Biochimica et Biophysica Acta, 776 (1984) 113–121
Elsevier

BBA 72200

SULFATE TRANSPORT BY MOUSE RENAL CORTICAL SLICES DOES NOT REPRESENT UPTAKE BY BRUSH-BORDER MEMBRANE *

DAVID E.C. COLE **, MAGDOLNA KOLTAY and CHARLES R. SCRIVER ***

Department of Biology, The Medical Research Council Genetics Group, and deBelle Laboratory for Biochemical Genetics, McGill University-Montreal Children's Hospital Research Institute, 2300 Tupper Street, Montreal, Quebec H3H 1P3 (Canada)

(Received April 6th, 1984)

Key words: Sulfate transport; Na^+ gradient; Brush-border membrane; (Mouse kidney)

We measured uptake of isotopically ^{35}S-labelled sulfate anion by slices and by brush-border membrane vesicles prepared from mouse renal cortex to identify: (i) whether metabolic incorporation of anion influences net transport; (ii) which membrane is primarily exposed in the renal cortex slice. Slices accumulated sulfate without significant incorporation into metabolic pools. Net uptake of sulfate at 0.1 mM by the slice occurred against an electrochemical gradient as determined by measurement of free intracellular sulfate concentration, the isotopic distribution ratio at steady-state, and the distribution of lipophilic ions (TPP^+ and SCN^-). Carrier mediation of sulfate transport in the slice was confirmed by observing concentration-dependent saturation of net uptake and counter-transport stimulation of efflux. Anion uptake was Na^+-independent, K^+- and H^+-stimulated, and inhibited by disulfonated stilbenes. Brush-border membrane vesicles accumulated sulfate by a saturable mechanism dependent on a Na^+ gradient (outside > inside); others have shown that uptake of sulfate by brush-border membrane vesicles is insensitive to inhibition by disulfonated stilbenes. These findings indicate that different mechanisms serve sulfate transport in renal cortex slice and brush-border membrane vesicle preparations. They also imply that the slice exposes an epithelial surface different from the brush-border, presumably the basolateral membrane, or its equivalent, since sulfate transport by slices resembles that observed with isolated basolateral membrane vesicles.

Introduction

Renal transport is an important determinant of sulfate anion homeostasis in vertebrates. In the mammal, sulfate transport occurs in the proximal tubule. Reabsorption from filtrate involves transport inward at the brush-border membrane, diffusion through cytosol, and either metabolic 'run-out' or efflux at the basolateral membrane [1–3]. The process clearly favors net reabsorption in mammals [4], although a secretory flux of sulfate can also occur. Transport at the brush-border membrane in the intact tubule [5–7], and in the isolated membrane vesicle preparation [8–10], is saturable, concentrative, apparently electroneutral and depen-

---

* Publication No. 84021 from the McGill University-Montreal Children's Hospital Research Institute.

** Present address: I.W. Killam Hospital, Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia B3J 3G9, Canada.

*** To whom correspondence should be addressed at: deBelle Laboratory for Biochemical Genetics, Montreal Children's Hospital, 2300 Tupper Street, Montreal, Quebec H3H 1P3, Canada.

Abbreviations: DIDS, 4,4-diisothiocyanostilbene-2,2′-disulfonate; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate; Mes, 2-(*N*-morpholino)ethanesulfonate; NAP-taurine, *N*-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonate); SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate; TPP^+, tetraphenylphosphonium ion.

dent on a transmembrane sodium gradient (outside > inside); it is not inhibited by disulfonated stilbenes. Secretion of sulfate anion would require an initial uptake at the basolateral membrane, a process which has been delineated in the intact tubule [5,7,10] and in the isolated vesicle preparation [10–12]. Basolateral membrane transport of sulfate is sodium-independent, concentrative, participates in self-exchange, and is inhibited by disulfonated stilbenes. These findings indicate that renal sulfate transport in the mammal is mediated by carriers with different properties at the brush-border and basolateral membrane.

We studied renal sulfate transport in the mouse, a species not previously examined. We reported earlier [13] that a mutation impairing renal phosphate transport in the mouse spared sulfate, implying, as expected, that homeostasis of these two anions is regulated by independent renal mechanisms. In the work described here, we used both brush-border membrane vesicles and renal cortex slices to study sulfate transport in mouse kidney. We show that metabolic runout is not an important determinant of net sulfate uptake in the slice. Brush-border membrane vesicles and slices exhibit different sulfate uptake mechanisms, and uptake by the latter resembles transport by isolated basolateral membrane vesicles. These findings clearly corroborate, at a functional level, preceding work with enzyme markers [14], in which we participated, showing that slice preparations of renal cortex primarily expose basolateral membranes.

