Published in final edited form as:

Eur J Pharmacol. 2012 August 15; 689(1-3): 17–24. doi:10.1016/j.ejphar.2012.05.032.

Phospholipase C not protein kinase C is required for the activation of TRPC5 channels by cholecystokinin

Laurel A. Grisanti, Lalitha Kurada, Nicholas I. Cilz, James E. Porter, and Saobo Lei*

Department of Pharmacology, Physiology and Therapeutics, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND58203, USA

Abstract

Cholecystokinin (CCK) is one of the most abundant neuropeptides in the brain where it interacts with two G protein-coupled receptors (CCK1 and CCK2). Both types of CCK receptors are coupled to Gq/11 proteins resulting in increased function of phospholipase C (PLC) pathway. Whereas CCK has been suggested to increase neuronal excitability in the brain via activation of cationic channels, the types of cationic channels have not yet been identified. Here, we co-expressed CCK2 receptors and TRPC5 channels in human embryonic kidney (HEK) 293 cells and studied the effects of CCK on TRPC5 channels using patch-clamp techniques. Our results demonstrate that activation of CCK2 receptors robustly potentiates the function of TRPC5 channels. CCK-induced activation of TRPC5 channels requires the functions of G-proteins and PLC and depends on extracellular Ca^{2+} . The activation of TRPC5 channels mediated by CCK2 receptors is independent of IP3 receptors and protein kinase C. CCK-induced opening of TRPC5 channels is not store-operated because application of thapsigargin to deplete intracellular Ca^{2+} stores failed to alter CCK-induced TRPC5 channel currents significantly. Bath application of CCK also significantly increased the open probability of TRPC5 single channel currents in cell-attached patches. Because CCK exerts extensive effects in the brain, our results may provide a novel mechanism to explain its roles in modulating neuronal excitability.

Keywords

cholecystokinin; G protein; protein kinase; channel; calcium; TRP

1. Introduction

Cholecystokinin (CCK) is a neuropeptide that is abundantly distributed in the brain (Beinfeld et al., 1981). CCK interacts with two G protein-coupled receptors: CCK1 and CCK2 (Wank, 1995). Activation of both CCK receptors increases the activity of phospholipase C (PLC) resulting in the hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP2) into inositol trisphosphate (IP3) to increase intracellular Ca^{2+} release, and diacylglycerol to activate protein kinase C (PKC) although CCK1 receptors have also been shown to increase the activity of adenylyl cyclase which enhances the generation of cyclic

© 2012 Elsevier B.V. All rights reserved.

*Corresponding author: Saobo Lei, Department of Pharmacology, Physiology and Therapeutics, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND58203, USA. 701-777-4745 (phone) 701-777-4490 (fax) Saobo.lei@med.und.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

AMP and subsequent activation of protein kinase A (Wank, 1995). CCK1 receptors are distributed in the peripheral tissues and in limited brain regions including the area postrema, interpeduncular nucleus and nucleus tractus solitarius (Hill et al., 1987; Hill et al., 1990; Moran et al., 1986). CCK2 receptors are the predominant CCK receptors in the brain (Van Dijk et al., 1984). CCK serves as a neuromodulator in the brain either by inhibiting K⁺ channels (Branchereau et al., 1993; Chung et al., 2009; Cox et al., 1995; Deng and Lei, 2006; Deng et al., 2010a; Meis et al., 2007; Miller et al., 1997) or by activating cationic channels (Chung and Moore, 2009; Meis et al., 2007; Thorn and Petersen, 1992; Tsujino et al., 2005; Wang and Sims, 1998; Wang et al., 2011; Wu and Wang, 1996a; b; Zhao et al., 2011). Recently, it has been suggested that CCK activates the canonical transient receptor potential (TRPC) channels in amygdaloid (Meis et al., 2007) and entorhinal (Wang et al., 2011) neurons. However, direct evidence that CCK activates TRPC channels has not been available. The aim of the present study is to provide direct evidence that activation of CCK receptors activates TRPC channels. We co-transfected CCK2 receptors and TRPC5 channels in HEK293 cells and tested the effects of CCK on TRPC5 channels using patch-clamp recording techniques. Our results demonstrate that activation of CCK2 receptors results in robust activation of TRPC5 channels via activation of PLC without the requirement of PKC.

