A REPRESSOR SUBSTANCE OF CARBONIC ANHYDRASE INDUCTION IS PRESENT IN THE EYESTALKS OF THE EURYHALINE CRAB, *CARCINUS MAENAS*, BUT NOT THE STENOHALINE CRAB, *CANCER IRRORATUS*

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Carbonic anhydrase (CA) activity is induced tenfold (from approximately 120 to 1,200 umol CO_2 mg protein^{-1} min^{-1}) in the posterior, ion transporting gills (G7-9) of the euryhaline green crab, *Carcinus maenas*, after transfer from 33 to 10 ppt salinity (Henry et al., Bull. Mt. Desert Island Biol. Lab. 38:55, 1999). This induction is a result of salinity-mediated gene activation and increased expression of CA mRNA (Gehnrich et al., *Bull. Mt. Desert Island Biol. Lab.* 40:114-115, 2001). The increase in mRNA expression occurs at 24 hours after low salinity transfer and immediately precedes the initial increase in CA activity. Induction of CA activity is enhanced by removal of the major endocrine complex of the crab, the eyestalk. Eyestalk ablation (ESA) resulted in a doubling of CA activity in posterior gills in crabs acclimated to 32 ppt and not given a low salinity stimulus, and it potentiates the salinity-stimulated response by about 20% (Henry et al., *Bull. Mt. Desert Island Biol. Lab.* 39:21-22, 2000). Anterior gills served as control tissues since CA activity in this tissue does not respond to either low salinity or ESA.

These data served as the basis for hypothesizing the presence of a repressor substance in the eyestalks of *C. maenas* acclimated to high salinity, which inhibits CA expression and keeps CA activity at baseline levels. The effect of this putative repressor is either reduced or removed upon exposure to low salinity, allowing CA induction to occur. Furthermore, injection of extracts taken from the eyestalks of crabs acclimated to 33 ppt, inhibits CA induction by 50% in intact (untreated) crabs transferred from 33 to 10 ppt (Henry, *Bull. Mt. Desert Island Biol. Lab.* 40:35-36, 2001). To test this idea further, a series of experiments were designed to determine if the putative repressor was present in eyestalks of *C. maenas* after low salinity acclimation and if this putative compound was also present in a stenohaline, osmotic and ionic conforming species, *Cancer irroratus*.

C. maenas and *C. irroratus* were collected from Frenchman s Bay, maintained at 33 ppt in running seawater, and fed mussels. *C. maenas* were transferred directly to a 75 gallon recirculating aquarium of 10 ppt salinity either untreated (control) or after treatment with ESA. After 4 and 7 days post-transfer, CA activity was measured in anterior (G3) and posterior (G7) gills. At 4 days after transfer to low salinity, there was a doubling of CA activity in G7 and no change in G3 (Table 1). ESA enhanced this increase by about 50%. At 7 days post-transfer, CA activity in G7 was 1,241 umol CO_2 mg protein^{-1} min^{-1} (a sixfold increase in this case), and this was potentiated by about 20% by treatment with ESA (Table 1).

In a second set of experiments, *C. maenas* were acclimated to 10 ppt for a period of 14 days. At that point, crabs were either left untreated (controls) or subjected to ESA. Both groups were left at 10 ppt for another 7 days and then assayed for CA activity in G3 and G7. There was no change in CA activity in G3, and there was no significant difference in activity in G7 between the control and the ESA-treated crabs (Table 1). ESA, which potentiates CA induction in the

acute phase of low salinity acclimation, has no effect on CA activity after acclimation is complete. This suggests that whatever repressor is present in the eyestalk at high salinity is gone once the crab has acclimated to low salinity, as removal of the eyestalk has no further effect on CA activity.

In a third set of experiments, *C. maenas* acclimated to 33 ppt were then transferred to 10 ppt and given a daily injection of eyestalk extract taken from either *C. maenas* or *C. irroratus*, both also acclimated to 33 ppt. Crabs were injected through the arthroidial membrane covering the hemolymph sinus at the base of the walking leg. Two eyestalks were homogenized in 0.5 ml of filtered seawater and centrifuged at 10,000 g for 10 min at 4°C. Each crab was given a 400 l injection of the supernatant immediately before transfer to 10 ppt and daily for a period of 7 days thereafter, at which point CA activity in G3 and G7 was measured. Eyestalk extract from *C. maenas* reduced CA induction by 50% compared to untreated crabs (Table 1). However, eyestalk extract from *C. irroratus* had no effect on salinity-stimulated CA induction in the gills of *C. maenas*.

Table 1. Effects of salinity transfer, eyestalk ablation, and injection of eyestalk extracts on CA activity in anterior (G3) and posterior (G7) gills of the green crab, *Carcinus maenas*. Injections were performed daily for a period of 7 days. CA activity reported as umol CO_2 mg protein^{-1} min^{-1}. Mean ± SEM (N). T = 12°C.

	CA Activity	
Treatment	Anterior (G3)	Posterior (G7)
33 ppt acclimated	97 ± 12 (11)	210 ± 16 (11)
33-10 ppt transfer, untreated, 4 days	99 ± 17 (6)	422 ± 64 (6)
33-10 ppt transfer, ESA, 4 days	124 ± 11 (6)	612 ± 72 (6)
33-10 ppt transfer, untreated, 7 days	71 ± 13 (6)	1,241 ± 76 (6)
33-10 ppt transfer, ESA, 7 days	90 ± 5 (7)	1,465 ± 152 (7)
10 ppt acclimated, untreated, 7 days	88 ± 13 (5)	1,983 ± 155 (5)
10 ppt acclimated, ESA, 7 days	106 ± 18 (4)	1,706 ± 227 (4)
33-10 ppt transfer, untreated, *Carcinus* eyestalk injected	61 ± 5 (10)	593 ± 53 (10)
33-10 ppt transfer, untreated, *Cancer* eyestalks injected	123 ± 4 (7)	1,244 ± 93 (7)

These results confirm and extend the original hypothesis of the presence and action of a carbonic anhydrase repressor in the eyestalk of *C. maenas*. The effects of this substance are removed quickly after exposure to low salinity, as the greatest potentiation in CA induction occurs during the acute phase of low salinity acclimation. In green crabs acclimated to low salinity, the substance and/or its effects are absent, as evidenced by ESA having no effect on CA induction in crabs acclimated to 10 ppt. Additionally, this putative repressor appears to be absent from stenohaline crabs (e.g., *C. irroratus*) that do not possess the mechanism for CA induction in low salinity.

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