Receptors & Clinical Investigation 2014; 1: e127. doi: 10.14800/rci.127; © 2014 by Maria Albrizio, *et al.*
http://www.smartscitech.com/index.php/rci

RESEARCH HIGHLIGHT

Stress and mu opioid receptor in the management of gilthead sea bream (*Sparus aurata*) aquaculture

Maria Albrizio*, Antonio Ciro Guaricci*, Giulio Aiudi*

Department of Emergency and Organs Transplantation, Veterinary Clinics and Animal Productions Unit, University of Bari-Aldo Moro, Valenzano, Bari; Strada Prov. per Casamassima Km3, Valenzano (BA), 70010, Italy

*These authors ccontributed equally to this work.
Correspondence: Maria Albrizio
E-mail: maria.albrizio@uniba.it
Received: March 20, 2014
Published online: May 05, 2014

The growing consumption of aquaculture products requires always new techniques to increase the production yield. Generally, the intensification of aquaculture practices is associated with a stress level rise of bred fishes. Sensitivity to stress, leading to disease, reduced growth and mortality, is higher in larvae than in adult fish. The stress induced effects can be reduced acting on opioid receptors. In this light we evaluated the efficacy of naloxone, an opioid receptor antagonist, directly added to the water during *Sparus aurata* larval development. We found that in larvae subjected to artificial induced stressors, such as overcrowding, reduced pH, increased temperature and salinity, naloxone was useful to decrease the negative effects caused. In this Research highlight we discuss the finding of our recent study and research advancements.

To cite this article: Albrizio M, et al. Stress and mu opioId receptor in the management of gilthead sea bream (*Sparus aurata*) aquaculture. Receptor Clin Invest 2014; 1: e127. doi: 10.14800/rci.127.

Fish farming has a great impact on the world food market and, in Mediterranean area, Gilthead sea bream (*Sparus aurata*) is probably the most important species farmed from decades [1]. Reproduction in captivity is still challenging because of problems in gamete maturation, spawning and larval rearing [2] associated with overcrowding, difficulty in satisfying fish feeding requirements and other technical management issues. Stress is one of the main causes of reproductive failure that in turn is responsible of severe economic loss.

Temperature, salinity and pH are some of the factors that mainly affect the survival of aquatic organisms during the first phases of development for the intense produced stress. Sensitivity to stress, leading to disease, reduced growth and mortality is higher in larval stage thus influencing fitness and the adult phenotype [3]. The opioidergic system is directly involved in the modulation of stress throughout endogenous peptides acting on specific receptors. In stressful conditions the release of endogenous opioid peptides (EOP) is increased with consequent calcium (Ca^{2+}) metabolism alteration and a specific membrane Ca^{2+} channels block [4]. The widely studied opioidergic receptors belong to three main classes: mu, delta and kappa; among them the mu opioid receptor (MOR) seems particularly involved in modulating reproduction. It is triggered by both endogenous/ exogenous agonists and inactivated by antagonists. Its

involvement in fish reproduction is well known since 1989 when a study on goldfish *Carassius auratus* demonstrated that naloxone (Nx), a mu opioid receptor-antagonist [5], influences GtH release [6]. More recently the discovered localization of MOR on the reproductive tract [7,8] and gametes [9,10,11,12,13,14] of different species strengthens this role.