Materials and Methods

Animals. We used adult male mice (25–30 g) of the inbred C57Bl/6J strain purchased from the Jackson Laboratory, Bar Harbour, ME. Animals were housed in facilities conforming to Canadian standards for animal care. They were fed Purina Rodent Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water ad libitum.

Chemicals. Methoxy[^{14}C]inulin (M_r 5000, 15 mCi/g), poly(ethylene glycol), (M_r 4000, 0.7 mCi/g), α-amino[1,2-^{3}H]isobutyric acid isobutyric acid (53 mCi/mmol), D-[1-^{3}H]glucose (18 mCi/mmol) and [5′-$^{35}SO_4$]PAPS (0.7 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Labelled [^{14}C]thiocyanate (59 mCi/mmol) and [^{3}H]TPP$^+$ (4.3 Ci/mmol) were a gift from Dr. Ronald Poole, Department of Biology, McGill University. SITS, DIDS and NAP-taurine were purchased from Pierce Chemical Co., Rockford, IL. Furosemide and amiloride were gifts from Hoechst Pharmaceuticals Ltd., Montreal, Quebec and the Upjohn Co., Kalamazoo, MI, respectively. All others chemicals (reagent grade) were obtained from standard commercial sources.

Renal cortex slice preparations. Thin cortex slices (approx. 200 μm thick; wet wt. approx. 5 mg) were prepared as described by Chesney et al. [15] and incubated in Erlenmeyer flasks at 37°C under 100% O_2 in a Dubnoff shaking incubator. Water spaces of slices were determined with radiolabelled [^{14}C]inulin according to the method of Rosenberg et al. [16] or with ^{3}H-labelled PEG as described by Chesney et al. [15].

Various media (all at 300 mosM) of specified composition (mM) were used for incubation of slices: (i) a modified Krebs-Ringer buffer (pH 7.4): NaCl, 124/KCl, 5/$MgCl_2$, 2.1/$CaCl_2$, 5.3/D-glucose, 2.6/Tris-HCl, 27; (ii) a sucrose medium, modified after Deyrup [17], (pH 6.5) containing: sucrose, 280/Mes-Tris buffer, 10; (iii) a sucrose-KCl medium (pH 6.5) containing: sucrose, 240/KCl, 20/Mes-Tris, 10, or Pipes, 10.

Experiments to measure distribution and net uptake of solute by cortex slices were performed in 2 ml volumes as described previously [18]. The isotopic distribution ratio (cpm per ml ICF : cpm per ml ECF), net uptake (transport) rate (mmol/l ICF per unit time), efflux constant (k^{-1}) and apparent kinetic constants ($K_{\frac{1}{2}}$ and V_{max}) were calculated as described previously [13,18].

Efflux of sulfate from slices was measured after preincubation to steady-state with sulfate (1.0 mM) in the sucrose-KCl medium; slices were then removed, blotted and transferred into 10 ml 'transequilibrium' sucrose-KCl medium (containing sulfate at preincubation concentration) or 'trans-zero' medium (containing no sulfate). Labelled sulfate was measured in tissue and medium at 2-min intervals up to 30 min beginning at 6 min.

Tissue sulfate was measured by controlled-flow anion chromatography [19]. Organification of labelled sulfate after uptake by renal cortex slices was measured by a procedure that minimizes PAPS

degradation [20]. Ten slices (approx. 50 mg) were incubated either with or without labelled sulfate, removed and placed in homogenizing vessels; [^{35}S]PAPS was then added to the slices incubated with unlabelled sulfate. 10 vol glycine-NaOH buffer (in 50% (v/v) methanol-water, pH 9.2) were then added to both sets of flasks. After standing in a boiling water bath for 3 min, the preparations were chilled in an ice bath and homogenized. After centrifugation (12000 × *g*, 30 min), 50 μl samples were spotted on 20 × 20 cm DEAE-cellulose thin-layer plates and developed in 0.3 M ammonium bicarbonate until the solvent front had advanced 18 cm. The plates were dried at room temperature and 1-cm strips of cellulose scraped into vials for liquid-scintillation counting to identify distribution of accumulated $^{35}SO_4$ in the intracellular pools and in situ stability of the [^{35}S]PAPS standard.

Brush-border membrane vesicle preparation. We prepared brush-border membrane vesicles from renal cortex of 6–10 adult mice by a slight modification [21] of the method using Mg^{2+} precipitation and differential centrifugation described by Booth and Kenny [22]. Enrichment of the brush-border membrane, estimated by the increase in alkaline phosphatase specific activity, was 10-fold relative to whole cortex homogenate (10.7 ± 0.8, mean ± S.E., $n = 12$). Contamination of brush-border membrane vesicles by the basolateral membrane was not measured specifically in this work but we and others [22] have found no enrichment of the basolateral membrane marker ($(Na^+ + K^+)$-ATPase) in the brush-border membrane vesicle preparation.

