

## Characterization of a novel vasopressin/oxytocin superfamily peptide and its receptor from an ascidian, *Ciona intestinalis*

Kawada T, Sekiguchi T, Itoh Y, Ogasawara M, Satake H

*Peptides* 29, 1672-1678 (2008)

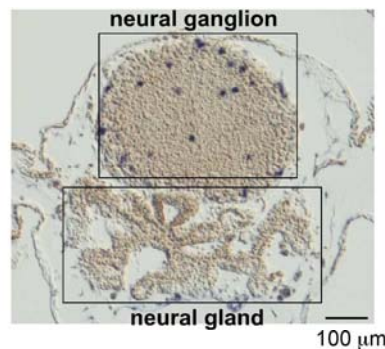
The Vasopressin (VP)/oxytocin (OT) superfamily peptides are one of the most widely distributed neuropeptides and/or neurohypophysial hormones, but have ever not been characterized from any deuterostome invertebrates including protochordates, ascidians. In the present study, we show the identification of a novel VP/OT superfamily peptide and its receptor in the ascidian, *Ciona intestinalis*. Intriguingly, the *Ciona* VP/OT-related peptide (Ci-VP), unlike other 9-amino acid and C-terminally amidated VP/OT superfamily peptides, consists of 13 amino acids and lacks a C-terminal amidation. Mass spectrometry confirmed the presence of the 13-residue Ci-VP in the neural complex. Furthermore, 10 of 14 cysteines are conserved in the neurophysin domain, compared with other VP/OT counterparts. These results revealed that the VP/OT superfamily is conserved in ascidians, but the Ci-VP gene encodes an unprecedented VP/OT-related peptide and neurophysin protein. Ci-VP was also shown to activate its endogenous receptor, Ci-VP-R, at physiological concentrations, confirming the functionality of Ci-VP as an endogenous ligand. The Ci-VP gene was expressed exclusively in neurons of the brain, whereas the Ci-TK-R mRNA was distributed in various tissues including the neural complex, alimentary tract, gonad, and heart. These expression profiles suggest that Ci-VP, like other VP/OT superfamily peptides, serves as a multifunctional neuropeptides. Altogether, our data revealed both evolutionary conservation and specific divergence of the VP/OT superfamily in protochordates. This is the first molecular characterization of a VP/OT superfamily peptide and its cognate receptor from not only ascidians but also deuterostome invertebrates.

### (A) Amino acid sequences of Ci-VP and OT/VP family peptides

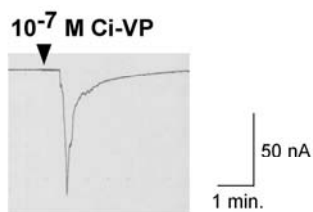
PEPTIDE	SEQUENCE
<b>Ci-VP</b>	<b>CFFRDCSNMDWYR</b>
Vasopressin	CYFQNCPRGa
Vasotocin	CYIQNCPRGa
Oxytocin	CYIQNCPLGa
Mesotocin	CYIQNCPIGa
Isotocin	CYISNCPIGa
Annetocin	CFVRNCPTGa
Lys-conopressin	CFIRNCPKGa

'a' denotes C-terminal amide.

### (B) *In situ* hybridization of Ci-VP gene in neural complex of *Ciona intestinalis*



### (C) Interaction of Ci-VP with Ci-VP receptor



We identified a novel vasopressin (VP) / oxytocin (OT) peptide and its receptor from an ascidian, *Ciona intestinalis*. This is the first molecular characterization of a VP/OT superfamily peptide and its cognate receptor from not only ascidians but also deuterostome invertebrates. The *Ciona* VP/OT superfamily peptide (Ci-VP) is composed of 13 amino acids featured by extension of four C-terminal amino acids, compared with all known 9-residue VP/OT superfamily peptides, and non-amidated C-terminus due to the absence of a C-terminal Gly amidation signal (A). The *Ci-VP* gene was expressed exclusively in neurons of the brain (B), whereas the Ci-VP-receptor mRNA was distributed in various tissues including the neural complex, alimentary tract, gonad, and heart. Application of Ci-VP to the Ci-VP-receptor-expressing *Xenopus* oocytes evoked a typical calcium-dependent inward chloride (C), revealing that Ci-VP is undoubtedly an endogenous ligand of Ci-VP-receptor.