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Mechanisms of Organic Acid and Monosaccharide Transport in the Kidney of the Brush-tailed Possum, *Trichosurus vulpecula*

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Abstract

The mechanisms of organic acid and monosaccharide transport in the kidney of *T. vulpecula* were investigated using the renal cortical slice preparation. The kinetics, the sensitivity to inhibitors of metabolism and sodium transport, and the specificity of the concentrative uptake of *p*-aminohippurate and α-methyl-D-glucoside were examined and compared with published results from studies in eutherian mammals. Some minor differences between metatherians and eutherians were noted with regard to the specificities of the renal slice uptake systems. Penicillin G, a competitor of organic acid transport in eutherian kidneys, did not interact with the marsupial uptake system, and sodium acetate, which stimulates transport in other mammals, inhibited *p*-aminohippurate uptake in the slice of the possum kidney. 2-Deoxy-D-glucose, which interacts with the phlorizin-sensitive monosaccharide transport system in the dog, rat and rabbit kidney, had no effect on α-methyl-D-glucoside uptake in the possum, and the magnitude of the interaction of D-fructose resembled that reported in the dog and rat but was greater than the inhibition reported in the rabbit. D-Glucuronic acid and D-glucuronic acid lactone inhibited α-methyl-D-glucoside uptake in the possum but had no effect on uptake in rat renal slices. In consideration of the reported variability of these parameters between different classes of eutherians, it was concluded that the primary mechanisms involved in organic solute transport by the proximal nephron of metatherians and eutherians were not significantly different.

Introduction

Previous investigations on marsupial renal function indicate that the metatherian kidney has typical mammalian function and morphology (Reid and McDonald 1968, 1969; Tyndale-Biscoe 1973). Using *in vivo* renal clearance techniques in the Australian brush-tailed possum, *Trichosurus vulpecula*, Reid and McDonald (1968) demonstrated an eutherian type of proximal nephron function with respect to glucose reabsorption and organic acid secretion. Analysis of these two transport systems at the biochemical level could reveal mechanistic differences between the metatherians and eutherians that might not be apparent from *in vivo* clearance results. The present study was undertaken to examine in more detail the biochemical mechanisms involved in the renal transport of organic acids and monosaccharides by the metatherian kidney of *T. vulpecula*.

Organic acid secretion by the proximal tubule of the eutherian kidney is known to involve a carrier-mediated, active transport system in the basolateral plasma membrane of the cell (Weiner 1973; Ullrich 1976). Glucose reabsorption by the mammalian proximal tubule occurs via a carrier-mediated, Na^+-dependent, phlorizin-sensitive active transport system that resides in the brush-border plasma membrane

(Mudge *et al.* 1973; Silverman 1976; Ullrich 1976). Using the *in vitro* renal cortical slice preparation, an extensive analysis was made of the kinetics, specificities and inhibitor sensitivities of the uptake systems for the organic acid *p*-aminohippurate (PAH) and the non-metabolizable sugar α-methyl-D-glucoside (αMDG).

Materials and Methods

Animals

Adult and juvenile possums weighing 1·5–3·0 kg were collected in the field by cage trapping and were maintained on a diet of rat pellets (diet 86, Wairarapa Stock Foods, Wairarapa) and assorted fruits and vegetables. No distinction was made with regard to sex or coat-colour variety.

Renal Slice Preparation

Possums were killed by a sharp blow to the head, and both kidneys were immediately removed, decapsulated, quartered and placed in oxygenated, ice-cold Ringer solution. Cortical slices of approximately 0·3 mm thickness were prepared using a Stadie-Riggs microtome (A. H. Thomas Co., Philadelphia). The slices were cut by hand into small pieces 1–2 mm square and kept on ice in oxygenated Ringer solution.

PAH and αMDG Uptake Assay

The standard Ringer solution for incubation of the slices contained 115 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1 mM $CaCl_2$, 1·2 mM $MgSO_4$, 1·2 mM NaH_2PO_4, 10 mM sodium acetate and 5 mM glucose. Glucose was omitted from the medium for studies on αMDG uptake. Continuous aeration of the solution with 95% O_2, 5% CO_2 served to stir the solution and maintain a pH of 7·4. Ion, substrate and drug concentrations were altered in the incubation medium according to the experimental protocol. Incubations were routinely performed at 25°C rather than body temperature to prevent the development of anoxia and necrosis in the central region of the tissue (Balaban *et al.* 1980).