In our recent paper entitled: "Mu opioid receptor in spermatozoa, eggs and larvae of gilthead sea bream (sparus aurata) and its involvement in stress related to aquaculture" [15] we brought to light the involvement of MOR in counteracting the negative effects of stress during larval development of sparus aurata reared in captivity. After the demonstration of the presence and localization of MOR on eggs, spermatozoa and larvae at different developmental stages (10, 20 and 30 days old) by immunoblot and indirect immunofluorescence, we undertook several experiments to evidence that stress related to aquaculture facilities can be reduced by adding the Ca^{2+}/Nx pharmacological association. More in detail we divided larvae in two experimental groups that received two different daily doses (0.125 ml/l or 0.250 ml/l) of Ca^{2+}/Nx for thirty days and one control group. The pharmacological association of Ca^{2+}/Nx was composed by 0.4 mg/ml of Nx (Naloxone Hydrochloride; Dyosint®, Holland), dissolved in a solution of 20% Ca^{2+} gluconate (New ICC; Upjohn®, Italy). After thirty days of treatment, larvae of each group underwent to stress tests by variations of temperature (from 19 to 13°C), salinity (from 37 to 60 g/l) and pH (from 7.6 to 4.5).

While no statistical difference was evidenced among groups in the pH stress test, in the other two assays, the group treated with the higher concentration of Ca^{2+}/Nx association showed a lower statistically significant larval mortality compared to the control ($p<0.01$). Previously a larval mortality analysis was conducted at the end of thirty days of treatment showing how a higher survival rate occurred in group treated with 0.125 ml/l of Ca^{2+}/Nx association. Altogether these results confirm how the efficacy of the pharmacological administration of Ca^{2+}/Nx is dose-dependent as demonstrated in other works conducted in vivo and in vitro [16,17].

Clearly appears in this study that during larval growth, MOR is always expressed in all considered periods with a protein production increase during larval growth, thus confirming a primary role of the opioidergic system in the modulation of fish development. Moreover the efficacy of the Ca^{2+}/Nx association in determining resistance in stressed larvae confirms that antagonizing opioids, thus normalizing Ca^{2+} turnover in cellular membranes, it is possible to recover the physiological dynamic balance of the cells and consequently of the entire organism.

From the molecular point of view it appears how the third extracellular loop of MOR, the binding site for agonists/antagonists, is a region highly conserved between species, so that the downstream actions originated upon receptor activation are of primary importance for cell physiology. This is particularly evident in those mammals that undergo seasonal anestrous; they show, in their oocytes and cumulus cells, a more abundant expression of MOR during the blocking period [18].

While wild fish is mainly exposed to acute stress at which may follow adaptive responses, in intensive aquaculture stress, due to the management, is acute repetitive (hexogen food, weaning, handling etc.) and/or chronic (water deterioration quality, overcrowding). In fish farming it is important to discover sub-lethal and chronic stress conditions, especially if not clearly evident, because their effects fall down on larval growth and mortality determining a dramatic loss [19]. The Ca^{2+}/Nx association looks a good candidate to overcome these problems, Nx has a good water solubility, a very short half-life (15-20 min) [20], a LD50 in mice of 1 g/kg b.w. and is light sensitive. The dosage employed (0.05-0.1 mg/l) is 10^4 times lower compared to the LD50 and, because of short half-life of the drug, it could be assumed that no dangerous residues will be found in edible meat as well as in wastewater tanks of farmed fish thus embracing legislation on animal welfare and environmental preservation.

Recently our group started a new study to test the use of the Ca^{2+}/Nx association in subsequent larval growing stages (over 30 days) and to improve the survival rates of fry transported to other locations (pre-fattening and fattening) at high density, in order to optimize the management of the whole fish farming production. Moreover the quality of meat and water in terms of Nx residues is under evaluation.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Duarte CM, Marba N, Holmer M. Rapid domestication of marine species. Science 2007; 316:382-383.
2. Araki H, Ardren W, Olsen R, Cooper B, Blouin MS. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood River. Conserv Biol 2007; 21:181-190.
3. McKim JM. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J Fish Res Board Can 1977; 34:1148-1154.
4. Minoia P, Sciorsci RL. Metabolic control through L calcium channel, pkc and opioid receptors modulation by an association

of naloxone and calcium salts. Curr Drug Targets Immune Endocr Metabol Disord 2001; 1:131-137.

