CORTISOL ALTERS CARBONIC ANHYDRASE-DEPENDENT SULFATE SECRETION BY PRIMARY CULTURES OF WINTER FLOUNDER (*PLEURONECTES AMERICANUS*) RENAL EPITHELIUM

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Teleost plasma sulfate is stable at about 0.6 mM, and renal sulfate clearance ratios may exceed twelve (Renfro, J.L., *Fish Physiology*, Academic Press, New York, vol. 14:147-172, 1996). This tubular secretory process is vital for excretion of the large inorganic sulfate load gained from ingestion of seawater. Transport studies on isolated flounder renal tubule basolateral (BLM) and brush-border membrane (BBM) vesicles revealed that sulfate entry, interstitium-to-cell, across the BLM is in exchange for OH^-, whereas the exit, cell-to-lumen, at the BBM is in exchange for HCO_3^-. Our recent studies demonstrated that carbonic anhydrase (CA) facilitates renal sulfate secretion in the marine winter flounder (Renfro, J.L. et al. Am. J. Physiol. 276:F288-F294, 1999). CA apparently enhances this process through dehydroxylation of HCO_3^-.

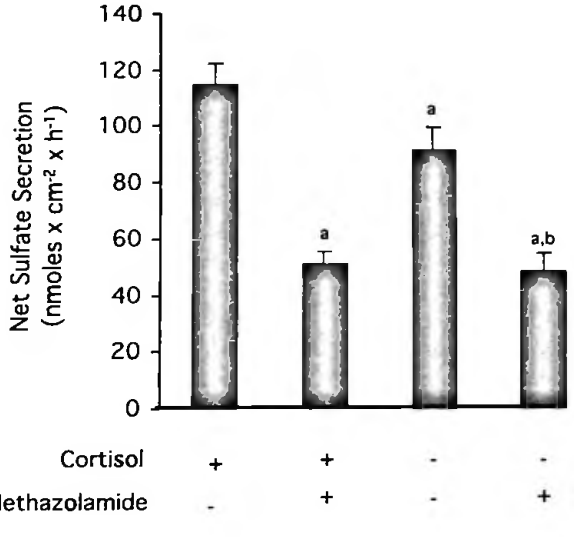

Figure 1. Net sulfate secretion calculated from unidirectional isotopic fluxes in flounder proximal tubule primary monolayer cultures. Vertical lines are standard errors, n = 6 individual culture preparations. Cortisol was 5 µg/ml hydrocortisone; 100 µM methazolamide was added at the initiation of the flux measurement. Mean fluxes at 90 min are shown. [a]Significantly different from cortisol treatment alone. [b]Significantly different from values with no added cortisol.

Teleosts that tolerate varying salinities obviously have the capability to regulate renal excretion. Bern (Am. Zool. 15:937-948, 1975) implicated cortisol in the adaptation of teleosts to seawater; indeed it was termed the "seawater adapting" hormone. Renfro (Am. J. Physiol. 257:R511-R516, 1988) showed that adaptation of seawater winter flounder to 10% seawater (SO_4-free) resulted in sulfate clearance ratios less than one and decreased BBM $HCO_3^-:SO_4^{2-}$ exchange. Clearance ratios greater than one and enhanced BBM $HCO_3^-:SO_4^{2-}$ exchange in these animals were restored by daily injec-

tions of the long-lived cortisol analog, dexamethasone (60 µg/100 g bd. wt.), for 5 days. This profound effect of glucocorticoid on renal sulfate secretion together with the recent observation that renal sulfate secretion was CA dependent prompted the present examination of the influence of cortisol on CA-dependent renal sulfate secretion.

Table 1. Effect of 100 µM methazolamide on transepithelial electrical resistance (TER), phloridzin-sensitive glucose current (PS-I_{glu}) and transepithelial electrical potential (PD) in flounder proximal tubule primary monolayer cultures after 5 days with or without cortisol in the tissue culture medium.

Treatment	TER (Ω x cm^2)	PS-I_{glu} (µA x cm^{-2} x h^{-1})	PD (mV)
Cortisol	36.06 ± 4.40	-3.08 ± 0.27	-0.31 ± 0.05
Cortisol + Methazolamide	31.13 ± 2.95	-2.68 ± 0.27	-0.26 ± 0.04
No added Cortisol	32.60 ± 4.15	-2.34 ± 0.21	-0.20 ± 0.03
No added Cortisol + Methazolamide	40.94 ± 4.08	-2.30 ± 0.17	-0.26 ± 0.04

Values are mean ± standard error, n = 12. These are the same tissues used to obtain the data shown in Figure 1. Treatments had no significant effect on electrical parameters.

Flounder renal epithelial cells were isolated as previously described (Dickman, K.G. and Renfro, J.L., Am. J. Physiol. 251:F424-F432, 1986) and plated to confluence on native rat tail collagen. Unidirectional $^{35}SO_4^{2-}$ fluxes were determined for 14-day-old cultures mounted in Ussing chambers. Net secretion rates shown in Figure 1 were calculated from unidirectional fluxes. Control tissues had complete tissue culture medium with 5 µg/ml hydrocortisone (cortisol). Removal of cortisol 5 days prior to flux determination caused a significant 20% reduction in net secretion. Methazolamide (100 µM), a specific CA inhibitor, reduced net secretion to about 43% in both control and no-added-cortisol tissues. The methazolamide-insensitive component of flux was unaffected by cortisol removal. Table 1 shows that these treatments had no significant effect on the transepithelial electrical properties of the tissues.

These preliminary data indicate that cortisol may control the methazolamide-sensitive component of renal sulfate secretion raising the possibility that renal carbonic anhydrase functional activity may be hormonally controlled.

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