Diffusion of sulfate and D-glucose was measured by replacing Na^+ by K^+ in the external medium. Each incubation medium contained approx. 10^6 cpm (as $^{35}SO_4$) per 90 μl. To correct for variations in vesicle size between experiments, initial uptakes at 15 s were expressed as percent of uptake at equilibrium (60 min). Protein was measured by the method of Lowry et al. [23] using bovine serum albumin as standard.

Statistics. We used the methods of Sokal and Rohlf [24] to compare means and dispersions of experimental and control values. Where there was a priori reason to believe that the data were not normally distributed, nonparametric methods were used.

Results

Water spaces and distribution of isotopic sulfate in renal cortex slices

Postincubation (60 min) dry weights in the various incubation media were similar (pooled values: 29.9 ± 0.3% of wet wt.; mean ± S.E., $n = 26$); extracellular volumes in the different media, measured with either inulin or poly(ethylene glycol), were also comparable (pooled values, 37.8 ± 0.4% of wet wt., mean ± S.E., $n = 10$).

Time-course for net sulfate uptake by slices

The isotopic sulfate distribution ratio (at 0.1 mM extracellular sulfate) increased to attain a steady-state (Fig. 1), reaching 7.9 ± 0.5 (mean ± S.D., $n = 27$) in the sucrose-KCl medium at pH 6.5, and 2.4 ± 0.2 in the modified Krebs-Ringer medium at pH 7.4 (Table I). The uptake ratio was not different when Pipes or Mes-Tris buffers were used in the sucrose-KCl medium; we used Pipes in the DIDS experiments, outlined below, to minimize interaction between stilbene and buffer components.

Sulfate content of renal cortex slices and fate of extracellular sulfate during uptake

We measured the intracellular content of slices to determine whether uptake at 0.1 mM extracellular sulfate in vitro occurred against a chemical gradient. We also measured incorporation of sulfate anion into organic pools to discern whether

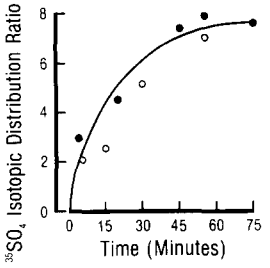

Fig. 1. Time-course of sulfate ($^{35}SO_4$) uptake by mouse renal cortex slices. The isotopic distribution ratio (^{35}S in ICF: ^{35}S in ECF) was determined at specified times in sucrose-KCl medium containing 0.1 mM SO_4 using either Mes-Tris(○) or Pipes (●) buffers at pH 6.5.

TABLE I

SIMULTANEOUS DISTRIBUTION RATIOS FOR LIPID-PERMEABLE IONS AND SULFATE IN MOUSE RENAL CORTEX SLICES

Ion	Concn. (μM)	Distribution ratio	
		Krebs-Ringer medium (pH 7.4)	Sucrose-KCl medium (pH 6.5)
TPP^+	2	37.70 ± 5.60 (7)	35.30 ± 3.50 (11)
SCN^-	2	0.18 ± 0.06 (4)	0.22 ± 0.05 (5)
SO_4^{2-}	100	2.40 ± 0.20 (9)	7.90 ± 0.50 (27)

metabolic runout was a significant determinant of net uptake.

Intracellular sulfate content in freshly excised renal cortex was approx. 1.1 mmol/l in adult mouse renal cortex, implying that sulfate uptake in vitro took place against a chemical gradient.

We measured the labelled soluble cellular products in the presence of $^{35}SO_4$ in sucrose-KCl and modified Krebs-Ringer after incubation of slices for 60 min. Labelled intracellular material, identified by DEAE-cellulose thin-layer chromatography, was inorganic sulfate (R_f, 0.95); only trace amounts of label were identified in the region of PAPS and other sulfoesters (R_f, 0.10). The labelled PAPS standard used as an internal control showed no more than 10% degradation to inorganic sulfate in situ during processing of the slices. Taken together, our measurements of cellular sulfate concentration, net uptake in vitro, and labelling of intracellular sulfate pools imply that slices accumulate sulfate anion from the extracellular space against a chemical gradient without metabolic 'runout'.

Distribution of lipophilic ions (TPP^+, SCN^-) in cortex slices

We measured the distribution ratio of each lipophilic ion simultaneously with that of sulfate at steady-state (60 min) to determine whether sulfate was accumulated by slices against an electrical gradient.

