Role of tubular secretion and carbonic anhydrase in vertebrate renal sulfate excretion

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Pelis, Ryan M., and J. Larry Renfro. Role of tubular secretion and carbonic anhydrase in vertebrate renal sulfate excretion. Am J Physiol Regul Integr Comp Physiol 287: R491–R501, 2004; 10.1152/ajpregu.00084.2004.—The renal proximal tubule of vertebrates performs an essential role in controlling plasma SO\textsuperscript{4}\textsuperscript{2}\textsuperscript{-} concentration ([SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}]). Although net tubular SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} reabsorption is the predominate control process in terrestrial vertebrates, a facilitated secretory flux is also present. In contrast, marine teleosts obtain excess SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} from drinking, and increased plasma [SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}] is prevented predominately through net tubular secretion. Tubular SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} secretion is accomplished by at least two electroneutral anion exchange processes in series. Movement of SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} into the cell across the basolateral membrane is pH dependent, suggesting SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}/OH\textsuperscript{-} exchange. Luminal HCO\textsubscript{3}\textsuperscript{-} and Cl\textsuperscript{-} can facilitate SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} movement out of the cell across the brush-border membrane. The molecular identities of the anion exchangers are unknown but are probably homologues of SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} transporters in the mammalian SLC26 gene family. In all species tested, glucocorticoids increase renal SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} excretion. Whereas glucocorticoids downregulate SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} reabsorptive mechanisms in terrestrial vertebrates, they may also stimulate a mediated secretory flux. In the marine teleost, cortisol increases the level of SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange at the brush-border membrane, tubular carbonic anhydrase (CA) activity, CAII protein, and a proportion of tubular SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} secretion that is CA dependent. CA activity is required for about one-half of this net SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} secretion but is also required for about one-half of the net reabsorption in bird proximal epithelium. A CA-SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}/anion exchanger metabolon arrangement is proposed that may speed both the secretory and reabsorptive processes.

INORGANIC SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} IS THE SECOND most abundant anion in the sea and one of the most abundant anions in vertebrate plasma, with concentrations varying among species from 0.3 to 1.8 mM. This large tetrahedral divalent anion can be obtained from the diet (83) or oxidation of the sulfur-containing amino acids cysteine and methionine (77). Sulfoconjugation reactions are the initial detoxification process for many endogenous (e.g., steroid hormones) and xenobiotic compounds and are required for the activation of numerous biological molecules (e.g., heparin, cholecystokinin, and gastrin) (59). SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} is a major component of glycosaminoglycans (dermatan sulfate, chondroitin sulfate, keratan sulfate, and heparan sulfate), which are structural components of numerous tissues, including cartilage, bone, myelin, and basal lamina (40). SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} deficiency in chondrocytes causes phenotypes (chondrodysplasias) that are characterized by severe growth abnormalities (24). Elevated plasma SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} can cause diuresis and acidosis (18), and increased SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} concentration ([SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}]) in the renal tubule lumen reduces Ca\textsuperscript{2}\textsuperscript{+} and Mg\textsuperscript{2}\textsuperscript{+} reabsorption (66).

RENA L SULF A T E CLR EA RNCE

The kidneys are the major avenue for SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} excretion in vertebrates. Nearly all of plasma SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} is ultrafilterable in mammals (4), whereas 80% and 47% of plasma SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} are ultrafilterable in birds (71) and fish (75), respectively. Variation in the estimates of the ultrafilterable fraction of plasma SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} in vertebrates may arise from species differences or variations in method of determination. Urine content is determined by glomerular filtration, tubular reabsorption, and tubular secretion. This review will focus on the latter and will provide only a brief overview of tubular reabsorption because it has been described in detail elsewhere (2, 51, 52, 57).

Reabsorption of filtered SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} occurs primarily in the proximal tubule (35) and is required for SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} homeostasis in mammals and presumably all other terrestrial and freshwater vertebrates. SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} clearance ratios [SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} clearance/glomerular filtration rate (GFR)] >1 have not been measured in any terrestrial vertebrate, suggesting that net renal tubular SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} secretion does not occur in these animals. SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} loading apparently has no effect on the saturable reabsorptive transport maximum for SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} (TmSO\textsubscript{4}\textsuperscript{2}\textsuperscript{-}; ) in the renal tubule of the human, dog, and frog (3, 28, 48). In contrast, TmSO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} in rats is reduced ~50% after plasma SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} loading, indicating that adaptive mechanisms are in place to depress reabsorption, thus increasing excretion (27). Similarly, in domestic fowl, TmSO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} decreases with plasma SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} loading, leading to the conclusion that a fraction of the excreted SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} is the result of tubular secretion (30). Thiosulfate, an inhibitor of SO\textsubscript{4}\textsuperscript{2}\textsuperscript{-} transporters,
reduces an SO$_4^{2-}$ secretory flux in perfused renal tubules of the rabbit (13) and in renal proximal tubule epithelial monolayers (PTCs) prepared from the domestic chicken (Gallus gallus) (22) (Fig. 1), Brazy and Dennis (13) further demonstrated inhibition of the SO$_4^{2-}$ secretory flux in rabbit tubules by SITS, suggesting an anion exchange mechanism. Renal tubular thiosulfate secretion in dogs may share a common secretory mechanism with SO$_4^{2-}$ (6). The marine herring gull (Larus argentatus) can produce urine containing 77 mM SO$_4^{2-}$ in the lumen (7), and the transepithelial concentration and electrical potential (−1.9 mV, lumen negative) differences indicated that net SO$_4^{2-}$ secretion was active (10). Flounder PTCs in Ussing chambers were used to definitively demonstrate net active SO$_4^{2-}$ secretion (21). These epithelial sheets exhibit differentiated properties of proximal tubule cells, including ciliary activity, structural polarity, low transepithelial resistance, greater permeability to Na$^+$ than Cl$^-$, and phlorizin-sensitive, sodium-coupled glucose transport. In the presence of flounder saline (1 mM SO$_4^{2-}$), typical measurements yield unidirectional secretory fluxes of 98.4 ± 6.67 nmol·cm$^{-2}$·h$^{-1}$, unidirectional reabsorptive fluxes of 4.8 ± 1.08 nmol·cm$^{-2}$·h$^{-1}$, and net fluxes of 93.6 ± 7.64 nmol·cm$^{-2}$·h$^{-1}$ under short-circuited conditions (69). Because freshwater generally contains very low levels of SO$_4^{2-}$, and teleosts limit their drinking in freshwater, ingested food items should provide the only external source of SO$_4^{2-}$ available to freshwater teleosts. Although it has not been demonstrated, the kidney of freshwater teleosts should be a site of net SO$_4^{2-}$ reabsorption.

