GILTHEAD SEA BREAM *(SPARUS AURATA)* FERTILIZED EGGS EXPRESS THE MU-OPIOID RECEPTOR

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Introduction

Reproduction of fishes is negatively affected in captivity by stressful conditions (e.g. confinement, handling, overcrowding, etc.) (Schreck et al., 2001). Stress response is also mediated by the endogenous opioid peptide (EOP) system (Arends et al., 1999). Although a large amount of work has been done on opioidergic receptors in mammals, little information are available on other vertebrate and invertebrate species. A close correlation has been found between opioid peptides and reproductive system of different fish species (Facchinetti et al., 1997; Sakharkar et al., 2006). The activation of the mu-opioid receptors (MOR) by endogenous ligands, such as β-endorphin, causes a deep alteration of membrane potential and permeability and consequently a damage of some cellular functions (Sciorsci et al., 2000). Presence of opioid receptors was shown on oocytes and spermatozoa of several mammalian species (Dell'Aquila et al., 2002; Albrizio et al., 2005) and on sperm cells of *Dicentrarchus labrax* and *Sparus aurata* (Aiudi et al., 2004). The aim of the present study was to investigate, by a molecular approach, on the presence of MOR in eggs of gilthead sea bream after fertilization.

Materials and methods

To evaluate MOR expression, we performed a western blot. Freshly fertilized gilthead sea bream eggs were collected from an hatchery. To recover crude plasma membrane proteins, eggs were lysed in ice-cold homogenizing buffer and processed as previously reported by Albrizio et al. (2005). Western blot analysis was performed using a polyclonal anti-MOR antibody against the third extracellular loop of the receptor that selectively binds mu-agonists. Before loading, 60μg isolated proteins were denatured for 4min at 90°C and run on a 12% (w/v) polyacrylamide gel. Separated proteins were transferred onto an Immobilon-P membrane (Millipore, Bedford, MA, USA) as previously described and incubated with the rabbit polyclonal anti-MOR antibody (Chemicon Int. Temecula, CA, USA) diluted 1:7500. After washing, the membrane was incubated for 1h with peroxidase-conjugated goat anti-rabbit IgG antibody (Sigma, Milan, Italy) diluted 1:15000 and then revealed for horseradish peroxidase activity by SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA).

Results

The expression of MOR on eggs of gilthead sea bream was evidenced by a positive immunoreactive doublet of approximately 50/65 KDa molecular mass (Fig.1, lane A). The negative control, obtained incubating the blot with a solution of the primary

antibody preadsorbed with a molar excess of the immunizing peptide, doesn't show any signal (Fig. 1, lane B).

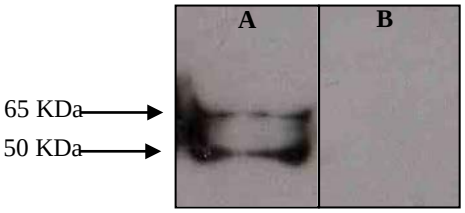

Fig 1. Immunoblotting analysis of MOR in plasma membranes from fertilized eggs of *Spaurus aurata*. A)Two bands of 50 and 65 KDa were observed (arrows) B) Negative control blot where plasma membranes were incubated with a solution where the primary antibody was adsorbed with a molar excess of the immunizing peptide.

Discussion and conclusions

This is the first study showing the expression of MOR on fertilized fish eggs employing the western blot technique. In this species, as in mammals (Albrizio et al., 2005), MOR appeared as a doublet with a molecular mass of approximately 50-65 KDa. The positive signal obtained on fertilized eggs together with the previous evidences on sea bream and sea bass sperm cells, suggests a role for the opioidergic system in the regulation of teleost reproduction. In view of the fact that stress affects not only reproduction but also the early stages of life, studying the expression of MOR is critical to better understand the effects that stress condition could have on the survival and quality of fish embryos.

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