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Short Communication

Fresh water acclimation elicits a decrease in plasma corticosteroids in the euryhaline Atlantic stingray, *Dasyatis sabina*

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ABSTRACT

It is thought that the elasmobranch corticosteroid hormone 1α-hydroxycorticosterone (1α-B) functions as both a glucocorticoid (GC) and mineralocorticoid (MC). Classical antinatriuretic MC activities would run counter to the osmoregulatory strategy of euryhaline elasmobranchs acclimating to fresh water (FW). Therefore we hypothesize that FW acclimation will be accompanied by a decrease in plasma corticosteroids in these animals. However, events that activate the "fight-or-flight" response could mask changes associated with acclimation to lower salinities. To better define the MC role of corticosteroids in elasmobranchs, we designed a transfer system that allows the acclimation of Atlantic stingrays (*Dasyatis sabina*) from seawater (SW) to FW over 12 h while minimizing other extraneous stressors. Blood and interrenal glands were sampled from one group of stingrays 24 h after FW transfer, while another group was sampled two weeks after FW transfer. Two other groups served as mock-transfer controls in that they were treated and sampled in the same way, but remained in SW for the entire period. Plasma corticosteroids, osmolality, chloride, and urea were significantly lower in FW-acclimated stingrays (compared to mock-transfer stingrays) 24 h after FW transfer. This pattern remained after two weeks in FW, with the exception that plasma corticosteroids returned to pre-acclimation levels. There were no significant differences between experimental groups in interrenal levels of mRNAs encoding key steroidogenic proteins (steroidogenic acute regulatory protein and cholesterol side chain cleavage enzyme). Temporally decreased corticosteroid levels during FW acclimation are consistent with the unique strategy of euryhaline elasmobranchs, whereby lower plasma osmolality is maintained in FW vs. SW environments to reduce hydromineral gradients.

1. Introduction

Aquatic organisms living in coastal environments may experience frequent and significant changes in the salinity of the water in which they are living. In fishes, there are two primary physiological strategies to deal with changes in environmental osmolality. Euryhaline teleosts expend energy to maintain fairly constant osmolality by drinking water and secreting excess salt while in seawater (SW), and producing dilute urine while retaining and scavenging salt when salinity decreases (Smith, 1930; Keys, 1931; Krogh, 1937; Evans, 1980). On the other hand, the elasmobranch fishes retain high levels of nitrogenous compounds such as urea and trimethylamine oxide, increasing their osmolality equal to or just greater than that of SW, so that while in SW they do not need to drink, as water is gained osmotically (Smith, 1931b; Thorson, 1962). In fresh water (FW), however, elasmobranchs lessen the osmotic gradient by actively reducing plasma levels of solutes e.g. ions and nitrogenous compounds (Smith, 1931a; Piermarini and Evans, 1998; Pillans and Franklin, 2004; Hammerschlag, 2006).

Adrenocortical tissues in terrestrial vertebrates produce specific steroids to serve as glucocorticoid (GC; cortisol or corticosterone depending on the species) and mineralocorticoid (MC; aldosterone) steroid hormones. However, in fishes, interrenal tissue produces a dominant steroid thought to have both GC and MC properties. In elasmobranchs this steroid is 1α-hydroxy corticosterone (1α-B), a corticosteroid unique to this vertebrate subclass (Idler and Truscott, 1966). The putative GC and MC actions of 1α-B are supported by *in vitro* studies, which demonstrate that interrenal production of 1α-B is stimulated by both the stress hormone adrenocorticotropic hormone (ACTH; Hazon and Henderson, 1985; Klesch and Sage, 1975; Nunez and Trant, 1999;

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O'Toole et al., 1990) and the osmoregulatory hormone angiotensin II (Armour et al., 1993a; Evans et al., 2010). Direct antinatriuretic MC actions of 1α-B have also been demonstrated using heterologous bioassays, including *in vitro* stimulation of sodium transport across isolated toad bladders and *in vivo* sodium retention in adrenalectomized mice following 1α-B administration (Grimm et al., 1969; Idler et al., 1967).

Additional *in vivo* support for a MC role of 1α-B in elasmobranchs includes reduced plasma osmolality, sodium and urea in interrenalectomized Atlantic stingrays (de Vlaming et al., 1975) as well as elevated plasma 1α-B in dogfish (*Scyliorhinus canicula*) fed a low protein diet (Armour et al., 1993b). In the latter study *S. canicula* were unable to maintain plasma urea due to low protein availability but maintained osmolality via increased NaCl, leading to the hypothesis that the elevated 1α-B may be responsible for increased ion retention. In the single study that has examined changes in circulating 1α-B during salinity acclimation, 1α-B was higher in stenohaline *S. canicula* 14 days after gradual (10% every 10 days) acclimation to 50% SW (Hazon and Henderson, 1984). Chronically elevated 1α-B may therefore facilitate ion retention in reduced salinities; however, the MC actions of 1α-B would be counterproductive during the initial phase of acclimation characterized by decreasing concentrations of plasma ions.