2. Materials and Methods

2.1. Co-transfection of HEK293 cells with CCK2 receptors and TRPC5 channels

Detailed experimental procedures for the transfection of HEK293 cells were described previously (Deng et al., 2007; Deng et al., 2009; Xiao et al., 2009a). Briefly, a cDNA construct containing TRPC5 channels (GenBank accession number NM 012471) subcloned into pCMV6-XL4 was purchased from OriGene (Rockville, MD). A cDNA construct containing CCK2 receptors (GenBank accession number AY 322551) subcloned into pcDNA3.1 was purchased from UMR cDNA Resource Center (www.cdna.org). The green fluorescent protein (GFP, pGreenLantern; Invitrogen) was co-transfected with TRPC5 channels plus CCK2 receptors for the identification of transfected cells. Dulbecco's minimum essential medium (DMEM) and fetal bovine serum (FBS) was purchased from Atlanta Biologicals (Atlanta, GA). Cell culture grade penicillin and streptomycin was purchased from Mediatech, Inc (Herndon, VA). HEK 293 cells obtained from American Type Culture Collection (Manassas, VA) were maintained in DMEM containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 U/ml). Confluent HEK293 cells were washed in Hank's Balance Salt Solution (HBSS), trypsinized and seeded at the appropriate density in 35 mm dishes to ensure 50–80% cell confluence within 24 h. Transient transfection of the cDNA constructs was performed after 24 h with GeneJammer transfection reagent according to the manufacturers' protocol (Stratagene, La Jolla, CA) using a 2.7 µl reagent per 1 µg cDNA ratio for the transfection cocktail. A ratio of 2:4:4 was used for GFP, CCK2 and TRPC5 channels, respectively. Transfected HEK293 cells were subsequently used for electrophysiological recordings 24–48 h post-transfection.

2.2. Electrophysiological recordings from HEK293 cells

Whole-cell recordings were made from transfected HEK293 cells using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in voltage-clamp mode as described previously (Deng et al., 2010b; Wang et al., 2012; Xiao et al., 2009b). The extracellular solution contained (in mM) 130 NaCl, 3 KCl, 2 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 10 HEPES and 10 glucose (pH 7.4) unless stated otherwise. Recording electrodes had resistances of 5–8 MΩ after being filled with the solution containing (in mM) 100 Cs-gluconate, 0.6 EGTA, 5 MgCl₂, 8 NaCl, 2 ATP2Na, 0.3 GTPNa and 40 HEPES (pH 7.3). Holding current at –60 mV with a ramp protocol from –100 mV to +60 mV (1 mV/ms, interval: 7 s) was recorded from the HEK293 cells that showed fluorescence under a

fluorescence microscope (Olympus 1X70). The capacitances of the recorded cells were measured by the readings of the amplifier after compensation.

Single-channel currents were recorded from HEK293 cells transfected with CCK2 receptors together with TRPC5 channels using the cell-attached patch configuration as described previously (Xiao et al.). The pipettes contained (in mM) 140 CsCl, 1 MgCl₂, 2 CaCl₂, 10 glucose and 10 HEPES (pH 7.4). The holding potential of patches was usually +60 mV, but various potentials were used when acquiring data to calculate the single-channel conductance. Single-channel currents were sampled at 10 kHz and filtered at 2 kHz. Only patches with stable basal activities for at least 2 min were analyzed to ensure that the changes in activity were not attributable to random fluctuations. Open probability was determined off-line using the 50% crossing thresholds method. Slope conductance was computed by linearly fitting the amplitudes recorded at different holding potentials.

2.3. Data analysis

Data were presented as the means $\pm$ S.E.M. To exclude the influence of cell size on the effects of CCK, we normalized the current to the capacitance of the recorded cell to calculate the current density. Student's paired or unpaired *t* test or analysis of variance (ANOVA) was used for statistical analysis as appropriate; *P* values were reported throughout the text and significance was set as *P*<0.05. *N* number in the text represents the cells examined unless stated otherwise.

2.4. Chemicals

Sulfated CCK-8 (abbreviated as CCK thereafter) was purchased from American Peptide Company (Sunnyvale, CA). Guanosine-5'-O-(2-thiodiphosphate) (GDP- β -S), GF109203X, U73122 and U73343 were products of Enzo Life Sciences International, Inc. (Plymouth Meeting, PA). Inositol trisphosphate (IP3) was purchased from Echelon Research Laboratories (Salt Lake City, UT). Thapsigargin, heparin, 1,2-bis-(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) and 2-aminoethyldiphenyl borate (2-APB) were from Tocris Cookson Inc. (Ellisville, MO). The other chemicals were products of Sigma-Aldrich (St. Louis, MO).

