systems.

References

Burdick, J, Varkarakis, M J and Murphy, G P (1973) Journal of Medicine, 4, 202
Collins, G M, Bravo-Shugarman, M and Terasaki, P I (1969b) Transplantation, 8, 821
Leonard, C D and Scribner, B H (1971) Cryobiology, 8, 290
Pegg, D E, Calne, R Y, Pryse-Davies, J and Brown, F L (1964) Annals of the New York Academy of Sciences, 120, 506
Varkarakis, M J, Sampson, D, Abramczyk, J and Murphy, G P (1973) American Surgeon, 39, 295