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Evolution of Glucocorticoid and Mineralocorticoid Responses: Go Fish

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In the pre-molecular biology era, before the cloning of the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), more was known about the binding of aldosterone, cortisol, corticosterone and other corticosteroids to the GR than was known about their binding to the MR. The GR had advantages of being both present in reasonable quantities and sufficiently stable in tissue homogenates for steroid competition analyses. Moreover, establishment of the rat hepatoma HTC cell line in Tomkins' laboratory (1) provided the opportunity for parallel studies of the binding of cortisol, corticosterone and other corticosteroids to the GR and their effects on GR-mediated induction of tyrosine amino transferase. In addition, HTC cell extracts did not contain corticosteroid binding globulin (CBG), which was present in tissue homogenates, and skewed measurements of the binding of endogenous glucocorticoids to the GR.

In contrast, the MR was much more difficult to study due to low levels of MR in the kidney, the main target organ for aldosterone, and MR instability in kidney homogenates. Moreover, CBG in kidney homogenates made it difficult to study the competitive binding of cortisol corticosterone, deoxycorticosterone and other glucocorticoids to the MR (2). With effort CBG was removed from kidney homogenates, and there was a surprise: aldosterone, corticosterone and cortisol have similar affinity for the MR. Even more surprising was the demonstration that hippocampus, a non-traditional aldosterone target tissue, contained an MR with specificity similar to that in the kidney MR (3,4).

The cloning of the human MR by Arriza et al. (5) established that aldosterone, corticosterone and cortisol also had similar affinity for the human MR, although in the cell cultures studied, aldosterone was active at a 10-fold lower concentration than cortisol in stimulating MR-mediated transcription (6). The presence of mRNA for MR was verified in brain and heart, as well as traditional tissues, kidney and colon.

These studies (2-6) raised important questions about aldosterone action. First, because free levels of glucocorticoids are 100-fold higher in blood than those of aldosterone, how could aldosterone bind to MR and regulate the mineralocorticoid response? Second, what is the function of a mineralocorticoid receptor in the brain and heart?

A year later, two papers resolved the first question (7,8) and in the process, added the mechanism of tissue-specific expression of enzymes as a partner with steroid receptors in regulating the actions of corticosteroids and other steroid hormones (9-11). MR in the distal tubule of the kidney are not activated by cortisol due to co-expression of 11β-hydroxysteroid dehydrogenase, now called 11β-HSD-2, which oxidizes cortisol to cortisone, an inactive steroid (7,8,12). Aldosterone forms an 11,18 hemiacetal in solution, and thus is not a substrate for 11β-HSD-2; it can thus occupy the MR and regulate electrolyte transport in the presence of 11β-HSD-2. However, this mechanism does not apply to hippocampus or heart muscle cells, which contain MR and lack 11β-HSD-2. Indeed, understanding what the MR is doing in non-traditional tissues has been a lively area of research in the last decade (13).

In this issue, Greenwood et al. (14) report the cloning from the cichlid fish of one MR and three GR isoforms, their response to aldosterone and cortisol, and their expression in various tissues, which both clarifies our understanding of the actions of the MR and GR and raises additional questions about the evolution of responses to corticosteroids. Their work illustrates Dobzhanzky's epigram "Nothing in biology makes sense except in the light of evolution" (15), which is even more relevant now than in 1973. Indeed, evolutionary analyses of complete genomes from bacteria and eukaryotes have determined when various genes and regulatory networks evolved, and how they diversified as different life forms evolved. In this regard, on the time scale of 3.8 billion years for life on earth, the adrenal and sex steroid response is relatively young, as GR, MR, and receptors for androgens, estrogens and progestins are not found in invertebrates such as the fruit fly and worm (16,17). These receptors appear to have evolved at the base of the vertebrate line, some time before the Cambrian explosion, which occurred 545 million years ago (17). The earliest evidence for steroid receptors is in lamprey, a jawless fish, which contains an ER, PR, and corticosteroid receptor (18); however, their ligands and the responses that they mediate are not yet known.

Steroid binding studies to a partial MR sequence (19) and full GR from trout (20) have provided some information on corticosteroid receptors in fish. However, with the newly cloned full length MR and three GRs from cichlid (14), we have the necessary tools to study transcriptional activity of different corticosteroids and the functions of the MR and GR in fish. Common features between mammalian and fish receptors are likely to predate the divergence of fish and land vertebrates, which moves our understanding corticosteroid actions back about 450 million years, when mammals and fish last shared a common ancestor.

