TRANSCRIPTOMIC AND NON-TRANSCRIPTOMIC SIGNALING MECHANISMS OF CORTISOL IN FISHES

Brototi Roy and Umesh Rai *

Abstract

Corticosteroid hormones regulate diverse physiological functions in vertebrates. In fishes, molecular characterization of cortisol receptors was initiated in the last decade. Various cytosolic GR isoforms as well as MR mediating slow transcriptomic effect of cortisol have been described. Moreover, several genes have been identified that are altered transiently on corticosteroid receptor activation. In addition to classical transcriptomic effect, the rapid non-transcriptomic effect of cortisol has been recently demonstrated in tilapia and spotted murrel. The study conducted in our laboratory suggest that the rapid effect of cortisol in *Channa punctatus* is mediated by activation of membrane-bound cortisol receptor coupled to adenylate cyclase/cAMP/protein kinase A pathway. Here, we provide a brief review focused on the transcriptomic as well as novel non-transcriptomic mechanisms of cortisol actions in teleosts.

Introduction

Corticosteroids in fishes are secreted from the interrenal tissues, the homologue of mammalian adrenal cortex. Although interrenal cells in teleosts are of diffused nature, they are embedded in the most anterior portion of kidney known as the head kidney. The general pattern of corticosteroidogenesis in fishes is slightly different from that in higher vertebrates. The major corticosteroid released from teleost interrenal is cortisol, and not the corticosterone (Fig. 1).

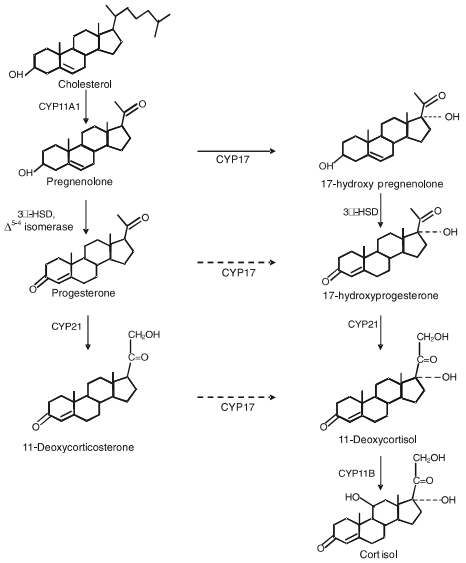

Fig. 1. Cortisol biosynthesis in teleosts. Two major groups of enzymes are involved, hydroxysteroid dehydrogenase (HSD) and cytochorome P450 (CYP).

**Corresponding author: Umesh Rai, Department of Zoology, University of Delhi, Delhi-110 007*

The most important difference between the mammalian and the teleost corticosteroid secretion is the absence of mineralocorticoid due to absence or very low activity of the 18-hydroxylating system. Thus in fishes, cortisol regulates energy metabolism as well as hydromineral balance. Over the past decades, it has become increasingly apparent in mammals that glucocorticoid exerts its effect through non-genomic as well as classical genomic mode of actions. In recent years, evidences suggest that a similar scenario of trancriptomic/genomic as well as fast non-transcriptomic/non-genomic mechanisms of cortisol action is operational in teleosts. These reports in fishes are summarized in this review.

Signaling Mechanisms

Transcriptomic mechanism of cortisol action **:** Cortisol mediates it classical genomic action via the cytosolic corticosteroid receptors (CRs). The CR has five functional domains; the amino terminal A/B domain responsible for transcriptional activation, C domain for DNA binding and receptor dimerization, D domain is involved in conformational changes, and E domain at the carboxyl terminal end is involved in hormone binding and ligand sensitive transactivation (Prunet *et al.,* 2006). Although mineralocorticoid is absent, both glucocorticoid as well as mineralocorticoid receptors are present in teleosts. It is hypothesized that mineralocorticoid receptor (MR) phenotype appeared prior to the evolution of mineralocorticoid (Bridgham *et al.,* 2006). There is substantial overlap in pharmacology of these two receptor types. In fact, MR has ten fold higher affinity to cortisol than glucocorticoid receptor (GR). This led to the characterization of MR and GR as type I and type II GR, respectively. Moreover, GR and MR bind to the same glucocorticoid response elements (GREs). Despite these similarities, GR and MR regulate different cellular processes by virtue of distinct cellular localization, and by their interaction of specific A/B domain to different transcription factors (Lim-Tio *et al.,* 1997; Pearce and Yamamoto, 1993). As fishes have 2 copies of each steroid receptor, the duplication of whole genome is hypothesized in the teleosts lineage (Jaillon *et al.,* 2004). This is corroborated by the existence of multiple CRs in some fishes such as rainbow trout, cichlid, common carp and puffer fish.

