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PRESERVATION OF AMINO ACID ACCUMULATION BY FROZEN RENAL CORTICAL TISSUE

PHILIP G. HOLTZAPPLE,* WILLIAM BUCHANAN and STANTON SEGAL

Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, Philadelphia, Pa, and Department of Pediatrics, Medical School of the University of Pennsylvania, Philadelphia, Pa., U.S.A.

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Abstract—Accumulation *in vitro* of α-aminoisobutyric acid by rat renal cortical slices was studied in the presence of cryoprotective agents. The uptake was normal in the presence of 15% dimethylsulfoxide, but was impaired by 15% glycerol. In the presence of dimethylsulfoxide, cortical slices could be frozen to −30° and thawed with no impairment of α-aminoisobutyric acid accumulation. Freezing to −70° was associated with a reduction of amino acid uptake. Assessment of membrane transport phenomena may be applied as a viability assay of successful preservation of frozen renal tissue.

PRESERVATION of cellular function by freezing has been the object of many studies. The ultimate goal of many of these studies has been the preservation *in vitro* of whole organs for transplantation. However, the most reproducible studies involving viable tissue have been those of cellular suspensions to which have been added a low molecular weight additive designed to reduce cellular and membrane susceptibility to the effects of high electrolyte concentrations during the freezing process. Extension of the optimal conditions defined for freezing and thawing of cellular suspensions are not always applicable to the successful preservation of frozen tissues or whole organs.[1]

Because of the increasing experimentation of organ preservation by storage and freezing methods, we have attempted to evaluate the effects of freezing and the addition of two known cryoprotective agents on the preservation of cellular membrane function in renal cortex. Our results indicate that membrane function as assayed by intracellular accumulation of amino acids can be maintained after freezing to −70°.

METHODS

Male Sprague–Dawley rats weighing 150–200 mg were sacrificed by stunning and decapitation. Renal cortical slices weighing 10–20 mg were prepared by a Stadie–Riggs microtome following removal of the kidneys. The technique for the study of amino acid uptake in renal cortical slices with assessment of intracellular and extracellular radioactivity has been described in detail elsewhere.[2,3] Total tissue water was determined by the difference between the tissue weight after blotting on filter paper and the weight after drying at 105° for 24 hr in a vacuum oven. Extracellular fluid space was estimated with [^{14}C]inulin as previously described.[4] The Krebs–Ringer bicarbonate (KRB) buffer containing either dimethylsulfoxide (DMSO) or glycerol was prepared so that the ionic concentration and pH were equal to that of the Krebs–

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Ringer bicarbonate buffer without the added cryoprotective agent. Renal cortical slices were equilibrated in buffer alone or in buffer containing the cryoprotective agent at room temperature for 30 min prior to the incubation and uptake of amino acid at 37° in a Dubnoff shaker.

In the freezing experiments, slices from 3 separate rats were placed in a polyethylene flask with 2 ml of the Krebs–Ringer bicarbonate buffer containing the cryoprotective agent; the flask was gassed with 95% O_2–5% CO_2, stoppered, and the contents were frozen at a freezing rate of 1°/min in a Linde biological freezer, model 1 BF 3-R. Upon reaching −70°, the flask was removed from the freezer, the media and tissue were rapidly rewarmed to 37° in less than 1 min, 0·2 μc of α-[^{14}C]aminoisobutyric acid was added to the flask (final concentration 0·065 mM), which was then re-gassed with 95% O_2–5% CO_2 and incubated at 37°. The assessment of radioactive amino acid uptake was then carried out in a similar fashion to unfrozen tissues. The data are expressed as the distribution ratio, i.e. the ratio of the counts per minute per milliliter of intracellular fluid to the counts per minute per milliliter of incubation media. A distribution ratio greater than one represents accumulation of radioactivity against a concentration gradient.

Alpha [^{14}C]aminoisobutyric acid (AIB) and [^{14}C]inulin were obtained from the New England Nuclear Corp. Unlabeled α-aminoisobutyric acid was obtained from the Mann Research Laboratories. Both compounds were chromatographically pure by paper chromatography. Analytical grade dimethylsulfoxide (DMSO) and glycerol were obtained from Fisher Scientific Products.

RESULTS

Initially the effects of the cryoprotective agents upon the parameters of total tissue water, extracellular fluid space and amino acid uptake in non-frozen cortical slices were examined. In Table 1 the effects of dimethylsulfoxide and glycerol upon the percentage wet weight and extracellular fluid space are shown. There is no significant difference in the total water content of slices equilibrated either in 15 or 20% DMSO in KRB buffer or in KRB buffer alone. There is a significant reduction of tissue water in cortical slices equilibrated in 5, 15 or 20% glycerol in KRB buffer.

