BBA 75719

EFFECTS OF HEAVY METALS ON RENAL ASPARTATE TRANSPORT AND THE NATURE OF SOLUTE MOVEMENT IN KIDNEY CORTEX SLICES

E. C. FOULKES

Departments of Environmental Health and Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45219 (U.S.A.)

(Received March 15th, 1971)

SUMMARY

1. This paper explores effects of uranium poisoning and of *p*-chloromercuribenzoate (PCMB) on renal amino acid transport in the rabbit. Characteristics of translocation of amino acids as well as of sugars are compared *in vitro* (cortical slices) and *in vivo* (double indicator dilution analysis of artery-to-vein transit kinetics, clearance studies) in order to evaluate the physiological significance of slice studies.

2. Dicarboxylic amino acids interact with the peritubular cell membrane *in vivo* by a specific mechanism which is sensitive to uranyl ions and PCMB. However, PCMB fails to depress tubular reabsorption of filtered aspartate; this makes it unlikely that the PCMB-sensitive process at peritubular cell membrane can form part of the reabsorptive process.

3. Aspartate uptake *in vitro* is inhibited by PCMB. It follows that uptake of aspartate by slices is a function of peritubular cell membrane, and not of the PCMB-insensitive reabsorptive mechanism.

4. Evidence is also presented for the occurrence of sugar transport across peritubular cell membrane *in vivo*. The specificity of this transport resembles that described for sugar uptake by slices. Activity at the peritubular membrane may suffice therefore to account for sugar accumulation *in vitro*. As suggested previously there is thus no need to invoke participation of luminal cell membranes in solute transfer by kidney cortex slices.

INTRODUCTION

Thin slices of kidney cortex have proven extremely useful for the study of renal transport processes[1]. There are reasons to believe, however, that the slice system may not be equally suitable for the analysis of all transport events. Thus, we previously suggested that only the basement side or peritubular membrane of epithelial cells participates to a significant extent in solute translocation by kidney cortex slices[2,3]. This conclusion was based on the dual consideration of the long diffusion path through the length of any open tubule in the absence of flow of tubular fluid, coupled with the collapse of most tubular lumina in slices. If one accepts these argu-

Abbreviation: PCMB, *p*-chloromercuribenzoate.

ments it follows that slice studies can shed little light on the properties of luminal cell membranes and can, therefore, provide only limited information on the mechanism of solute reabsorption from tubular urine.

A different view was taken by KLEINZELLER *et al.*[4] who interpreted their data on sugar uptake by rabbit kidney cortex slices in terms of transport across luminal cell membranes. Similarly it has been suggested that accumulation of amino acids by kidney may also relate to the mechanism responsible for amino acid reabsorption[5,6]. It must be pointed out here that the recent work of SILVERMAN *et al.*[7] has rendered less compelling the reasons for assigning amino acid and sugar transport in slices to luminal cell membranes. These authors demonstrated the existence of specific transport mechanisms for sugars on the peritubular side of cortical cells in the dog kidney. A primary role of peritubular cell membrane in solute movement *in vitro* can therefore not easily be excluded, and it remains an open question whether kidney slices are suitable for the study of luminal transport mechanisms.

The present experiments aimed at localizing the functional lesion responsible for aminoaciduria, a well-known symptom of heavy metal intoxication. As part of this work effects of mercury and uranium on renal amino acid transport were investigated. Our results provide direct evidence against the equation of the process of amino acid uptake by slices with reabsorptive mechanisms. The significance of sugar accumulation *in vitro* is also further explored and the conclusion is reinforced that movement of solute into and out of renal cortical slices reflects primarily properties of the peritubular cell membrane.