**MECHANISMS OF TUBULAR REABSORPTION**

Although the emphasis of this review is tubular secretion, the reabsorptive process is mentioned briefly to draw attention to a newly discovered role of CA in the process. Cellular phospholipid bilayers have a very low passive permeability to SO$_4^{2-}$, and, therefore, transport proteins are required to efficiently move SO$_4^{2-}$ across the plasma membrane (38). Reabsorptive transport of SO$_4^{2-}$ across the brush-border membrane (BBM) is Na$^+$ dependent in the intact proximal tubule (88) and in isolated BBM vesicles from mammalian (80, 87) and avian (71) species. The Na$^+$-SO$_4^{2-}$ cotransporter (NaSi-1) has been identified from a rat renal cortex cDNA library by expression

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**Fig. 1.** Effects of thiosulfate on the unidirectional reabsorptive and secretory fluxes of SO$_4^{2-}$ in perfused rabbit proximal tubule and chicken renal proximal tubule epithelium in primary culture (chick PTCs). Data are presented as a percentage of the control unidirectional reabsorptive flux. Thiosulfate was added to the interstitial bath, luminal bath, or both. *Significantly different from control. Rabbit and chicken data are from Refs. 13 and 22, respectively.
RENAL TUBULAR SULFATE SECRETION IN VERTEBRATES

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Table 1. Anions that trans-stimulate sulfate transport across renal tubular basolateral and BBM of various species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Membrane</th>
<th>Substrates</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammal</td>
<td>BLM</td>
<td>HCO$_3^-$; oxalate; OH$^-$; S$_2$O$_3^{2-}$</td>
<td>(44, 65)</td>
</tr>
<tr>
<td>Mammal</td>
<td>BBM</td>
<td>HCO$_3^-$; Cl$^-$; OH$^-$; SCN$^-$; NO$_3^-$; I$^-$</td>
<td>(64)</td>
</tr>
<tr>
<td>Chicken</td>
<td>BLM</td>
<td>HCO$_3^-$; S$_2$O$_3^{2-}$</td>
<td>(71)</td>
</tr>
<tr>
<td>Chicken</td>
<td>BBM</td>
<td>HCO$_3^-$; OH$^-$; SCN$^-$; S$_2$O$_3^{2-}$</td>
<td>(71)</td>
</tr>
<tr>
<td>Marine teleost</td>
<td>BLM</td>
<td>OH$^-$</td>
<td>(75)</td>
</tr>
<tr>
<td>Marine teleost</td>
<td>BBM</td>
<td>HCO$_3^-$; Cl$^-$; SCN$^-$; S$_2$O$_3^{2-}$</td>
<td>(76)</td>
</tr>
</tbody>
</table>

Substrates were tested using either renal basolateral membrane (BLM) or brush-border membrane (BBM) vesicles isolated from rat, rabbit, chicken, or marine teleost.

cloning (54). NaSi-1 protein localizes to the BBM of renal proximal tubule cells (20).

Electroneutral Na$^+$-independent SO$_4^{2-}$/HCO$_3^-$ exchange has been demonstrated in renal basolateral membrane (BLM) vesicles isolated from the rat (65), chicken (71), and rabbit (44) (Table 1). In addition to SO$_4^{2-}$/HCO$_3^-$ exchange, Kuo and Aronson (44) found oxalate/HCO$_3^-$ exchange in rabbit renal BLM vesicles and concluded that all three anions (SO$_4^{2-}$/HCO$_3^-$/oxalate) are substrates for the same transporter. A transporter exhibiting SO$_4^{2-}$/oxalate exchange similar to that found in renal BLM vesicles from mammals was cloned from rat liver and called sat-1 (11). Markovich et al. (53) injected rat kidney cortex mRNA into Xenopus oocytes and demonstrated Na$^+$-independent SO$_4^{2-}$ uptake that was inhibited by oxalate and thiosulfate, which is similar to that found for liver sat-1. Size fractionation of the mRNA followed by expression in Xenopus oocytes and Northern hybridization provided further evidence for sat-1 in the renal cortex. Sat-1 was later localized to the BBM of proximal tubule cells with an anti-sat-1 antibody (41).

The involvement of CA in renal tubular SO$_4^{2-}$ reabsorption has recently been demonstrated in the chicken proximal tubule (22). CA catalyzes the reversible dehydroxylation of HCO$_3^-$ and hydroxylation of CO$_2$ (HCO$_3^-$ → OH$^-$ + CO$_2$). Both CA biochemical activity and CA isoform II (CAII) protein are present in chick PTCs, and net SO$_4^{2-}$ reabsorption is inhibited ~50% after CA inhibition. It is not known whether intracellular and/or extracellular CA activity is required for tubular SO$_4^{2-}$ reabsorption, because ethoxzolamide, a soluble CA inhibitor with a relatively high lipid-to-water partition coefficient, was used in the aforementioned study.

