Experimental Panel 7.1 Sulfate secretion by the proximal tubule of marine teleost fish

Background

In marine teleosts and seawater-dwelling euryhaline teleosts, the excretion of sulfate (SO_4^{2-}) is crucial for maintaining the low concentrations in extracellular fluids. Figure 7.28 shows a model of the mechanisms by which SO_4^{2-} secretion is thought to occur in proximal tubules, which hypothesises:

- Sulfate exchange with hydroxyl ions on the basolateral membranes of the epithelium, maintained by the activity of **carbonic anhydrase**.
- Efflux of SO_4^{2-} in exchange for Cl^- and/or bicarbonate ions (HCO_3^-) on the luminal border of the epithelium.

These hypotheses have been investigated using monolayers of proximal tubular epithelial cells from winter flounders (*Pseudopleuronectes americanus*).

In the mammalian kidney, several SO_4^{2-} transporter proteins that belong to the large family of solute carriers (Slc) have been identified, which provided an incentive to search for similar proteins in fish kidneys. Eels (*Anguilla japonica*) are an ideal species in which to investigate SO_4^{2-} transporters because they acclimate to different salinities and during their lifetime migrate from fresh water to seawater to breed; movement from fresh water to seawater requires activation of SO_4^{2-} secretion to excrete the excess.

Experimental series 1 – Sulfate secretion by proximal tubular epithelial cells of winter flounder

Renal tubules of the winter flounder consist almost entirely of proximal tubule. Once the epithelial cells are separated and dispersed in saline, the cells settle as a monolayer and will attach to a membrane at their basolateral borders. The SO_4^{2-} fluxes (or transport of other ions) across monolayers of cells can be investigated in experimental chambers in which pharmacological agents can be added to the fluids.

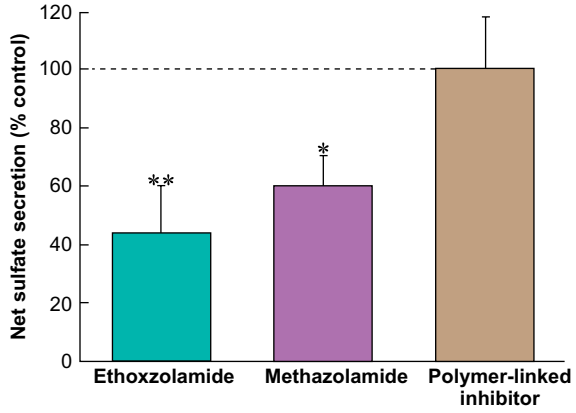

Figure A Inhibition by carbonic anhydrase inhibitors of transepithelial sulfate secretion by proximal tubule monolayers of winter flounders (*Pseudopleuronectes americanus*)

Rates of sulfate secretion in the presence of two carbonic anhydrase inhibitors, ethoxzolamide and methazolamide are compared to control preparations incubated in the presence of a polymer-linked inhibitor of carbonic anhydrase. The dashed line at 100% indicates the control value. The inhibitors were added to the extracellular side of the monolayer. Data are means + standard errors; *P<0.05, ** P<0.01 compared to control preparations.

Source: Pelis RM and Renfro JL (2004). Role of tubular secretion and carbonic anhydrase in vertebrate renal sulfate excretion. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 287: R 491–501.

Methods

To test the hypothesis that carbonic anhydrase is involved in the process of SO_4^{2-} secretion, the rates of SO_4^{2-} secretion were measured in the presence and absence of the carbonic anhydrase inhibitors ethoxzolamide and methazolamide. Control preparations were incubated in the presence of a polymer-linked inhibitor of carbonic anhydrase that is unable to penetrate the cells.

To test the hypothesis that SO_4^{2-} secretion via the luminal border of proximal tubular cells occurs in exchange with Cl^- and/or HCO_3^-, the composition of the physiological saline overlying the cells was manipulated by replacing Cl^- or HCO_3^- with gluconate anions. Physiological saline is normally gassed with mixtures of oxygen and carbon dioxide, but in solutions lacking HCO_3^- the solutions were gassed with 100 per cent O_2.

Results and conclusions

Figure A shows that both of the carbonic anhydrase inhibitors, ethoxzolamide and methazolamide, reduced SO_4^{2-} secretion by almost 60 per cent and 40 per cent respectively compared to control preparations. These studies support the hypothesis that carbonic anhydrase plays a significant role in SO_4^{2-} secretion, presumably by generating OH^-, which is then exchanged with SO_4^{2-} on the basolateral border. However, a significant rate of SO_4^{2-} secretion continues even in the presence of the carbonic anhydrase inhibitors, and may result from uncatalysed formation of OH^-, or use of an alternative exchange anion.