METHODS

The application of the double indicator dilution technique[7] to the rabbit has been described elsewhere[8]. In brief, New Zealand white rabbits weighing on the average 2 kg were used throughout this study. The method involves cannulation of a renal vein and close arterial injection of the ^{14}C-labeled test substance (1 μC) mixed with glomerular marker (15 μC [^{3}H]methoxyinulin) in 0.15 ml saline. Total venous effluent is collected in serial 2- or 3-sec fractions for 60–90 sec, obviating any significant recirculation of tracer. Measurements of renal blood flow refer to collected venous effluent. Blood loss is replaced with 6 % Dextran in saline. By comparison of the ratio of $^{14}C/^{3}H$ in the injected bolus and in venous blood the fractional recovery of ^{14}C can be computed in relation to non-filtered inulin. As pointed out by SILVERMAN *et al.*[7] a fractional recovery of ^{14}C in any sample less than that of inulin can be attributed to a larger postglomerular volume of distribution of ^{14}C than of inulin. In control experiments no evidence could be obtained for significant amino acid uptake by red cells during periods as long as 10 min, *i.e.* intervals of time much longer than the 60–90 sec involved in renal transit studies. In other words, the loss of ^{14}C from the post glomerular inulin space may be interpreted in terms of penetration through or adsorption on the peritubular cell membranes of the renal cortex. While the present experiments cannot distinguish between these two possibilities, the fact that the interaction is not abolished by loading animals with the appropriate amino acid to a plasma level of approx. 5 mM perhaps supports the assumption that the interaction represents uptake by cells.

When the recovery of ^{14}C is less than that of the reference substance (^{3}H-labeled

inulin), the area between the two recovery courves measures the percent loss of ^{14}C from the inulin space. Reduction in this area, *i.e.* depression of the relative loss of ^{14}C, may be caused either by inhibition of ^{14}C movement, or by expansion of the inulin space.

In experiments on the effect of *p*-chloromercuribenzoate (PCMB) the drug was injected intravenously at the end of the first (control) period, and 15–20 min later a second transit study was performed. Uranium was administered as in previous experiments[9,10] in the form of uranyl nitrate (1 μmole/kg body weight) 2 days prior to the experiments.

To study effects of PCMB on aspartate uptake by slices, the drug was injected into anesthetized animals. 15 min later each rabbit was heparinized, blood was collected by cardiac puncture and the kidneys removed and placed on ice. Slices were cut free hand and suspended in the plasma from the appropriate animal. After 15 min equilibration at 37° on a Dubnoff shaker in an atmosphere of 95 % O_2–5 % CO_2 [^{14}C]aspartate, ^{3}H-labeled inulin and unlabeled aspartate to a final concentration of 1 mM were added. Following short periods of incubation (1–2 min), the slices were rapidly decanted, blotted and weighed, then extracted with 5 % trichloroacetic acid at 100° for 5 min, and the extracts counted as usual.

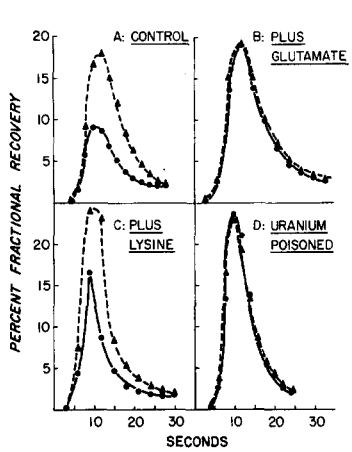
In order to avoid excessive metabolic breakdown of [^{14}C]aspartate during long clearance periods, the determination of fractional reabsorption of the filtered compound was carried out essentially by the same technique as the renal transit studies. A tracer bolus containing 1 μC [^{14}C]aspartate and 15 μC ^{3}H-labeled inulin was injected intraarterially and the reabsorption of filtered ^{14}C calculated from the ratio of the two isotopes in a 3–5 min urine sample. Urine was collected through ureteral catheters and urine flow was stimulated by the infusion of 1 ml/kg per min of 10 % mannitol in saline. All radioactive samples were counted in BRAY's[11] solution on a Tricarb Liquid Scintillation Spectrometer with application of the appropriate quench corrections. Radioactive compounds were procured commercially from the following sources: 2-amino[1-^{14}C]isobutyric acid, L-[$^{14}C_4$]aspartic acid, methyl-α-D-[$^{14}C_6$]glucopyranoside, Amersham/Searle; [1-^{14}C]glutamic acid, 2-deoxy[$^{14}C_6$]glucose, International Chemical and Nuclear Corporation; [^{3}H]methoxyinulin, New England Nuclear. *p*-Aminohippurate analyses were performed by the diazotization procedure of BRATTON AND MARSHALL[12]. Plasma amino acid levels were estimated by the technique of TROLL AND CANNON[13]. In unloaded animals total amino acid concentration averaged 4 mM; this figure was subtracted from values obtained in animals loaded with lysine or glutamate to yield the approximate level of the specific amino acid.

