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THE EFFECT OF STORAGE AT 4° ON AMINO ACID TRANSPORT BY RAT KIDNEY CORTEX SLICES

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SUMMARY

Amino acid transport was investigated in stored kidney tissue. Kidney cortex slices and whole kidneys stored at 4° in Krebs–Ringer–bicarbonate buffer under oxygen, actively concentrated lysine, arginine, α-aminoisobutyric acid, and valine at the same rate as in fresh tissue. Periods of storage up to 8 days resulted in a gradual diminution of uptake for all the amino acids studied except lysine. The time curve of uptake for α-aminoisobutyric acid and lysine in slices stored for 24 h was identical to that of fresh tissue. Other characteristics of active transport of the amino acids tested—exchange diffusion, inhibition of uptake by lack of O_2 and Na^+-deficient media—were also the same in slices stored for 24 h as in fresh kidney slices.

Slices made from whole kidneys stored at 4° for 24 h also exhibited active accumulation of amino acids and exchange diffusion. Electron micrographs showed that renal proximal tubular cells, after storage for 24 h or storage and subsequent incubation for 1 h at 37°, appeared normal, with intact brush border membranes, normal mitochondria, and normal basal membranes. These studies indicate that the intricate functioning of the cell membrane with regard to amino acid transport is present in the stored renal slices.

INTRODUCTION

Some biochemical functions have recently been studied in stored renal tissue. Low temperatures during storage lessen the metabolic activity and O_2 demands of renal tissue[1–3]. Despite this, after storage at 4° only a small percentage of kidneys resume function on subsequent transplantation[4–7]. There has been no analysis of the effect of storage on specific membrane processes in renal tissue, processes which are pertinent not only to biochemistry but to transplantation biology. The present study reports the effect of storage at 4° on a specific transport function in the kidney, that of amino acid transport.

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METHODS AND MATERIALS

Incubation methods

Male Sprague–Dawley rats, weighing 150 g, were killed by stunning and decapitation. Their kidneys were removed and cortical slices, 0.4 mm thick, were cut with a Stadie–Riggs tissue slicer. 6–8 slices, weighing 20 mg each, were placed in flasks with 5 ml of Krebs–Ringer–bicarbonate buffer lacking glucose, were gassed with O_2–CO_2 (95:5, v/v) stoppered and stored at 4° without shaking. After time periods up to 8 days, 3 slices, one from the stored kidneys of each of 3 rats, weighing a total of 60 mg, were incubated together at 37° under O_2–CO_2 (95:5, v/v) in 25-ml plastic flasks, with 2 ml of Krebs–Ringer–bicarbonate buffer and the appropriate ^{14}C-labeled amino acid. The tissue amino acid concentration and calculation of the distribution ratio, the ratio of the concentration of the amino acid in the intracellular fluid to that in the medium were determined by previously reported methods[8]. Anaerobiosis was maintained with N_2–CO_2 (95:5, v/v) and Tris buffer was substituted for NaCl to make Na^+-deficient media[9]. Exchange diffusion experiments were performed as reported previously[10]. In some of the experiments, whole kidneys were placed in 5 ml Krebs–Ringer–bicarbonate buffer, gassed with O_2–CO_2 (95:5, v/v), and stored at 4°. The kidneys were removed from storage at successive time periods; slices from the kidneys were used in the experiments. Sterile precautions were not taken during preparation of the tissues. Several experiments were performed adding 100 mg of streptomycin and 100 units of penicillin to each ml of storage medium.

For light microscopy, cortical slices were fixed in 10% neutral phosphate-buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. For electron microscopy the slices were fixed in 3% phosphate-buffered glutaraldehyde, washed in phosphate-buffered saline and post-fixed in 1% phosphate-buffered osmic acid. After dehydration in graded ethanol they were embedded in epoxy resin. Thin sections were stained with lead citrate and uranyl acetate.