For each assay, approximately 50 mg of tissue was placed in a glass vial containing 5 ml of Ringer solution, and the tissue was pre-incubated at 25°C for 15 min (PAH) or 45 min (αMDG). The uptake period was started by addition of 0·015–0·15 mM ^{14}C-labelled PAH or 0·1–1·0 mM ^{14}C-labelled αMDG (0·1–0·2 μCi). In some cases, ^{3}H-labelled methoxy inulin (0·5–1·0 μCi) was included in the incubation to permit the measurement of the extracellular fluid volume of the slices. At the end of the incubation period, the medium was removed by aspiration, and the tissue was washed with 10 ml of ice-cold, non-radioactive Ringer solution. The slices were blotted on Whatman No. 1 filter paper and their wet weight determined. Dry weights were measured following overnight drying in a 90°C oven. Radioactive label of the tissue was extracted overnight at 4°C into 1 ml of 3% trichloroacetic acid (TCA). A 0·3-ml aliquot of TCA supernatant or incubation medium was added to 5 ml of liquid scintillation fluid containing 85% toluene, 15% Triton X-100; 4 g l^{-1} 2,5-diphenyloxazole (PPO), and 0·1 g l^{-1} 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP). The radioactivity of the samples was measured in a Beckman LS-100C liquid scintillation counter. All samples with radioactivity above background level were counted for a sufficient time to give an error of less than 5%. Counting efficiencies for ^{14}C and ^{3}H averaged 59 and 15%, respectively. No significant loss of ^{14}C-labelled PAH, ^{14}C-labelled αMDG or ^{3}H-labelled methoxy inulin was evident following overnight drying of medium samples at 90°C. Na^+ and K^+ concentrations in the medium and in the TCA extracts of the tissue were monitored using an EEL flame photometer (model A; Evans Electroselenium Limited, Essex, England). Medium osmolality was determined with an osmometer (model 3W; Advanced Instruments, Needham Heights, Ma.).

PAH Efflux Assay

Renal cortical slices were pre-loaded with isotope in medium containing 0·15 mM ^{14}C-labelled PAH and ^{3}H-labelled methoxy inulin by incubation at 25°C for 2 h. During the uptake period, the slices were suspended in the medium in separate stainless steel baskets to facilitate rapid changes of medium during the subsequent efflux periods. After the slices were pre-loaded with isotope, they

were quickly washed in ice-cold Ringer and transferred to 5 ml of non-radioactive Ringer solution. Subsequent transfers to 5-ml volumes of non-radioactive Ringer were made during the period of efflux at 7, 16, 26, 36 and 48 min. Media and tissue radioactivity were determined as described above. Efflux rate constants (k') were calculated from the equation:

$$k' = \ln(R_1/R_2)/T,$$

where R is rate and T is time (min).

Effects of Transport Inhibitors on Uptake

Using standard uptake assay procedures, transport inhibitors were added at the start of the pre-incubation period (Table 1). For the PAH uptake in acetate- and glucose-free media, a 30-min pre-incubation was used instead of the standard 15-min pre-incubation. Anaerobic slices were continuously gassed with 95% N_2, 5% CO_2 in covered vials. Choline and K^+ salts were used to replace Na^+ in Na^+-free medium giving final concentrations of 0 m-equiv. Na^+ and 36·2 m-equiv. K^+. Na^+ salts were used to replace K^+ in K^+-free medium giving final concentrations of 156·2 m-equiv. Na^+ and 0 m-equiv. K^+. NaCl (10 mM) was substituted for sodium acetate in acetate-free media. The percentage inhibition of total uptake was calculated from the ratios of tissue water to medium concentration (T/M) of treated and control samples.

Specificity of Uptake

Assorted organic acids and bases and monosaccharides (Table 2) were added at the start of the uptake period for PAH or αMDG. The percentage inhibition of uptake due to the presence of unlabelled competitors was calculated from the T/M values of experimental and control using total uptake for PAH and 0·2 mM phlorizin-sensitive uptake for αMDG. A competitor K_m' value was estimated from two-point Lineweaver–Burk plots using the V_{max} values from the data of Fig. 1*b* and assuming V_{max} to be constant. These K'_m values and the K_m values obtained from the data of Fig. 1*b* were used to calculate an estimated K_i value from the following equation in which I represents the competitor concentration (mM):

