DISTRIBUTION AND CELLULAR LOCALIZATION OF SEA BREAM ESTROGEN RECEPTORS IN CALCIFIED TISSUES

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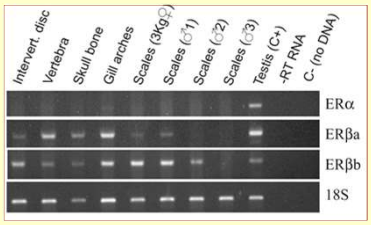
INTRODUCTION

In fish, estradiol (E_2) appears to control calcium metabolism at several levels: it increases whole body calcium influx (Guerreiro *et al.* 2002, J.Endocrinol.173(2):377-85) and promotes calcium mobilization from internal stores, particularly the scales (Armour *et al.* 1997, FEBS Lett.411(1):145-8). The presence of high-affinity estrogen binding sites and of estrogen receptor a (ERa) mRNA in scales (Persson *et al.* 2000, Gen.Comp.Endocrinolol.120(1):35-43) suggests that calcium mobilization is mediated by the direct action of E_2 binding to its receptor in this tissue. However, the mechanism by which E_2 brings about its effect in fish scales remains to be established and information about receptor type and cellular distribution would help resolve this question. The objectives of the study were to investigate the expression of sea bream ER transcripts (sbERα, sbERβa and sbERβb) in scales by RT-PCR and to localize sbER proteins by immunohistochemistry using ER isoform-specific antibodies.

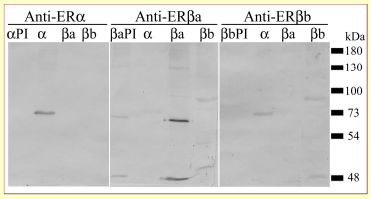
RESULTS

RT-PCR of sbERs in calcified tissues of adult sea bream



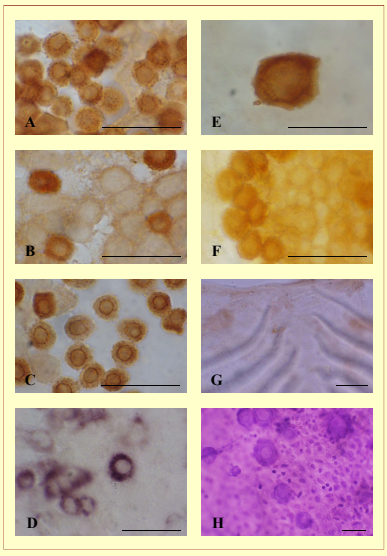
The expression of sbERα is generally very low and limited to gill arches. The two sbERβ isoforms are expressed in most tissues analysed, with expression in scales showing high variability among individuals. 18S was used as internal control.

Western blot of recombinant sbER proteins with ER isoform specific antibodies



Antisera raised against synthetic peptides specific for each sbER protein were tested for the detection of each recombinant ER protein expressed in *E. coli*. PI is the pre-induced control for each ER. Anti-sbERa and anti-sbERβa were highly specific for sbERa and sbERβa recombinant proteins, respectively, while anti-sbERβb cross reacted with sbERa.

Whole mount immunohistochemistry with anti-ER antibodies in sea bream scales



Scale bars: 25 µm.

Expression of ERa (**A**), ERβa (**B**) and ERβb proteins (**C**) was detected in groups of big rounded cells in the posterior region of the scale of juvenile sea bream.

The same type of cells also express TRACP mRNA, as detected by *in situ* hybridization (**D**), suggesting that they are putative osteoclasts.

Panel H shows the morphology of these cells in the posterior region of a scale stained by H&E.

Expression was also identified for ERα in isolated cells in the anterior region of adult sea bream scales (**E**) and for ERβa in rounded cells near the scale posterior margin in juvenile sea bream (**F**).

A negative control was carried out without the primary antibody (**G**) and no signal was detected.

CONCLUSIONS

- The sea bream ERβa and ERβb mRNAs are expressed in a number of different calcified tissues including scales, while sbERa expression was almost undetectable.
- Immunohistochemistry using specific sbER antisera showed that the three ER forms were expressed in scales from both juvenile and adult sea bream. However the signal localization and intensity varied with the age of animals and the type of receptor.
- The ERs were co-localized in cells which also express TRACP, suggesting that the calcium mobilizing action of E_2 on scales is via a direct action on putative osteoclasts.

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