RESULTS

Change of inulin space with storage

Inulin space, which is 27% of the wet tissue weight in fresh cortical slices[11] was found to increase to 35% of wet tissue weight in slices stored 24 h, 37% at 48 and 72 h and 49% at 8 days. At the same time total tissue water changed only slightly (80% of wet tissue weight to 82% wet tissue weight).

Effect of storage on amino acid accumulation

All amino acids tested were actively accumulated by slices that were stored for 24 h and then incubated for 1 h at 37°. The distribution ratio was the same in stored as in fresh tissue (Fig. 1). The distribution ratio of arginine accumulation averaged 3.9 in fresh tissue and remained at this level for 48 h. It then fell gradually, until on day 8 it averaged 2.8. The distribution ratio for α-aminoisobutyric acid accumulation began at 3.25 in normal tissue and fell gradually during the 8-day storage period until on the 8th day the distribution ratio was 1.25. Valine uptake also fell gradually until on the 8th day it was accumulated passively by diffusion. Lysine accumulation nearly equaled that in fresh tissue for all of the 8-day storage period.

Following a 24-h storage period, the time curve of α-aminoisobutyric acid uptake was similar to that of fresh tissue (Fig. 2). Equilibrium was reached within 60 min on both the fresh and the stored tissue. The time curve of lysine uptake was similar

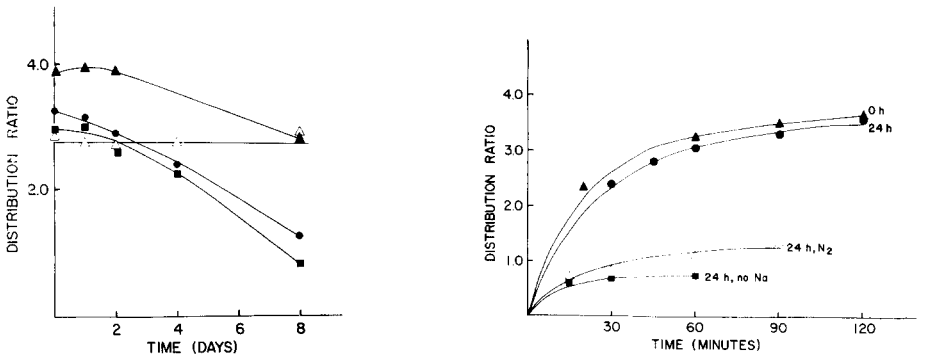

Fig. 1. The effect of storage on amino acid uptake. Slices of renal cortex, weighing 20 mg each, were stored in Krebs–Ringer–bicarbonate buffer at 4° for varying time periods, then incubated in fresh Krebs–Ringer–bicarbonate buffer at 37° for 1 h, with 0.2 μC of one of the following ^{14}C-labeled amino acids, final concn. 65 μM: ▲, arginine; ●, α-aminoisobutyric acid; ■, valine; or △, lysine. The uptake of the amino acid into the slices was measured. Each point represents the mean value of 9–18 rats. Ordinate: ratio of intracellular to extracellular concentration of the labeled amino acid (distribution ratio). Abscissa: time of storage (days) at 4° prior to incubation.

Fig. 2. Uptake of α-aminoisobutyric acid in stored renal cortical slices. Slices of renal cortex, weighing 20 mg each, were stored in Krebs–Ringer–bicarbonate buffer at 4° for 24 h. They were then incubated for varying time periods at 37° in 2 ml fresh Krebs–Ringer–bicarbonate buffer (pH 7.4), gassed with O_2–CO_2 (95:5, v/v) (●), in buffer with the Na^+ replaced by Tris (■) or in buffer gassed with N_2–CO_2 (95:5, v/v) (△). The results are compared with fresh tissue incubated in Krebs–Ringer–bicarbonate buffer (▲). Each flask contained 0.2 μC aminoisobutyric acid, final concn. 65 μM. Each point represents the mean value of 9–18 rats. Ordinate: ratio of intracellular to extracellular concentration of labeled aminoisobutyric acid (distribution ratio). Abscissa: time of incubation (min).

in both fresh and stored tissue (Fig. 3). After 48-h storage, the time curve for lysine uptake was slightly lower than the uptake at 24 h.