RESULTS

Interaction of peritubular cell membrane with aspartic acid in vivo

Experiments (E. C. FOULKES, unpublished results) to be reported at a later date have shown that a variety of amino acids interact with peritubular cell membrane in the rabbit. For the present purpose primary attention will be focused on the behavior of aspartic acid. Fig. 1A shows a typical double indicator dilution experiment illustrating the disappearance of ^{14}C-labeled aspartic acid from the postglomerular inulin space of a control rabbit. By analogy with the work of SILVERMAN *et al.*[7] this is interpreted as transfer of aspartate across peritubular cell membrane.

The fact that this transfer cannot be ascribed to simple diffusion can also be deduced from Fig. 1. Here it will be seen first that unlabeled glutamate completely inhibits aspartate uptake (Fig. 1B); presumably the glutamate effect represents a competitive inhibition, as an equimolar concentration of lysine exerts no such effect on movement of aspartate across peritubular cell membrane (Fig. 1C). Finally the observation that administration of small amounts of uranyl salts abolish the ability of peritubular cell membrane to react with aspartate (Fig. 1D) reinforces the view that this interaction represents a specific mechanism. In contrast to the interaction of aspartate with peritubular cell membrane, repeated experiments failed to demonstrate such a reaction with α-aminoisobutyric acid. In six studies, not further illustrated here, α-aminoisobutyric acid and inulin showed essentially identical artery-to-vein transit characteristics.



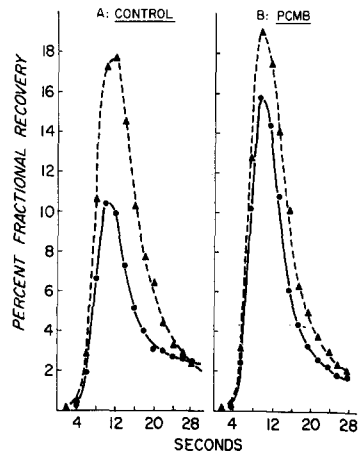


Fig. 1. Renal transit patterns of aspartate. Venous recoveries of [^{3}H]inulin (▲) and ^{14}C injected as aspartate (●) are shown as function of time. (A) Control MM No. 3, renal blood flow 23.6 ml/min. (B) Animal Asp. No. 7, renal blood flow 19.2 ml/min, plasma glutamate 33 mM. (C) Animal Asp. No. 7, renal blood flow 22.4 ml/min, plasma lysine 36 mM. (D) Rabbit U-Asp. No. 1, injected intravenously 48 h prior to experiment with 1 μmole uranyl nitrate per kg body wt., renal blood flow 20.0 ml/min; *p*-aminohippurate extraction 37%, compared to control > 90%. Results shown are typical of the following number of studies: (A) approx. 30; (B) 4; (C) 4; (D) 3.

Fig. 2. Effect of PCMB. As for Fig. 1. Rabbit PCMB (Asp.) No. 4. (A) Control period, renal blood flow 29.2 ml/min. (B) 20 min after intravenous injection of 9 mg PCMB per kg body weight, renal blood flow 30.4 ml/min.