3. Results

3.1. CCK activates TRPC5 channels in HEK293 cells co-transfected with CCK2 receptors and TRPC5 channels

Cells were held at -60 mV and a ramp protocol from -100 mV to +60 mV at the rate of 1 mV/ms was applied every 7 s. In this condition, bath application of CCK (0.3 μ M) induced an inward current assessed by calculating the net change of the current density (-26.6 ± 5.8 pA/pF, *n*=7, *P*=0.004, Fig. 1A1-A4). The CCK-induced inward current showed considerable desensitization (Fig. 1A1 and 1A4). The voltage-current relationship of the net current induced by CCK at its maximal effect resembled that of the TRPC5 channels activated by M1 muscarinic receptors (Strubing et al., 2001) indicating that activation of CCK2 receptors activates TRPC5 channels. As a negative control, we transfected HEK293 cells with TRPC5 channels alone. Bath application of CCK at the same concentration to these cells transfected with TRPC5 alone failed to induce significant changes in current density (0.2 ± 0.1 pA/pF, *n*=6, *P*=0.08, Fig. 1B1-B4). These data together indicate that activation of CCK2 receptors opens TRPC5 channels.

3.2. CCK-induced activation of TRPC5 channels is G protein-dependent and requires the function of PLC

CCK2 receptors are G protein-coupled (Wank, 1995). We next tested whether G proteins are required for CCK-mediated activation of TRPC5 channels. We included GDP- β -S (2 mM), a G protein inactivator, in the recording pipettes and waited for ~15 min after the formation of whole-cell configuration to allow the dialysis of GDP- β -S into cells. Under these circumstances, bath application of CCK (0.3 μ M) did not significantly induce an inward current in HEK293 cells co-transfected with CCK2 receptors and TRPC5 channels (-0.42 ± 0.40 pA/pF, $n=6$, $P=0.34$, Fig. 2A-2D) demonstrating that G proteins are necessary for CCK-induced activation of TRPC5 channels.

Because activation of CCK2 receptors activates PLC (Wank, 1995), we then tested whether the function of PLC is required for CCK-induced activation of TRPC5 channels. HEK293 cells co-transfected with CCK2 receptors and TRPC5 channels were pretreated for 20 min with the PLC inhibitor U73122 (5 μ M) dissolved in the extracellular solution and the bath was continuously perfused with the same concentration of U73122. Whole-cell recordings were then conducted from the pretreated cells. For controls, the HEK293 cells transfected with both CCK2 receptors and TRPC5 channels were pretreated with the inactive analog U73343 (5 μ M) for the same time. Under these circumstances, bath application of CCK (0.3 μ M) failed to significantly induce an inward current in HEK293 cells treated with U73122 (-3.2 ± 2.4 pA/pF, $n=12$, $P=0.21$, Fig. 3A1-A4) whereas application of the same concentration of CCK still induced an inward current in HEK293 cells pretreated with the same concentration of the inactive analog U73343 (-25.3 ± 4.8 pA/pF, $n=5$, $P=0.006$, Fig. 3B1-B4). These data demonstrate that PLC is required for CCK-induced activation of TRPC5 channels.

3.3. CCK-mediated activation of TRPC5 channels is dependent on extracellular Ca^{2+} , blocked by intracellular BAPTA but independent of intracellular Ca^{2+} depletion induced by thapsigargin

Activation of CCK2 receptors increases PLC which further hydrolyzes PIP2 to generate IP3 to increase intracellular Ca^{2+} release and diacylglycerol to activate PKC (Wank, 1995). Furthermore, potentiation of TRPC5 channels induced by muscarinic receptors has been shown to rely on extracellular Ca^{2+} (Blair et al., 2009). We therefore tested whether CCK-mediated activation of TRPC5 channels is Ca^{2+} -dependent or not. We tested the roles of extracellular Ca^{2+} by omission of Ca^{2+} in the extracellular solution. In the nominally Ca^{2+} -free extracellular solution, application of CCK failed to significantly induce an inward current (-0.9 ± 1.1 pA/pF, $n=5$, $P=0.45$, Fig. 4A1-A4) demonstrating that CCK-mediated activation of TRPC5 channels is dependent on extracellular Ca^{2+} . We then tested the roles of intracellular Ca^{2+} . We included BAPTA (10 mM) in the recording pipettes and waited for ~15 min after the formation of whole-cell configuration to permit its dialysis into cells. In this condition, application of CCK did not significantly change the holding current (-2.7 ± 1.1 pA/pF, $n=5$, $P=0.07$, Fig. 4B1-B4) suggesting that CCK-mediated activation of TRPC5 channels is dependent on intracellular Ca^{2+} concentration.