$$K_i = (K_m \cdot I)/(K_m' - K_m).$$

Chromatography

Ascending paper chromatography (Whatman No. 1 chromatography paper) using a solvent system of n-butanol:acetic acid:water (25:12:10) was used to assess whether ^{14}C-labelled PAH accumulated by cortical slices was metabolized by the tissue. For ^{14}C-labelled αMDG metabolism, silica gel thin-layer chromatography was used with a solvent system of n-butanol:acetic acid:ether:water (9:6:3:1). After standard uptake assays, medium and tissue TCA extracts were chromatographed, and the isotope mobilities were measured by counting separate fractions in a liquid scintillation counter. Co-chromatographed standards were assessed either colorimetrically or by scintillation counting. The chromatography results indicated that no significant metabolism of either isotope occurred in the cortical slice preparation.

In vivo *Clearance Measurements*

In order to determine if αMDG interacts *in vivo* with the monosaccharide reabsorptive system of the kidney, clearance measurements for D-glucose and ^{14}C-labelled αMDG were performed using a modification of the technique of Reid and McDonald (1968). Two possums were anaesthetized by intravenous injection of 80 mg kg^{-1} 5,5-diallylbarbituric acid. A cannula inserted into the jugular vein was used to infuse a solution of 10% (w/v) mannitol, 0·09% (w/v) NaCl at a rate of 0·76 ml min^{-1}. After a steady-state osmotic diuresis was established, a priming dose of 5 ml of 0·4% (w/v) ^3H-labelled methoxy inulin in 0·9% (w/v) NaCl was administered, and the initial infusion solution was immediately replaced with a solution containing 10% mannitol, 0·09% (w/v) NaCl, 0·04% (w/v) ^3H-labelled methoxy inulin and either 20 mg ml^{-1} D-glucose (possum No. 1, male, 2·2 kg) or 20 mg ml^{-1} ^{14}C-labelled αMDG (possum No. 2, female, 3·4 kg). At 5-min intervals for the next 45 min, urine and arterial blood samples were collected from indwelling cannulae positioned in the two ureters and the carotid artery, respectively. A single bolus injection of phlorizin (4 mg kg^{-1}) was then administered, and fractions of urine and blood were collected for an additional 15 min.

Glucose concentrations in the urine and plasma were measured colorimetrically by the method of Middleton and Griffiths (1957). Samples containing ^{14}C-labelled αMDG and ^{3}H-labelled methoxy inulin were counted in a liquid scintillation counter. The fractional excretion of glucose or αMDG was calculated by dividing the clearance of sugar (ml min^{-1}) by the methoxy inulin clearance (ml min^{-1}).

Statistical Analysis

All data are expressed as the mean ±s.e.m. Statistical significance of the data was determined by the Student's *t*-test for unpaired observations. Standard statistical methods were used for linear regression analysis based on the least squares method.

Reagents

All reagents were of analytical grade. Competitive and metabolic inhibitors were obtained from Sigma Chemical Company (St Louis, Mo.) with the exception of furosemide, which was a gift from Hoechst N.Z. Ltd (Auckland). ^{14}C-labelled PAH (*glycyl*-1-^{14}C) at 49 mCi mmol^{-1}, ^{14}C-labelled αMDG (D-[U-^{14}C]glucose) at 300 mCi mmol^{-1} and ^{3}H-labelled methoxy inulin (*methoxy*-^{3}H) at 210 mCi g^{-1} were supplied by New England Nuclear (Boston).

Results

αMDG Reabsorption in vivo

Clearance studies on two possums indicated that both D-glucose and αMDG undergo net reabsorption by the kidney of *T. vulpecula*, although the mean fractional excretion of ^{14}C-labelled αMDG in possum No. 2 during the 45-min infusion period ($0{\cdot}504 \pm 0{\cdot}051$, $n = 8$) was considerably higher than that of glucose for possum No. 1 ($0{\cdot}004 \pm 0{\cdot}001$, $n = 8$) and possum No. 2 ($0{\cdot}047 \pm 0{\cdot}003$, $n = 7$). The measured clearance values in millilitres per minute per gram of kidney for possum No. 1 were $0{\cdot}104 \pm 0{\cdot}075$ for inulin ($n = 9$) and $0{\cdot}0004 \pm 0{\cdot}0001$ for glucose ($n = 8$). Clearance values for possum No. 2 were $0{\cdot}656 \pm 0{\cdot}186$ for inulin ($n = 9$), $0{\cdot}031 \pm 0{\cdot}002$ for glucose ($n = 7$) and $0{\cdot}335 \pm 0{\cdot}040$ for ^{14}C-labelled αMDG ($n = 8$).