The molecular characterization of fish GR began with the cloning of rainbow trout GR by Ducouret *et al.,* 1995. It was found that fish GR resembled mammalian GR in many aspects but with one major exception. In case of fishes, there are 9 additional amino acids between the two zinc fingers present in the DNA binding domain (Ducouret *et al.,* 1995; Greenwood *et al.,* 2003, Takeo *et al.,* 1996; Tokuda *et al.,* 2005). The insertion of 9 amino acids in between the two zinc fingers changes the protein structure. It is suggested that this change in structure affects the receptor dimerization and their interaction with the DNA (Wickert and Selbig 2002). Conventionally, the GRE contains two palindromic half sites separated by 3 nucleotides. But, there are some non-classical GREs which have alternate spacing, or may have multiple independent half sites in tandem with other regulatory sites. It has been suggested that the change in protein structure of fish CR due to the amino acids insertion might help in binding to these non-classical GRE (Greenwood *et al.,* 2003).

Nuclear Localization and CR signaling: All steroid receptors act as ligand-inducible transcription factors. In the absence of ligand, CRs exist in the cytoplasm as a multi-protein complex involving chaperone proteins such as HSP 90 (heat shock protein 90) and other HSPs, HOP (HSP 70/HSP

90 organizing proteins) and different immunophilins (Tissing *et al.,* 2005). In mammals it has been proposed that the ratio of HSP 90/GR may be a key regulator of steroid action (Kang *et al.,* 1999). It was shown that high intracellular HSP 90 levels increase the HSP 90/GR ratio and prevents GR binding to its DNA responsive element. As glucocorticoids are lipophilic in nature, it can easily pass through the cell membrane and bind with cytosolic GR. Thereafter, GC/GR complex is translocated to the nucleus where it binds as a homodimer to specific DNA binding sites (GRE) in the promoter region of glucocorticoid-regulated genes. The binding of GC/GR complex to the GRE results in activation of transcription and translation processes. On the other hand, CRs may repress gene expression by interaction with other transcription factors or co-factors rather than by direct interaction of CR with the DNA (Dostert and Heinzel, 2004; Glass and Rosenfeld, 2000). Expression of several genes involved in hepatic metabolism, immune system and reproduction have been shown to be altered by cortisol in fishes (Aluru and Vijayan, 2009). In general, transcriptomic action requires time in hours. More than 30 min exceed before marked changes are visible at the level of regulatory proteins, but it usually takes hours to days before the changes become evident at the cellular, tissue or organism level.

The rapid non-transcriptomic mechanism of cortisol action*:* In addition to transcriptomic, non-transcriptomic rapid action of cortisol is demonstrated in recent years in fishes though the studies are limited. In tilapia *Oreochromis mossambicus*, cortisol rapidly reduced the prolactin release in a dose-dependent manner from anterior pituitary cells *in vitro* (Borski *et al.,* 1991). Further, the rapid effect of cortisol on prolactin release was shown insensitive to translation inhibitor, cycloheximide. Recently, we have also demonstrated the rapid immunosuppressive effect of cortisol on phagocytes collected from the spleen of spotted murrel *Channa punctatus* (Roy and Rai, 2009). The rapid inhibitory effect of cortisol on phagocytosis remained unaltered when splenic phagocytes were pre-treated with transcription or translation inhibitors. It is worth mentioning that these protein synthesis inhibitors attenuated the long-term transcriptomic effect of cortisol on phagocytes. Based on these observations, non-transcriptomic action of cortisol is implicated in regulation of physiological mechanism in fishes. The pleiotropic signaling mechanisms have been proposed to explain the rapid non-transcriptomic effect of cortisol in mammals (Stahn *et al.,* 2007).

(a) Non-specific interactions: It has been observed that GCs at very high concentrations intercalate into membranes, thereby changing their physicochemical properties and the activities of membrane associated proteins (Buttgereit and Scheffold, 2002; Buttgereit *et al.,* 2004). This results in altered calcium and sodium cycling across plasma membrane which contributes to rapid action of GC.

