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Expression and distribution of the glucocorticoid receptor *Dl*GR1 in the teleost *Dicentrarchus labrax* brain

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ABSTRACT - Cortisol is the main corticosteroid secreted by the interrenal cells of the head kidney and it exerts a role in mantaining the omeostatic status in fish. In teleosts its effects are mediated through intracellular receptors expressed in several tissues, that are ligand-dependent transcription factors by binding to specific tissue DNA sequences. In *Dicentrarchus labrax* we previously cloned and sequenced a glucocorticoid receptor, *Dl*GR1, isolated from leukocytes of peritoneal cavity. In this work we showed mRNA expression and tissue immunohistochemical localization of brain *Dl*GR1 by *in situ* hybridization assays, with a riboprobe with *Dl*GR1 cDNA trascriptional activation domain, and by immunohistochemical methods, using a specific antibody for a selected sequence of the receptor transcriptional domain. The mRNA and the protein are expressed in pyramidal cells of the optic lobe and in the small globular neurons of the diencephalon.

Key words: D. labrax, Immunohistochemistry, *In situ* hybridization, Glucocorticoid receptor.

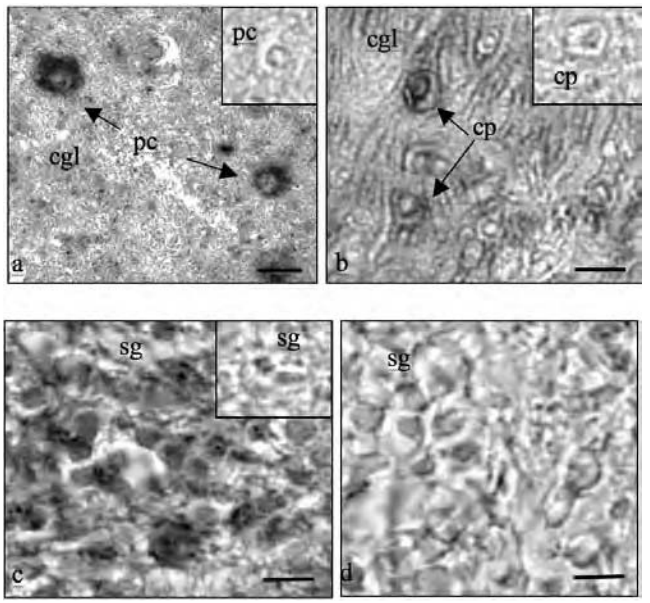
Introduction - Cortisol is the main glucocorticoid hormone of the teleost fishes, secreted by the interrenal glands under the control of the hypotalamic-pituitary-interrenal axis, it has intracellular receptors that act as ligand-dependent transcription factors with a cytoplasmic versus nuclear distribution in many tissues (Vazzana *et al.*, 2008; Di Bella *et al*., 2008; Greenwood *et al.,* 2003). Cortisol is considered the "stress" hormone and in the brain it has a neuromodulatory role, in fact it regulates the main neuronal populations of the caudal telencephalon/anterior preoptic region and diencephalon, which are involved in the regulation and secretion of gonadotropins (Huang et al. 1999) and it is related in spawning behaviour (Satou *et al*., 1984). Fish under aquaculture conditions are often subject to environmental changes or stressors, such as handling, crowding, transporting, and changing water quality. This stressors have been associated with increases in plasma cortisol concentration (Vazzana *et al*., 2002). Vizzini *et al*. (2007) isolated and sequenced the cDNA (2592 bp) of leukocytes taken from the peritoneal cavity of the sea bass, that encodes for a receptor named *Dl*GR1. Hybridization *in situ* assays with the specific riboprobe and immunohistochemical methods with a specific antibody have revealed *Dl*GR1 in the brain of *D. labrax.*

Material and methods - Animals coming from a fish farm were treated with a lethal dose of 3-aminobenzoic acid ethyl ester, the brain explanted, fixed in Bouin's solution for 24 hours, then included in paraffin and cut at 7 µm. Hybridization *in situ* assays were performed according to Le Guellec (1998), the sections deparaffined and rehydrated were incubated with the digoxigenin-11-UTP-labeled riboprobe (1 µg/ml), and then treated with the BCIP/NBT liquid-substrate system. The immunohistochemical assays were performed with a specific policlonal antibody raised in rabbit, by using the hydrophilic peptide designed from the deduced amino acid sequence of the transcriptional activation domain of *Dl*GR1 (Sigma Genosys). The sections, deparaffined and rehydrated, were incubated with

the primary antibody diluted 1:500 in a phosphate buffer with 0,1% of Tween 20 (PBS-T), and then with a secondary antibody anti-rabbit IgG (diluted 1:10000 in PBS-T), and the reaction developed with the cromogen BCIP/NBT liquid substrate system (Sigma). Control experiments were performed by using the corresponding sense cRNA (1 μg/ml) for hybridization *in situ* assays and omitting the primary antibody or substituting it with the preimmune rabbit antiserum for immunohistochemical assays.

