Cloning and Expression of Guanylin-like Peptides in Teleost Fish.

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Abstract

Homologues of the guanylin peptide family are expressed in teleost fish. Using the eel as a model euryhaline species, cDNAs for three peptides, guanylin, uroguanylin and renoguanylin were cloned and found to be expressed within both renal and intestinal epithelia. Seawater (SW) acclimation resulted in up-regulation of uroguanylin mRNA expression in the intestinal epithelia of both immature "yellow" and sexually mature "silver" eels. SW-acclimation also resulted in an increase in guanylin mRNA in silver eel intestine whereas there was no change in renoguanylin mRNA expression under any condition. No changes in expression were found in the kidney following SW acclimation.

Introduction

Guanylin and uroguanylin are members of a small family of heat-stable, cysteine-rich peptides that have been identified in mammals and reported to be

extracellular regulators of trans-epithelial chloride secretion.[1] The peptides exhibit both paracrine and endocrine functions, being released from specialised cells within the intestine to act on guanylate cyclase (GC-C) receptors located within apical membranes of intestinal and renal epithelial cells. The physiological roles of these peptides in mammals include the stimulation of intestinal fluid secretion to facilitate digestion and the activation of renal tubular natriuresis and diuresis with concomitant reduction in blood volume and blood pressure. This report demonstrates that this peptide-signalling system, first described in mammals, appears to be present in lower vertebrates, including teleost fish and that these peptides may play a role in osmoregulation when fish move between environments of changing salinity.

Material and methods

Total RNA was extacted from the intestinal epithelia and the renal kidney and cDNAs encoding the complete coding regions for the guanylin-like peptide prohormones were amplified using degenerate inosine containing primers in RT-PCR and cloned into the pCR4 -TOPO vector (Invitrogen Ltd) as described previously.[2] Specific DNA probes were used in Northern blot analyses to quantify guanylin-like peptide mRNA expression.[2]

Results and Discussion

Complementary DNAs for guanylin-like peptides were isolated from eel, cod, salmon, and trout and cDNAs encoding uroguanylin-like peptides were

isolated from eel, plaice, flounder, cod and sculpin. Although the prohormone sequences exhibited very low homologies to their mammalian counterparts the putative active peptides, located at the C-terminal of the prohormones exhibited relatively high amino acid homology to the known mammalian peptides (Fig. 1). As previously reported for the Japanese eel,[3] a third, and novel guanylin-like peptide was identified in the European eel. The peptide was named renoguanylin, as expression was higher in the kidney than the intestine. These peptides may mediate their actions by binding to different isoforms of the particulate guanylate cyclase C (GC-C) receptor subtype which we have previously shown to be expressed in both in the eel intestine and kidney.[4] In freshwater-acclimated yellow and silver eels, mRNAs for all peptides were found throughout the whole length of the intestinal epithelium with relative levels of expression being renoguanylin ≥ guanylin > uroguanylin. In the kidney, mRNA expression levels were renoguanylin >> uroguanylin >> guanylin. Three weeks after transfer to SW, there was an approximate 4-fold increase in the expression of uroguanylin mRNA within the intestinal epithelium of both immature "yellow" and sexually mature "silver" eels (Fig.2). Messenger RNA for guanylin was also increased by approximately 50%, but only in silver eel intestine. Intestinal guanylin mRNA in yellow eels and renoguanylin mRNA in both yellow and silver eels did not change after SW acclimation. There was also no significant change in the abundance of mRNAs for all three peptides within the kidney following SW acclimation.

These results suggest that both guanylin and particularly uroguanylin, are important regulators of fluid balance within the intestine of SW-acclimated fish. When fish are in the marine environment a major role for the intestine is fluid absorption. In this situation, the guanylin peptides may stimulate bicarbonate secretion into the intestinal lumen, which would enhance the precipitation of the imbibed divalent cations such as calcium and magnesium as carbonate salts which are often found as soft white deposits within the gut of most marine teleosts.

References

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Guanylin sequences:

Human	PGTCEICAYAACTGC
Eel guanylin	YDECEICMFAACTGC
Eel renoguanylin	ADLCEICAFAACTGCL
Salmon/trout	MDICEICAFAACTGC
Cod	MDVCEICAYAACTGC

Uroguanylin sequences:

Human	NDDCELCVNVACTGCL
Eel	PDPCEICANAACTGCL
Plaice/flounder	SDPCEICANPSCFGCLT
Cod	RDACDICANPSCFGCRQ
Sculpin	MDPCEICANPSCFGCLK

FIGURE 1. Amino acid sequences of teleost guanylin-like peptides

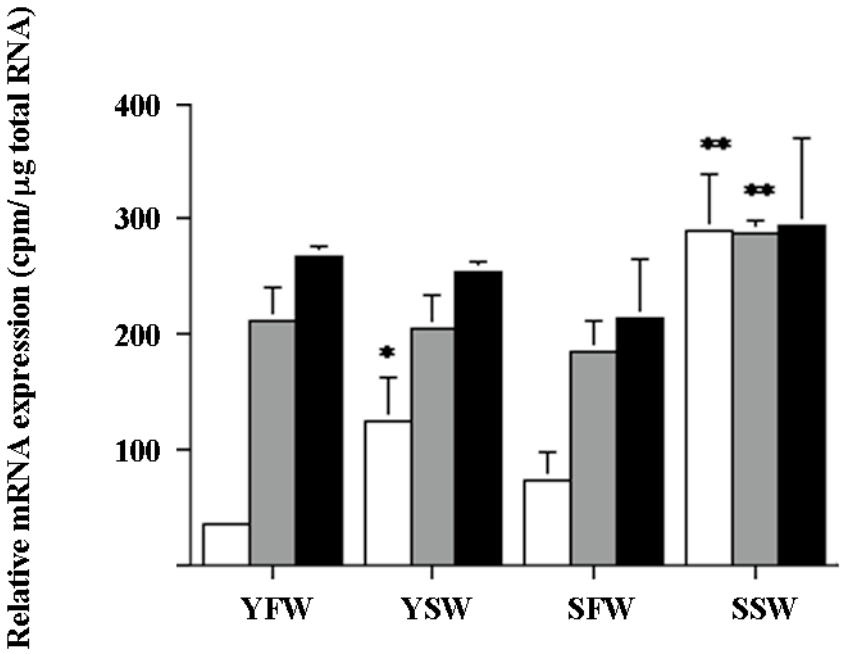

FIGURE 2. Relative expression of uroguanylin (open bars), guanylin (grey bars) and renoguanylin (black bars) mRNAs in the intestinal epithelium of yellow (Y) and silver (S) eels acclimated for 3 weeks in freshwater (FW) or seawater (SW). Statistically significant differences between FW- and SW-acclimated fish are indicated (* $p < 0.05$; ** $p<0.01$).