The effect of the cryoprotective agents on the distribution of the tissue water in the renal slices is also shown in Table 1. The inulin distribution is expressed as the percentage of the total tissue weight. The extracellular fluid space is reduced in cortical slices equilibrated in either dimethylsulfoxide or glycerol buffer. The extracellular fluid space in slices incubated in 15% DMSO or glycerol is 18 per cent of the total tissue weight compared to 28 per cent for control slices.

The accumulation of α-aminoisobutyric acid is shown in relation to various times of incubation in Fig. 1. There is no difference in tissue uptake of α-aminoisobutyric acid by renal cortical slices for short term periods of incubation in 15% DMSO–KRB buffer when compared to slices incubated in control KRB buffer. The steady state attained is not different from control uptake. In contrast, both the early uptake and the steady state accumulation of α-aminoisobutyric acid is reduced in renal cortical slices incubated in 15% glycerol–KRB buffer.

A Lineweaver–Burk plot of the reciprocals of the substrate and the velocity of uptake for control slices and slices incubated in 15% glycerol–KRB buffer is shown in Fig. 2. The apparent K_m of the uptake system for α-aminoisobutyric acid is the same

TABLE 1. EFFECT OF DIMETHYLSULFOXIDE-GLYCEROL ON TISSUE WATER AND EXTRACELLULAR FLUID SPACE IN RAT RENAL CORTEX*

		DMSO in buffer		Glycerol in buffer		
	KRB buffer	15%	20%	5%	15%	20%
Tissue water as % of wet weight	78·8 ± 0·96† (27)	79·8 ± 0·93 (27)	81·2 ± 0·17 (9)	74·0 ± 0·7‡ (9)	74·8 ± 1·8‡ (9)	74·7 ± 0·17‡ (9)
Inulin space as % of wet weight	28·5 ± 0·82 (36)	18·2 ± 0·82§ (27)	15·4 ± 0·94§ (27)	21·7 ± 0·08§ (9)	18·0 ± 0·95§ (9)	15·0 ± 1·7§ (9)

* Kidney cortical slices pooled from three rats were incubated in 2 ml of buffer in an atmosphere of 95% O_2–5% CO_2 for 30 min at 37°. Extracellular fluid space was estimated by the addition of 0·5 μc [^{14}C]inulin.

† Mean ± 1 S.E. Total number of animals in parentheses.

‡ $P < 0{\cdot}01$.

§ $P < 0{\cdot}001$.

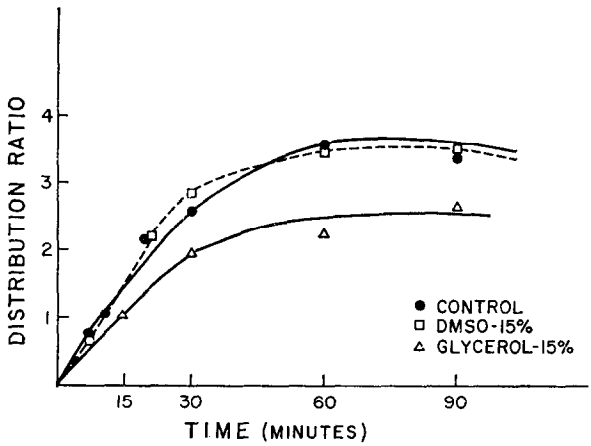

FIG. 1. Timed uptake of 0·065 mM α-aminoisobutyric acid into rat kidney cortical slices incubated at 37° with 95% O_2–5% CO_2 either in 2 ml of Krebs–Ringer bicarbonate buffer alone or buffer prepared with 15% dimethylsulfoxide or glycerol. The symbols represent the distribution ratios determined in triplicate flasks, one slice from each of three rats per flask, and a total of at least nine animals per experiment. Glycerol-inhibited uptake at 30, 60 and 90 min is statistically significant at P values less than 0·001, 0·05 and 0·01 respectively.

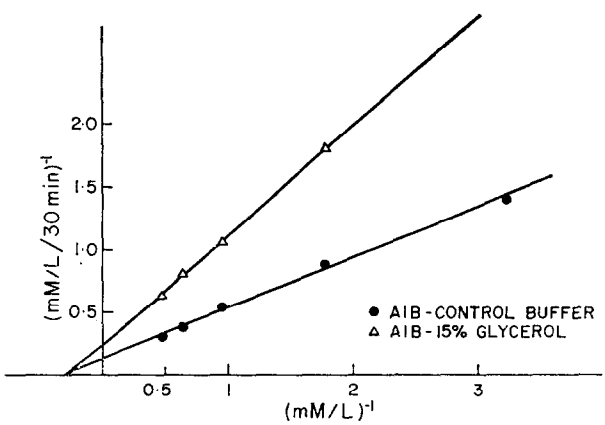

FIG. 2. Lineweaver–Burk plot of α-aminoisobutyric acid uptake by rat renal cortical slices in KRB buffer and 15% glycerol–KRB buffer. The velocity of α-aminoisobutyric acid uptake is in micromoles per milliliter of intracellular fluid per 30 min and has been corrected for the diffusion component. The substrate (S) concentration for AIB ranged from 0·25 to 2 mM.