Results and conlusions - Hybridization *in situ* assays revealed the expression of mRNA in the pyramidal cells of the central gray layer of the optic lobe, in the cytoplasm and in the nucleus (Figure 1a), and in the nucleus of the diencephalon small globular neurons (Figure 1c). By using the same concentration of the corresponding sense cRNA in the control experiments we showed a negative reaction. The immunohistochemistry staining identified *Dl*GR1 protein distributed in the cytoplasm of the pyramidal cells fibres and around the nucleus (Figure 1b), while the sections treated with the preimmune didn't show any reaction. The protein wasn't revealed in the diencephalon small globular neurons (Figure 1d), maybe because it has a low concentration to react with the specific antibody.

Figure 1. Hybridization and immunohistochemical assays.



a - optic lobe, DlGR1 mRNA in pyramidal cells nucleus and cytoplasm, insert: control section. b - optic lobe, DlGR1 in the fibres of the pyramidal cells and around the nucleus; insert: control section. c - diencephalon, DlGR1 mRNA in small globular neurons nucleus, insert: control section. d - diencephalon, the section treated with the primary antibody didn't show the expression of DlGR1 protein. Longitudinal sections, bar: 20μm. cgl: central gray layer, sg: small globular neurons, pc: pyramidal cells.

In conclusion we revealed through hybridization *in situ* and immunohistochemical assays the expression and distribution of *Dl*GR1 in the teleost *Dicentrarchus labrax* brain. The mRNA and the protein showed the same expression pattern in the optic lobe. The protein was not revealed in the diencephalon because it couldn't be revealed by the utilized method or, maybe, an isoform, cloned and sequenced from the liver tissue of the sea bass by Terova *et al.* (2005), could be expressed, as shown in

the brain of adult *Haplochromis burtoni* (Greenwood *et al*. 2003) where the receptor *Hb*GR2 is more expressed in the brain than *Hb*GR1. Stress in fish can cause immunosuppression and results in an increased susceptibility to diseases. The expression of cortisol is strictly related to the environmental stressors like handling, stocking density, transportation, confinement, netting and hypoxia that fishes suffered in fish culture. Therefore, understanding of the physiology of fish and the careful management of the environment is required in intensive aquaculture.

REFERENCES - Di Bella, M.L., Vazzana, M., Vizzini, A., Parrinello, N., 2008. Glucocorticoid receptor (*Dl*GR1) is expressed in pre-larval and larval stages of the teleost fish *Dicentrarchus labrax*. Cell Tissue Research, 333: 39-47. **Greenwood, A.K.**, Butler, P.C., White, R.B., De Marco, U., Pearce, D., Russel, D.F., 2003. Multiple corticosteroid receptors in a teleost fish: distinct sequences, expression patterns, and transcriptional activities. Endocrinol. 144**:** 4226–4236. **Huang, Y-S.**, Rousseau, K., Sbaihi, M., Le Belle, N., Schmitz, M., Dufour, S., 1999. **Cortisol selectively stimulates pituitary gonadotropin b-subunit in a primitive teleost,** *Anguilla anguilla*. Endocrinology, 40:1228–1235. **Le Guellec, D**., 1998. Ultrastructural *in situ* hybridization: A review of technical aspects. **Biology of** the Cell, 90: 297-306. **Satou, M.**, Oka, Y., Kusunoki, M., Matsushima, T., Kato, M., Fujita, I., Ueda, K., 1984. Telencephalic and preoptic areas integrate sexual behavior in hime salmon (landlocked red salmon, *Oncorhynchus nerka*): results of electrical brain stimulation. **Physiol. Behav.,** 33:441–447. **Terova, G**., Gornati, R., Rimoldi, S., Bernardini, G., Saroglia, M., 2005. Quantification of a glucocorticoid receptor in sea bass (*Dicentrarchus labrax*, L.) reared at high stocking densitiy. Gene, 357: **144-151. Vazzana, M.,** Cammarata, M., Parrinello, N., 2002. Confinement stress in sea bass (*Dicentrarchus labrax*) depresses peritoneal leukocyte cytotoxicity. Aquaculture, 210: 231-243. **Vazzana, M**., Vizzini, A., Salerno, G., Di Bella M.L., Celi, M., Parrinello, N., 2008. Expression of a glucocorticoid receptor (*Dl*GR1) in several tissue of the teleost fish *Dicentrarchus labrax*. **Tissue and Cell, 40: 89-94. Vizzini, A.,** Vazzana, M., Cammarata, M., Parrinello, N., 2007. Peritoneal cavity phagocytes from the teleost sea bass express a glucocorticoid receptor (cloned and sequenced) involved in gen**omic modulation of the** *in vitro* chemiluminescence response to zymosan. Gen. Comp. Endocrinol., 150: 114-123.