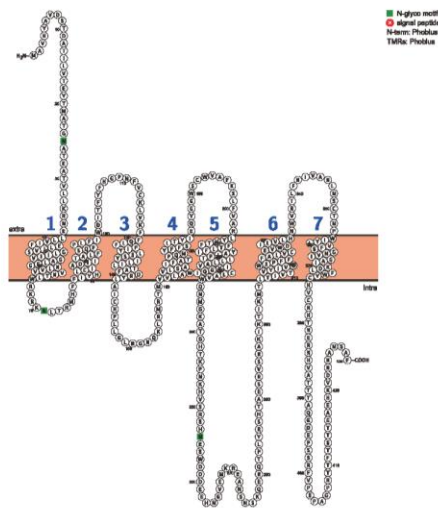


[illegible]

294 V G A R A K I K A I K M G L T V T V V Y
1141 GGGTATGACGGGCTAAGATTAAACATCAAGTGAAGCTCACCATASTAACTGGTATATA
334 T M 6 (306-334)
1201 V C W P F F A L C E V E W D L I L
1201 TGTCTGCTGGAGTCCTCTCTTGTGCAATGGTGTGAAGCTGGGACATACTGGTAT
334 H N W V R T L F N I V A N L C T N
1261 TACATTAATTGGGTGTCTAGCTATTCAATATTGGGCCAACCTGAAAGCTGTGACCAACT
374 T M 7 (354-374)
1354 I Y I V L V F S G N V I G A T K D L F W Y
1321 CATGATGATACCTGATTTCAGTCGGCAATGTGCTGGTGCTTACCAAGTATCTCTGCTG
374 C H V C R H T N E R H V A T A Q G
1381 ACTGCATGTCTCTGTGCACCAATAGAGGAATCAOSTGGCCTACTGCAGACGCCAAG
394 D E P S R F F P A G R T F T T
1441 GGGAGCAACGTCFAGTGCATTGTTCCTACCCCGCTGGACGGAGGACTATTATTCCT
414 G A E H K V D N N N S A F *
1501 CCACGCGACGGCGGACCAATAGGTGGACATACGCTAATTTCGCCGATTTCGTGCTGACTA
1561 cgaatgcgtgaatcagctaaatgcgaatgaacacaaacaccccaagatctcagctgt
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B



C

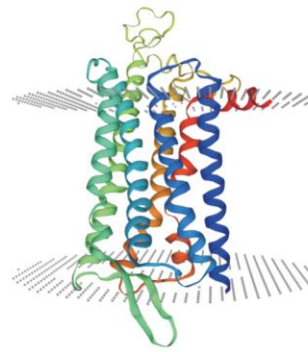


Figure S2. Molecular structure and characterization of *AjHOR* in *A. japonicus*. **(A)** *AjHOR* cDNA sequence (GenBank accession number: PIK60987.1) and the deduced amino acid sequence. ORF were denoted in uppercase; 3'UTR and 5'UTR were shown in lowercase. The asterisk (*) indicated the termination codon. The square indicated phosphorylation sites. The seven transmembrane domains (TM1-TM7) were labeled in blue underline. **(B)** Seven transmembrane domains of *AjHOR*. The number showed the position of seven transmembrane domains. **(C)** Protein tertiary structure of *AjHOR*.

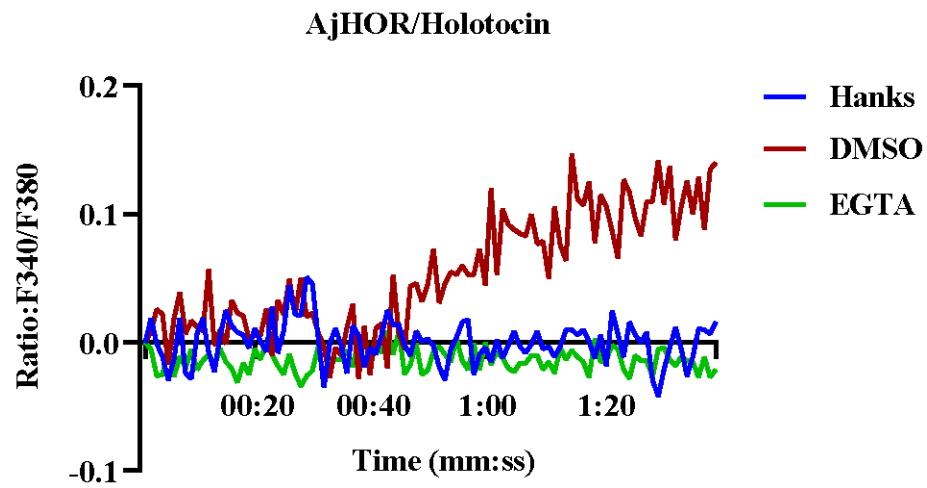


Figure S5. Calcium chelators EGTA (5 mM) on holotocin (1 μ M) in DMSO-mediated Ca^{2+} influx in HEK293T cells. All experiments were repeated independently three times with similar results.