Anaerobiosis

Anaerobiosis and Na^+ lack are known to be effective inhibitors of the uptake of α-aminoisobutyric acid in fresh slices of renal cortex[8,9]. Slices of cortex, stored for 24 h, were tested for α-aminoisobutyric acid accumulation in O_2-deficient or Na^+-deficient media. As is shown in Fig. 2, lack of Na^+ and O_2 abolished the active accumulation of α-aminoisobutyric acid. The distribution ratios did not approach normal at any point on the time curve.

Exchange diffusion

Exchange diffusion, a characteristic of carrier-mediated transport, was studied in stored slices by determining the uptake of lysine into pre-loaded and unloaded cells. Exchange diffusion, as manifested by an increase in the distribution ratio in cells pre-loaded with lysine, was present in slices stored for 24 and 48 h (Table I). The distribution ratios for pre-loaded or the control slices were similar to those of fresh slices (Fig. 4). The peak of active accumulation in the stored, pre-loaded slices

occurred 20 min after the onset of incubation. The distribution ratios of both the pre-loaded and unloaded slices became similar after 60 min of incubation. These data are identical to previously published studies on fresh cortical slices[10].

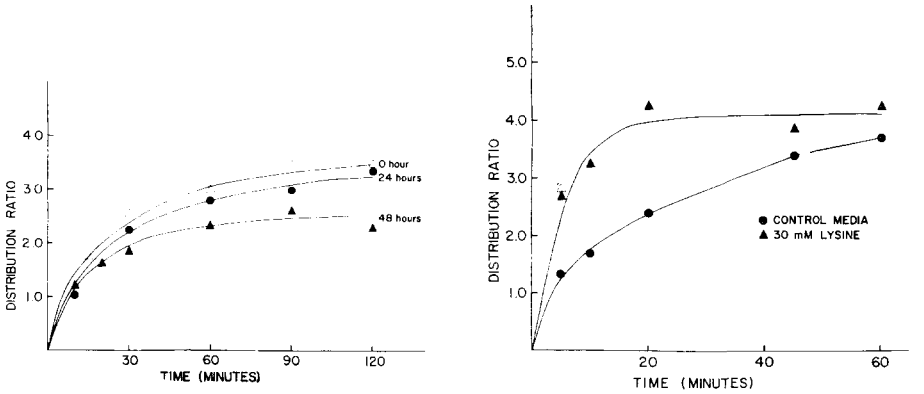

Fig. 3. Uptake of lysine by stored renal cortical slices. Slices of renal cortex, weighing 20 mg each, were stored in Krebs–Ringer–bicarbonate buffer at 4° for 24 h (●) or 48 h (▲), then incubated in 2 ml fresh Krebs–Ringer–bicarbonate buffer (pH 7.4) under O_2–CO_2 (95:5, v/v) for various time periods up to 120 min. Each flask contained 0.2 μC of [^{14}C]lysine, final concn. 65 μM. Each point represents the mean value of 9–18 rats. Ordinate: ratio of intracellular to extracellular concentration of labeled lysine (distribution ratio). Abscissa: time of incubation (min). △, fresh slices incubated without prior storage.

Fig. 4. Exchange diffusion of lysine in stored kidney slices. Slices of renal cortex, weighing 20 mg each, were stored in Krebs–Ringer–bicarbonate buffer at 4° for 24 h. They were then placed in either fresh Krebs–Ringer–bicarbonate buffer or the buffer with lysine added to a final concentration of 30 mM. The tissues were preincubated at 37° under 95% O_2–5% CO_2, for 30 min, transferred to flasks containing 2 ml Krebs–Ringer–bicarbonate buffer *plus* 0.2 μC [^{14}C]lysine, final concentration 65 μM and incubated for the times indicated. Each value represents the average of 9 rats, pooled into groups of 3. Ordinate: ratio of intracellular to extracellular concentration of labeled lysine. Abscissa: time of incubation (min). ●, tissues preincubated in Krebs–Ringer–bicarbonate buffer; ▲, preincubated in 30 mM lysine; △, value of fresh tissue preincubated in 30 mM lysine media; ○, value of fresh tissue preincubated in Krebs–Ringer–bicarbonate buffer control media.

