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Identification of two functional guanylin receptors in eel: Multiple hormone-receptor system for osmoregulation in fish intestine and kidney

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Abstract

Guanylyl cyclase C (GC-C) is a single transmembrane receptor for a family of intestinal hormones, guanylins. In the eel, we previously identified three guanylins, whose gene expression was enhanced in the intestine after transfer from fresh water to seawater. However, only limited information is available about the structure and function of their receptor(s). In the present study, we cloned full-length cDNAs encoding two isoforms of GC-C, named GC-C1 and GC-C2, from eel intestine. The predicted GC-C proteins consisted of extracellular ligand-binding domain, membrane-spanning domain, kinase-like domain and cyclase catalytic domain, in which GC-C-specific sequences were largely conserved. Phylogenetic analyses showed that the cloned membrane GCs are grouped with the GC-C of other vertebrates but not with GC-A and GC-B. However, eel GC-Cs appear to have undergone unique structural evolution compared with other GC-Cs. The three eel guanylins (guanylin, uroguanylin and renoguanylin), but not eel atrial natriuretic peptide, stimulated cGMP production dose-dependently in COS cells expressing either of the cloned cDNAs, providing functional support for assignment as eel guanylin receptors. The potency order for cGMP production was uroguanylin > guanylin ⩾ renoguanylin for GC-C1; guanylin ⩾ renoguanylin > uroguanylin for GC-C2. The distinctive ligand selectivity was consistent with the low homology (53%) of the extracellular domain of the two GC-Cs compared with that observed for other domains (74–90%). Both GC-C genes were expressed in the alimentary tract (esophagus, stomach and intestine) and kidney, and their expression was higher in the intestine of seawater-adapted eels than that of freshwater eels just as observed with the guanylin genes. However, the expression of the receptor genes was unchanged for 24 h after transfer of eels from fresh water to seawater or vice versa, showing slower response of the receptors to salinity changes than their ligands. Collectively, the multiple guanylin—GC-C system may be involved as a paracrine factor in seawater adaptation at the intestine and kidney of the eel.

Keywords: Eel; *Anguilla japonica*; Guanylyl cyclase C; Guanylin; Uroguanylin; Renoguanylin; Seawater adaptation

1. Introduction

Guanylyl cyclases (GCs) are a group of enzymes that catalyze the production of cGMP, leading to important regulations of body fluid homeostasis, smooth muscle contraction, photoreception and others (Yamagami and Suzuki, 2005). They are classified into two major forms, membrane GC and cytoplasmic GC. Guanylyl cyclase C (GC-C), one of the membrane GCs (Schulz et al., 1990), is a receptor for a group of intestinal small peptide hormones, guanylin (Currie et al., 1992) and uroguanylin (Hamra et al., 1993). In mammals, both guanylins bind to a single GC-C, inducing secretion of water, Cl^- and HCO_3^- into the lumen of the intestine (Guba et al., 1996; Joo et al., 1998) and natriuresis and diuresis in the kidney (Fonteles et al., 1998; Forte et al., 2000). An exogenous

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ligand, heat-stable enterotoxin (STa), also binds to GC-C (Schulz et al., 1990) and has stronger effects on the intestine than guanylin and uroguanylin, resulting in acute secretory diarrhea (Hamra et al., 1997; Forte et al., 2000). Both guanylin and uroguanylin are produced in the intestine and kidney, but uroguanylin shows more significant effect on the kidney than guanylin (Fonteles et al., 1998; Forte et al., 2000). GC-C is located primarily on the apical surface of the intestinal epithelia and renal proximal tubule (Forte et al., 2000). Thus, guanylin and uroguanylin are secreted into the lumen of the intestine and renal proximal tubule to act locally, as well as into the circulation to act in an endocrine fashion (Guba et al., 1996; Fonteles et al., 1998; Joo et al., 1998; Forte et al., 2000).

Guanylin(s)/GC-C seems to exist in various non-mammalian vertebrates as suggested by immunohistochemical evidence (birds, Krause et al., 1995; reptiles, Krause et al., 1997; see also Forte, 2004). In eels, the hormone family includes guanylin and uroguanylin, and a third member named renoguanylin that were identified from the molecular analysis (Comrie et al., 2001a; Yuge et al., 2003; Cramb et al., 2005). Renoguanylin has characteristics of both guanylin and uroguanylin in its structure and site of production. Concerning the receptors, two GC-C cDNAs, named OlGC6 (Mantoku et al., 1999) and OlGC9 (Iio et al., 2005), have been identified in the medaka fish (*Oryzias latipes*) (see also Nakauchi and Suzuki, 2005), and partial sequences of two GC-C-like cDNAs have been reported in the European eel (Comrie et al., 2001b). Thus, the guanylin-GC-C system appears to have diversified in fish compared with that of mammals.