Figure B shows the results of experiments in which Cl^- and HCO_3^- (or both) were absent from the saline overlying the cells. When both Cl^- and HCO_3^- are absent, secretion of SO_4^{2-} almost ceases. These results support the hypothesis that Cl^- and HCO_3^- together can be exchanged with SO_4^{2-}, allowing its secretion across the luminal border

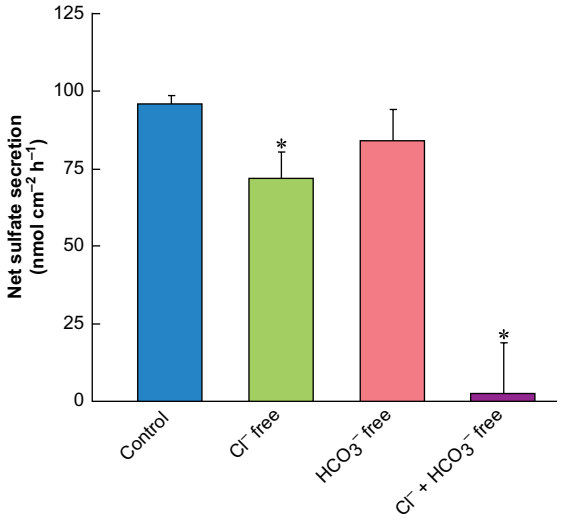

Figure B Sulfate secretion by cultured monolayers of proximal tubular epithelium of winter flounders (*Pseudopleuronectes americanus*)

The epithelial layers were incubated with a physiological saline solution on the luminal surface that mimics tubular fluid (control). The composition of the physiological saline was manipulated in the chloride (Cl^-) free preparations by replacing Cl^- by gluconate anions. In bicarbonate (HCO_3^-) free preparations, HCO_3^- was replaced by gluconate anions and the saline was gassed with 100 per cent O_2. Data are means + standard errors; * P<0.05 relative to control preparations.

Source: Pelis RM and Renfro JL (2004). Role of tubular secretion and carbonic anhydrase in vertebrate renal sulfate excretion. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 287: R 491–501.

of the proximal tubule. Absence of HCO_3^- alone has no significant effect on SO_4^{2-} secretion, presumably because of the ready availability of the alternative ion, Cl^- (at 150 mmol L^{-1}), to exchange with SO_4^{2-}. An absence of Cl^- alone in the luminal fluid slightly reduces SO_4^{2-} secretion; in such circumstances, HCO_3^- could exchange with SO_4^{2-}, allowing a large part of the secretion to continue.

Experimental series 2 – Identification and localisation of SO_4^{2-} transporters in eel kidneys

Methods

RNA isolated from kidneys of the Japanese eel (*Anguilla japonica*) was used to isolate genes encoding SO_4^{2-} transporter proteins based on their similarity with the gene sequences of solute carriers (Slc) available in databases. The expression of these genes was investigated in freshwater-acclimated eels and at various intervals after their transfer to seawater.

In the second part of the studies, specific antisera raised against Slc proteins identified in eels were applied to sections of kidney tissue to localize binding of the antisera to Slc proteins in the luminal and/or basolateral borders of the first and second segments of the proximal tubule[1].

Results and Conclusions

Figure C shows the expression of Slc genes in freshwater-acclimated eels and after their transfer to seawater. Three of the genes: *Slc26a1*, *Slc26a6b* and *Slc26a6c*, are equally expressed in the kidneys of eels acclimated to seawater and fresh water. These results suggests that the three proteins encoded by these genes are less significant in the process of acclimation to differing environmental salinities than the proteins encoded by *Slc13a1* and *Slc26a6a*, which show large changes in expression after transfer between salinities.

Notice in Figure C that the *Slc13a1* gene *decreases* in expression after transfer of freshwater-acclimated eels to seawater. In contrast, the *Slc26a6a* gene *increases* in expression within one day of being transferred to seawater. Hence, Slc26a6a may be particularly important in stimulating SO_4^{2-} secretion.

Figure D shows the localisation of four Slc proteins in eel kidneys. In the second proximal segment (proximal II), Slc26a1 occurs on the basolateral membrane, where it could transport SO_4^{2-} into the cells, while Slc26a6b occurs on the luminal membrane, where it could transport SO_4^{2-} into the tubular fluid.