TABLE I

EXCHANGE DIFFUSION OF LYSINE IN STORED TISSUES

Kidneys were stored whole or as slices 0.4 mm thick, weight 20 mg, in Krebs–Ringer–bicarbonate buffer at 4° for 24 h. The whole kidney was removed and slices were made. These and the stored slices were placed in Krebs–Ringer–bicarbonate buffer, the control media, or Krebs–Ringer–bicarbonate buffer loaded with 30 mM lysine. The tissues were incubated at 37° in O_2–CO_2 (95:5, v/v) for 30 min, then incubated for 5 min in flasks containing the respective control media *plus* 0.2 μC of [^{14}C]lysine at 65 μM. The initial uptake of 30 mM lysine was determined by incubating slices for 30 min in 30 mM lysine with 2 μC of [^{14}C]lysine added. Each value represents the average of 9 rats, pooled into groups of 3.

Time of storage (h)	*Whole kidney*			*Slices*		
	Initial uptake (mM lysine)	*Distribution ratio*		*Initial uptake (mM lysine)*	*Distribution ratio*	
		Control	*Loaded*		*Control*	*Loaded*
0	60	1.07	4.02	60	1.07	4.02
24	53.4	2.43	4.35	70.1	1.01	2.69
48	31	1.14	1.96	77.5	1.34	4.60

Amino acid transport in stored whole kidney

The uptake of several amino acids was measured in kidneys stored whole in Krebs–Ringer–bicarbonate buffer, and then sliced immediately prior to the incubation period. After 24 h of storage, the active accumulation of valine, cycloleucine, and α-aminoisobutyric acid were slightly less than in fresh tissue slices (Table II). After further periods of storage, the accumulation of valine continued to diminish as it had in the stored slices. Lysine uptake remained similar in stored and fresh tissue.

TABLE II

ACCUMULATION OF AMINO ACIDS IN SLICES OF WHOLE KIDNEY

Whole kidneys were placed in 5 ml Krebs–Ringer bicarbonate buffer, lacking glucose, at 4° for various storage periods. They were then removed and incubated in 2 ml of Krebs–Ringer–bicarbonate with added 0.2 μC of ^{14}C amino acids, at a final concentration 65 μM. The distribution ratio was calculated as the ratio of concentration of labeled amino acid in the intracellular fluid to that in the extracellular fluid. Each value represents the average of 9–18 rats, pooled in groups of 3.

Time of storage (h)	*Distribution ratio*				
	Valine	*Lysine*	*Arginine*	*Cycloleucine*	*α-Amino-isobutyric acid*
0	2.94	2.80	3.90	2.51	3.25
24	1.61	2.81	3.95	1.73	2.57
48	1.94	2.87			
72	1.30	2.67			

Exchange diffusion of lysine was also present in whole kidney stored for 24 h as it was in stored slices. However, after 48 h of storage, the amount of lysine exchanged fell by 50%.

Morphology of stored slices

After 24 h of storage little change from normal was observed in the proximal tubules under light microscopy but the distal tubules and Henle's loops exhibited some loss of cytoplasm. Subsequent incubation of the stored tissues for 1 h at 37° resulted in reticulated cytoplasm but little evidence of necrosis could be found. After 48-h storage, focal necroses were present.

Electron micrographs of tissue slices stored for 24 h showed little change in the proximal tubules (Fig. 5). The brush border and the junctional complex appeared normal, as did the mitochondria. The interspaces between the infoldings of the plasma membranes were widened and the endothelia of the capillaries was lost. Subsequent incubation of the stored slices for 1 h at 37° resulted in swollen mitochondria and protoplasmic feet, adjacent to the basal membrane, in a number of cells of the proximal tubules (Fig. 5).