in both incubation systems. However, the maximal velocity of the uptake of α-aminoisobutyric acid in 15% glycerol–KRB buffer is one-half that of the uptake in control slices. No consistent change in either the K_m or V_{max} was seen for α-aminoisobutyric acid in the DMSO–KRB incubation media.

The results of α-aminoisobutyric acid uptake by frozen slices are shown in Table 2. The distribution ratio for amino acid uptake by control slices incubated in 15% DMSO–KRB buffer for 30 min is 2·75. Renal cortical slices which had been frozen in the same cryoprotective buffer at 1°/min were able to accumulate α-aminoisobutyric acid after thawing. The distribution ratio of amino acid uptake by slices frozen to −30° is 2·44, not significantly different from control slices. When slices were frozen

TABLE 2. ACCUMULATION OF α-AMINOISOBUTRYIC ACID BY RAT RENAL CORTICAL SLICES*

	Control	−30°	−70°
Distribution ratio†	2·75 ± 0·13‡ (36)	2·44 ± 0·16 (27)	1·64 ± 0·11§ (45)

* Cortical slices from three rats were frozen in 2 ml of 15% DMSO–KRB buffer at −1°/min until reaching either −30° or −70°. The contents of the flasks were rapidly thawed, 0·13 μM of α-aminoisobutyric acid was added and the flasks regassed with 95% O_2–5% CO_2 and incubated for 30 min at 37°.

† Distribution ratio of (counts/min/ml intracellular fluid)/counts/min/ml media).

‡ Mean ± 1 S.E. Total number of animals in parentheses.

§ $P < 0·001$.

to a temperature of −70° there was impairment of uptake of α-aminoisobutyric acid, the distribution ratio being 1·64. This does not represent non-specific binding to an altered membrane since anaerobic incubation prevented the accumulation of amino acid against its concentration gradient. The distribution ratio for control slices and slices frozen to −70° but incubated in 95% N_2–5% CO_2 was 0·88 and 0·72 respectively

DISCUSSION

These studies provide evidence that the determination of amino acid accumulation can be used to evaluate the preservation of kidney tissue function after freezing. Other tests for viability have been advocated, including oxygen consumption or the appearance of various intracellular enzymes, lactate or potassium ions in the incubation media after the freezing–thawing cycle.[5,6] In Abbott's studies the use of 15% DMSO had no effect on the efflux of transaminases, alkaline and acid phosphatases and α-glucuronidase from rabbit renal cortical slices into the media. Freezing unprotected kidney slices to −70° resulted in a diminution of oxygen consumption and the increased appearance of the intracellular enzymes in the media. A controlled rate of freezing at −1°/min and cryoprotection with 15% DMSO was better than rapid freezing. The studies we have described indicate not just another measure of viability, but rather the preservation of a specific important function of the tubule cell transport of amino acid against a concentration gradient.

In most studies thus far utilizing renal tissue, the presence of cryoprotective agents during the freezing procedure results in better function of cellular processes than that of unprotected tissue. Glycerol and dimethylsulfoxide have been the agents most extensively used. Dimethylsulfoxide does not result in significant reduction in oxygen consumption of rabbit renal cortical slices either in unfrozen or frozen–thawed conditions.[5] In contrast to these findings, oxygen consumption in frozen glycerolated mouse renal cortical slices was reduced approximately one-third when compared to control glycerolated slices.[7] Our results indicate that renal tubular function in glycerol equilibrated kidney slices is altered even in the absence of the freezing procedure. Our study supports the findings of Abbott,[5] that dimethylsulfoxide, at concentrations of 2 M, can support basic metabolic functions.

Although the immediate concern of the function of a transplanted kidney is for the

adequacy of renal blood flow and glomerular filtration, ultimate success depends upon preservation of renal tubular function as well.[8] Deterioration of tubular function has been proposed as a parameter to follow for evidence of kidney rejection.[9] In the intact cortical slice, strenuous conditions such as freezing–thawing does not result in complete deterioration of tubular function.

Although a large technological gap exists between the study of renal cortical accumulation of α-aminoisobutyric acid in frozen slices and preservation of the intact organ by freezing methods, the findings that we have reported indicate the inherent feasibility of maintaining certain intrinsic cellular processes during the storage of intact whole organs.

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