TPP^+ cation readily penetrated the intracellular space along with sulfate in slices incubated in either sucrose-KCl or modified Krebs-Ringer medium (Table I), whereas thiocyanate anion was excluded. We found no binding of lipophilic ions to tissue sites after disruption of cellular integrity

TABLE II

EFFECT OF CATIONS ON SULFATE UPTAKE BY MOUSE RENAL CORTICAL SLICES

Slices were incubated in sucrose-Tris/Hepes medium (pH 6.5) containing 0.1 mM SO_4^{2-} anion for 60 min at 37 °C. Tissue water spaces were measured with ^{3}H-labelled PEG. There was no significant change in intracellular water with substitution or addition of various cations. All cations were added as the chloride salt. Uptake values are expressed as mean ± S.E., $n = 3$.

Cation added	Final concn. of cation in medium (mmol/l)	Uptake (% of maximum)
None	–	33 ± 2
K^+	20	100 ± 5
	40	82 ± 3
	80	80 ± 4
	5	53 ± 2
$K^+ + Na^+$	20 + 20	57 ± 7
Na^+	20	46 ± 3
$Choline^+$	20	54 ± 4
Li^+	20	42 ± 1
Rb^+	20	98 ± 5
Cs^+	20	95 ± 4

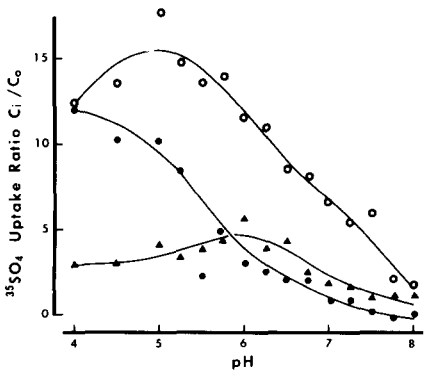

Fig. 2. Effect of extracellular pH on sulfate by mouse renal cortex slices incubated in sucrose (●), sucrose-KCl (○) (with 20 mM KCl added), and Krebs-Ringer media (▲). Buffers added to adjust pH were 10 mM Mes-Tris (pH 4.0–6.0) and 10 mM Tris-Hepes (pH 6.25–8.0). Each point is the mean (± S.E.) of triplicate values.

TABLE III

EFFECT OF INHIBITORS ON UPTAKES OF SULFATE AND α-AMINOISOBUTYRIC ACID BY MOUSE RENAL CORTICAL SLICES

Values are expressed as mean ± S.E., n shown in parentheses. n.s., not significant.

Inhibitor added (Concentration)	Uptake (% of control)			
	Sulfate [a]	p [c]	AIB [b]	p [c]
None	100 ± 5 (3)		100 ± 6 (3)	
Amiloride (1 mM)	151 ± 9 (3)	< 0.01	67 ± 7 (3)	< 0.01
Furosemide (1 mM)	111 ± 12 (3)	n.s.	84 ± 5 (3)	< 0.05
Ouabain (1 mM)	108 ± 2 (3)	n.s.	29 ± 2 (3)	< 0.01
NAP-taurine (1 mM) [c,d]				
before photoreaction	82 ± 15 (6)	n.s.	112 ± 16 (4)	n.s.
after photoreaction	56 ± 11 (7)	< 0.05	84 ± 20 (4)	n.s.
DIDS (1 mM) [c]	52 ± 6 (6)	< 0.01	89 ± 6 (5)	n.s.
SITS (1 mM) [c]	43 ± 9 (5)	< 0.01	92 ± 7 (5)	n.s.

[a] Sulfate uptake at 0.1 mM extracellular concentration was measured in the sucrose-KCl buffer (pH 6.5) as the isotopic distribution ratio at steady-state, and distribution ratio of 7.9 represents 100%.

[b] α-Aminoisobutyric acid (AIB) uptake at 0.1 mM extracellular concentration was measured in modified Krebs-Ringer buffer (pH 7.4) as the isotopic distribution ratio at steady-state; a distribution ratio of 7.3 represents 100%.

[c] Pipes buffer (10 mM, pH 6.5) was used to avoid interaction of Tris with the disulfonate stilbene.

[d] Photoreaction performed as described by Cheng and Levy [25].

[e] Relative to control (no inhibition).

by freeze-thaw treatment of slices. These observations imply that sulfate is accumulated by renal cortex slices against an inside-negative membrane electrical potential.

Influence of extracellular environment on sulfate accumulation by slices.

