Characterization of a Cholecystokinin/Sulfakinin-like pathway in a Lophotrochozoa, the oyster *Crassostrea gigas.*

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Extended knowledge of the repertoires of neuropeptides and G Protein-coupled receptors (GPCRs) in the oyster *C. gigas* offers the opportunity to identify ligand/receptor pairs in a Lophotrochozoan animal. In *C gigas*, we identified a couple of related GPCRs (Cg-CCKR3.6 and Cg-CCKR4.6) displaying phylogenetic proximity with both the vertebrate Gastrin/cholecystokinin (G/CCKR) and the insect sulfakinin (SKR) receptors. A unique transcript encoding the precursor for two peptides exhibiting only subtle similarity with vertebrate CCKs and SKs was considered as the oyster CCK/SK homologue. The predicted peptides (Cg-CCK1: pQGAWDY**DY**GLGGG**RF**a; Cg-CCK2: F**DY**NFGGG**RWa**) share the C-terminal RF(W)amide with the SKs and the DY motif common to the CCK/SK peptide family. The Y residue of this conserved motif being often subjected to sulfatation.

To investigate the actual coupling of Cg-CCKRs with these peptides, we challenged Cg-CCKR3.6 and Cg-CCKR4.6 expressed in a mammalian (HEK) cell line with the oyster non-sulfated CCKs. Using a calcium mobilization assay, only Cg-CCK1 induced both receptors in a dose dependent manner. Since the peptide concentrations required to elicit a half maximal activation were pretty high (0.3 µM and 0.8 µM), sulfated peptides forms were tested. Interestingly, sulfated CgCCK1s and to a lesser extend Cg-CCK2s induced a maximal activation of the oyster receptors at nM concentrations. This suggests that Cg-CCKs may be sulfated and represent a physiologically active peptide form. This hypothesis is currently being tested.

Receptor expression pattern was obtained using FPKM values of the corresponding transcripts in oyster tissue transcriptome libraries. Cg-CCKR4.6 was very weakly expressed in a variety of tissues. In contrast, Cg-CCKR3.6 was significantly expressed in nervous tissues, the mantle and the gonad, suggesting a distinct role for these two receptors. Experiments are underway in order to clarify the physiological involvement of oyster CCK signaling by studying the temporal expression patterns or expression related to environmental constraints (temperature, salinity, food ...) of the genes encoding the CCK receptors and its ligands.