Cholecystokinin (CCK) and gastrin are vertebrate brain/gut peptides featured by a sulfated tyrosine residue and a C-terminally amidated tetrapeptide consensus sequence. Cionin, identified in the ascidian, Ciona intestinalis, the closest species to vertebrates, harbors two sulfated tyrosines and the CCK/gastrin consensus tetrapeptide sequence. While a putative cionin receptor, CioR, was cloned, the ligand-receptor relationship between cionin and CioR remains unidentified. Here, we show the identification of two cionin receptors, CioR1 and CioR2, which are the aforementioned putative cionin receptor and its novel paralog, respectively. Phylogenetic analysis revealed that CioRs are homologous to vertebrate CCK receptors (CCKRs) and diverged from a common ancestor in the Ciona-specific lineage. Cionin activates intracellular calcium mobilization in cultured cells expressing CioR1 or CioR2. Mono-sulfated and non-sulfated cionin exhibited less potent or no activity, indicating that CioRs possess similar pharmacological feature to the vertebrate CCK-specific receptor, CCK1R rather than its subtype CCK2R, given that a sulfated tyrosine in CCK is required for binding to CCK1R, but not to CCK2R. Collectively, the present data reveal that CioRs share a common ancestor with vertebrate CCKRs, and indicate that CCK and CCK1R form the ancestral ligand-receptor pair in the vertebrate CCK/gastrin system. Cionin is expressed in the neural complex, digestive organs, oral siphon and atrial siphons, whereas the expression of CioRs was detected mainly in these tissues plus the ovary. Furthermore, cioninergic neurons innervate both of the siphons. These results suggest that cionin is involved in the regulation of siphonal functions.

Figure 1. Activation of CioRs by cionin. A concentration-response curve in CioR1-expressing cells (A) and CioR2-expressing cells (B) is shown. The Ca²⁺ levels elicited by cionin and its derivatives are plotted. The Ca²⁺ level caused by 1μM cionin was defined as 100%. The error bars denote S.E. (n = 5). Y indicates sulfated tyrosine.