Experiments were performed to delineate the effect on sulfate uptake of various cations and agents known to interact at the basolateral membrane. Net uptake of sulfate was stimulated by extracellular K^+, Rb^+ and Cs^+ in the sucrose-KCl medium; maximum stimulation was obtained with 20 mM K^+ (Table II). Addition of Na^+ to K^+-containing medium inhibited sulfate uptake. Uptake was maximal at pH 5 in the sucrose-KCl medium and at pH 6 in the modified Krebs-Ringer medium (Fig. 2).

Sulfate uptake in sucrose-KCl-Pipes medium at pH 6.5 and α-aminoisobutyric acid uptake in Krebs-Ringer buffer at pH 7.4 were measured in the presence of inhibitors of renal transport activity. Ouabain and furosemide inhibited α-aminoisobutyric acid uptake but had no effect on sulfate (Table III), whereas amiloride stimulated sulfate and inhibited α-aminoisobutyric acid uptakes. Inhibitors of anion-exchange systems (DIDS, SITS and NAP-taurine) impaired net uptake of sulfate only.

Evidence that net sulfate uptake by slices is saturable

Net sulfate uptake by slices was concentration-dependent at steady-state in the sucrose-KCl medium (Fig. 3). Eadie-Hofstee transformation of the uptake rates corrected for diffusion (U vs. U/S) yielded a single regression line ($r = 0.95$, $p = 0.01$). The apparent V_{max} for sulfate uptake was 5.34 mmol/l ICF per h and the apparent $K_{\frac{1}{2}}$, 0.76 mM.

Efflux of sulfate from slices

We examined counter-transport under conditions of sulfate efflux to confirm that transport of sulfate was carrier-mediated in the slice preparation. Efflux of radiolabelled sulfate from preloaded slices observed first-order rate kinetics with or without counter-transport stimulation in the sucrose-KCl medium (Fig. 4). Sulfate efflux was enhanced when sulfate anion was present in the external medium, indicating that efflux of sulfate is mediated by a carrier.

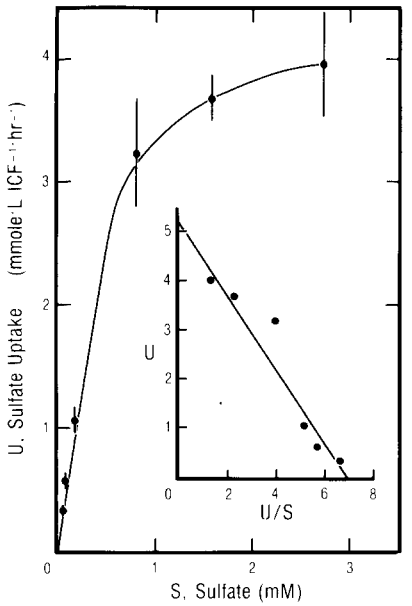

Fig. 3. Concentration dependence of $^{35}SO_4$ uptake by mouse renal cortex slices incubated in sucrose-KCl medium. Each point is the mean (±S.E.) of at least three replicates. (Inset: Eadie-Hofstee transformation of values; best fit was obtained by least-squares regression). The apparent constants were: $K_{\frac{1}{2}}$ = 0.76 mM; V_{max} = 5.34 mmol/l ICF per h.

Sulfate transport in brush-border membrane vesicles

We studied sulfate uptake by mouse renal brush-border membrane vesicles only to identify whether it was different from uptake identified in the slice preparation.

We ascertained first whether there was any nonspecific binding of anion to brush-border membrane vesicles. Measurements of uptake were made with vesicles whose volume was modified by extravesicular osmolarity (Fig. 5). We also calculated the intravesicular volumes for sulfate and

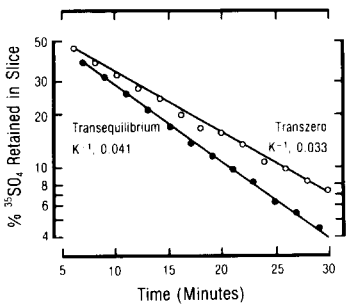

Fig. 4. Efflux of $^{35}SO_4$ from mouse renal cortex slices preloaded with 1 mM sulfate to steady-state. The exponential rate is rectified on this semilogarithmic plot. When measured under trans-equilibrium conditions (●) with 1.0 mM SO_4 in the efflux buffer, sulfate efflux was significantly faster, relative to the corresponding trans-zero condition (○) without sulfate in the efflux buffer. Data shown are for a single experiment; the finding was corroborated in five replicate experiments: k^{-1} ($min^{-1} \cdot 10^{-2}$) in trans-zero condition, 4.06±0.39; in trans-equilibrium condition, 4.35±0.35 (p = 0.0029 by χ^2 procedure (p. 623, Ref. 24).