MECHANISMS OF TUBULAR SECRETION

Tubular SO$_4^{2-}$ secretion is the emphasis of this review, because the process is seldom discussed in other reviews of SO$_4^{2-}$ homeostasis. Although a facilitated SO$_4^{2-}$ secretory flux has been observed in birds and mammals, it is perhaps best expressed in marine teleosts. Anion exchange mechanisms for SO$_4^{2-}$ were identified in the renal tubular BBM and BLM of two species of marine flounder (75, 76). Concentrative SO$_4^{2-}$ uptake into renal BLM vesicles is pH dependent, suggesting SO$_4^{2-}$/OH$^-$ exchange (Table 1) (75). SO$_4^{2-}$ transport by BBM vesicles is stimulated by HCO$_3^-$, Cl$^-$, SCN$^-$, and S$_2$O$_3^{2-}$, with HCO$_3^-$ serving as most effective counteranion (76). Anion exchange at the BBM is not pH dependent, distinguishing it from the basolateral SO$_4^{2-}$/OH$^-$ exchanger (75). Unlike renal BBM vesicles from avian and mammalian species, an Na$^+$ gradient has no effect on concentrative SO$_4^{2-}$ uptake into marine teleost renal BBM vesicles (75). Both basolateral SO$_4^{2-}$/OH$^-$ exchange and brush-border SO$_4^{2-}$/anion exchange are electroneutral processes that are inhibited by the anion exchange inhibitor DIDS. It is not clear for either pole of flounder proximal tubule epithelial cells whether a single anion exchanger or multiple exchangers facilitate SO$_4^{2-}$ transport.

Anion exchange mechanisms behaving similarly to those in the BBM and BLM of the marine teleost proximal tubule have also been found in the renal tubule of birds and mammals (Table 1). Although less effective than HCO$_3^-$, OH$^-$ was shown to trans-stimulate SO$_4^{2-}$ uptake into rat renal BLM vesicles (65). SO$_4^{2-}$ uptake into rat renal BBM vesicles is trans-stimulated by HCO$_3^-$, Cl$^-$, OH$^-$, SCN$^-$, NO$_3^-$, and I$^-$, with HCO$_3^-$ being most effective (64). HCO$_3^-$-stimulated SO$_4^{2-}$ uptake into chick BBM vesicles is inhibited by OH$^-$, SCN$^-$, and S$_2$O$_3^{2-}$, suggesting that these anions can act as substrates (71). The aforementioned anion exchange processes in the terrestrial vertebrate proximal tubule may perform a role similar to those in the marine teleost renal tubule.

SO$_4^{2-}$/anion exchangers important for SO$_4^{2-}$ secretion most likely represent one or more of the SO$_4^{2-}$/anion transporters within the mammalian SLC26 gene family. There have been 11 SLC26 genes (SLC26A1-SLC26A11) identified and seven of these have been shown to transport SO$_4^{2-}$ when expressed in heterologous systems (58). Of the seven that transport SO$_4^{2-}$, five (SLC26A1, SLC26A2, SLC26A6, SLC26A7, and SLC26A11) are expressed in the mammalian kidney. SLC26A1 encodes the SO$_4^{2-}$/HCO$_3^-$ exchanger (sat-1) identified in the BLM of mammalian renal proximal tubule cells (41) (Table 2). In addition, SLC26A1 exhibits Cl$^-$ transport activity when expressed in Xenopus oocytes (45). SLC26A2 (DTDST), which appears to be expressed ubiquitously (32), has affinities for many anions, including SO$_4^{2-}$, HCO$_3^-$, Cl$^-$, oxalate, and S$_2$O$_3^{2-}$ (79). The putative Cl$^-$/formate exchanger SLC26A6 (PAT-1, CFEX) has been localized to the BBM of mammalian renal proximal tubule cells (43). In addition to Cl$^-$ and formate, SLC26A6 has affinities for oxalate, HCO$_3^-$, and SO$_4^{2-}$ (39, 93). SLC26A7 has been localized to the BLM of rat outer medullary collecting duct cells where it has been hypothesized to function in HCO$_3^-$ reabsorption (63). When expressed in Xenopus oocytes, SLC26A7 exhibits HCO$_3^-$, Cl$^-$, oxalate, and SO$_4^{2-}$ transport activity (47, 63). SLC26A11 mRNA transcript is present in the mammalian kidney and displays DIDS-sensitive Na$^+$-independent SO$_4^{2-}$ transport when expressed in insect Sf9 cells (92).

