Calcitonin in a protochordate, Ciona intestinalis – the prototype of the vertebrate calcitonin/calcitonin gene-related peptide superfamily

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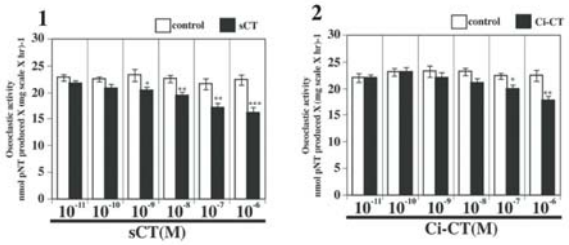
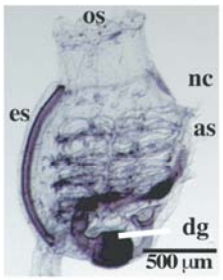
The calcitonin (CT)/CT gene-related peptides (CGRPs) constitute a large peptide family in vertebrates. However, no CT/CGRP superfamily members have so far been identified in invertebrates, and the evolutionary process leading to the diverse vertebrate CT/CGRP superfamily members remains unclear. In this study, we have identified an authentic invertebrate CT, Ci-CT, in the ascidian Ciona intestinalis, which is the phylogenetically closest invertebrate chordate to vertebrates. The amino acid sequence of Ci-CT was shown to display high similarity to those of vertebrate CTs and to share CT consensus motifs, including the N-terminal circular region and C-terminal amidated proline. Furthermore, the Ci-CT gene was found to be the only Ciona CT/CGRP superfamily gene. Ci-CT also exhibited less potent, but significant, activation of the human CT receptor, as compared with salmon CT. Physiological analysis revealed that Ci-CT reduced the osteoclastic activity that is specific to vertebrate CTs. CD analysis demonstrated that Ci-CT weakly forms an a-helix structure. These results provide evidence that the CT/CGRP superfamily is essentially conserved in ascidians as well as in vertebrates, and indicate that Ci-CT is a prototype of vertebrate CT/CGRP superfamily members. Moreover, expression analysis demonstrated that Ci-CT is expressed in more organs than vertebrate CTs in the cognate organs, suggesting that an original CT/CGRP superfamily member gene was also expressed in multiple organs, and each CT/CGRP superfamily member acquired its current specific tissue distribution and physiological role concomitantly with diversification of the CT/CGRP superfamily during the evolution of chordates. This is the first report on a CT/CGRP superfamily member in invertebrates.

A. A comparision of the amino acid sequences of CT from different chordate species

B. Effect of Ci-CT on CT activity in cultured scales from gold fish



C. Whole mount *in situ* hybridization of Ci-CT in *Ciona* juveniles



We identified a novel Calcitonin (CT)/CT gene-related peptide (CGRP) superfamily peptide from an ascidian, *Ciona intestinalis*. This is the first identification of a CT/CGRP superfamily member in invertebrates. The *Ciona* CT/CGRP superfamily peptide (Ci-CT) was composed of 30 amino acids. Ci-CT almost conserves a C-terminal amidated proline and an N-terminal circular region formed by disulfide bondbetween Cys1 and Cys7, which are requisite for biological activities of vertebrate CTs (A). Suppression of the osteoclastic activity was detected in goldfish scales treated with salmon CT (sCT) (B-1) or Ci-CT (B-2) at 18h. These physiological assays verified the CT-specific activity of Ci-CT. The Ci-CT gene was shown to be expressed in the neural complex, stigumata cell of gill, gastrointestinal tract, blood cells, and endostyle, suggesting that Ci-CT is involved in multiple physiological functions (C). nc, neural complex; en, endostyle; dg, digestive gland; as, atrial siphon; os, oral siphon