Effects of mercurials on aspartate transport in vivo

Injection of 5 mg mercury in the form of the non-diuretic mercurial PCMB per kg body weight inhibited the disappearance of aspartate from the post-glomerular inulin space. This fact is illustrated in Fig. 2 by the reduction in the area between the recovery curves for ^{14}C and ^{3}H; the range of inhibition of 3 studies amounted to 29–51 %. In three further identical experiments PCMB also inhibited the reaction of [^{14}C]glutamate with peritubular cell membrane (mean inhibition 56 %).

Under the conditions under which PCMB depressed movement of dicarboxylic amino acid across peritubular cell membrane, no effects could be discerned on reabsorption of aspartate filtered at the glomerulus. Thus in 5 experiments tubular reab-

sorption of filtered ^{14}C after rapid intraarterial injection of labeled aspartate and inulin was $82 \pm 3\%$ (S.D.) during control studies, compared to $82 \pm 2\%$ after PCMB injection (10 mg Hg per kg body weight). In repeated similar control studies, over 80% of filtered ^{14}C-labeled α-aminoisobutyric acid was reabsorbed.

Action of PCMB in vitro

In order to study the effects of PCMB in the amounts administered above on the transport of aspartate by slices the mercurial was injected into anesthetized animals. 15 min later heparinized blood was collected by cardiac puncture. The kidneys were then rapidly removed, sliced into freshly separated plasma from the same animal and incubated as shown in Table I for one of three similar such experiments. Note that PCMB had no significant effect on the inulin space of the slices, but depressed the intracellular uptake of aspartate.

TABLE I

UPTAKE OF [^{14}C]ASPARTATE BY SLICES

Slices were obtained from control and poisoned (10 mg Hg per kg body wt.) animals whose own heparinized plasma provided the incubation medium. Each value represents the mean of 6 measurements on separate slices. Duration of incubation after addition of aspartate and inulin: 90 sec. Mean inulin space in controls was $18.6 \pm 2.2\%$ (S.D.), compared to $17.4 \pm 2.1\%$ in poisoned slices. Standard deviations from the calculated means for intracellular accumulation did not exceed $\pm 16\%$.

μmoles aspartate taken up per g			
Control		*PCMB*	
Total	*Intracellular*	*Total*	*Intracellular*
0.36	0.17	0.26	0.09

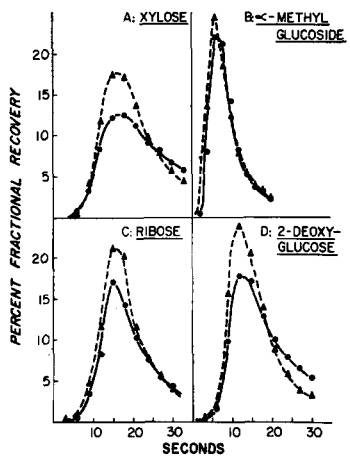


Fig. 3. Renal transit patterns of sugars. As for Fig. 1; the symbol (●) refers to ^{14}C injected as the appropriate sugar. (A) Rabbit XYL No. 2, renal blood flow 12.8 ml/min. (B) Rabbit MGL No. 1B, renal blood flow 27.6 ml/min. (C) Rabbit RIB No. 3, renal blood flow 12.8 ml/min. (D) Rabbit DOG No. 4, renal blood flow 18.8 ml/min. Results shown are typical of the following number of studies: (A) 6; (B) 2; (C) 5; (D) 7.