We next tested whether CCK-induced inward current was store-operated. HEK293 cells transfected with CCK2 receptors and TRPC5 channels were pretreated with extracellular solution containing 0 Ca^{2+} and thapsigargin (5 μ M) for 10 min. The bath was continuously perfused with the extracellular solution containing normal concentration of Ca^{2+} and thapsigargin (5 μ M). In this condition, application of CCK still induced a comparable inward current (-24.5 ± 5.9 pA/pF, $n=7$, $P=0.006$, Fig. 4C1-C4) demonstrating that CCK-mediated inward current is not store-operated.

To test whether IP3 receptors are required for CCK-mediated activation of TRPC5 channels, we first included heparin (5 mg/ml), an IP3 receptor inhibitor, in the recording pipettes and waited for ~15 min after the formation of whole-cell configuration to allow this compound to diffuse into the cells. In the presence of heparin, bath application of CCK (0.3 μ M) still opens TRPC5 channels (-26.7 ± 4.5 pA/pF, $n=6$, $P=0.002$, Fig. 5A1–A4). Similarly, intracellular application of 2-APB (100 μ M), another IP3 receptor inhibitor, failed to change significantly CCK-induced inward current (-25.1 ± 2.1 pA/pF, $n=5$, $P<0.001$, Fig. 5B1–5B4). Furthermore, we included IP3 (100 μ M) in the recording pipettes and waited for 15 min after the formation of whole-cell configuration. Under these circumstances, bath application of CCK (0.3 μ M) still induced a comparable inward current density (-21.7 ± 3.4 pA/pF, $n=8$, $P<0.001$, Fig. 5C1–5C4). These data together suggest that IP3 receptors are dispensable for CCK-induced activation of TRPC5 channels.

3.4. PKC is not involved in CCK-mediated activation of TRPC5 channels

We then tested the roles of PKC by applying the specific PKC inhibitor, GF109203X. HEK293 cells cotransfected with CCK2 receptors and TRPC5 channels were pretreated with GF109203X (1 μ M) and the same concentration of GF109203X was continuously bath-applied. Under these circumstances, application of CCK (0.3 μ M) still induced a comparable inward current (-19.4 ± 4.1 pA/pF, $n=11$, $P<0.001$, Fig. 6A–6D).

3.5. CCK increases single channel open probability

We next tested the effects of CCK on the single channel properties of TRPC5 channels cotransfected with CCK2 receptors and TRPC5 channels using cell-attached patches. No single channel events were observed at +60 mV in mock cells (cells without fluorescence, $n=10$ patches). In transfected cells (cells showing fluorescence), 6 out of the 10 patches showed constitutive single channel activity at +60 mV. However, these single channel events became unpredictable and quite a lot of them disappeared when we tried to change the holding potentials to measure their slope conductance. We therefore included CCK (0.3 μ M) in the pipette solution and still used cell-attached patches to measure the slope conductance as performed previously (Strubing et al., 2001). Under these circumstances, a slope conductance of 37 ± 3 pS ($n=5$ patches, Fig. 7A1–7A2) was obtained suggesting that the single channel events were mediated by opening of TRPC5 channels (Strubing et al., 2001). We then used cell-attached patch (without CCK in the pipette solution) and determined the effects of CCK on TRPC5 single channels. Bath application of CCK (0.3 μ M) increased the open probability of the single channel events by 9.2 ± 3.4 folds ($n=6$ patches, $P=0.03$, paired t-test, Fig. 7B1–7B2) indicating that TRPC5 channels respond to CCK2 receptor stimulation.

4. Discussion

Whereas CCK has been demonstrated to increase neuronal excitability via activating cation channels (Chung and Moore, 2009; Meis et al., 2007; Thorn and Petersen, 1992; Tsujino et al., 2005; Wang and Sims, 1998; Wang et al., 2011; Wu and Wang, 1996a; b; Zhao et al., 2011) including TRPC (Meis et al., 2007; Wang et al., 2011) and TRPV (Zhao et al., 2011) channels, direct evidence that CCK opens cation channels still lacks. Because CCK has been shown to increase neuronal excitability in a variety of neurons, identification of the involved cation channels likely has broad physiological significance. Because previous studies indicate that activation of CCK2 receptors facilitates neuronal excitability via activation of TRPC channels in amygdaloid (Meis et al., 2007) and entorhinal (Wang et al., 2011) neurons, we cotransfected HEK293 cells with CCK2 receptors and TRPC5 channels and examined the effects of CCK on TRPC5 channels. We provided direct evidence that

activation of CCK2 receptors activates TRPC5 channels indicating a novel mechanism that can explain the roles of CCK in the brain.