Table 2. Renal localization and substrate specificity of sulfate transporters in the mammalian SLC26 gene family

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Localization</th>
<th>Substrates</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>SLC26A1</td>
<td>BLM of PT cells</td>
<td>SO$_4^{2-}$; oxalate; HCO$_3^-$; Cl$^-$</td>
<td>(41, 44, 45, 53)</td>
</tr>
<tr>
<td>SLC26A2</td>
<td>?</td>
<td>SO$_4^{2-}$; HCO$_3^-$; Cl$^-$; oxalate; S$_2$O$_3^{2-}$</td>
<td>(32, 79)</td>
</tr>
<tr>
<td>SLC26A6</td>
<td>BLM of PT cells</td>
<td>SO$_4^{2-}$; oxalate; HCO$_3^-$; Cl$^-$; formate</td>
<td>(39, 43, 93)</td>
</tr>
<tr>
<td>SLC26A7</td>
<td>BLM of OMCD cells</td>
<td>SO$_4^{2-}$; HCO$_3^-$; Cl$^-$; oxalate</td>
<td>(47, 63)</td>
</tr>
<tr>
<td>SLC26A11</td>
<td>?</td>
<td>SO$_4^{2-}$</td>
<td>(92)</td>
</tr>
</tbody>
</table>

OMCD, outer medullary collecting duct; ?, renal localization unknown; PT, proximal tubule.
Whereas anion exchangers seem certain to play a role in tubular SO$_4^{2-}$ secretion, exactly how they are involved in vivo is less certain. Luminal [SO$_4^{2-}$] in perfused renal tubules of the winter flounder is $\sim$10 mM (7). With the use of estimates of luminal volume (42) and measurements of [SO$_4^{2-}$] in the luminal (10) and tubular fluid spaces (73), Renfro (70) estimated total intracellular [SO$_4^{2-}$] to be 2 mM. Hence, the driving force for SO$_4^{2-}$ exit across the BBM to be questioned because the anion exchange mechanism is electroneutral and SO$_4^{2-}$ appears to move against a chemical gradient. Although HCO$_3^-$ can trans-stimulate SO$_4^{2-}$ uptake into BBM vesicles (Table 1), it is not apparent whether luminal concentrations in vivo are high enough to facilitate SO$_4^{2-}$ exit across the BBM. BBM vesicle experiments have shown that Cl$^{-}$ can trans-stimulate SO$_4^{2-}$ transport (Table 1). Cl$^{-}$ appears to be a plausible substrate to drive SO$_4^{2-}$ exit from the cell because luminal [Cl$^{-}$] is the same as or slightly higher than in plasma ($\sim$160 mM) (19) and the Nernstian concentration of intracellular Cl$^{-}$ would be $\sim$15 mM at membrane potential difference of $\sim$60 mV. The data presented in Fig. 2 show that in the absence of luminal HCO$_3^-$, Cl$^{-}$ is able to maintain transepithelial SO$_4^{2-}$ secretion. The movement of Cl$^{-}$ down its chemical gradient into the cell likely energizes the uphill movement of SO$_4^{2-}$ out of the cell across the BBM.

Our unpublished data also show that in the absence of luminal Cl$^{-}$, 5 mM HCO$_3^-$ is able to maintain transepithelial SO$_4^{2-}$ secretion by flounder PTCs (Fig. 2), albeit at a reduced level. In contrast, complete removal of luminal Cl$^{-}$ and HCO$_3^-$ together and reduction of luminal SO$_4^{2-}$ to 0.1 mM to prevent SO$_4^{2-}$/SO$_4^{2-}$ exchange completely abolishes net SO$_4^{2-}$ secretion (Fig. 2). With the use of a conservative estimate of 7.3 for intracellular pH and a dissociation constant (pK) for OH$^-$ + CO$_2$ ⇌ HCO$_3^-$ of 6.3 at 20°C, and assuming that intracellular P$_{CO_2}$ is identical to extracellular P$_{CO_2}$ (4 mM Hg), the calculated intracellular HCO$_3^-$ concentration would be $\sim$2 mM. The tubular fluid in the early proximal tubule of marine teleosts would have a [SO$_4^{2-}$], pH ($\sim$7.7), and [HCO$_3^-$] ($\sim$5 mM) identical to plasma, and thus [HCO$_3^-$] in the initial filtrate should be high enough to energize SO$_4^{2-}$ exit across the BBM. The final urine of marine teleosts is acidic ($\sim$6.6) and, therefore, contains low levels of HCO$_3^-$ (<1 mM) (33, 50, 74). Assuming that the proximal tubule is responsible for most HCO$_3^-$ reabsorption and proton secretion, the late proximal tubule luminal [HCO$_3^-$] should be greatly reduced compared with the calculated intracellular [HCO$_3^-$]. Luminal [HCO$_3^-$] in the Japanese bullfrog (Rana catesbeiana) proximal tubule is slightly lower than intracellular [HCO$_3^-$] (29). It can be concluded, therefore, that whereas HCO$_3^-$ may drive SO$_4^{2-}$ secretion in early proximal tubule segments, it cannot energize SO$_4^{2-}$ exit across the BBM in the late segments where the Cl$^{-}$ gradient may be essential.

Uptake of SO$_4^{2-}$ into flounder renal BLM vesicles is electroneutral (75). The OH$^-$ gradient should be unfavorable for SO$_4^{2-}$ uptake via SO$_4^{2-}$/OH$^-$ exchange given that intracellular pH in marine teleosts is $\sim$6.0 pH units lower than plasma (15). Similarly, the SO$_4^{2-}$ gradient across the BLM should also be unfavorable because plasma [SO$_4^{2-}$] in the flounder is $\sim$0.6 mM, whereas total intracellular [SO$_4^{2-}$] is $\sim$2 mM. Even so, there exists the possibility that the level of free intracellular SO$_4^{2-}$ available for exchange is much less than the estimated 2 mM. Intracellular Cl$^{-}$ can bind to proteins and phospholipid surfaces and can be sequestered in organelles making the exchangeable [Cl$^{-}$] much less than the total (38). If SO$_4^{2-}$ behaves similarly, then there exists the possibility that the exchangeable intracellular [SO$_4^{2-}$] is less than the interstitial [SO$_4^{2-}$]. Another possibility, however, is that the pH of an unstirred layer of fluid in the peritubular interstitites may be lower than intracellular pH thus providing an OH$^-$ gradient to drive the process.