Specificity of interaction of peritubular cell membrane with sugars

The experiments of SILVERMAN *et al.*[7] on artery-to-vein transit patterns of various sugars in the dog can readily be confirmed in the rabbit; this is shown in Fig. 3. There is seen clear evidence for interaction between some sugars and peritubular cell membrane. Although only a limited number of compounds was tested it must be emphasized that the specificity of the reaction in rabbits differs from that reported in the dog[7]. In particular, 2-deoxy-D-glucose does not react with peritubular cell membrane in the dog but readily does so in the rabbit. The relative inability of α-methyl glucoside to cross peritubular cell membrane *in vivo* contrasts with the strong accumulation of this compound by slices *in vitro*[14]. We could readily confirm the latter finding but further observed that in the presence of the same concentration of D-glucose as obtained during the *in vivo* studies the ability of slices to accumulate α-methyl glucoside was strongly depressed. Details of one such experiment are shown in Table II. Control flasks contained mannitol instead of glucose. The action of the sugar was specific to the extent that simultaneous accumulation of *p*-aminohippurate was not significantly affected. Competition between D-glucose and α-methyl glucoside was also reported by KLEINZELLER[14].

TABLE II

ACCUMULATION OF α-METHYL GLUCOSIDE BY SLICES

Each value represents mean of 4 separate analyses. Incubations were carried out at 30°, in an atmosphere of O_2–CO_2 (95:5, v/v) under the conditions of KLEINZELLER[14]. *p*-Aminohippurate (PAH, final concentration 0.1 mM) and ^{14}C-labeled glucoside (4 μC/100 ml) were added after 40 min preincubation designed to reduce endogenous glucose; incubation then continued for 60 min. Mannitol and glucose were added to a final concentration of 8 mM. Standard deviations rom the calculated mean slice to medium (S/M) concentration ratios did not exceed ±7%.

	Mannitol	*Glucose*
(S/M^{14}C	2.6	1.2
(S/M) PAH	11.2	13.6

DISCUSSION

The experiments reported in the present paper have extended earlier work[7] from the dog to the rabbit. Results obtained demonstrate for this species also a specific interaction of selected sugars with the peritubular cell membrane. In all probability this interaction leads to uptake by the cells but on this point the present experiments cannot be conclusive. A similar reaction between peritubular cell membrane and a wide variety of amino acids has also been found in the rabbit (E. C. FOULKES, unpublished results). Details are here provided for L-aspartic and L-glutamic acids. These dicarboxylic acids are seen to penetrate beyond the post-glomerular inulin space in the rabbit kidney; the competition between the two compounds further suggests that a common carrier system may be involved.

In the uranium-poisoned rabbit the artery-to-vein transit characteristics of aspartate become identical with those of inulin. Such a finding could theoretically result from inhibition of aspartate transfer across peritubular cell membrane, or from an increased permeability of peritubular cell membrane to inulin. Results

published elsewhere[8] show, however, that uranium poisoning of the rabbit does not alter the intrarenal volume of distribution of inulin. It follows then that the reduced loss of [^{14}C]aspartate from the postglomerular inulin space in such intoxicated animals (see Fig. 1D) must indicate actual inhibition of aspartate movement. We have previously described a similar lesion caused by uranium at peritubular cell membrane inhibiting the accumulation of *p*-aminohippurate by proximal tubular cells[9]. The present experiments therefore confirm the occurrence of damage to peritubular cell membrane in uranium-poisoned animals.

As in the case of uranium, no evidence could be obtained to suggest that the volume of distribution of inulin in kidney is altered by PCMB (see Table I). Effects of the organic mercurial on aspartate transit across the kidney (see Fig. 2) therefore presumably also reflect an inhibitory action at the peritubular membrane. The fact that PCMB depresses the transfer of both aspartate and glutamate across peritubular-cell membrane again points to the existence of a common carrier for the two dicarboxylic acids. It is interesting to note that, in contrast with the PCMB sensitivity of this carrier, no effects of PCMB could be seen on the reaction of peritubular cell membrane with phenylalanine or methionine (unpublished experiments).

The concentration of PCMB which inhibited uptake of aspartate across peritubular cell membrane did not alter fractional reabsorption of filtered aspartate. The fact that amino acid reabsorption is independent of peritubular activity is further illustrated by the fact that α-aminoisobutyric acid does not react with peritubular cell membrane in the rabbit, yet is as efficiently reabsorbed from glomerular filtrate as is aspartate. It follows that whatever may be the physiological significance of the specific mechanisms at the peritubular membrane, they do not directly participate in the process of reabsorption of amino acids. It is possible that the mechanisms are primarily involved in the maintenance of intracellular composition and supply of nutrients. It may further be concluded that the amino aciduria seen in heavy metal poisoning does not result from the type of peritubular lesion demonstrated here.

