THE CELL CONTEXT INFLUENCES RAINBOW TROUT GONADOTROPIN RECEPTORS' SELECTIVITY

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Introduction:

Vertebrate reproduction is tightly regulated by glycoprotein hormones produced by the pituitary gland. Two gonadotropins, FSH and LH are present in tetrapod vertebrates and the duality of gonadotropins has become an accepted principle also for fishes, the largest group of vertebrates. The presence of two distinct gonadotropin receptors (GtHRs) in a single fish species was confirmed by the molecular cloning of two different cDNAs in several fish species: salmon, catfish, zebrafish, sea bass, eel and trout. The ligand selectivity of mammalian GtHRs is well defined: FSHR and LHR bind their respective ligands specifically and show little cross-activation (0.01–0.1%). In contrast to the situation in mammalian species, the bioactivity of fish gonadotropins seems to be less well separated as a result of promiscuous hormone–receptor interactions. Depending on the species, hormones, and hormone concentrations used, a promiscuous activation of one or the other fish GtHRs was reported in functional studies using mammalian cell lines expressing fish receptors. In African catfish, recombinant cfFSH and cfLH activated FSHR with a similar biopotency [1] whereas in amago salmon, only FSH was able to activate FSHR. In zebrafish, recombinant zfFSH stimulated only FSHR, whereas recombinant zfLH stimulated both FSHR and LHR [2]. In trout, gonadotropins purified from trout or salmon pituitaries specifically activate their cognate receptor [3]. In summary, data from studies using different bioassays do not allow drawing general conclusions on the responsiveness of the piscine receptors to GtHs. In the present study, we report that the apparent discrepancy in fish gonadotropin receptors cross-selectivity originates mainly in the choice of the cell line used for receptor expression, and also from the heterologous or homologous origin of the hormones tested. The COS-7 cell line led to a highly selective responsiveness of the GtHRs whereas HEK cells show strong cross-reactivity.

Methods:

Rainbow trout FSH and LH receptor expression vectors were constructed using pcDNA3.1/V5-His-TOPO expression vector (Invitrogen) and the GtHR cDNA placed upstream from the polyadenylation site of the bovine growth hormone gene and downstream the cytomegalovirus (CMV) promoter. The HEK293/CREB-Luc cells (Panomics), and COS -7 cells were used for transient transfection assays. HEK293 cells were cultured in presence of 0.2 % hygromycin B (50mg/ml) in the DMEM culture medium in 24 well culture plate at a density of 20000 cell/well for 96 hours. COS-7 cells were cultured in a 24 well culture plate at a density of 70,000 cells/well for 24 hours. After that cells were transfected with either pcDNA3.1/V5-His-FSHR or pcDNA3.1/V5-His-LHR (10ng/well). Cells were stimulated with purified chinook FSH (cFSH), chinook LH(cLH), recombinant zebrafish FSH (zfFSH), recombinant zebrafish LH (zfLH), human FSH (hFSH), human LH (hLH) and human chorionic gonadotropin (hCG) for six hours. Luciferase activity was measured from 40µl lysates using the luciferase assay kit (Promega). Cultures of testicular tissue explants were carried out to analyse the effect of different salmonid and non-salmonid hormones on 11-ketotestosterone (11-KT) production.

Results and discussion:

Functional characteristics of trout GtHRs were analysed in two different cell contexts: COS-7 and HEK-293 cell lines. FSH receptor was efficiently activated by both cFSH and cLH in the HEK293 cell line and similar inductions of FSHR were obtained at 800 ng/ml (maximal fold induction of the luciferase reporter gene was 6.4 with cFSH vs. 6.6 with cLH). The cLH potency appeared to be lower, with an effective half-maximum concentration (EC_{50}) equal to 349 ng/ml versus 111 ng/ml for cFSH. In contrast, in the COS-7 cell line, FSH receptor was only activated by cFSH, with an effect similar to that observed in HEK cells (5.9 fold induction at 800 ng/ml and EC_{50} =133 ng/ml). These results reveal that FSHR response to LH is strongly dependent on the cell context.

LH receptor was mainly stimulated by cLH in both HEK293 and COS-7 cell lines. The maximal fold induction was significantly higher in HEK293 cell compared to COS-7 cell (x79 vs. x14). In addition, the potency of cLH appeared almost twice higher in HEK293 cells than in COS-7 cells, with an EC_{50} value of 5 ng/ml versus 9.8 ng/ml, respectively. This indicates

that HEK cells were more favorable to LH receptor response, as compared to COS cells. Although trout LHR was mainly stimulated by cLH, high doses of cFSH (1600 ng/ml) efficiently activated LHR in the HEK293 cell line but not in the COS cell line. So, our results indicate that the cellular context modulates the selectivity and the amplitude of the LH receptor response.

To address further whether the cell context could modulate ligand-receptor selectivity, we tested the trout GtHRs responsiveness to mammalian and zebrafish gonadotropins. In COS-7 cells, FSH receptor was activated by salmonid FSH but not by the zebrafish or human gonadotropins tested. In HEK cells, FSH receptor was activated not only by salmonid FSH and LH, but also by all heterologous LH and hCG. In COS cells, LH receptor was efficiently stimulated by salmonid LH and moderately by non-salmonid LH but never by any FSH or hCG. On the contrary, in HEK cells, LH receptor was activated by all types of LH and hCG. Interestingly, it was also induced by salmonid and non-salmonid FSH, although to a lower magnitude.

Finally, additional studies revealed that mammalian hormones including hCG, up to 1600 ng/ml, did not induce 11-KT production from rainbow trout testicular explants cultured *ex vivo*.

Conclusions:

We demonstrate that cross-selectivity of the trout GtHRs' responsiveness depends on the cellular context. Trout GtHRs show high ligand selectivity when expressed in COS-7 cells, but not when expressed in HEK cells. Trout receptors selectivity in COS-7 cells seems to reflect better the *ex vivo* conditions. Altogether, we propose that trout GtHRs are highly selective and that the mammalian cell lines used reflect only partially this high selectivity.

References:

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