Whereas activation of Gq/11-coupled receptors is generally considered to activate TRPC channels, a limited number of receptors have been demonstrated to activate TRPC channels directly. Most of the studies about TRPC channels especially for the TRPC5 channels were conducted by using muscarinic receptors (Kim et al., 2008; Strubing et al., 2001; Zhu et al., 2005). To our knowledge, we provided the first piece of evidence demonstrating that activation of CCK2 receptors, a peptide receptor belonging to the family B G protein-coupled receptors, activates TRPC5 channels when they are expressed in HEK293 cells. The mechanism underlying the activation of TRPC5 channels by G protein-coupled receptors is still an unsolved mystery. It is generally accepted that PLC is required for the opening of TRPC5 channels activated by Gq/11-coupled receptors. Consistent with this scenario, we have shown that CCK-induced inward current in HEK293 cells co-transfected with CCK2 receptors and TRPC5 channels is dependent on PLC because treatment of the transfected cells with the PLC inhibitor, U73122, blocked CCK-induced inward currents. Activation of PLC hydrolyzes PIP2 to generate IP3 to increase intracellular Ca^{2+} release and diacylglycerol to activate PKC. We further tested the roles of Ca^{2+} and PKC in CCK-induced inward currents. Our results demonstrate that CCK-mediated activation of TRPC5 channels depends on extracellular Ca^{2+} because omission of extracellular Ca^{2+} or buffering of intracellular Ca^{2+} by BAPTA blocked CCK-induced inward currents, consistent with muscarinic receptor-mediated activation of TRPC5 channels (Blair et al., 2009; Plant and Schaefer, 2003; 2005). However, CCK-mediated activation of TRPC5 channels is not dependent on intracellular Ca^{2+} release because treatment of cells with thapsigargin to deplete intracellular Ca^{2+} stores failed to alter significantly CCK-induced inward currents suggesting that CCK-mediated activation of TRPC5 channels is not store-operated. Consistent with our results, TRPC5 channels are not store-operated (Okada et al., 1998; Schaefer et al., 2000) although there is one report suggesting that TRPC5 channels are store-operated (Philipp et al., 1998). The other downstream target of PLC is PKC. However, we have not observed significant effects when PKC was inhibited by application of GF109203X, a specific PKC inhibitor suggesting that PKC is not required for CCK-mediated activation of TRPC5 channels. Consistent with the effects of CCK on TRPC5 channels, activation of CCK1 receptors increases intracellular Ca^{2+} concentration via activation of TRPV channels in nodose neurons and PLC and extracellular Ca^{2+} are required for the effects of CCK on TRPV channels whereas the common downstream PLC-activated pathways, PKC and the generation of IP3 and subsequent release of intracellular Ca^{2+} , are not involved in the responses to CCK (Zhao et al., 2011).

5. Conclusion

In summary, our results demonstrate that activation of CCK2 receptors robustly opens TRPC5 channels in HEK293 cells cotransfected with CCK2 receptors and TRPC5 channels. CCK2 receptor-mediated activation of TRPC5 channels requires the functions of G proteins and PLC and depends on extracellular Ca^{2+} but does not rely on IP3 receptors and PKC. We have also shown that TRPC5 channels activated by CCK2 receptors are not store-operated. CCK increases TRPC5 single channel open probability. Our results provide a novel mechanism that can explain the effects of CCK in the brain.

Acknowledgments

This work was supported by National Institutes of Mental Health (MH082881)