In mammals, H$^+$ secretion indirectly drives proximal tubular HCO$_3^-$ reabsorption, and 40% of tubular HCO$_3^-$ reabsorption is CA dependent (67). Proximal tubular CA is also required for a fraction (50%) of tubular SO$_4^{2-}$ reabsorption in chickens (22). Although the role of CA in the marine fish kidney has long been questioned because CA inhibition has no effect on urine pH (see Table 3) or [HCO$_3^-$] (37), it is now recognized that CA activity subserves proximal tubular SO$_4^{2-}$ secretion in this tissue (74). CA inhibition in vivo reduces renal tubular SO$_4^{2-}$ secretion $\sim$40% (Table 3). CA activity is localized to the

![Fig. 2. Effects of luminal anion removal on net transepithelial SO$_4^{2-}$ secretion by primary cultures of winter flounder renal proximal tubule epithelium (flounder PTCs). Normal flounder saline always bathed the interstitial side of the epithelium and contained in (mM) 150 NaCl, 4 KCl, 0.5 NaH$_2$PO$_4$, 4.2 NaHCO$_3$, 1.0 Na$_2$SO$_4$, 25.0 HEPES, pH 7.5. Glucosinate salts substituted for the removed anions. Cl$^{-}$ and HCO$_3^-$-free saline (Cl$^{-}$ + HCO$_3^-$ free) contained 0.1 mM Na$_2$SO$_4$. All solutions were gassed with 99% O$_2$ - 1% CO$_2$, except solutions lacking HCO$_3$ which were gassed with 100% O$_2$. Transport studies were conducted in Ussing flux chambers under open circuited conditions. Each bar is means + SE of $n = 4$ preparations. Values shown were obtained at $t = 1.5$ h. *Significantly different from paired control ($P < 0.05$, paired $t$-test). These data have not been previously published.

| Table 3. Effect of methazolamide on renal function in winter flounder |
|-------------------------|---------|---------|---------|---------|
|                         | GFR     | Flow Rate | Serum pH | Urine pH |
|                         | (mL/min) | (mL/h)   | (pH)    | (pH)    |
| Control                 | 2.1 ± 0.61 | 0.7 ± 0.19 | 7.8 ± 0.05 | 6.7 ± 0.09 |
| Methazolamide           | 1.7 ± 0.55 | 0.5 ± 0.15 | 7.6 ± 0.04* | 6.7 ± 0.13 |
|                          | 24.7 ± 7.13 | 15.3 ± 6.54* |

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Values are means ± SE; $n = 8$ animals. Values shown are paired and taken $t$-h before (Control) and 1 h after administration of methazolamide. GFR, glomerular filtration rate; $Q_{SO_4^{2-}}$: sulfate secretion rate; *$P < 0.001$, †$P < 0.05$ compared with control. Significant differences determined by paired $t$-test comparisons. Data taken from Ref. 74.
pathway that allows metabolites to move efficiently between active sites by limiting the loss of intermediates to diffusion (84, 85). Even for enzymes that approach “kinetic perfection,” such as CA, the rate-limiting step in enzymatic catalysis is diffusion (19). Copeland (19) uses the electron transport system as an example of an enzyme pathway that has overcome the diffusion limitation by stating, “Because of the proximity of the enzymes in the membrane, the product leaves the active site of one enzyme and is presented to the active site of the next enzyme without the need for diffusion through solution.” A CA transport metabolon can be envisioned in the same manner, with presentation of the product of enzymatic catalysis (HCO\(_3^-\) or OH\(^-\)) in close proximity to the anion-binding site on the transporter or, alternatively, enzymatic catalysis may remove substrate from the vicinity of the anion-binding site. For anion exchange, this association may effectively create a functional asymmetry, which favors the transport of a particular substrate (HCO\(_3^-\) or OH\(^-\)) in one direction.

The association of mammalian CAII and AE1 occurs between a histidine-rich region in the NH\(_2\) terminal of CAII and a hydrophobic residue followed by 2–3 acidic residues in the COOH terminal of AE1 (85, 90, 91). In addition to CAII acceleration of transport activity, the COOH-terminal motif of AE1 (LDADD) increases CAII activity (81). Numerous other ion transporters, including Pendrin (SLC26A4), a member of the mammalian SLC26 gene family, possess potential binding sites for CAII (85). Figure 4 shows that the SO\(_4^{2-}\) transporters SLC26A1, SLC26A2, SLC26A7, and SLC26A11, which are expressed in the mammalian kidney, also possess potential CAII binding sites at their COOH terminals. SLC26A6, the putative Cl\(^-\)/formate exchanger in the BBM of mammalian proximal tubule cells does not appear to contain a CAII binding motif, at least in the last 40 amino acids of the COOH terminal. Because the cytoplasmic portion of the COOH-terminal tail could not be confidently identified it cannot be ruled out that SLC26A6 contains binding sites for CAII.

CAII has been cloned from numerous fish species and the amino terminus contains histidine residues that may form an electrostatic interaction with either glutamate or aspartate residues. A portion of CAII (329 bp) was recently cloned from the winter flounder proximal tubule, and CAII protein was shown to be both cytosolic and membrane associated (61). Furthermore, CAII protein migrated differently than purified bovine

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Fig. 3. Comparison of inhibitory effects of 3 sulfonamides at 10 μM on net transepithelial SO\(_4^{2-}\) secretion by primary cultures of winter flounder renal proximal tubule epithelium (flounder PTCs). Polymer-linked inhibitor was polyoxyethylene-aminobenzolamide, which is restricted to the extracellular space. Data are means + SE (n = 3 preparations) and are presented as a percentage of net SO\(_4^{2-}\) secretion by paired controls. Hatched line at 100% mark represents SO\(_4^{2-}\) transport in the paired controls. *P < 0.05 and **P < 0.01 compared with paired control. Data taken from Ref. 74.

