

PLASMA LEVELS OF ATRIAL NATRIURETIC PEPTIDE IN ANGUILLA ROSTRATA  
DURING ADAPTATION TO FRESHWATER (FW) AND SEAWATER (SW).

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Because cardiac peptides might play a role in the adaptation of euryhaline teleosts to changes in external salinity, we measured the levels of immunoreactive atrial natriuretic peptide (ANP) in the plasma of American eels (Anguilla rostrata) when their environment was acutely altered from freshwater to seawater, and the reverse.

Eels weighing 150 to 390 grams (mean  $\pm$  s.e. =  $221 \pm 36$ ), trapped in the estuary of Northeast Creek or purchased from a commercial supplier in Nova Scotia, were adapted to freshwater or seawater for at least 3 weeks before transfer. Blood was drawn from the dorsal aorta of the tail into a heparinized syringe at 2, 4, 8 and 20 days after transfer from fresh to salt water, and on the first day after transfer to fresh water. For determination of plasma ANP levels, 0.4 - 1.0 ml of blood were collected utilizing a 3 ml heparinized syringe and transferred to iced 5 ml polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (500 KIU/ml). The blood was then centrifuged at 4500 rpm for 45 minutes at 4°C. The plasma was removed, acidified with 2.0 N HCl (0.25 ml/ml plasma), then extracted via Sep-pak C<sub>18</sub> cartridge with 15 ml 10 mM trifluoroacetic acid (TFA) and eluted with 2.5 ml of 60% acetonitrile containing 10 mM TFA. The extracts were fast frozen with dry ice and acetone, then lyophilized. All samples were stored at -70°C prior to assay. Samples were reconstituted in 250  $\mu$ l of assay buffer (50 mM sodium phosphate, pH 7.4 containing 0.2% (w/v) bovine serum albumin, 10 mM EDTA, 0.1% (v/v) Triton X-100 and 0.01% sodium azide). Radioimmunoassay was performed using a double antibody technique with rabbit anti-human alpha ANP (Amersham, UK). Synthetic alpha-hANP, 28 amino acid (Peninsula Laboratories, Belmont, CA) was used to construct standard curves.

The level of ANP in plasma of eels chronically adapted to seawater ( $154 \pm 29$  picograms/ml; n=8) did not differ significantly from that in freshwater-adapted eels ( $127 \pm 20$ ; n=12). When 6 freshwater eels were transferred to seawater, they lost weight within the first 24 hours ( $14 \pm 5\%$ ,  $p < 0.01$ ), as expected. Serum sodium rose to  $188 \pm 8$  mEq/L by the fourth day but returned to  $165 \pm 3$  mEq/L by 10 days after the transfer. Plasma ANP, however, was not significantly altered by acute transfer to seawater (FW control:  $80 \pm 7$ ; 2 days SW  $99 \pm 21$ , 4 days SW  $70 \pm 5$ ; 8 days SW  $75 \pm 12$ ; 10 days SW  $70 \pm 12$  picograms/ml).

Transfer of 4 eels from seawater to freshwater resulted in weight gain ( $13.5 \pm 5\%$ ) and a fall in serum sodium from  $162.4 \pm 4$  mEq/L to  $100.8 \pm 7.7$  mEq/L in 24 hours. Plasma ANP levels did not change consistently ( $148 \pm 39$  in SW;  $99.7 \pm 17$  after 24 hours in FW).

In summary, ANP immunoreactivity is present in the plasma of unanesthetized specimens of Anguilla rostrata, in easily measured amounts, but did not change consistently during adaptation to fresh or salt water.

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