FEBS Letters 582 (2008) 229–232

TRPA1 channel activation induces cholecystokinin release via extracellular calcium

A.K. Purhonen[a], L.M. Louhivuori[b,c], K. Kiehne[d], K.E.O. Åkerman[b,c], K.H. Herzig[a,e,f,*]

[a] Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland
[b] Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland
[c] Institute of Biomedicine, University of Helsinki, Helsinki, Finland
[d] I. Department of Internal Medicine, Universitätsklinikum Schleswig Holstein (UKSH), Kiel, Germany
[e] Department of Internal Medicine, Kuopio University Hospital, Kuopio, Finland
[f] Department of Physiology, University of Oulu, P.O. Box 5000, FIN-90014, Oulu, Finland

Received 18 October 2007; revised 30 November 2007; accepted 3 December 2007

Available online 17 December 2007

Edited by Maurice Montal

Abstract TRPA1 channels are non-selective cation channels activated by plant derived pungent products including allyl isothiocyanate (AITC) from mustard. Therefore, possible intestinal secretory functions of these channels were investigated. We detected TRPA1 mRNA in mouse and human duodenal mucosa and in intestinal mouse neuroendocrine STC-1 cells. Stimulation of STC-1 cells with AITC increased intracellular calcium ($[Ca^{2+}]_i$) and significantly stimulated cholecystokinin secretion by 6.7-fold. AITC induced cholecystokinin release was completely blocked by TRPA1 antagonist ruthenium red and depletion of extracellular calcium and reduced by 36% by nimodipine and nifedipine. This suggests that spices in our daily food might stimulate digestive functions.

Keywords: TRP channel; TRPA1; Cholecystokinin release; Calcium

1. Introduction

Transient receptor potential ankyrin 1 (TRPA1) channels, members of transient receptor potential (TRP) superfamily, are calcium permeable non-selective cation channels originally reported to sense noxious cold temperatures [1]. Since their discovery TRPA1 channels have also been implicated in a number of sensory functions including the mediation of nociceptive and inflammatory signals in response to pungent ingredients [2–5], involvement in the development of cold hyperalgesia following inflammation and nerve injury [6], as well as a role in mechanosensation [7,8].

Several plant derived structurally diverse compounds can activate TRPA1 channels, including isothiocyanates (allyl isothiocyanate, AITC) from mustard, α,β-unsaturated aldehydes (cinnamaldehyde) from cinnamon, thiosulfinates (allicin) from garlic and cannabinoids (Δ^9-tetrahydrocannabinol) [2,3]. As many of the activators are pungent by their nature and TRPA1 channels are known to be expressed in sensory nociceptive neurons, these channels have been implicated as sensors of pain induced by spicy foods.

TRPA1 channels are expressed in subsets of nociceptive neurons of dorsal root, trigeminal and nodose ganglia, in other sensory neurons and in the stereocilia of hair cells of the inner ear and mechanosensory neurons in mice [9]. In addition, TRPA1 are present in sensory terminals of nociceptive fibers in their target organs like trigone of the bladder and the cornea [9,10]. Interestingly, TRPA1 channels are expressed on the protein level in several non-neuronal human tissues including gastrointestinal mucosa of small intestine and colon [11]. However, the physiological role of TRPA1 in these tissues is unknown. In this study, we show that TRPA1 channel is expressed in the human and mouse duodenum. In order to investigate the possible function we used mouse intestinal neuroendocrine cell line STC-1 as a well-established model for intestinal hormone release (CCK, GLP-1, GIP) [12–15] and show that activation of TRPA1 channels with mustard oil derived AITC elevates the intracellular Ca^{2+} levels and stimulates cholecystokinin release.

2. Materials and methods

2.1. Materials

Nifedipine, nimodipine, ruthenium red and AITC were from Sigma (Sigma–Aldrich, St. Louis, MO, USA).

2.2. Cell culture

STC-1 cells are derived from an intestinal endocrine tumor in a double-transgenic mouse [16]. Cells (passages 32–50) were cultured as described previously [17].

2.3. Animals

CD2 male mice were sacrificed by cervical dislocation, proximal 5 cm of duodenum dissected and mucosa scraped. The sample was snap frozen in liquid nitrogen and stored −70 °C until mRNA extraction.

2.4. Human duodenal mucosa samples

Duodenal mucosa was obtained from intestinal endoscopy biopsies from patients without intestinal disease. Samples were snap frozen in liquid nitrogen. The study was approved by the Ethics Committees

*Corresponding author. Address: Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. Fax: +358 17 163030.
E-mail addresses: herzig@uku.fi, karl-heinz.herzig@oulu.fi (K.H. Herzig).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.12.005

of the University of Kiel, Germany. Total RNA was extracted using RNeasy Kit (Qiagen, Hilden, Germany). Genomic DNA was digested by deoxyribonuclease I (Qiagen). Reverse transcription of RNA was performed using the You-prime First-strand cDNA synthesis kit (Amersham Biosciences).