**Table:**

<table>
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<th>Transporter</th>
<th>GenBank Accession #</th>
<th>C-Terminal Amino Acid Sequence</th>
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Fig. 4. Alignment of amino acid sequences from the COOH termini of various ion transport proteins including members of the mammalian SLC26 gene family and the Na\(^+\)/H\(^+\) exchanger isoform 1 (NHE-1) from the human (h) and winter flounder (f). Potential CAII binding sites (underlined) consist of a stretch of at least 2–3 acidic residues (aspartate or glutamate) usually preceded by a hydrophobic residue. Last 40 amino acids in the COOH-terminal tail of the SLC26 transporters were assumed to be cytoplasmic because these sequences were rich in hydrophilic residues.
CAII on non-denaturing gels, suggesting that it associates with another membrane component. Unpublished data from our laboratory show that CAII immunolocalizes to the cytosol, as well as in or near the BBM and BLM of intact flounder proximal tubule cells (Fig. 5).

**IS THERE A REGULATED RELATIONSHIP BETWEEN SO\(_4^{2-}\) TRANSPORT AND CA?**

Dietary SO\(_4^{2-}\) and K\(^+\) availability, acid/base status, heavy metals, and membrane fluidity affect renal SO\(_4^{2-}\) handling (2, 51, 52, 57). Hormones that stimulate SO\(_4^{2-}\) reabsorption include growth hormone, insulin-like growth factor I, progesterone, estradiol, vitamin D\(_3\), and triiodothyronine (2, 51, 52, 57).

In all species tested, glucocorticoids stimulate renal SO\(_4^{2-}\) excretion. Renal SO\(_4^{2-}\) clearance is elevated in rats treated (in vivo) with the glucocorticoid methylprednisolone (78). Furthermore, a reduced \(V_{\text{max}}\) value for Na\(^+\)-dependent SO\(_4^{2-}\) transport in renal BBM vesicles and reductions in NaSi-1 mRNA and protein levels occur concurrently with the increase in renal SO\(_4^{2-}\) excretion. The \(V_{\text{max}}\) for Na\(^+\)-dependent SO\(_4^{2-}\) transport in renal BBM vesicles is similarly reduced in domestic chickens (\(G.\ gallus\)) treated with the long-lived cortisol agonist dexamethasone (72). Treatment of chick PTCs with supraphysiological concentrations of cortisol for 24 h selectively reduces net transepithelial SO\(_4^{2-}\) reabsorption \(\sim 50\%\) (22). The decrease in net SO\(_4^{2-}\) reabsorption is the result of an increase in the unidirectional secretory flux and decrease in the unidirectional reabsorptive flux (Fig. 6). This study provided evidence that both the facilitated SO\(_4^{2-}\) reabsorptive and secretory fluxes in the avian proximal tubule are regulated by glucocorticoids.

In teleosts, cortisol is essential for osmoregulation in both freshwater and seawater (23, 25, 55, 56). Net renal tubular SO\(_4^{2-}\) secretion ceases (SO\(_4^{2-}\) clearance ratio <1) after acclimation of winter flounder to 10% seawater (69). The cessation in net SO\(_4^{2-}\) secretion is associated with the complete inhibition of SO\(_4^{2-}\)/HCO\(_3^-\) exchange in the BBM. Treatment of 10% seawater-acclimated winter flounder with dexamethasone in vivo restores SO\(_4^{2-}\)/HCO\(_3^-\) exchange in the subsequently isolated BBM vesicles to levels comparable to seawater-acclimated controls. Interestingly, intravenous SO\(_4^{2-}\) infusion into flounder acclimated to 10% seawater stimulated net renal SO\(_4^{2-}\) secretion after 2.5 h, indicating that the secretory mechanism can be elicited rapidly. The mechanism by which SO\(_4^{2-}\) secretion is acutely stimulated has yet to be determined.

Reduction of flounder PTC culture medium cortisol to 10% of normal serum concentration for 9–12 days reduces SO\(_4^{2-}\) secretion to 33% of the control value (69). Nonspecific effects, however, could not be ruled out because transepithelial resistance and phlorizin-sensitive glucose current, measures of proximal tubule-like function, were also significantly altered by cortisol removal. Cortisol is a known differentiating factor (26), and altered electrical properties in this study imply partial dedifferentiation of the epithelium.

Cortisol has recently been shown to alter CA expression in flounder PTCs (61). Control flounder PTCs were supplemented with high levels of cortisol (7.3 \(\times\) 10\(^{-6}\) M), and removal of cortisol for 5 days resulted in cortisol concentrations (6.3 \(\times\) 10\(^{-9}\) M) at the lower end of the physiological range (8.4 \(\times\) 10\(^{-8}\)-3.5 \(\times\) 10\(^{-7}\) M). The 5-day exposure was chosen knowing that longer treatments cause partial dedifferentiation of this
epithelium (69). Both CA biochemical activity (30%) and CAII protein abundance (65%) are reduced in flounder PTCs maintained in reduced cortisol for 5 days. In addition, net active \( \text{SO}_4^{2-} \) secretion is 28% lower after the reduced cortisol treatment (Fig. 7). Methazolamide treatment sufficient to block 100% of the CA biochemical activity and methazolamide in combination with reduced cortisol inhibit net active \( \text{SO}_4^{2-} \) secretion to the same extent (~60%). The reduced cortisol level does not alter transepithelial resistance or phlorizin-sensitive glucose current, indicating that the epithelium remains in a differentiated state. The observations that reduction of cortisol to very low levels 1) lowers CA expression (biochemical and protein), 2) inhibits net active \( \text{SO}_4^{2-} \) secretion, but 3) has no effect on the CA-independent fraction of \( \text{SO}_4^{2-} \) secretion led the authors to conclude that cortisol influences a component of \( \text{SO}_4^{2-} \) transport that is CA dependent.