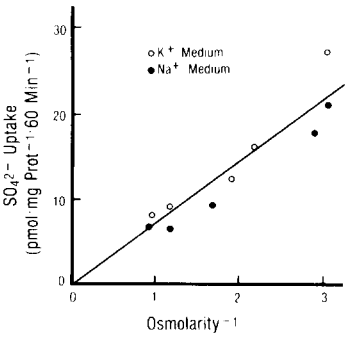

Fig. 5. Effect of increasing external osmolarity on sulfate distribution volume in mouse renal cortex brush-border membrane vesicle at equilibrium. The impermeable solute was sucrose, at external concentrations to attain 300–1000 mosM in the incubation medium. Experiments were performed with potassium-containing medium (○) or sodium-containing medium (●). Each point is the mean of triplicate values for sulfate distribution at equilibrium (60 min).

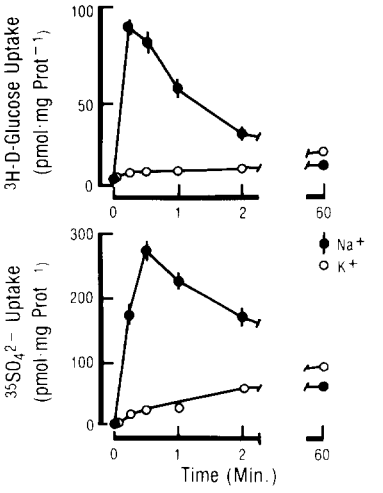

Fig. 6. Time-course for uptakes of D-glucose and sulfate by mouse renal cortex brush-border membrane vesicles measured simultaneously in potassium-containing (○) and sodium-containing (●) incubation media. Each point is mean ± S.E. of five replicates.

D-glucose at equilibrium; they were the same (2.0 $\pm$ 0.2 μl/mg protein). These findings exclude significant binding of sulfate to brush-border membrane vesicles.

Simultaneous uptakes of sulfate and D-glucose by brush-border membrane vesicles were both dependent on an inward-directed transmembrane sodium gradient (Fig. 6). The maximum 'overshoot' in uptake was observed at 15 s for D-glucose and at 30 s for sulfate. Both solutes attained equilibrium by 60 min, sulfate doing so more slowly than D-glucose. Uptake of 50 μM sulfate at 15 s was inhibited 14% by 10 μM D-glucose ($n = 6$, $p = 0.005$, Student t-test) perhaps by partial dissipation of the Na^+ gradient during the more rapid uptake of D-glucose. Accordingly, estimates of kinetic values for sulfate uptake were obtained in the absence of D-glucose. Concentration-dependent uptake of sulfate anion was measured by subtracting the nonsaturable component of uptake, measured in the K^+ medium, from total uptake measured in the Na^+ medium. Saturable sodium-dependent uptake of sulfate was observed in renal brush-border membrane vesicles. The apparent kinetic values for sulfate uptake were: V_{max}, 780 pmol/mg protein at 15 s; $K_{\frac{1}{2}}$, 0.36 mmol/l (coefficient of variation, 3.9%).

Discussion

Endogenous sulfate anion, whether available from cysteine oxidation, sulfoester hydrolysis or diet, is the cosubstrate for many sulfoconjugation reactions and biosynthetic pathways [26]. Accordingly, the role of kidney in maintaining homeostasis of extracellular sulfate to serve these important metabolic events is of interest. We used renal brush-border membrane vesicles and slice preparations in the work described here for two reasons: first, to define the importance of metabolic runout in net renal reabsorption of sulfate; second, to determine which membrane (brush-border membrane or basolateral membrane) is exposed in the slice. The slice preparation retains metabolic activity, and we studied metabolism of sulfate in this preparation. Since sulfate transport has been well characterized in both renal brush-border membrane vesicle preparations [8–10,12], this anion is now a useful probe to delineate which membrane is operating functionally in the slice preparation; therefore, we compared the characteristics of sulfate uptake in slices with the brush-border membrane vesicle preparation. The use of mouse kidney in this context is a novelty.

Sulfate was not rapidly incorporated into organic pools in the slice preparation. Accordingly, we conclude that metabolic incorporation of anion is not an important determinant of net reabsorption across brush-border membranes or of net uptake across basolateral membrane. This finding has not previously been reported to our knowledge.

Sulfate transport by brush-border membranes involves no significant binding of anion. It is dependent on the Na^+-gradient (outside > inside) and is saturable; elsewhere [13], we showed that it is competitively inhibited by molybdate. These findings corroborate the work of others [8–10], where it was also shown that stilbenes do not inhibit sulfate transport by brush-border membrane vesicles. Our value for the apparent $K_{\frac{1}{2}}$ in mouse renal brush-border membrane vesicles (approx. 0.28 mM) is one-third of that reported by Lucke et al. [8] for rat renal brush-border membrane vesicles and half of that reported by Brazy and Dennis [11] for the isolated perfused rabbit proximal tubule segment. Differences in species in preparation may account for these apparent discrepancies.