Figure C Time course of changes in expression of renal SO_4^{2-} transporter genes after transfer of freshwater-acclimated eels (*Anguilla japonica*) to seawater (SW)

β-actin was used as a control in the reverse transcriptase (RT)-PCR reactions.
Source: Watanabe T, Takei Y (2011). Molecular physiology and functional morphology of SO_4^{2-} excretion by the kidney of seawater-adapted eels. Journal of Experimental Biology 214: 1783–1790.

The first proximal segment (proximal I) has been suggested to be the more important part for SO_4^{2-} secretion, but in these studies no Slc protein was identified on the basolateral border of this segment (although it is suspected that one exists, and that it transports SO_4^{2-} into these cells). On the luminal border of the proximal I, two transporter proteins occur: Slc26a6a and Slc26a6c, as shown in Figure D.

The increased expression of the *Slc26a6a* gene during seawater acclimation, shown in Figure C, coupled with location of the encoded protein on the luminal border strongly supports the idea that this Slc protein is particularly important in SO_4^{2-} secretion into the tubular fluid.

Find out more:

Kato A, Chang M-H, Kurita Y, Nakada T, Ogoshi M, Nakazato T, Doi H, Hirose S, Romero MF (2009). Identification of renal transporters involved in sulfate excretion in marine teleost fish. American Journal of Physiology – Regulatory and Integrative Comparative Physiology 297: R1647–R1659.

Nakada T, Zandi-Nejad K, Kurita Y, Kudo H, Broumand V, Kwon C Y, Mercado A, Mount DB, Hirose S (2005). Roles of Slc13a1 and Slc26a1 sulfate transporters of eel kidney in sulfate homeostasis and osmoregulation in freshwater. American Journal of Physiology – Regulatory and Integrative Comparative Physiology 289: R575–R585.

Pelis RM, Renfro JL (2004). Role of tubular secretion and carbonic anhydrase in vertebrate renal sulfate excretion. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 287: R 491–501.

Renfro JL, Maren TH, Zeien C, Swenson ER (1999). Renal sulfate secretion is carbonic anhydrase dependent in a marine teleost, *Pleuronectes americanus*. American Journal of Physiology – Renal Physiology 276: F288–F294.

Watanabe T, Takei Y (2011). Molecular physiology and functional morphology of SO_4^{2-} excretion by the kidney of seawater-adapted eels. Journal of Experimental Biology 214: 1783–1790.

[1] There are two proximal segments in euryhaline teleosts, as illustrated in Figure 7.11.

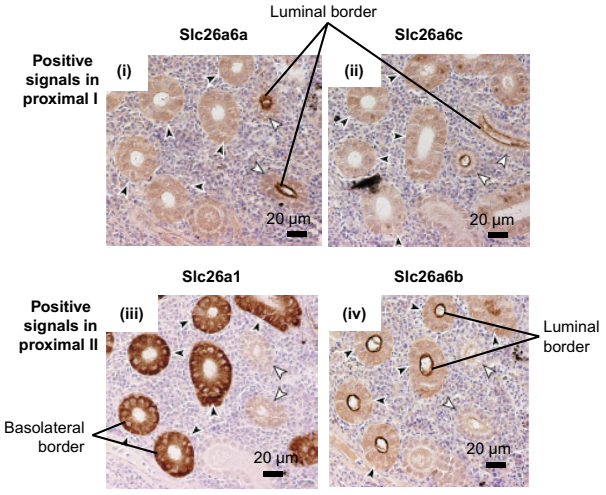

Figure D Immunohistochemical staining of kidneys of seawater-acclimated eels after exposure to specific antisera to SO_4^{2-} transporters.

On each micrograph, white arrowheads indicate sections across proximal I of the nephron; black arrowheads indicate sections across proximal II of the nephron. Intense brown staining indicates binding of the antisera to the specific sulfate transporter (positive signals). Panels (i) and (ii) show positive results for the first part of proximal tubules (proximal I) in the luminal border with antisera to (i) Slc26a6a (strongest signal) and (ii) Slc26a6c. Panels (iii) and (iv) show positive results for the second part of proximal tubule (proximal II) in the basolateral borders with antisera to Slc26a1 and in the luminal border with antisera to Slc26a6b.
Source: Watanabe T, Takei Y (2011). Molecular physiology and functional morphology of SO_4^{2-} excretion by the kidney of seawater-adapted eels. Journal of Experimental Biology 214: 1783–1790.