References

- Beinfeld MC, Meyer DK, Eskay RL, Jensen RT, Brownstein MJ. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. *Brain Res.* 1981; 212:51–57. [PubMed: 7225864]
- Blair NT, Kaczmarek JS, Clapham DE. Intracellular calcium strongly potentiates agonist-activated TRPC5 channels. *J Gen Physiol.* 2009; 133:525–546. [PubMed: 19398778]
- Branchereau P, Champagnat J, Denavit-Saubie M. Cholecystokinin-gated currents in neurons of the rat solitary complex in vitro. *J Neurophysiol.* 1993; 70:2584–2595. [PubMed: 7509860]
- Chung L, Moore SD. Cholecystokinin excites interneurons in rat basolateral amygdala. *J Neurophysiol.* 2009; 102:272–284. [PubMed: 19386755]
- Chung L, Moore SD, Cox CL. Cholecystokinin action on layer 6b neurons in somatosensory cortex. *Brain Res.* 2009; 1282:10–19. [PubMed: 19497313]
- Cox CL, Huguenard JR, Prince DA. Cholecystokinin depolarizes rat thalamic reticular neurons by suppressing a K⁺ conductance. *J Neurophysiol.* 1995; 74:990–1000. [PubMed: 7500167]
- Deng PY, Lei S. Bidirectional modulation of GABAergic transmission by cholecystokinin in hippocampal dentate gyrus granule cells of juvenile rats. *J Physiol.* 2006; 572:425–442. [PubMed: 16455686]
- Deng PY, Poudel SK, Rojanathammanee L, Porter JE, Lei S. Serotonin inhibits neuronal excitability by activating two-pore domain k⁺ channels in the entorhinal cortex. *Mol Pharmacol.* 2007; 72:208–218. [PubMed: 17452494]
- Deng PY, Xiao Z, Jha A, Ramonet D, Matsui T, Leitges M, Shin HS, Porter JE, Geiger JD, Lei S. Cholecystokinin facilitates glutamate release by increasing the number of readily releasable vesicles and releasing probability. *J Neurosci.* 2010a; 30:5136–5148. [PubMed: 20392936]
- Deng PY, Xiao Z, Lei S. Distinct modes of modulation of GABAergic transmission by Group I metabotropic glutamate receptors in rat entorhinal cortex. *Hippocampus.* 2010b; 20:980–993. [PubMed: 19739246]
- Deng PY, Xiao Z, Yang C, Rojanathammanee L, Grisanti L, Watt J, Geiger JD, Liu R, Porter JE, Lei S. GABA(B) receptor activation inhibits neuronal excitability and spatial learning in the entorhinal cortex by activating TREK-2 K⁺ channels. *Neuron.* 2009; 63:230–243. [PubMed: 19640481]
- Hill DR, Campbell NJ, Shaw TM, Woodruff GN. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. *J Neurosci.* 1987; 7:2967–2976. [PubMed: 3625281]
- Hill DR, Shaw TM, Graham W, Woodruff GN. Autoradiographical detection of cholecystokinin-A receptors in primate brain using 125I-Bolton Hunter CCK-8 and 3H-MK-329. *J Neurosci.* 1990; 10:1070–1081. [PubMed: 2329368]
- Kim BJ, Kim MT, Jeon JH, Kim SJ, So I. Involvement of phosphatidylinositol 4,5-bisphosphate in the desensitization of canonical transient receptor potential 5. *Biol Pharm Bull.* 2008; 31:1733–1738. [PubMed: 18758068]
- Meis S, Munsch T, Sosulina L, Pape HC. Postsynaptic mechanisms underlying responsiveness of amygdaloid neurons to cholecystokinin are mediated by a transient receptor potential-like current. *Mol Cell Neurosci.* 2007; 35:356–367. [PubMed: 17482476]
- Miller KK, Hoffer A, Svoboda KR, Lupica CR. Cholecystokinin increases GABA release by inhibiting a resting K⁺ conductance in hippocampal interneurons. *J Neurosci.* 1997; 17:4994–5003. [PubMed: 9185537]
- Moran TH, Robinson PH, Goldrich MS, McHugh PR. Two brain cholecystokinin receptors: implications for behavioral actions. *Brain Res.* 1986; 362:175–179. [PubMed: 3002550]
- Okada T, Shimizu S, Wakamori M, Maeda A, Kurosaki T, Takada N, Imoto K, Mori Y. Molecular cloning and functional characterization of a novel receptor-activated TRP Ca²⁺ channel from mouse brain. *J Biol Chem.* 1998; 273:10279–10287. [PubMed: 9553080]
- Philipp S, Hambrecht J, Braslavski L, Schroth G, Freichel M, Murakami M, Cavalie A, Flockerzi V. A novel capacitative calcium entry channel expressed in excitable cells. *EMBO J.* 1998; 17:4274–4282. [PubMed: 9687496]