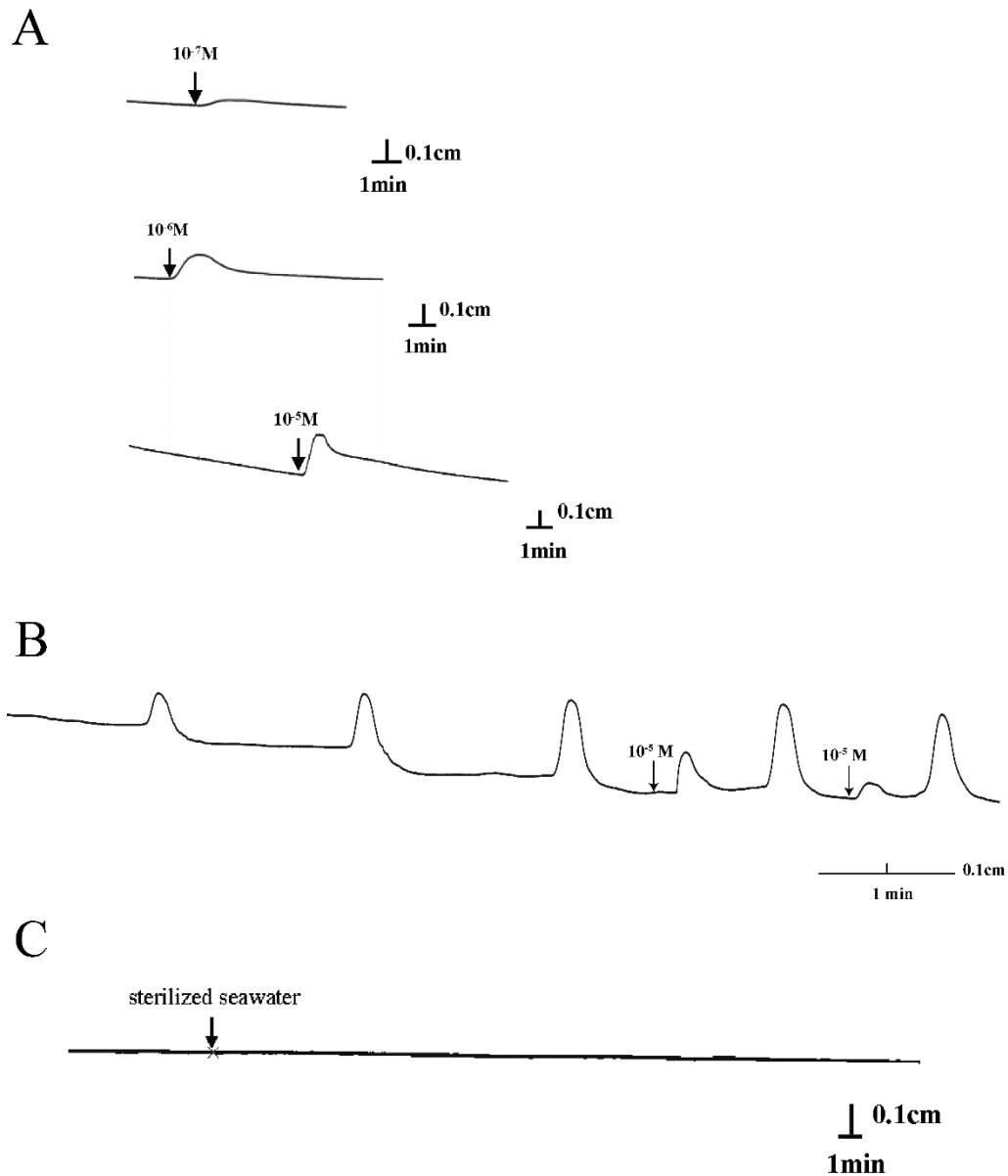


Figure S6. Negative and positive controls of *in vitro* pharmacological experiments and the necessity detection of calcium ions during longitudinal muscle contraction caused by holotocin. **(A)** NGIWyamide caused tonic contraction on longitudinal muscle preparations from *A. japonicus*. ($n = 3$). **(B)** A representative recording showed that holotocin ($10^{-5}M$) caused contraction of tentacle preparation. **(C)** Sterile seawater was used as the negative control and it could not cause the contraction of preparations.