Teleost fish suffer from dehydration in seawater (SW) because their body fluid osmolality is lower (one-third) than that of the environment (Potts, 1968). To compensate for water lost osmotically from the body, marine teleost fish drink SW and absorb water together with NaCl through the intestine. Thus, the intestine plays an important role in SW teleost fish as does the gill in NaCl excretion (Hirano et al., 1976b; Tsukada et al., 2005). In particular, euryhaline fish such as eels that can survive in both fresh water (FW) and SW, must alter intestinal function drastically according to the environmental salinity (Hirano et al., 1976b). Hence, the eel provides an excellent model for the study of intestinal hormones such as guanylins for water and ion regulation. In fact, the morphology of eel intestine changes enormously after transfer from FW to SW; blood vessels develop and the epithelia become thinner to facilitate absorptive activity. In mammals, guanylin secretion in the intestine and uroguanylin gene expression in the kidney increase in response to NaCl load to the intestinal lumen (Kita et al., 1999; Potthast et al., 2001). In the eel, Cl^- is sensed by the intestine, which leads to the secretion of unknown humoral factor(s) for body fluid regulation (Ando and Nagashima, 1996). Guanylins may be candidates for such humoral factors as their gene expression increased in SW eel intestine (Yuge et al., 2003).

To further elucidate the physiology of the eel guanylin family, we attempted to obtain all cDNAs encoding GC-C type receptors from the intestine and kidney of the Japanese eel, *Anguilla japonica*. As a result, we obtained full-length cDNAs of two distinct guanylin receptors that exhibit different ligand selectivities to the three eel guanylins. Subsequently, we examined the tissue distribution of the two GC-C mRNAs to identify the target tissues of guanylins. Finally, changes in GC-C gene expression were examined after acute transfer of eels from FW to SW or vice versa, and between FW- and SW-adapted fish to assess a role for the guanylin system in SW adaptation in this euryhaline fish.

2. Materials and methods

2.1. Animals

Fish experiments were performed in accordance with the Guidelines for Care and Use of Animals in the University of Tokyo and approved by the Committee for Animal Experiments. Cultured immature Japanese eels, *A. japonica*, were purchased from a local dealer. They were maintained in aquaria where FW or SW was continuously circulated, aerated and regulated at 18 °C. Eels were adapted for at least 2 weeks in each condition and were henceforth defined as FW eels (189 ± 3.1 g, $n = 34$) or SW eels (186 ± 3.3 g, $n = 37$). They were not fed after purchase to avoid the effect of luminal food on the intestinal receptors. In order to examine the receptors and hormonal ligands in the same sample specimens, the fish used in the ligand experiments were also utilized in this study (Yuge et al., 2003) except for the SW to FW transfer experiment where new eels were prepared.

2.2. cDNA cloning and construction of the expression vectors

Total RNA was extracted from the brain, gill, heart, liver, esophagus, stomach, anterior and posterior intestines, kidney, head kidney (including interrenal tissue), and spleen with Isogen (Nippon Gene, Tokyo, Japan). After purification of poly A^+ RNA from the intestine and kidney, the cDNA pool was constructed by SMART cDNA Library Construction Kit or SMART RACE cDNA Amplification Kit (BD Biosciences Clontech, Franklin Lakes, NJ). All partial cDNAs were cloned by PCR using Ex *Taq* DNA polymerase (TaKaRa Bio., Otsu, Japan) under the following condition: initial denaturation at 94 °C for 30 s, then 35–40 cycles of 94 °C for 1 min, 55–64 °C for 30 s and 72 °C for 1.5–3.25 min, and final extension at 72 °C for 7 min. Primers and modified PCR conditions were shown in Table 1A. Based on the sequences of all known vertebrate GCs, degenerate primers were designed to amplify all GC-C type genes present across this group.

Firstly, a partial cDNA for GC-C1 and GC-C2 was cloned using degenerate primers, GCC-S11 and GCC-A1, and GCC-S3 and GCC-A1, respectively. Secondly, 3′-rapid amplification of cDNA ends (RACE) for GC-C1 and GC-C2 was performed using GCC-S5 and GCC-S7, respectively, for sense primer, and CDS III/3′ for antisense primer. Finally, 5′-RACE for GC-C1 and GC-C2 was performed using 5′ UPM for sense primer and GCC-A11 and GCC-A6 for antisense primers, respectively, followed by nested PCR using 5′ NUP for sense primer, and GCC-A21 and GCC-A5 for antisense primers. All amplified products were subcloned into pT7blue vector (Novagen, Madison, WI) and sequenced in a 3100 DNA sequencer (Applied Biosystems, Foster City, CA). Each analysis was repeated independently at least three times to eliminate sequencing errors.

A full coding region of GC-C1 and GC-C2 was then amplified by PCR using a higher fidelity DNA polymerase, *Pyrobest* (TaKaRa Bio.) and specific primers shown in Table 1B for the subsequent experiment of the transient expression under the following conditions: initial