We hypothesize that, in the absence of other stressors, plasma corticosteroids including 1α-B will decline during FW acclimation as part of the elasmobranch strategy to reduce plasma osmolytes during acclimation to lower salinities. However, as there is evidence that 1α-B may function as both GC and MC, studies attempting to isolate the MC actions of 1α-B may be hampered by the confounding effects of stress on experimental animals. In such studies, care must be taken to eliminate handling stress and other stressors that might stimulate the release of ACTH, leading to changes in plasma corticosteroid concentrations that are not related to osmoregulatory challenges. We therefore designed a transfer system that allowed the acclimation of SW-adapted Atlantic stingrays (*Dasyatis sabina*) to FW with minimal stress. We used this system to examine how acclimation from SW to FW alters blood corticosteroid concentrations and other blood chemistries. To see if such acclimation impinges on the capacity of the Atlantic stingray to produce corticosteroids, we also examined the expression of mRNAs encoding the rate-limiting steroidogenic proteins StAR (steroidogenic acute regulatory protein) and cytochrome P450scc (cholesterol side-chain cleavage enzyme) in interrenal tissue. This study indicates that acclimation to FW results in significant changes in several plasma chemistries (including corticosteroids) within 24 h. However, levels of plasma corticosteroids in animals remaining in FW for two weeks are not significantly different from animals in seawater.

2. Materials and methods

2.1. Animals

Sexually mature (>22 cm disk width; Tricas et al., 2000) *D. sabina* were captured using hand nets in shallow bays near Port Aransas, Texas. Thirty stingrays were used for this study (14 male, 16 female; disk width: 22–31.5 cm; mass: 0.4–1.0 kg). Animals were transferred to the laboratory, maintained in a 550 L tank with ambient flow-through SW (30–33 ppt) and fed chopped shrimp ad libitum. Animals were acclimated to captivity for at least two weeks prior to experimentation and were sacrificed by cervical dislocation using methods approved by the University of Texas Animal Care and Use Committee (IACUC protocol #06030101).

2.2. Fresh water acclimation

For FW acclimation, four animals at a time were transferred by net to a tank with recirculating, filtered SW (170 gallons, 30 ppt salinity) and allowed to acclimate for 48 h. Animals were not fed once moved to the acclimation tank and for at least 24 h after transfer to FW. The acclimation tank was surrounded by an opaque curtain and equipped with external valves so that salinity adjustments could be made without disturbing the animals, eliminating the effects of additional handling stress. Two biological filter boxes were connected to the tank to allow for the recirculation of either SW or FW. Following the 48 h acclimation period, recirculation through the SW filter box was closed and dechlorinated, buffered FW (pH 8.2–8.4; Malawi/Victoria Buffer; Seachem) was transferred from a 600-gallon tank into the experimental tank using a submersible pump. FW flow was monitored and adjusted to result in a controlled salinity change from 30 ppt to 0 ppt over 12 h. All FW acclimations were conducted from 9 PM to 9 AM to further reduce external stressors such as human activity. Following salinity change to FW, valves were then adjusted to allow recirculation through the FW filter box. Matched controls followed the above methods, except that 30 ppt SW was used in place of FW. Animals were terminally sampled during separate experimental runs at the following time points: 48 h after introduction to the experimental tank (pre-acclimation, $n = 10$), 24 h after salinity change to FW ($n = 8$), 24 h after salinity "change" to SW ($n = 4$), 14 days following acclimation to FW ($n = 4$) and 14 days in SW ($n = 4$). At each experimental time point, stingrays were removed individually from the tank by net, a blood sample (200 μL) was quickly collected from the caudal vein using a 25-gauge needle and heparinized syringe (lithium heparin; Sigma) and animals were sacrificed by cervical dislocation. All stingrays from the tank were captured and sampled within a single 5 min period to reduce variation in stress due to capture time, with blood samples placed on ice. Interrenal glands were then collected, rapidly frozen on dry ice and stored at −80 °C. Following hematocrit determination as described in Section 2.3, blood samples were centrifuged for 5 min at 5000×*g* to separate plasma from the cellular fraction, with plasma transferred to a new microcentrifuge tube and stored at −80 °C.

2.3. Plasma analyses

To examine the effects of acute and chronic salinity acclimation on plasma components, osmolality, chloride, urea and hematocrit were quantified. Plasma osmolality (mOsm kg^{-1}) was quantified in duplicate using a Wescor 5130B vapor pressure osmometer (Wescor Incorporated, Logan, UT). Chloride and urea (mM) were quantified by colorimetric improved Fried (Yokoi, 2002) and Jung (Jung et al., 1975) methods, respectively, using commercial kits (QuantiChrom Chloride and Urea Assay Kits; BioAssay Systems) and a spectrophotometer (SpectraMAX 190; Molecular Devices). For osmolality (1:2) and chloride/urea (1:50) quantification, 5 μL of each sample was diluted in ddH_2O. Hematocrit was determined by drawing approximately 50 μL of blood into 75 mm ammonium-heparin hematocrit tubes, centrifuging these tubes in a microhematocrit centrifuge (IEC Micro-MB, Thermo Scientific) and determining the percentage of packed red blood cells using a microhematocrit capillary tube reader disk.

2.4. Corticosteroid enzyme-linked immunoassay

There is currently no specific antibody against 1α-B, and therefore plasma corticosteroids were quantified using a commercial corticosterone ELISA (Cayman Chemical) previously validated for 1α-B (Evans et al., 2010), with the exception that samples were not subjected to HPLC fractionation. It has previously been