The renal cortex slice accumulates sulfate, at low extracellular concentrations (less than 1 mM), against an electrochemical gradient (Fig. 1, Table I); we used the simultaneous distribution of lipophilic ions [28] and the analysis of tissue sulfate anion to confirm this fact. In contrast to our findings with the brush-border membrane vesicle preparation, sulfate uptake by slices is sodium-independent (Table II) and inhibited by disulfonated stilbenes (Table III). This is sufficient evidence that the mechanisms for sulfate uptake are clearly different in slice and brush-border membrane vesicle preparations. Our additional evidence for carrier-mediated uptake (vs. binding) of sulfate anion by the slice includes: saturability (Fig. 3) and trans-equilibrium stimulation of efflux (Fig. 4). If we assume that the sulfate carrier exposed in the slice is not located in the brush-border membrane, it is of interest to know whether

it resembles any of the anion-exchange systems assigned to the basolateral membrane.

Pritchard and Renfro [10] have characterized sulfate transport in a rat renal cortex basolateral membrane vesicle preparation. They found that sulfate transport is: Na^+-independent, stimulated by K^+ or H^+, saturable, dependent on membrane electrical potential, inhibited by stilbene and Hg^{2+}, and stimulated by counter-transport exchange. They present strong evidence for HCO_3^- or OH^- exchange on the sulfate carrier and propose that this inorganic anion exchanger is different from the organic anion-exchange system of renal tubule; they further deduce, largely from data published by Ullrich and Murer [7] that the inorganic anion-exchange system is located in late proximal tubule.

It was not our intention to delineate sulfate anion transport in detail in brush-border membrane vesicles or slices. Nonetheless, some interesting observations were made. Sulfate uptake by the slice is increased in the presence of furosemide and amiloride at relatively high concentrations (1 mM) of inhibitor (Table III). While furosemide can inhibit potassium flux specifically across the membrane, when its concentration is below 1 mM [29], and might thus inhibit K^+-dependent sulfate uptake, it can have a broader effect to include inhibition of $(Na^+ + K^+)$-ATPase at higher concentrations; amiloride also inhibits $(Na^+ + K^+)$-ATPase at equivalent concentrations [30]. Accordingly, the stimulatory effect of these agents on sulfate uptake by slices could reflect inhibition of $(Na^+ + K^+)$-ATPase activity with secondary perturbation of intracellular pH; the same agents inhibited the uptake of α-aminoisobutyric acid which, unlike sulfate, is Na^+-dependent in the slice [18]. In light of these findings, it is not surprising that ouabain also stimulated sulfate uptake slightly and inhibited α-aminoisobutyric acid uptake (Table III). The result obtained with ouabain is not unconventional. Smith et al. [31,32] reported 18% stimulation of the serosa-to-mucosa sulfate flux when serosal surface of rabbit ileum was exposed to 0.25 mM ouabain. Stilbenes and NAP-taurine, each at 1 mM, inhibited slice uptake of sulfate (Table III) and others [10–12] have observed this effect with isolated tubule segments and basolateral membrane vesicle preparations. High concentrations of inhibitor were required in the slice preparation, relative to the other preparations, presumably because of nonspecific binding of inhibitor.

The K^+ specificity for sulfate uptake by renal cortex slices observed here was reported initially by Deyrup and Ussing [33]. Pritchard and Renfro [10] observed stimulation of sulfate uptake by rat renal basolateral membrane vesicles with a 50 mM $KHCO_3$ gradient (inside > outside) relative to an equivalent $NaHCO_3$ gradient; the effect of $KHCO_3$ was not inhibited when 50 mM NaCl replaced mannitol in both vesicle and incubation buffers. Since bicarbonate stimulates sulfate uptake by basolateral membrane vesicles, whether K^+ is present or not [10], it is apparent that this cation is permissive but not obligatory for sulfate exchange on the anion carrier. The influence of pH on sulfate uptake by slices described here was also reported initially by Deyrup and Ussing [33]; and Pritchard and Renfro [10] noted that a pH gradient (OH^- inside > outside) may drive sulfate uptake by basolateral membrane vesicles. Whether the effects of K^+ and pH on sulfate uptake by slices could be related in common to cytosolic (type II) carbonic anhydrase activity is a matter of speculation. Thus, the important role of K^+ and H^+ on sulfate uptake in the slice preparation has not yet been explained.