The fine structure of the majority of the tissues stored for 48 h showed gross alterations. The cell was in an advanced necrotic stage. The microvilli of the brush border, mitochondria and all of the orderly fine structure of the cytoplasma had disappeared.

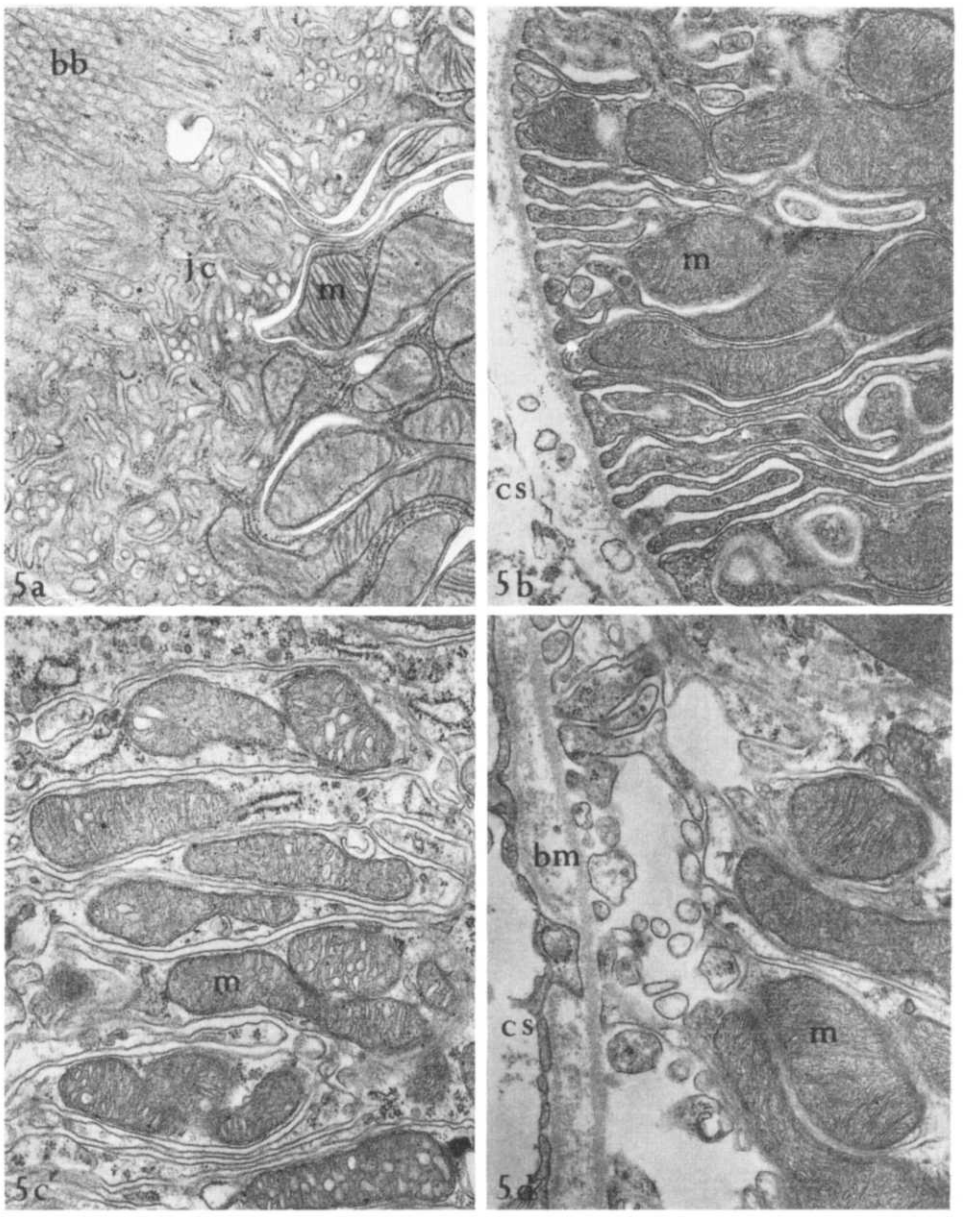

Fig. 5. A. Cell of convoluted proximal tubule. Control tissue. Brush border (bb), junctional complex (jc), and mitochondria (m) appear normal. Magnification 18000 ×. B. Proximal tubule after 24-h storage (4°). Some cells show a widening of interspaces between infoldings of plasma membrane and loss of endothelium. The capillary space appears at lower left (cs). Magnification 20000 ×. C and D. Proximal tubule after 24-h storage (4°), and incubation for 1 h at 37°. Changes in mitochondria and protoplasmic processes are apparent. The mitochondria are distorted with widened christae (C). Pseudopods adjacent to the basal membrane (bm) show gross alterations (D). Magnification 20000 ×.

Sterility

Sterile precautions were not taken during storage and the question arose if the amino acid uptake occurred in bacteria rather than in the stored tissue slices. However, colony counts on saline extracts of the stored slices were negligible (1 colony/50 mg of tissue) in tissues (1) stored at 4°, and (2) in tissues stored at 4° without antibiotics then incubated at 37° for 1 h. In several experiments the tissues were stored in media containing antibiotics. The amino acid uptake in slices stored in antibiotic media did not differ from that of slices stored in regular media without antibiotics.

DISCUSSION

The uptake of amino acids probably occurs *via* the same transport systems in both stored and fresh tissue. This was suggested by the present experiments in which the inhibitors of amino acid uptake in fresh tissue, anaerobiosis and Na^+-deficient media, affected amino acid uptake in stored tissues in the same manner as in fresh tissue. Also, exchange diffusion, one of the characteristics of carrier-mediated transport, was similar in both fresh and stored renal tissue. The decrease in active accumulation with increasing storage time may represent either a general metabolic deterioration of the slices or a specific membrane defect, starting with an increased efflux of the amino acid. It is of interest that the storage media did not contain glucose nor any energy-yielding substrate like succinate, so that the slices survived by utilizing their own energy stores.

