11ß-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE ELECTROLYTE EXCRETORY ORGANS OF THE EEL (Anguilla Anguilla).

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Corticosteroids play an important role in the maintenance of water and electrolyte homeostasis. In mammals the main regulator of electrolyte excretion is the mineralocorticoid aldosterone. However, most fishes are incapable of synthesizing aldosterone, and cortisol (the main glucocorticoid in man) functions both as a glucocorticoid, regulating classical glucocorticoid functions such as carbohydrate, protein and lipid metabolism, as well as a mineralocorticoid, regulating electrolyte balance. Given this dual function of cortisol, it seems logical to assume that its bio-availability is differentially regulated in organs involved in glucocorticoid-regulated functions versus those involved in electrolyte balance.

Mammalian mineralocorticoid target cells possess the enzyme 11ß-hydroxysteroid dehydrogenase (11-OHSD), which, by degrading glucocorticoids, allows aldosterone to bind to mineralocorticoid receptors that have the same affinity for aldosterone and endogenous glucocorticoids. Recently we have identified a new species of 11-OHSD in the renal collecting duct which is kinetically and antigenically distinct from the previously characterized liver isoform and utilizes NAD instead of NADP as cofactor (Rusvai and Náray-Fejes-Tóth, J. Biol. Chem. 268: 10717-10720, 1993). The aim of this study was to determine whether either isoform (NAD or NADP-dependent) 11-OHSD is present in the electrolyte excretory organs of eels and if so, whether the enzyme activity changes when the eel is transferred from fresh water to seawater.

The gill, kidney, colon and liver were harvested from eels adapted to either fresh water or seawater and homogenized in 0.25 M sucrose, 20 mM Tris (pH 7.4) containing and 1.5 mM EDTA on ice. The homogenate was centrifuged at 10,000 g for 5 min and the supernatant used. 11-OHSD activity was determined by measuring the rate of conversion of [^{3}H]corticosterone to 11-dehydrocorticosterone at 18 °C for 15-120 min in the presence of 0.1-1 mM NAD or NADP. The reaction was stopped by adding 2.5 volumes of 70% methanol containing corticosterone and 11-dehydrocorticosterone at 50 µg/ml. Metabolites of corticosterone were separated by HPLC using a Beckman Ultrasphere ODS 5-µm column and isocratic elution with 70% methanol.

Two different 11-OHSD activities were detected. The liver -- a glucocorticoid target organ-- expressed the NADP-dependent isoform, similarly to the mammalian liver. On the other hand, the gills, kidney and colon expressed the recently described NAD-dependent 11-OHSD species, present in mammalian mineralocorticoid target cells. 11-OHSD activity was 0.021 ± 0.004 fmol.min^{-1}.mg protein^{-1} in gill tissue (SEM; n=6) without exogenous cofactors; this activity was increased more than 10-fold in the presence of 1 mM NAD (0.234 ± 0.005 fmol/min/mg protein) whereas 1 mM NADP did not activate the enzyme significantly. On the other hand, in the liver 11-OHSD activity was NADP-dependent (0.78 fmol.min^{-1}.mg protein^{-1} with 1 mM NADP). An NAD-dependent 11-OHSD activity was also present in the kidney (0.57 vs. 0.1 fmol.min^{-1}.mg protein^{-1} with 1 mM NAD vs. NADP, respectively) and in the colon (0.25 ± 0.07 vs. 0.11 ± 0.03 fmol.min^{-1}.mg protein^{-1}; n=4). 11-OHSD activity was also present in the gill and colon of eels maintained in seawater. However, 11-OHSD activity did not change significantly in either organ.

Our results demonstrate for the first time, the presence of the NAD-specific 11-OHSD isoform in a lower vertebrate, that does not possess specific mineralocorticoid hormones or receptors. These data support the hypothesis that local mechanisms operate in salt excretory organs to regulate the concentration of corticosteroids.

Supported by the Milbury Fellowship. The authors are grateful for the continuous help of Dr. Stephen Gehnrich.