SUPPRESSION OF BRANCHIAL CARBONIC ANHYDRASE INDUCTION BY A COMPOUND IN THE EYESTALK OF THE GREEN CRAB, *CARCINUS MAENAS*

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Carbonic anhydrase (CA) activity is induced nearly tenfold (from approximately 150 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. CA induction is enhanced by the removal of the major endocrine complex of the crab, the eyestalks. Eyestalk ablation (ESA) results 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 CA induction 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 does not change in response to salinity in anterior gills, and it also did not respond to ESA.

Based on these initial data, it was hypothesized that there was active suppression of CA expression in crabs acclimated to high salinity, the substance responsible was localized in the eyestalk, and the effect was removed upon transfer to low salinity. To further test this idea, a series of experiments were designed in which the supernatant from crude eyestalk homogenate was injected back into the crabs in an attempt to inhibit CA induction. Crabs acclimated to 33 ppt were injected with filters seawater (SW), subjected to ESA, or ESA followed by daily injections of eyestalk extract over a 7 day period. For the eyestalk extract injections, a pair of eyestalks were homogenized in 0.5 ml of filtered SW and centrifuged at 10,000 g for 10 min at 4°C. The crabs were given a 400 uL injection of the supernatant into the hemolymph sinus at the base of the walking legs. As second set of crabs were transferred from 33 to 10 ppt for 7 days, either untreated, subjected to ESA or ESA plus injection of eyestalk extract.

CA activity was low and not significantly different in anterior and posterior gills of crabs acclimated to 33 ppt (Table 1). SW injection had no effect on branchial CA activity. ESA resulted in an approximate 50% increase in CA activity in G7, but not G3, over a 7 day period. Injection of eyestalk extract into crabs that had been subjected to ESA completely abolished that increase. Transfer of intact crabs from 33 to 10 ppt resulted in the typical large induction of branchial CA activity in G7; injection of eyestalk extract inhibited this induction by approximately 50% (Table 1). Salinity transfer combined with ESA resulted in CA induction that was potentiated by about 18%. Preliminary results on two crabs treated with ESA followed by low salinity transfer indicated that CA induction was inhibited by close to 70% (Table 1). The combination of these two treatments also resulted in high mortality that was not seen in any other experiment.

Table 1. Effects of salinity transfer, eyestalk ablation, and injection of eyestalk extract 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	165 ± 28 (6)	197 ± 22 (6)
33 ppt acclimated, SW injected sham	97 ± 9 (10)	176 ± 8 (10)
33 ppt acclimated, ESA	115 ± 8 (7)	286 ± 49 (8)
33 ppt acclimated, ESA/eyestalk injected	96 ± 10 (7)	153 ± 16 (7)
33-10 ppt transfer	71 ± 13 (6)	1,241 ± 76 (6)
33-10 ppt transfer, eystalk injected	60 ± 5 (7)	640 ± 67 (7)
33-10 ppt transfer, ESA	90 ± 5 (7)	1,465 ± 152 (7)
33-10 ppt transfer, ESA/eyestalk injected	156 (2)	458 (2)

These results confirm and extend the original hypothesis of the presence a mechanism of suppression of CA gene expression in the eyestalk, which exerts its effect at high salinity. Removal of the eyestalks results in CA induction even in the absence of a low salinity stimulus, and that effect is completely inhibited by injection of eyestalk extract. Furthermore, injection of eyestalk extract also at least partially inhibits the normal low salinity mediated CA induction. Finally, injection of eyestalk extract into crabs treated with both ESA and low salinity exposure severely retards CA induction. This strongly suggests the presence of a substance, localized to the eyestalk, that acts as a suppressor of the expression of CA activity. This substance, or signal, is removed after low salinity transfer to allow induction of CA expression, and if it is added back to the crab s hemolymph, it inhibits the process that leads to CA induction.

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