- Plant TD, Schaefer M. TRPC4 and TRPC5: receptor-operated Ca^{2+} -permeable nonselective cation channels. *Cell Calcium*. 2003; 33:441–450. [PubMed: 12765689]
- Plant TD, Schaefer M. Receptor-operated cation channels formed by TRPC4 and TRPC5. *Naunyn Schmiedeberg Arch Pharmacol*. 2005; 371:266–276. [PubMed: 15902430]
- Schaefer M, Plant TD, Obukhov AG, Hofmann T, Gudermann T, Schultz G. Receptor-mediated regulation of the nonselective cation channels TRPC4 and TRPC5. *J Biol Chem*. 2000; 275:17517–17526. [PubMed: 10837492]
- Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron*. 2001; 29:645–655. [PubMed: 11301024]
- Thorn P, Petersen OH. Activation of nonselective cation channels by physiological cholecystokinin concentrations in mouse pancreatic acinar cells. *J Gen Physiol*. 1992; 100:11–25. [PubMed: 1380978]
- Tsujino N, Yamanaka A, Ichiki K, Muraki Y, Kilduff TS, Yagami K, Takahashi S, Goto K, Sakurai T. Cholecystokinin activates orexin/hypocretin neurons through the cholecystokinin A receptor. *J Neurosci*. 2005; 25:7459–7469. [PubMed: 16093397]
- Van Dijk A, Richards JG, Trzeciak A, Gillessen D, Mohler H. Cholecystokinin receptors: biochemical demonstration and autoradiographical localization in rat brain and pancreas using [^3H] cholecystokinin8 as radioligand. *J Neurosci*. 1984; 4:1021–1033. [PubMed: 6325601]
- Wang B, Sims SM. CCK regulates nonselective cation channels in guinea pig gastric smooth muscle cells. *Am J Physiol*. 1998; 274:G709–717. [PubMed: 9575853]
- Wang S, Chen X, Kurada L, Huang Z, Lei S. Activation of Group II Metabotropic Glutamate Receptors Inhibits Glutamatergic Transmission in the Rat Entorhinal Cortex via Reduction of Glutamate Release Probability. *Cereb Cortex*. 2012; 22:584–594. [PubMed: 21677028]
- Wang S, Zhang AP, Kurada L, Matsui T, Lei S. Cholecystokinin facilitates neuronal excitability in the entorhinal cortex via activation of TRPC-like channels. *J Neurophysiol*. 2011; 106:1515–1524. [PubMed: 21753024]
- Wank SA. Cholecystokinin receptors. *Am J Physiol*. 1995; 269:G628–646. [PubMed: 7491953]
- Wu T, Wang HL. The excitatory effect of cholecystokinin on rat neostriatal neurons: ionic and molecular mechanisms. *Eur J Pharmacol*. 1996a; 307:125–132. [PubMed: 8832213]
- Wu T, Wang HL. G $\alpha_q/11$ mediates cholecystokinin activation of the cationic conductance in rat substantia nigra dopaminergic neurons. *J Neurochem*. 1996b; 66:1060–1066. [PubMed: 8769867]
- Xiao Z, Deng PY, Rojanathammanee L, Yang C, Grisanti L, Permpoonputtana K, Weinshenker D, Doze VA, Porter JE, Lei S. Noradrenergic depression of neuronal excitability in the entorhinal cortex via activation of TREK-2 K^+ channels. *J Biol Chem*. 2009a; 284:10980–10991. [PubMed: 19244246]
- Xiao Z, Deng PY, Yang C, Lei S. Modulation of GABAergic transmission by muscarinic receptors in the entorhinal cortex of juvenile rats. *J Neurophysiol*. 2009b; 102:659–669. [PubMed: 19494196]
- Xiao Z, Jaiswal MK, Deng PY, Matsui T, Shin HS, Porter JE, Lei S. Requirement of phospholipase C and protein kinase C in cholecystokinin-mediated facilitation of NMDA channel function and anxiety-like behavior. *Hippocampus*.
- Zhao H, Kinch DC, Simasko SM. Pharmacological investigations of the cellular transduction pathways used by cholecystokinin to activate nodose neurons. *Auton Neurosci*. 2011; 164:20–26. [PubMed: 21664195]
- Zhu MH, Chae M, Kim HJ, Lee YM, Kim MJ, Jin NG, Yang DK, So I, Kim KW. Desensitization of canonical transient receptor potential channel 5 by protein kinase C. *Am J Physiol Cell Physiol*. 2005; 289:C591–600. [PubMed: 15843439]

Fig. 1.