In his classical review of the physiology of CA, Maren (49) pointed out that inhibitor concentrations sufficient to inhibit 99.2% of CA enzymatic activity have no physiological effect on functional parameters of kidney, eye, pancreas, and stomach. However, renal HCO\(_3^-\) excretion increases and intracellular pressure decreases at inhibitor concentrations that inhibit 99.7% of enzymatic activity, indicating that 1% of CA activity is required for normal physiological function in the majority of tissues. Despite this, a few investigations demonstrated that relatively small changes in CA activity can be physiologically relevant. Renal tubular CA activity is elevated 78% in rabbits undergoing metabolic acidosis, and increased renal acid excretion accompanies the induction of CA activity (14). As noted above, small changes in CA activity induced by cortisol alter the CA-dependent fraction of tubular \( \text{SO}_4^{2-} \) secretion (61).

CA is in such excess (~500-fold), why should relatively small changes in enzymatic activity cause physiologically important changes in tubular anion transport? This may occur if 1) CA physically associates with the anion transporter, 2) the association stimulates transport activity, and 3) the treatments (e.g., metabolic acidosis and cortisol) directly affect the number of functional associations.

HYPOTHETICAL MODELS OF TUBULAR SULFATE SECRETION

Consideration of the known properties of transepithelial \( \text{SO}_4^{2-} \) secretion gives rise to several hypothetical models (Fig. 8). In Fig. 8A, \( \text{SO}_4^{2-} \) enters the cell across the BBM in exchange for cellular OH\(^-\). At the opposite pole of the cell, inward HCO\(_3^-\) movement facilitates \( \text{SO}_4^{2-} \) exit across the BBM. Intracellular CA activity accelerates the formation of OH\(^-\) ions, the substrate for basolateral \( \text{SO}_4^{2-}/\text{OH}^- \) exchange. CO\(_2\) formed from the dehydroxylation of HCO\(_3^-\) diffuses across the BBM and reacts with OH\(^-\) to reform HCO\(_3^-\) in the interstitium.

It is well documented that mammalian CAII binds to and enhances the activity of the Cl\(^-/-\)HCO\(_3^-\) exchanger, AE-1 (68, 86). Thus it is entirely feasible that CAII binds to and enhances brush-border \( \text{SO}_4^{2-}/\text{HCO}_3^- \) exchange activity in marine teleosts as well. At normal intracellular pH, the ratio of HCO\(_3^-\) to CO\(_2\) is 20:1. Therefore, it should be questioned whether an association of CAII with the brush-border anion exchanger (\( \text{SO}_4^{2-}/\text{HCO}_3^- \)) would be stimulatory. On the other hand, an association of CAII with the basolateral \( \text{SO}_4^{2-}/\text{OH}^- \) exchanger would most likely enhance transport because CAII could cause rapid regeneration of transported OH\(^-\) ions from HCO\(_3^-\) near the intracellular OH\(^-\) binding site.

All cells are faced with a continuous acid load due to factors such as metabolic production of acid, passive HCO\(_3^-\) efflux, and passive proton influx (12). In the case of the marine teleost proximal tubule, the basolateral \( \text{SO}_4^{2-}/\text{OH}^- \) exchanger also contributes to the acid loading processes. The models in Fig. 8 show that Na\(^+\)/H\(^+\) exchange activity, as in all cells, is an important defense against the tendency for acid loading processes to cause intracellular acidification. Maintenance of normal pH assures normal intracellular [HCO\(_3^-\)]. Na\(^+\),K\(^+\)-ATPase activity is necessary to generate the Na\(^+\)-gradient needed to drive Na\(^+\)/H\(^+\) exchange. \( \text{SO}_4^{2-} \) uptake across the peritubular surface of flounder renal tubules is dependent on the Na\(^+\) gradient (73, 75), and there is precedent for Na\(^+\)/H\(^+\) exchange activity in teleost renal BBM vesicles (Anguilla anguilla) (89, 94). NHE-3 was recently cloned from Osorezan dace (Triobolodon hakonensis), and NHE-3 mRNA was found in its kidney (36). Although we hypothesized that an association between CAII and an \( \text{SO}_4^{2-} \) anion exchanger aids \( \text{SO}_4^{2-} \) secretion, a physical association between CAII and an Na\(^+\)/H\(^+\) exchanger may also be important as well. NHE-1 is a ubiquitous Na\(^+\)/H\(^+\) exchanger involved in intracellular pH regulation (82). Like hNHE-1, the COOH terminal of winter flounder NHE-1 contains numerous acidic residues that could serve as binding sites for CAII (Fig. 4). It remains to be seen whether an Na\(^+\)/H\(^+\) exchanger is required for maximum tubular \( \text{SO}_4^{2-} \) secretion. Na\(^+\)-HCO\(_3^-\) cotransport activity may also be important but has not yet been implicated in renal tubular \( \text{SO}_4^{2-} \) transport.
A typical value for tubular SO$_4^{2-}$ secretion rate in winter flounder is 20 μmol·kg$^{-1}$·h$^{-1}$, which exceeds the estimated maximum possible rate of tubular HCO$_3^-$ reabsorption (9 μmol·kg$^{-1}$·h$^{-1}$) (74). As luminal [HCO$_3^-$] decreases, continued SO$_4^{2-}$ secretion requires that another anion facilitate SO$_4^{2-}$ exit from the cell. In Fig. 8B, luminal Cl$^-$ energizes transfer of SO$_4^{2-}$ from cell to lumen. Cl$^-$ secretion has been demonstrated in proximal tubules of the dogfish shark (Squalus acanthias).