5. Reisine T, Pasternak G. Opioid analgesics and antagonists. In The pharmacological basis of therapeutics. Edited by Hardman JG, Limbird LE, Goodman Gilman A. New York USA, McGraw-Hill, 1996; 521-555.
6. Rosenblum PM, Peter RE. Evidence for the involvement of endogenous opioids in the regulation of gonadotropin secretion in male goldfish, Carassius auratus. Gen Comp Endocr 1989; 73:21-27.
7. Piva F, Limonta P, Maggi R, Dondi D, Messi E, Zanisi M, et al. Effect of aging on opioid and LHRH receptors in the brain, pituitary, and testis of the male rat. Neurobiol Aging 1994; 15:553-557.
8. Desantis S, Albrizio M, Ventriglia G, Deflorio M, Guaricci AC, Minoia R, et al. The presence of themuopioid receptor in the isthmus of mare oviduct. Histol Histopathol 2008; 23:555-564.
9. Albrizio M, Guaricci AC, Maritato F, Sciorsci RL, Mari G, Calamita G, et al. Expression and subcellular localization of the mu-opioid receptor in equine spermatozoa: evidence for functional role. Reproduction 2005; 129:39-49.
10. Agirregoitia E, Valdivia A, Carracedo A, Casis L, Gil J, Subiran N, et al. Expression and localization of δ κ and μ-opioid receptors in human spermatozoa and implications for sperm motility. J Clin Endocrinol Met 2006; 91:4969-4975.
11. Aiudi G, Albrizio M, Guaricci AC, Laricchiuta P, Lacalandra GM. Biochemical characterization of the mu-opioid receptor in Panthera Leo epididymal sperm cells. Biol Reprod 2006; 501:183.
12. Albrizio M, Guaricci AC, Calamita G, Zarrilli A, Minoia P. Expression and immunolocalization of the mu opioid receptor in human sperm cells. Fertil Steril 2006; 86:1776-1779.
13. Aiudi G, Bucci FA, De Sandro Salvati A, Guaricci AC, Albrizio M. Spermatozoa of Sharpsnout sea bream (Diplodus puntazzo) and European sea bass (Dicentrarchus labrax) express opioid receptors. Aquaculture 2010; 309:293-296.
14. Albrizio M, Lacalandra GM, Micera E, Guaricci AC, Nicassio M, Zarrilli A. Delta opioid receptor on equine sperm cells: subcellular localization and involvement in sperm motility analyzed by computer assisted sperm analyzer (CASA). Reprod Biol Endocrinol 2010; 8:78-89.
15. Albrizio M, Guaricci AC, Milano S, Macrı F, Aiudi G. Mu opioid receptor in spermatozoa, eggs and larvae of gilthead sea bream (Sparus Aurata) and its involvement in stress related to aquaculture. Fish Physiol Biochem 2013; DOI 10.1007/s10695-013-9900-9.
16. Sciorsci RL, Bianchi P, Minoia P. High levels of endorphins and related pathologies of veterinari concern. A review. Immunopharm Immunot 2000; 22:575-626.
17. DiSole F, Guerra L, Bagorda A, Reshkin SJ, Albrizio M, Minoia P, et al. Naloxone inhibits A6 Cell Na+/H+ exchange by activating protein kinase C via the mobilization of intracellular calcium. Exp Nephrol 2001; 9:341-348.
18. Dell'Aquila ME, Albrizio M, Guaricci AC, De Santis T, Maritato F, Tremoleda JL, et al. Expression and localization of the mu opioid receptor (MOR) in the equine cumulus-oocyte complex and its involvement in the seasonal regulation of oocyte meiotic competence. Mol Reprod Dev 2008; 75:1229-1246.
19. Schreck BC. Stress and rearing of salmonidis. Aquaculture 1982; 28:241-249.
20. Panerai A. Gli oppiacei. In: Neuropsicofarmacologia. Edited by Paoletti R, Nicosia S, Clementi F, Fumagalli G Torino Italy, UTET, 1998; 64-83.