Bath application of CCK induces an inward current in HEK293 cells cotransfected with CCK2 receptors and TRPC5 channels (**A₁-A₄**) whereas application of CCK had no effects in HEK293 cells transfected with TRPC5 channels alone (**B₁-B₄**). **A₁**, Current trace from a cell transfected with CCK2 receptors and TRPC5 channels held at -60 mV in response to a ramp protocol from -100 mV to +60 mV at a rate of 1 mV/ms every 7 s. The response to the ramp protocol was truncated (same for the rest figures) to show the effects of CCK on the inward current. The black bar indicates the period of application of CCK (0.3 μM). Note that CCK induced an inward current. **A₂**, Voltage-current relationship in response to the ramp protocol just before (**a**) and during (**b**) the application of CCK at its maximal effect. **A₃**, CCK-induced net current obtained by subtracting the current trace before (**a**) from that during (**b**) the application of CCK at the maximal effect. **A₄**, Pooled net current density from 7 cells by subtracting the average of the current density in control. **B₁-B₄**, Results from cells transfected with TRPC5 alone. The figures were arranged in the same fashion as **A₁-A₄**.

Fig. 2. CCK-induced inward current is dependent on G proteins. **A**, Current response recorded from a cell transfected with CCK2 receptors and TRPC5 channels in the intracellular solution containing GDP-β-S (2 mM). **B**, Voltage-current relationship before (**a**) and during (**b**) the application of CCK. **C**, CCK-induced net current obtained by subtracting the current just before the application of CCK (**a**) from that during the application of CCK (**b**). **D**, Summarized data from 6 cells.

Fig. 3.

CCK-induced inward current requires PLC activity. **A₁-A₄**, Application of the PLC inhibitor, U73122 (5 μ M), blocked CCK-induced inward current in HEK293 cells cotransfected with CCK2 receptors and TRPC5 channels. **A₁**, Pretreatment of cells with and continuous bath application of U73122 (5 μ M) blocked CCK-induced inward current. **A₂**, Voltage-current relationship before (**a**) and during (**b**) the application of CCK in the presence of U73122. **A₃**, Net current after subtraction of the voltage-current curve before the application of CCK (**a**) from that during the application of CCK (**b**) in the presence of U73122. **A₄**, Summarized current density obtained by subtracting the current density averaged in 2 min before the application of CCK in the presence of U73122. **B₁-B₄**, Application of the inactive analog, U73343 (5 μ M), failed to block CCK-induced inward current in HEK293 cells cotransfected with CCK2 receptors and TRPC5 channels.

Fig. 4. Effects of Ca^{2+} on CCK-induced inward current. **A₁-A₄**, Bath application of CCK ($0.3 \mu\text{M}$) failed to induce an inward current in the extracellular solution containing 0 Ca^{2+} . **B₁-B₄**, Intracellular application of BAPTA via the recording pipettes blocked CCK-induced inward current. **C₁-C₄**, Pretreatment of cells with and continuous bath application of thapsigargin ($10 \mu\text{M}$) did not change CCK-induced inward current.

Fig. 5.

CCK-induced inward current is independent of IP₃ receptors. **A₁-A₄**, Intracellular application of heparin (5 mg/ml) via the recording pipettes did not block CCK-induced inward current (n=6). **B₁-B₄**, Intracellular perfusion of 2-APB (100 μM) via the recording pipettes did not block CCK-induced inward current (n=5). **C₁-C₄**, Intracellular dialysis of IP₃ (100 μM) did not alter CCK-induced inward current (n=8).

Fig. 6.

CCK-induced inward current is not dependent on the activity of PKC. **A**, Pretreatment of HEK293 cells with and continuous bath application of GF109203X (1 μ M) did not block CCK-induced inward current. **B**, Voltage-current relationship in response to the ramp protocol before (**a**) and during (**b**) the application of CCK. **C**, CCK-induced net current obtained by subtracting the current trace before (**a**) from that during (**b**) the application of CCK. **D**, Pooled net current density from 11 cells by subtracting the average of the current density in control for 2 min.

Fig. 7.

CCK increases the open probability of TRPC5 single channels. **A₁**, Single channel currents recorded at different holding potentials in cell-attached patches. The pipette solution contained 0.3 μM CCK. **A₂**, Plot of the amplitudes of single channel currents versus the pipette holding potentials ($n=5$ patches). Dotted line denotes the linear fitting. **B₁**, Single channel currents recorded before and during bath application of CCK (0.3 μM) in cell-attached patches. **B₂**, Time course of the open probability averaged from 6 patches. Bin size equals 5 s.