Intravenous administration of phlorizin, a potent competitor of the glucose reabsorptive system in eutherians, caused a significant increase in the fractional excretion of both glucose and αMDG. The mean values for the fractional excretion of glucose during the 15-min period after phlorizin injection were $0{\cdot}331 \pm 0{\cdot}121$ for possum No. 1 ($n = 2$) and $0{\cdot}196 \pm 0{\cdot}003$ for possum No. 2 ($n = 3$). The mean fractional excretion value for ^{14}C-labelled αMDG after phlorizin injection was $0{\cdot}873 \pm 0{\cdot}028$ ($n = 3$).

Uptake Kinetics

The accumulation of PAH and αMDG in slices of renal cortex occurred against a medium to tissue concentration gradient (Fig. 1*a*). Values are expressed as the tissue water to medium concentration ratio (T/M). If the active transport systems for PAH and αMDG were abolished by competitive inhibitors, T/M values of less than unity were obtained. In the presence of 0·2 mM phlorizin, the 4-h T/M value for αMDG was decreased from 3·3 to 0·6 (Fig. 1*a*). In the presence of 0·15 mM bromcresol green (BCG), the 3-h T/M value for PAH was decreased from 11·3 to approximately 0·5 (data not shown). The equilibrium T/M value for inulin of 0·3 indicates that 30% of the tissue water was extracellular since inulin distributes passively and is unable to cross cell membranes.

Based on histological observations in this laboratory and by others, renal cortical slices from eutherians (Scholer and Edelman 1979; Balaban *et al.* 1980) and from the possum (Reid and McDonald 1968) consist predominantly of proximal tubular segments. The ability of possum tissue slices to accumulate PAH and αMDG against a concentration gradient is specific for renal cortex since renal medulla–papilla slices and liver slices from the possum give maximum T/M values of less than unity. For PAH uptake after 2 h at 25°C, the renal medulla–papilla T/M ratio was $0{\cdot}52\pm0{\cdot}12$ ($n = 7$) and the liver T/M ratio was $0{\cdot}58\pm0{\cdot}13$ ($n = 7$). For αMDG uptake after 1 h at 25°C, the renal medulla–papilla T/M ratio was $0{\cdot}65\pm0{\cdot}06$ ($n = 4$).

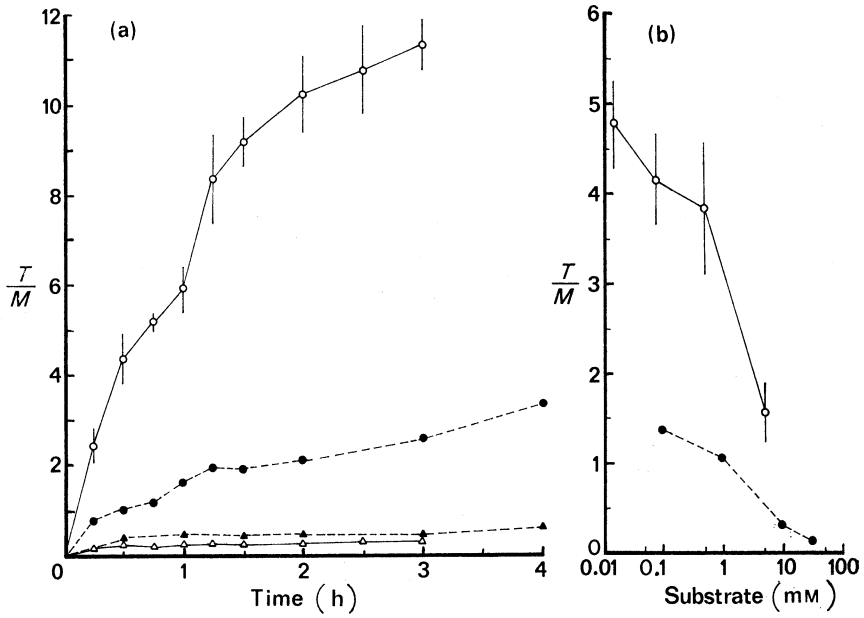

Fig. 1. Kinetics of uptake. (*a*) Time course of uptake. T/M data for PAH (○) and inulin (△) uptake at 25°C are presented as the mean ±s.e.m. of six separate experiments. The αMDG uptake data represent values obtained from a single experiment (one slice assay per time point) in the presence (▲) and absence (●) of 0·2 mM phlorizin. (*b*) Saturation kinetics of active uptake. Slices were incubated at 25°C for 1 h in standard media containing different PAH and αMDG concentrations. T/M values for PAH (○) and αMDG (●) represent BCG-sensitive and phlorizin-sensitive uptake only. Data for PAH are presented as the mean ±s.e.m. of four separate experiments. Data for αMDG are presented as the mean of duplicate samples from a single experiment.