(b) Receptor-mediated specific interactions: The cGR exists in the cytoplasm as a multi-protein complex. The binding of GC to cGR leads to the release of chaperone proteins. These proteins may act as signaling molecules and mediate the rapid action of GC (Croxtall *et al.,* 2000).

The other possibility could be through membrane-bound GR (mGR). The mGRs were first demonstrated in amphibian neuronal cells (Gametchu *et al.,* 1999). It was followed by the identification of mGR on the human peripheral blood mononuclear cells (Buttgereit *et al.,* 2004;

Song *et al.,* 2005; Bartholome *et al.,* 2004). Although molecular structure of the putative membrane receptor is unknown, evidences suggest that mGR is a variant of cGR produced by differential splicing or promoter switching, or by post-translational editing (Bartholome *et al.,* 2004). The non-transcriptomic action of GC at the membrane site might be mediated by activation of multiple second messenger systems.

Our study in *C. punctatus* provides the conclusive evidence of non-transcriptomic signaling mechanism of cortisol in phagocytes. First of all, we used membrane-impermeable BSA-conjugated cortisol which caused the rapid inhibition of phagocytosis. Since the effect was dose-dependent, the specific action of cortisol at the membrane level can be contemplated. Further, the mGRs in fish phagocytes were found to be pharmacologically similar to cGR as RU486, the well known cGR antagonist, abrogated the rapid action of cortisol at the membrane site. Also, we showed the mGR downstream signaling cascade of cortisol. Treatment of phagocytes with BSA-cortisol substantially increased the intracellular cAMP. Inhibitors of adenylate cyclase /protein kinase A (PKA) attenuated the rapid immuno-suppressive effect of cortisol. Taken together, mGR-coupled to the adenylate cyclase/cAMP/PKA second messenger system might be implicated in translating the rapid effect of cortisol on phagocytic activity of splenic phagocytes in *C. punctatus*.

Conclusion

Plasma cortisol levels rise dramatically within minutes following stress, cognitive as well as noncognitive including osmotic changes. Hence, it is not surprising that cortisol exerts rapid nontranscriptomic action in addition to the delayed effects on target tissues. However, even after 30 years of the discovery of membrane associated steroid receptor in mammals, very little attention has centered on delineating non-genomic action of cortisol in teleosts. So far, only two studies including ours have reported the non-transcriptomic effect of cortisol. We have precisely demonstrated the involvement of membrane-bound GR-coupled to adenylate cyclase/cAMP/PKA system in mediating the rapid effect of cortisol on phagocytic activity of fish splenic phagocytes. However, the characterization of mGR remains a big challenge. Nonetheless, extensive work is needed to establish the non-transcriptomic rapid action of cortisol in regulation of various physiological functions in fishes.

References

1. Aluru, N., Vijayan, M.M. (2009): Stress transcriptomics in fish: a role for genomic cortisol signaling. *Gen Comp Endocrinol.*, 164, 142-50.
2. Bartholome, B., Spies, C. M., Gaber, T., Schuchmann, S., Berki, T., Kunkel, D., Bienert, M., Radbruch, A., Burmester, G. R., Lauster, R,, Scheffold, A., Buttegereit, F. (2004): Membrane glucocorticoid receptors (mGCR) are expressed in normal human peripheral blood mononuclear cells and upregulated after *in vitro* stimulation and in patients with rheumatoid arthritis. *FASEB J*, 18, 70-80.
3. Borski, R. J., Helms, L. M. H., Richman, N. H., Grau, E. G. (1991): Cortisol rapidly reduces prolactin release and cAMP and 45 Ca2+ accumulation in the cichlid fish pituitary *in vitro*. *Proc. Natl. Acad. Sci. USA*, 88, 2758-62.
4. Bridgham, J.T., Carroll, S. M., Thornton, J. W. (2006): Evolution of hormone-receptor

complexity by molecular exploitation. *Science*, 312, 97-101.