Fig. 8. Hypothetical models of transepithelial SO$_4^{2-}$ secretion by the marine teleost renal proximal tubule. Movement of SO$_4^{2-}$ into the cell across the basolateral membrane is electroneutral and trans-stimulated by a pH gradient, suggesting SO$_4^{2-}$/OH$^-$ exchange (75). Because SO$_4^{2-}$ secretion is pH dependent, Na$^+$/H$^+$ activity, which is dependent on the Na$^+$-gradient generated by Na$^+$,K$^+$-ATPase, should be required to prevent the tendency for intracellular acidification. Luminal gradients for both HCO$_3^-$ and Cl$^-$ can facilitate SO$_4^{2-}$ exit across the brush-border membrane, a process that is also electroneutral (76). Intracellular CA activity accelerates the formation of OH$^-$ ions from HCO$_3^-$ that may be supplied by the brush-border SO$_4^{2-}$/HCO$_3^-$ exchanger (A). Through a physical association, CA may stimulate brush-border SO$_4^{2-}$/anion exchange, basolateral SO$_4^{2-}$/OH$^-$ exchange, and/or Na$^+$/H$^+$ exchange. When luminal HCO$_3^-$ has been depleted, SO$_4^{2-}$ secretion can continue through exchange for luminal Cl$^-$. Intracellular Cl$^-$ gained through SO$_4^{2-}$/Cl$^-$ exchange may be recycled to the lumen through the Cl$^-$ secretion process consisting of a Na$^+$-K$^+$-2Cl$^-$ cotransporter in the basolateral membrane and a Cl$^-$ channel (possibly CFTR) at the brush-border membrane (B and D) (8). CA activity is only required for half of SO$_4^{2-}$ secretion indicating the importance of CA-independent processes. Uncatalyzed dehydroxylation of HCO$_3^-$ (C), and the stimulation of SO$_4^{2-}$ uptake across the basolateral membrane by an anion other than OH$^-$, such as a metabolic intermediate (e.g., oxalate; D), may contribute to the non-CA-dependent fraction of SO$_4^{2-}$ secretion. Obviously there are other transport processes that are likely important (e.g., basolateral K$^+$ conductance) but are not indicated here.
and seawater and freshwater acclimated killifish (*Fundulus heteroclitus*) (9, 16, 17). The proposed mechanism includes the movement of Cl\(^-\) into the cell on an Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter, accumulation of intracellular Cl\(^-\) above electrochemical equilibrium, and exit of Cl\(^-\) across the BBM on a cAMP-activated conductance pathway (possibly CFTR) (8). Thus Cl\(^-\) gained through electroneutral SO\(_4\)^{2-}/Cl\(^-\) exchange at the BBM could be recycled to the lumen via this Cl\(^-\) secretory process (Fig. 8, B and D).

SO\(_4\)^{2-} secretion is reduced just 50% after CA inhibition, indicating that CA-independent mechanisms are responsible for a portion of SO\(_4\)^{2-} secretion. Although orders of magnitude slower than the CA catalyzed reaction, the uncatalyzed dehydroxylation of HCO\(_3^-\) may contribute OH\(^-\) ions for SO\(_4\)^{2-}/OH\(^-\) exchange (Fig. 8C). At present, the possibility cannot be ruled out that an anion other than OH\(^-\), such as a recyclable metabolic intermediate (e.g., oxalate), also facilitates SO\(_4\)^{2-} translocation across the BLM (Fig. 8D).

CONCLUDING REMARKS AND PERSPECTIVES

The renal proximal tubule is an important point of control for the maintenance of plasma [SO\(_4\)^{2-}] in vertebrates. Whereas a facilitated secretory flux exists in the terrestrial vertebrate proximal tubule the highly expressed reabsorptive flux dominates control of net excretion. In contrast, the marine teleost proximal tubule has highly expressed mechanisms that promote net tubular SO\(_4\)^{2-} secretion, with no evidence of facilitated reabsorption. Unique SO\(_4\)^{2-}/anion exchangers in the marine teleost proximal tubule are probably homologues of SO\(_4\)^{2-} transporters (SLC26A1, SLC26A2, SLC26A6, SLC26A7, and SLC26A11) in the mammalian SLC26 gene family. At least two of these transporters, SLC26A1 and SLC26A6, are present in the mammalian proximal tubule (41, 43).

CA has been shown to physically associate through an electrostatic interaction with numerous mammalian ion transporters, thus increasing their transport activities. CA activity is associated with a large fraction of SO\(_4\)^{2-} secretion in the marine teleost proximal tubule and SO\(_4\)^{2-} reabsorption in the avian proximal tubule, and a physical association of CA with transporters involved in SO\(_4\)^{2-} transport may be necessary for maximum transport activity. Many of the SO\(_4\)^{2-} transporters in the mammalian SLC26 gene family contain potential CAII binding motifs at their COOH termini, raising the possibility that these transporters may physically interact with CAII. Future work should be directed at identifying the SO\(_4\)^{2-}/anion exchangers in the marine teleost proximal tubule and assessing the functional interaction of CA with these SO\(_4\)^{2-} transporters.

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REFERENCES

RENAL TUBULAR SULFATE SECRETION IN VERTEBRATES