Carrier-mediated transport systems display saturation at high substrate concentrations. Increasing the PAH and αMDG concentrations in the incubation medium decreased the 1-h T/M values as the membrane carriers approached substrate saturation (Fig. 1*b*). The T/M values in Fig. 1*b* have been corrected for passive uptake by subtracting the 0·15 mM bromcresol green and 0·2 mM phlorizin uptake values from the total uptake of PAH and αMDG, respectively. Using these data, Michaelis–Menten kinetic analysis was applied to approximate the K_m and V_{max} for uptake. The true K_m and V_{max} values for the transport systems cannot be accurately measured in the slice preparation because of the problems of multiple cell types and uptake

compartments in addition to inaccurate initial uptake rates. The apparent K_m values for PAH and αMDG were 0·54 and 3·09 mM, respectively. The apparent V_{max} values were 2·65 mmol l^{-1} h^{-1} for PAH uptake and 4·37 mmol l^{-1} h^{-1} for αMDG uptake.

Efflux Kinetics

The efflux of PAH from pre-loaded cortical slices was measured under various conditions to determine if efflux occurs by a reversal of the carrier-mediated uptake pathway. An initial fast component of efflux (0–7 min) with a rate constant k' of $0{\cdot}093 \pm 0{\cdot}007$ min^{-1} ($n = 17$ slice assays) was assumed to be derived from the passive diffusion of PAH from the extracellular space into the medium. A later slow component of efflux ($k' = 0{\cdot}038 \pm 0{\cdot}002$; $n = 18$) followed first-order kinetics and was assumed to represent the movement of PAH from the cell to the medium. The k' for this slow efflux component was decreased $17 \pm 5\%$ ($n = 6$) when the temperature during the efflux period was lowered by incubation in an ice bath. The rate of efflux of PAH from the slices was stimulated, however, if inhibitors of active PAH uptake were added to the efflux medium. The resultant percentage increases of the k' values obtained for each inhibitor were $39 \pm 9\%$ for 1 mM unlabelled PAH, $101 \pm 21\%$ for 0·75 mM bromcresol green, $31 \pm 17\%$ for 1·5 mM probenecid, $18 \pm 9\%$ for 1 mM ouabain, $72 \pm 11\%$ for Na^+-free medium and $62 \pm 22\%$ for anaerobia ($n = 6$ for each inhibitor). The only PAH transport inhibitor that decreased the measured k' value was 2 mM iodoacetate ($72 \pm 11\%$ decrease, $n = 6$).

The efflux kinetics of ^{3}H-labelled methoxy inulin from renal slices showed a single fast component with first-order kinetics ($k' = 0{\cdot}106 \pm 0{\cdot}007$, $n = 18$). Low temperature decreased the k' value $24 \pm 8\%$ ($n = 6$); however, only one of the PAH transport inhibitors significantly altered the k' for inulin efflux, specifically, incubation in Na^+-free medium ($23 \pm 9\%$ inhibition, $n = 6$). The mechanism responsible for this unexpected inhibition of inulin efflux is unknown.

Transport Inhibitor Sensitivity

Various treatments that inhibit PAH or αMDG uptake in eutherian renal cortical slices were tested in the possum slice preparation (Table 1). Metabolic and Na^+ transport inhibition significantly decreased the uptake of PAH and αMDG, and the competitive inhibitors of glucose transport, phlorizin and phloretin, inhibited αMDG uptake in the possum slice in a manner quantitatively similar to that reported for eutherian transport systems. Deletion of glucose, acetate or both from the medium stimulated PAH uptake. In this case, the expected effect was an inhibition of uptake due to substrate limitation.

Substrate Specificity

The substrate specificities of the organic acid and monosaccharide transport systems were examined by testing the effects of transport analogues on PAH and αMDG uptake into slices (Table 2). The hierarchy of carrier affinity for the PAH uptake system was almost identical to that found in eutherians with the exception of the lack of interaction of penicillin G. Of the three organic bases tested, only *N*-methylnicotinamide showed any significant inhibition of PAH uptake. This

effect was presumably non-specific since no significant inhibition was obtained at lower concentrations ($-4\pm6\%$ inhibition at 10 times the PAH concentration, $n = 6$).