In summary, our findings indicate that the mouse renal cortex slice exposes a sulfate-transport system which is different from that in the isolated brush-border membrane; the results reported here are compatible with the hypothesis [14,27] that the slice preparation occludes the brush-border membrane from direct access to solute in the medium.

Acknowledgements

We thank Fazl Mohyuddin for help with some of the experiments, Michel Bergeron for helpful discussion, and Harriet Tenenhouse for her careful review of the manuscript. Ronald Poole generously provided the labelled lipophilic ions. Lynne Prevost and Kathryn Craig prepared the typescript. This work was supported in part by the Medical Research Council of Canada. D.E.C.C. held an MRC Fellowship and submitted parts of this study in partial fulfillment of the requirements for a

Doctorate degree (Department of Biology, McGill University).

References

1 Scriver, C.R., Chesney, R.W. and McInnes, R.R. (1976) Kidney Int. 9, 149–171
2 Schafer, J.A. and Barfuss, D.W. (1980) Am. J. Physiol. 238, F335–F346
3 Guidotti, G.G., Borghetti, A.F. and Gazzola, G.C. (1978) Biochim. Biophys. Acta 515, 329–366
4 Lotspeich, W.D. (1947) Am. J. Physiol. 151, 311–318
5 Ullrich, K.J., Rumrich, G. and Kloss, S. (1980) Pflügers Arch. 383, 159–163
6 Samarzija, I., Moker, V. and Fromter, E. (1981) in Advances in Physiological Science, Vol. 11, Kidney and Body Fluids (Takacs, L., ed.), pp. 419–423, Pergamon, Oxford
7 Ullrich, K.J. and Murer, H. (1982) Phil. Trans. R. Soc. Lond. B 299, 549–558
8 Lucke, J., Stange, G. and Murer, H. (1979) Biochem. J. 182, 223–229
9 Schneider, E.C., Durham, J.C. and Sacktor, B. (1980) Fed. Proc. Fed. Am. Soc. Exp. Biol. (Abstr.) 39, 1711
10 Pritchard, J.B. and Renfro, J.L. (1983) Proc. Natl. Acad. Sci. USA 80, 2603–2607
11 Brazy, P.C. and Dennis, V.M. (1981) Am. J. Physiol. 241, F300–F307
12 Grinstein, S., Turner, R.J., Silverman, M. and Rothstein, A. (1980) Am. J. Physiol. 238, F452–F460
13 Cole, D.E.C. and Scriver, C.R. (1984) Pediatr. Res. 18, 25–29
14 Arthus, M.-F., Bergeron, M. and Scriver, C.R. (1982) Biochim. Biophys. Acta 692, 371–376
15 Chesney, R.W., Jax, D.K., Mohyuddin, F. and Scriver, C.R. (1978) Renal Physiol. 1, 166–170
16 Rosenberg, L.E., Blair, A. and Segal, S. (1961) Biochim. Biophys. Acta 54, 479–488
17 Deyrup, I.J. (1964) Am. J. Physiol. 207, 84–87
18 Scriver, C.R. and Mohyuddin, F. (1968) J. Biol. Chem. 343, 3207–3213
19 Cole, D.E.C. and Scriver, C.R. (1981) J. Chromatogr. 225, 359–367
20 Wong, K.P. and Yeo, T. (1979) Biochem. J. 181, 107–110
21 Tenenhouse, H.S. and Scriver, C.R. (1978) Can. J. Biochem. 56, 640–646
22 Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581
23 Lowry, O.H., Rosebrough, N.J. Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
24 Sokal, R.R. and Rohlf, F.J. (1969) Biometry, W.H. Freeman, San Francisco
25 Cheng, S. and Levy, D. (1980) J. Biol. Chem. 255, 2637–2640
26 Mulder, G.J. (1981) Sulfation of Drugs and Related Compounds, pp. 131–185, CRC Press Inc., Boca Raton
27 Wedeen, R.P. and Weiner, B. (1973) Kidney Int. 3, 205–213
28 Goldinger, J.M., Duffey, M.E. and Hong, S.K. (1983) Proc. Soc. Expt. Biol. Med. 173, 281–287
29 Bakker-Grunwald, T. (1978) Biochim. Biophys. Acta 513, 292–295
30 Soltoff, S.P. and Mandel, L.J. (1983) Science 220, 957–959
31 Smith, P.L., Orellana, S.A. and Field, M. (1981) J. Membrane Biol. 63, 199–206
32 Langridge-Smith, J.E. and Field, M. (1981) J. Membrane Biol. 63, 207–214
33 Deyrup, I.J. and Ussing, H.H. (1955) J. Gen. Physiol. 38, 599–612