First, Greenwood et al. (14) find that aldosterone and cortisol have similar EC50s of 0.02 nM and 0.05 nM, respectively, for activation of cichlid MR. In parallel studies with rat MR, aldosterone and cortisol have EC50s of 0.04 nM and 0.3 nM, respectively, indicating less selectivity in fish for these two corticosteroids. As found in mammals, cichlid MR is highly expressed in brain and heart, which supports

an important function for the MR in these tissues. Unexpectedly, cichlid MR is not well expressed in kidney. Indeed, the levels in kidney are slightly below that in liver and spleen. Substantially more MR is expressed in the gill, which is a site for chloride transport in fish.

Cortisol has an EC50 of about 3 to 5 nM for all three GRs; aldosterone has about 100-fold lower potency. GR1 and GR2a have a DNA binding domain that is similar to other GRs. GR2b has an insert of 9 amino acids between each zinc finger domain that was first found in trout by Ducouret et al (20) and is also in flounder GR. A recent search of GenBank did not find this segment in other steroid receptors. Expression patterns of the three GRs are complex, which will require additional research to understand. GR2 is more highly expressed than GR1 in brain, heart, gill, kidney, liver and spleen. GR2b is the main GR2 receptor in gill and liver; GR2a is more highly expressed than GR2b in kidney and spleen; GR2a and GR2b are about equally expressed in brain and heart.

The report of Greenwood et al. (14) needs to be considered with that of Kusakabe et al. (21), who cloned trout 11β-HSD-2, which regulates two different endocrine responses. As in mammals, trout 11β-HSD-2 oxidizes cortisol to cortisone, controlling access of active glucocorticoids to fish MR and GRs. However, 11β-HSD-2 also catalyzes oxidation of 11β-testosterone to 11-keto-testosterone, the active androgen in fish. Thus, expression of 11β-HSD-2 can both increase 11-keto-testosterone and decrease cortisol levels. Due to its multiple biological activities, trout 11β-HSD-2 is highly expressed in kidney, gill, intestine, testis, ovary, heart, pituitary, liver and skin. However, 11β-HSD-2 is very weakly expressed in brain; Kusakabe et al. needed 8-fold more brain mRNA than was used for other tissues to see a weak signal in a Northern analysis. If other fish also have low expression of 11β-HSD-2 in brain, then cortisol will occupy the MR and GRs in fish brain.

The high affinity of aldosterone for the cichlid MR is relevant to the still unresolved question: Is aldosterone a fish steroid? This was a contentious issue even in 1967 (22); most attempts to find aldosterone in fish have been unsuccessful. In some fish, trace levels of aldosterone have been reported (22). However, given that Greenwood et al. (14) find that aldosterone has an EC50 of .03 nM for the MR, aldosterone could be biologically active at very low concentrations.

From an evolutionary perspective, aldosterone should not be the ancestral mineralocorticoid because synthesis of aldosterone is more complex than that of deoxycorticosterone and corticosterone, which have just as high an affinity as aldosterone for the mammalian MR (2-5). Parsimony suggests that corticosterone (or possibly deoxycorticosterone) was the ancestral ligand for the MR, and possibly for one or more fish GRs. Moreover, our search of the recently completed Fugu genome with human CYP11B2, aldosterone synthase, did not find a protein with greater than 45% sequence identity. This suggests that Fugu does not contain an aldosterone synthase, although it is possible that the Fugu genome is incomplete. Also, CYP11B2 may have been lost in Fugu, but be present in other fish.

Lastly, the complexity of corticosteroid action in cichlid uncovered by Greenwood et al. (14) makes a strong case for studying the role of serum proteins in steroid specificity for the MR and GR in fish (23); in particular, CBG, for which we do not find an ortholog in the Fugu genome. In the last decade, CBG has been overshadowed by the importance of 11β-HSD-2 in regulating corticosteroid action. However, as was shown thirty years ago (2), CBG regulates access of deoxycorticosterone, cortisol and corticosterone to MR and GR, with aldosterone escaping this protein (3,4), just as aldosterone escapes 11β-HSD-2. Aldosterone may have evolved along with a CBG that sequestered corticosterone and other glucocorticoids. Studies of MR, GR, CBG and aldosterone synthase in lobe-finned fish, such as lungfish and coelacanths, which belong to the fish class that is the ancestor of land vertebrates, should elucidate molecular events leading to the emergence of aldosterone as a mineralocorticoid in land animals.

As we celebrate the 50th anniversary of the discovery of aldosterone (13) it is comforting to know that its full story has not been told. More exciting discoveries, with important clinical applications, await the elucidation of the origins of corticosteroid action.

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