The sugar specificity for the αMDG uptake system was generally consistent with that reported for eutherians. The major differences in the possum were the total lack of interaction by 2-deoxy-D-glucose and the significant inhibition of αMDG uptake in the presence of D-glucuronic acid lactone and D-glucuronic acid (Table 2).

Table 1. Effects of transport inhibitors on uptake by renal cortical slices

Renal cortical slices were incubated with 0·15 mM PAH or 1·0 mM αMDG at 25°C in the presence and absence of inhibitors. Data are expressed as the mean ±s.e.m. * $P<0·05$; ** $P<0·01$; *** $P<0·001$

Inhibitor treatment	Inhibitor concn (mM)	Uptake period (h)	Percentage inhibition (*n*) PAH	αMDG
Anaerobia	—	2·0	93±1 (6)***	—
Cyanide	5·0	0·5	84±1 (6)***	—
Dinitrophenol	0·1	0·5	91±2 (3)***	—
	0·2	1·0	—	81±2 (6)***
Iodoacetate	2·0	0·5	84±1 (3)***	—
	0·2	0·5	40±0 (3)***	—
	0·2	1·0	—	46±3 (6)***
Ouabain	1·0	0·5	86±2 (3)***	—
	0·1	0·5	76±2 (3)***	—
	0·2	1·0	—	76±6 (6)***
Low temperature	—	1·0	86±4 (5)***	84±2 (6)***
Na^+-free medium	—	2·0	92±1 (6)***	—
	—	1·0	—	67±4 (6)***
K^+-free medium	—	2·0	66±5 (4)***	—
Acetate-free medium	—	2·0	−44±5 (6)***	—
Glucose-free medium	—	2·0	−27±10 (6)*	—
Acetate–glucose-free medium	—	2·0	−23±3 (6)***	—
Phlorizin	0·2	1·0	—	83±0 (9)***
	0·02	1·0	—	80±1 (2)**
Phloretin	0·2	1·0	—	42±3 (6)***
	0·02	1·0	—	9±1 (2)

In a comparable rat cortical slice experiment performed in this laboratory, neither 100 times the concentration of D-glucuronic acid lactone ($6\pm4\%$, $n = 5$) nor 100 times the concentration of D-glucuronic acid ($0\pm4\%$, $n = 5$) produced any significant percentage inhibition of phlorizin-sensitive αMDG uptake. Although not presented in Table 2, phlorizin and phloretin had the highest affinities for the αMDG transport system of the possum (see Table 1). The estimated K_i values for phlorizin (0·002 mM) and phloretin (0·2 mM) were similar to those reported for eutherian systems.

Discussion

Based on the results of this investigation and others (Reid and McDonald 1968, 1969), it is clear that the physiological mechanisms involved in proximal nephron function have undergone little evolutionary change since the initial divergence between the metatherians and eutherians. Renal clearance measurements in *T.*

vulpecula indicated almost complete reabsorbtion of glucose and partial reabsorption of αMDG. In eutherians, αMDG, unlike glucose, shows little interaction with the phlorizin-insensitive, Na^+-independent efflux pathway of the proximal tubular cell but is an effective competitor of glucose for the uptake pathway (Silverman 1976). This difference may account for the higher fractional excretion of αMDG in the possum since net reabsorption of sugar involves both uptake and efflux from the tubular cells. Other clearance measurements by Reid and McDonald (1968) have shown that PAH undergoes net proximal secretion in the possum in a manner analogous to that reported in eutherians.

Table 2. Specificity of uptake of organic acids and bases and monosaccharides by renal cortical slices

Renal cortical slices were incubated for 2 h at 25°C in the presence of 0·015 mM PAH, with and without 0·75 mM organic acid or base, or for 1 h at 25°C in the presence of 0·1 mM aMDG, with and without 10 mM monosaccharide. Data for percentage inhibition are expressed as the mean ± s.e.m.