ROCHMAN *et al.*[1] and others have found that the O_2 consumption of kidney slices fell to 68 % of normal after storage at 4–12° for 24 h. This diminished O_2 consumption has been attributed to changes in permeability of the mitochondrial membrane[12] or to loss of respiratory enzyme cofactors[13]. It may also reflect the lessened metabolic demands at 4° since renal slices stored at 38° will deteriorate within a few hours. Even greater than the fall in oxygen consumption is the fall in the phosphate esterification in whole kidneys stored under nitrogen for 24 h (ref. 14), indicating that specific metabolic functions may be differentially affected by the storage period.

It may be possible to characterize and separate the different transport systems of groups of amino acids by storage of renal cortex since each of the amino acid transport systems studied had a different rate of deterioration under storage. The uptake of valine and α-aminoisobutyric acid, neutral amino acids, were both markedly affected by storage while the two basic amino acids, arginine and lysine were less affected. Although lysine accumulation by cortical slices is resistant to anaerobiosis and Na^- lack[15] thus differentiating its transport characteristics from those of neutral amino acids, it seems rather amazing that there are normal concentration gradients formed after prolonged tissue storage. This is especially significant when microscopy reveals onset of necrosis at 48-h storage. The possibility must be entertained that the accumulation of lysine by necrotic tissue slices after 8 days of storage may be due to binding of the amino acid and not to active transport. Experiments with slices from whole kidneys stored 48 h indicate marked diminution of exchange diffusion properties of the membrane which are thought to be indicative of a mobile carrier (Table I) despite the fact that a normal concentration gradient is obtained (Table II). Further study of the dibasic amino acid uptake on prolonged storage of tissues appears indicated.

Besides contributing to the understanding of the metabolic function of stored tissue related to transplantation the present findings have important implications for the study *in vitro* of kidney cortex from patients or animals with transport diseases. The fact that kidney cortex slices can be stored at 4° for 24 h and still maintain histologic and transport integrity makes it possible for transport experiments to be performed in kidney cortex obtained in particular animals or human patients after shipment to centers performing these studies.

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