## EFFECTS OF EYESTALK ABLATION ON CARBONIC ANHYDRASE ACTIVITY IN THE GILLS OF CARCINUS MAENAS AND CANCER IRRORATUS

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The enzyme carbonic anhydrase (CA), a central molecular component of ion uptake mechanisms in euryhaline crustaceans, is induced by about 6 fold in the green shore crab, *Carcinus maenas*, when the animal is transferred from 32 to 10 ppt salinity (Henry et al., Bull. Mt Desert Island Biol. Lab. 38:55, 1999). The process of initiation and control of CA induction, however, is poorly understood. Recently, Lovett et. al. (Amer. Zool. 37:87A, 1997) suggested that methyl farnesoate (MF), a juvenile hormone analog, may have a role in transport enzyme induction. This idea was tested on *C. maenas* by determining the effects of eyestalk ablation (ESA; a means to elevate MF levels) on gill CA induction in combination with low salinity transfer.

In green crabs acclimated to 33 ppt, where CA activity is uniformly low, ESA resulted in a near tripling of activity over 7 days in posterior gills only (Table 1); anterior gills were unaffected. Eyestalk ablation also appeared to enhance the low salinity-stimulated CA induction, as ESA treated crabs had about 35% higher CA activity in posterior gills after a 4 day transfer from 33 to 10 ppt and 20% higher after 7 days (Table 1). Hemolymph MF levels were undetectable in control crabs acclimated to 33 ppt. After either ESA or salinity transfer, hemolymph MF concentrations were variable, ranging from 0 to 40 ng ml<sup>-1</sup>, but CA activity in posterior gills was not correlated with MF concentration in individual crabs.

Table 1. Effects of eyestalk ablation (ESA), low salinity transfer, and lovastatin injection (Lv) on CA activity in anterior (G3) and posterior (G7) gills of the green crab, *Carcinus maenas*. CA activity reported as  $\mu$ mol CO<sub>2</sub> mg protein<sup>-1</sup> min<sup>-1</sup>. Mean  $\pm$  SEM (N). T = 12°C.

CA activity

Treatment	Anterior	Posterior
33 ppt acclimated	$108 \pm 12 (5)$	119 ± 3 (5)
33 ppt ESA 7day	$140 \pm 29 (9)$	$300 \pm 52 (9)$
33-10 ppt 4 day	156 ± 19 (6)	$717 \pm 50 (6)$
33-10 ppt ESA 4 day	$132 \pm 20 (8)$	972 <u>+</u> 77 (6)
33-10 ppt 7day	$156 \pm 27 (7)$	$1,241 \pm 76 (8)$
33-10 ppt ESA 7 day	$90 \pm 5 (7)$	$1,465 \pm 52 (7)$
33 ppt sham injected	$67 \pm 27 (5)$	115 ± 11 (5)
33 ppt Lv 4 day	$102 \pm 25 (4)$	128 ± 35 (4)
33 ppt ESA Lv 4 day	$73 \pm 5(7)$	$144 \pm 7 (7)$
33-10 Lv 4 day	$79 \pm 8 (8)$	661 <u>+</u> 48 (8)

Injection of lovastatin (Lv) reduced hemolymph MF concentrations to 0 in most but not all crabs over a 4 day period. MF levels were highest in crabs that were given sham injections of the Lv adjuvant alone, but CA activity in either anterior or posterior gills of these animals were unaffected. There were no differences in CA activity in either G3 or G7 between control, sham injected, and Lv injected crabs either at 33 ppt or for crabs transferred from 33 to 10 ppt (Table 1).

In a stenohaline species, *Cancer irroratus*, CA induction does not occur in transfers from 33 to 18 ppt. CA activity in anterior gills was  $132 \pm 27$  (5) and  $166 \pm 28$  (4) at 33 and 18 ppt, respectively; values for posterior gills at those same salinities were  $138 \pm 12$  (5) and  $151 \pm 22$  (4), respectively. ESA had no significant effect on gill CA activity in crabs either acclimated to 33 ppt or transferred to 18 ppt.

It appears that in euryhaline crustaceans the eyestalks exert an effect on branchial CA regulation resulting in suppression of CA expression at high salinity. Removal of that effect causes CA to be expressed even in the absence of a low salinity stimulus, and ESA and low salinity appear to have additive effects on CA induction. The regulation of CA expression does not appear to involve MF.

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