* $P<0·05$; ** $P<0·01$; *** $P<0·001$

Competitor	Percentage inhibition (*n*)	K_i (mM)
Organic acids		
Bromcresol green	98±1 (6)***	0·01
Probenecid	94±1 (6)***	0·05
Ethacrynic acid	85±3 (6)***	0·13
Furosemide	81±10 (6)***	0·17
Phenol red	81±1 (6)***	0·17
Hippurate	56±12 (6)**	0·57
p-Aminohippurate	49±6 (8)***	0·76
Urate	41±5 (6)***	1·05
Penicillin G	4±7 (6)	—
D-Glucuronic acid	1±7 (4)	—
D-Glucoronic acid lactone	−5±3 (4)	—
Organic bases		
N-Methylnicotinamide	20±7 (6)*	—
Tetramethylammonium	−5±4 (6)	—
Creatinine	−2±11 (6)	—
Monosaccharides		
α-Methyl-D-glucoside	79±2 (5)***	2·6
D-Glucose	77±2 (5)***	2·9
D-Fructose	29±2 (5)***	23·6
D-Galactose	19±2 (5)***	41·1
D-Glucuronic acid	19±3 (5)**	41·1
D-Glucuronic acid lactone	19±3 (5)**	41·1
L-Glucose	7±4 (5)	—
D-Mannose	5±4 (5)	—
2-Deoxy-D-glucose	1±2 (5)	—
3-*O*-Methyl-D-glucoside	1±2 (5)	—
D-Xylose	0±3 (5)	—

The kinetics of PAH uptake and efflux and αMDG uptake into renal slices of the possum were comparable to reported kinetics in the rat (Segal *et al.* 1973; Park and Solomon 1977), rabbit (Kleinzeller *et al.* 1967; Welch and Bush 1970; Park *et al.* 1971; Podevin and Boumendil-Podevin 1977; Hong *et al.* 1978), and dog (Ross and Farah 1966; Silverman 1976). Calculated values for Arrhenius activation energies for PAH uptake (13·8 kcal mol^{-1}) and efflux (6·0 kcal mol^{-1}) in the

possum support a model of separate flux pathways. Although a facilitated, passive efflux system for PAH has been demonstrated *in vivo* (Foulkes 1977) and in brush-border vesicle preparations (Kinsella *et al.* 1979), no evidence for facilitated efflux was obtained in the present study.

One unique feature of transport in the possum slice preparation was the lack of stimulation of PAH uptake by addition of acetate to the medium. If acetate is taken up by the cells via the organic acid transport system as suggested by Kippen and Klinenberg (1978), then the quantitative differences in acetate effects observed between the possum (inhibition at 10 mM) and eutherians (stimulation at 10 mM, inhibition at higher concentrations) may result simply from differences in carrier affinity for acetate.

The substrate specificities of the PAH and αMDG uptake systems in possum slices were remarkably similar to those reported for eutherian systems with a few notable exceptions. Penicillin G, which competes for PAH secretion *in vivo* (Bito and Baroody 1978), showed no affinity for the PAH uptake system in the possum slice. In the phlorizin-sensitive sugar transport systems of the dog, rabbit and rat (Kleinzeller *et al.* 1967; Glossman and Neville 1972; Silverman 1976), 2-deoxy-D-glucose shows a slight but significant interaction, whereas no inhibition of αMDG uptake was observed in the possum. This difference may result from minor differences in the structural requirements for carrier binding. D-Fructose inhibited αMDG uptake to a greater extent than D-galactose, a result that differs from that reported for the rabbit by Kleinzeller *et al.* (1967) but is consistent with results obtained in the dog and rat (Glossman and Neville 1972; Silverman 1976; Ullrich 1976). The ability of glucuronic acid lactone and glucuronic acid to compete for phlorizin-sensitive αMDG uptake in the possum but not in the rat may again reflect minor differences in the structural requirements for binding to the glucose transporter. In this case it may be relevant to note that Hinks and Bolliger (1957) have shown that glucuronuria, while generally absent in eutherians, is physiologically normal in *T. vulpecula*. It seems unlikely, however, that significant net reabsorption of glucuronides occurs in the kidney of the possum since plasma levels of glucuronic acid and glucuronic acid lactone are negligible (Hinks and Bolliger 1957).

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References

Balaban, R. S., Soltoff, S. P., Storey, J. M., and Mandel, L. J. (1980). Improved renal cortical tubule suspension: spectrophotometric study of O_2 delivery. *Am. J. Physiol.* **238**, F50–9.

Bito, L. Z., and Baroody, R. A. (1978). Comparison of renal prostaglandin and *p*-aminohippuric acid transport processes. *Am. J. Physiol.* **234**, F80–8.