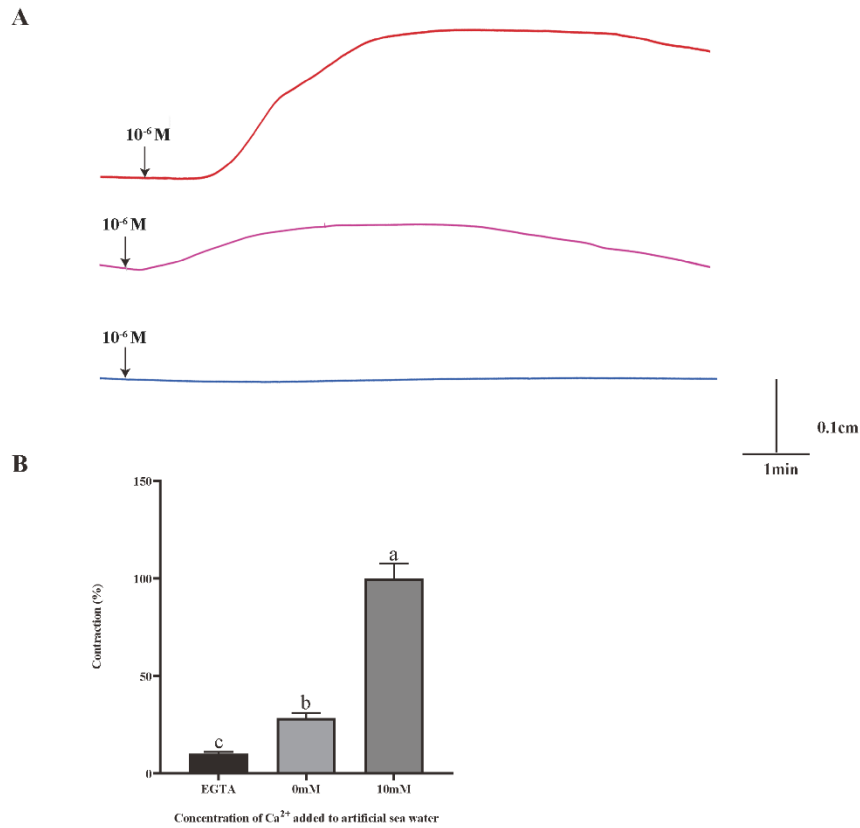


Figure S7. Dependence of holotocin contraction on different calcium ion concentration (Ca^{2+} -free solution containing 5 mM EGTA, 0 mM, 10 mM). **(A)** A representative recording showed that holotocin (10^{-6} M) caused longitudinal muscle contraction on artificial sea water with 0 mM or 10 mM Ca^{2+} . EGTA was used to chelate calcium ions and holotocin could not cause longitudinal muscle contraction. **(B)** Comparison of contraction efficiency of holotocin with different Ca^{2+} concentration, the contraction efficiency of holotocin in 10 mM Ca^{2+} was defined as 100%. The significant difference ($p < 0.05$) was shown by lowercase letters. Values were mean \pm S.E.M (n = 3 biological replicates).

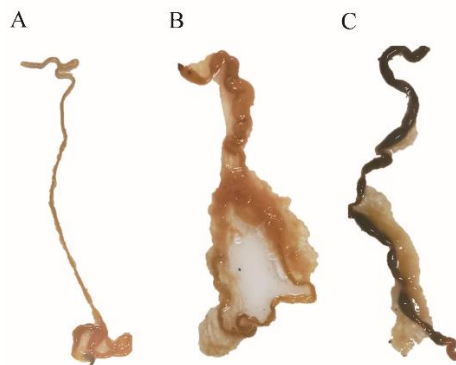


Figure S8. The anatomy of intestine during long-term injection in different groups. **(A)** The state of intestine in HOL. **(B)** The state of intestine in HOH. **(C)** The state of intestine after injection in CO.