5. Buttgereit, F., Scheffold, A. (2002): Rapid glucocorticoid effects on immune cells. *Steroids*, 67, 529-34.
6. Buttgereit, F., Straub, R. H., Wehling, M., Burmester, G. R. (2004): Glucocorticoids in the treatment of rheumatic diseases: an update on the mechanisms of action. *Arthritis Rheum.*, 50, 3408-17.
7. Croxtall, J. D., Choudhury, Q., Flower, R. J. (2000): Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptordependent, transcription-independent mechanism. *Br J Pharmacol.*, 130, 289-98.
8. Dostert, A., and Heinzel, T. (2004): Negative glucocorticoid receptor response elements and their role in glucocorticoid action. *Curr. Pharm. Des.*, 10, 2807-16.
9. Ducouret, B., Tujague, M., Ashraf, J., Mouchel, N., Servel, N., Valotaire, Y., Thompson, E.B. (1995): Cloning of a teleost fish glucocorticoid receptor shows that it contains a deoxyribonucleic acid-binding domain different from that of mammals. *Endocrinol.*, 136, 3774-83.
10. Gametchu, B., Chen, F., Sackey, F., Powell, C., Watson, C. S. (1999): Plasma membraneresident glucocorticoid receptors in rodent lymphoma and human leukemia models. *Steroids*, 64, 107-19.
11. Glass, C.K., and Rosenfeld, M.G. (2000): The coregulator exchange in transcriptional functions of nuclear receptors. *Genes. Dev.,* 14, 121-41.
12. Greenwood. A. K., Butler, P. C., White, R. B., DeMarco, U., Pearce, D., Fernald, R. D. (2003): Multiple corticosteroid receptors in a teleost fish: distinct sequences, expression patterns, and transcriptional activities. *Endocrinol.* 144, 4226-36.
13. Jaillon, O., Aury, J. M., Brunet, F., Petit, J. L., Stange-Thomann, N., Mauceli, E., Bouneau, L., et al. (2004): Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature.* 431, 946-57.
14. Kang, K.I., Meng, X., Devin-Leclerc, J., Bouhouche, I., Chadli, A., Cadepond, F., Baulieu, E.E., Catelli, M.G. (1999): The molecular chaperone Hsp90 can negatively regulate the activity of a glucocorticosteroid-dependent promoter. *Proc. Natl. Acad. Sci. U S A.* 96 (4), 1439-444.
15. Lim-Tio S. S., Keightley M. C., Fuller P. J. (1997): Determinants of specificity of transactivation by the mineralocorticoid or glucocorticoid receptor. *Endocrinol.* 138, 2537-43.
16. Pearce, D., Yamamoto, K. R. (1993): Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science.* 259, 1161-65.
17. Prunet, P., Sturm, A., Milla, S. (2006): Multiple corticosteroid receptors in fish: From old ideas to new concepts. *Gen. Comp. Endocrinol.* 147, 17-23.
18. Roy, B., Rai, U. (2009): Genomic and non-genomic effect of cortisol on phagocytosis in freshwater teleost *Channa punctatus*: an in vitro study. *Steroids*, 74, 449-55.
19. Song, I.H., Gold, R., Straub, R.H., Burmester, G.R., Buttgereit, F. (2005): New Glucocorticoids on the Horizon: Repress, Don't Activate! *J Rheumatol*, 32, 1199-207.
20. Stahn, C., Löwenberg, M., Hommes, D. W., Buttgereit, F. (2007): Molecular mechanisms of

glucocorticoid action and selective glucocorticoid receptor agonists. *Mol Cell Endocrinol.* 275, 71-8.

21. Takeo, J., Hata, J., Segawa, C., Toyohara, H., Yamashita, S. (1996): Fish glucocorticoid receptor with splicing variants in the DNA binding domain. *FEBS Lett.* 389, 244-48.
22. Tissing, W.J., Meijerink, J.P., den Boer, M.L., Brinkhof, B., Pieters, R. (2005): mRNA expression levels of (co)chaperone molecules of the glucocorticoid receptor are not involved in glucocorticoid resistance in pediatric ALL. *Leukemia* 19 (5), 727-33.
23. Tokuda, Y., Touhata, M., Kinoshita, M., Toyohara, H., Sagakuchi, M., Yokoyama, Y., Ichikawa, T., Yamashita, S. (2005): Sequence and expression of cDNA encoding Japanese flounder glucocorticoid receptor. *Fish Soc. (Tokyo)* 3, 466-71.
24. Wickert, L., Selbig, J. (2002): Structural analysis of the DNA-binding domain of alternatively spliced steroid receptors. *J Endocrinol.* 173(3), 429-36.