Foulkes, E. C. (1977). Movement of *p*-aminohippurate between lumen and cells of renal tubule. *Am. J. Physiol.* **232**, F424–8.

Glossman, H., and Neville, D. M. (1972). Phlorizin receptors in isolated kidney brush border membranes. *J. Biol. Chem.* **247**, 7779–89.

Hinks, N. T., and Bolliger, A. (1957). Glucuronuria in a herbivorous marsupial *Trichosurus vulpecula*. *Aust. J. Exp. Biol. Med. Sci.* **35**, 37–44.

Hong, S. K., Goldinger, J. M., Song, Y. K., Koschier, F. J., and Lee, S. H. (1978). Effect of SITS on organic anion transport in the rabbit kidney cortical slice. *Am. J. Physiol.* **234**, F302–7.

Kinsella, J. L., Holohan, P. D., Pessah, N. I., and Ross, C. R. (1979). Transport of organic ions in renal cortical luminal and antiluminal membrane vesicles. *J. Pharmacol. Exp. Ther.* **209**, 443–50.

Kippen, I., and Klinenberg, J. R. (1978). Effects of renal fuels on uptake of PAH and uric acid by separated renal tubules of the rabbit. *Am. J. Physiol.* **235**, F137–41.

Kleinzeller, A., Kolinska, J., and Benes, I. (1967). Transport of monosaccarides in kidney-cortex cells. *Biochem. J.* **104**, 852–60.

Middleton, J. E., and Griffiths, W. J. (1957). Rapid colourimetric micro-method for estimating glucose in blood and CSF using glucose oxidase. *Br. Med. J.* **2**, 1525–7.

Mudge, G. H., Berndt, W. O., and Valtin, H. (1973). Tubular transport of urea, glucose, phosphate, uric acid, sulfate, and thiosulfate. In 'Handbook of Physiology. Renal Physiology'. (Eds J. Orloff and R. W. Berliner.) pp. 587–652. (Am. Physiol. Soc.: Washington, D.C.).

Park, Y. S., and Solomon, S. (1977). pH–temperature dependence of organic acid transport in rat kidney slices. *Am. J. Physiol.* **233**, F382–7.

Park, Y. S., Yoo, H. S., and Hong, S. K. (1971). Kinetic studies on transport of organic acids in rabbit kidney slices. *Am. J. Physiol.* **220**, 95–9.

Podevin, R. A., and Boumendil-Podevin, E. F. (1977). Monovalent cation and ouabain effects on PAH uptake by rabbit kidney slices. *Am. J. Physiol.* **232**, F239–47.

Reid, I. A., and McDonald, I. R. (1968). Renal function in the marsupial *Trichosurus vulpecula*. *Comp. Biochem. Physiol.* **25**, 1071–9.

Reid, I. A., and McDonald, I. R. (1969). The renin–angiotensin system in a marsupial (*Trichosurus vulpecula*). *J. Endocrinol.* **44**, 231–40.

Ross, C. R., and Farah, A. (1966). *p*-Aminohippurate and *N*-methylnicotinamide transport in dog renal slices–an evaluation of the counter-transport hypothesis. *J. Pharmacol. Exp. Ther.* **151**, 159–67.

Scholer, D. W., and Edelman, I. S. (1979). Isolation of rat kidney cortical tubules enriched in proximal and distal segments. *Am. J. Physiol.* **237**, F350–9.

Segal, S., Rosenhagen, M., and Rea, C. (1973). Developmental and other characteristics of α-methyl D-glucoside transport by rat kidney cortex slices. *Biochim. Biophys. Acta* **291**, 519–30.

Silverman, M. (1976). Glucose transport in the kidney. *Biochim. Biophys. Acta* **457**, 303–51.

Tyndale-Biscoe, H. (1973). 'Life of Marsupials.' (E. Arnold: London.)

Ullrich, K. J. (1976). Renal tubular mechanisms of organic solute transport. *Kidney Int.* **9**, 134–48.

Weiner, I. M. (1973). Transport of weak acids and bases. In 'Handbook of Physiology. Renal Physiology'. (Eds J. Orloff and R. W. Berliner.) pp. 521–54. (Am. Physiol. Soc.: Washington, D.C.)

Welch, L. T., and Bush, M. T. (1970). Intracellular distribution and runout of *p*-aminohippurate in rabbit kidney slices. *Am. J. Physiol.* **218**, 1751–6.

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