

CORTISOL INCREASES CARBONIC ANHYDRASE ACTIVITY IN WINTER FLOUNDER RENAL PROXIMAL TUBULE PRIMARY CULTURES

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Carbonic anhydrase (CA) facilitates the active secretion of inorganic sulfate (S_i) by winter flounder (*Pleuronectes americanus*) renal proximal tubule epithelium in primary monolayer culture (PTC) (Renfro, J.L. et al. Am. J. Physiol. 276:F288-F294, 1999). Preliminary data indicate that the "seawater-adapting" hormone, cortisol, stimulates a methazolamide-sensitive component of active S_i secretion. In the present study CA activity was determined in PTCs continuously exposed to 100 μ g/ml cortisol vs. tissue maintained for five days with no added cortisol. Table 1 shows that CA activity was almost 30% lower when cortisol was removed. This decline in CA activity coincides with a 25% decrease in net S_i secretion by similarly treated PTCs (Renfro, J.L. et al., *Bull MDIBL* 39:88-89, 2000). Figure 1 shows that a polyclonal antibody to human CAII stains a specific fraction in rat as well as flounder RBCs, cells expected to be rich in CAII. A CAII band is also readily apparent in PTCs (two middle lanes). In tissues treated by cortisol removal, the pixel density ratio of CAII to actin (a reflection of variable extraction vol-

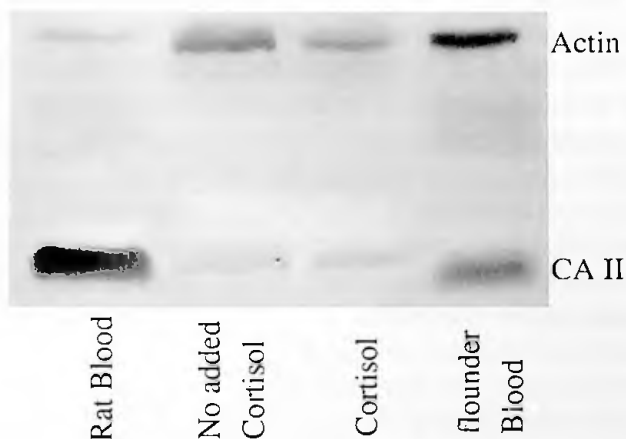


Figure 1. Western blot of carbonic anhydrase II activity in winter flounder PTCs (middle two lanes). Blots were probed with sheep anti-human CAII primary antibodies and monoclonal anti-mouse actin clone AC40.

Table 1. CA Activity in flounder renal proximal tubule primary monolayers is lowered by removal of cortisol.

Treatment	CA activity (U/ μ g protein)
Cortisol	16.0 \pm 3.36
No-added Cortisol	11.5 \pm 2.69*

Values are mean \pm standard error (n = 3 preparations). Cortisol was 0.1 mg/ml. No-added cortisol was begun 5 days before tissues were harvested for assay. *Significantly different at $P < 0.05$ from cortisol treated by paired t-test.

ume) was about one-half that of tissues undergoing continuous cortisol exposure. These preliminary data further support the possibility that cortisol stimulation of S_i secretion coincides with a cortisol stimulated increase in CA II enzyme content and activity in the renal epithelium. Supported by NSF-IBN9604070, NSF-IBN9808616 and NSF-IBN0078093.