The results of the present paper also bear on the problem of whether movement of solutes across luminal cell membranes can contribute to solute exchange between slices and incubation medium *in vitro*. In the case of sugars, of all the derivatives which are accumulated by rabbit kidney cortex slices[14] and whose transit characteristics through the dog kidney *in vivo* have also been studied[7] only 2-deoxy-D-glucose failed to react with peritubular cell membrane. The findings described here show, however, that the specificity of peritubular cell membrane in dog kidneys differs from that in rabbit where 2-deoxy-D-glucose strongly reacted with peritubular cell membrane (see Fig. 3). The absence of significant interaction of α-methyl glucoside with peritubular cell membrane *in vivo* may well be related to the high affinity of the sugar transport mechanism for glucose. In general it appears that sugars which can be accumulated by the rabbit kidney *in vitro* also react with peritubular cell membrane in that species *in vivo*. Clearly a primary role of peritubular cell membrane in sugar transport by slices can at present not be excluded.

A more compelling demonstration that translocation of certain solutes by slices involves primarily peritubular cell membrane and not the luminal side of the cells is provided by the findings on the PCMB inhibition of aspartate transport *in vivo* and *in vitro*. A concentration of mercurial which depressed the reaction of aspartate with peritubular cell membrane *in vivo* also inhibited uptake of aspartate by slices but

did not alter the reabsorption of filtered aspartate from tubular urine. It follows that, at least in the case of aspartate, uptake by slices proceeds via peritubular cell membrane and cannot serve as model for the process of reabsorption. Doubts must remain about the relevance of slice studies for the analysis of processes at the luminal cell membrane.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants HE 10346 and 5P10 ES 00159. It was a pleasure to have the skillful assistance of Mrs. Lory Greenland and Miss Sheila King.

REFERENCES

1 R. J. CROSS AND J. V. TAGGART, *Am. J. Physiol.*, 161 (1950) 181.
2 E. C. FOULKES AND B. F. MILLER, *Am. J. Physiol.*, 196 (1959) 86.
3 L. MURTHY AND E. C. FOULKES, *Nature* 213 (1967) 180.
4 A. KLEINZELLER, J. KOLINSKA AND I. BENES, *Biochem. J.*, 104 (1967) 843.
5 L. E. ROSENBERG, A. BLAIR AND S. SEGAL, *Biochim. Biophys. Acta*, 54 (1961) 479.
6 L. E. ROSENBERG, M. FOX, S. THIER AND S. SEGAL, *Proc. 2nd Intern. Congr. Nephrol., Prague, 1963*, Excerpta Medica Foundation, Amsterdam, 1964, p. 563.
7 M. SILVERMAN, M. A. AGANON AND F. P. CHINARD, *Am. J. Physiol.*, 218 (1970) 743.
8 E. C. FOULKES, *Toxicol. Appl. Pharmacol.*, (1971) in the press.
9 K. NOMIYAMA AND E. C. FOULKES, *Toxicol. Appl. Pharmacol.*, 13 (1968) 89.
10 F. J. BOWMAN AND E. C. FOULKES, *Toxicol. Appl. Pharmacol.*, 16 (1970) 391.
11 A. G. BRAY, *Anal. Biochem.*, 1 (1960) 279.
12 A. C. BRATTON AND E. K. MARSHALL, *J. Biol. Chem.*, 128 (1939) 537.
13 W. TROLL AND R. K. CANNAN, *J. Biol. Chem.*, 200 (1953) 803.
14 A. KLEINZELLER, *Biochim. Biophys. Acta*, 211 (1970) 264.