2.5. RT-PCR analysis of TRPA1 mRNA

mRNA from mouse duodenal mucosa and from STC-1 cells was isolated and DNAase treated as described above. First-strand cDNA was synthesized from 1 μg of mRNA by using TaqMan Reverse Transcriptase reagents (Applied Biosystems, Warrington, United Kingdom). PCR cocktail consisted of 2.5 μl 10× PCR-buffer, 1.5 mM $MgCl_2$, 250 μM dNTPs (each), 2 U Dynazyme II polymerase (Finnzymes, Finland), 0.5 μM sense and antisense primers and 5 μl of the above described first-strand cDNA.

TRPA1 mRNA was amplified with the following primers: 5′-agtggcaatgtggagcaa-3′ and 5′-tctgatccactttgcgta-3′. PCR profile (35 cycles): 5 min at 95 °C, 30 s at 48 °C, 30 s at 72 °C, 30 s at 94 °C, final extension 5 min at 72°C. PCR products were separated on 2% agarose gel, purified (Qiagen) and sequenced.

2.6. Quantitative fluorescence imaging

Coverslips with STC-1 cells were loaded with 4 μM fura-2-acetoxymethyl ester (fura-2-AM) in a buffer (in mM: 137NaCl, 5KCl, 1$MgCl_2$, 1$CaCl_2$, 10 glucose and 20 HEPES, pH 7.4) for 60 min in room temperature. Cells were rinsed with fura-2-AM free medium, placed on the bottom of an RC24-fast exchange chamber (Warner Instruments Inc.) and positioned on top of the microscope. For fura-2 excitation, cells were illuminated with two alternating wavelengths 340 and 380 nm through a dichroic mirror (DM430, Nikon) using a Polychrome IV monochromator (TILL Photonics GmbH, Gräfelfing, Germany). The emission was guided through a 510 nm cut-off filter then captured by a cooled 12-bit IMAGO CCD camera and digitized by a computer running the TILLvisION Multi-Color Ratio Imaging System (TILL Photonics GmbH, Gräfelfing, Germany). Ratio images were collected and saved for later analysis. Fluorescence from 340 and 380 nm exposures were imported into Microcal Software (Northampton, MA), and given as absolute calcium levels $[Ca^{2+}]_i$ or as changes in $[Ca^{2+}]_i$ levels ($\Delta[Ca^{2+}]_i$).

2.7. CCK secretion

STC-1 cells were plated on 6-well plates and cultured for 4–5 days. Cells were washed once with oxygenated HR-buffer (in mM: 130NaCl, 5KCl, 1.2$CaCl_2$, 1NaH_2PO_4, 1.2$MgSO_4$, 10 HEPES, 6.7 glucose, 0.4% bovine serum albumin, pH 7.4) and incubated for 20 min with buffer containing stimulants (1 ml/well). Supernatants were collected, spinned to remove cell debris and stored at −20 °C until analyzed with CCK-RIA (Euro-Diagnostica, Malmö, Sweden). Blockers (nifedipine 10 μM, nimodipine 5 μM and ruthenium red 10 μM) were preincubated for 30 min before stimulation of the cells. Nifedipine was diluted in DMSO and the final concentration of 0.1% DMSO was also applied to the control cells. For chelating extracellular calcium, 2 mM EGTA was added to the buffer, cells were washed once and stimulated as described above [18].

2.8. Statistical analysis

Data are presented as means ± S.E.M. unless otherwise stated. Statistical comparisons between groups were performed using One Way ANOVA with Tukey's Multiple Comparison Test (GraphPad Software Inc., San Diego, USA).

3. Results

3.1. TRPA1 channels are expressed in mouse and human duodenal mucosa and in STC-1 cells

TRPA1 channels have originally been described in sensory neurons. We detected TRPA1 mRNA in mouse duodenal mucosa, STC-1 cells (Fig. 1A) and in human duodenum (Fig. 1B). PCR product (509 bp) was confirmed by sequencing.

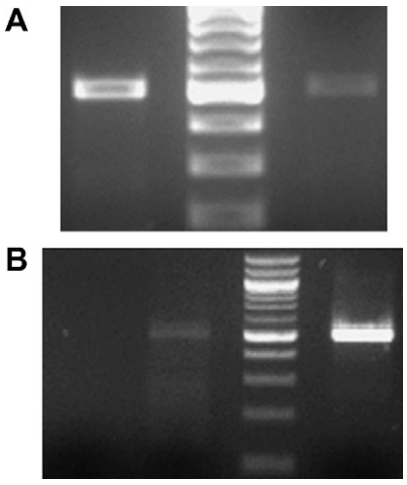

Fig. 1. (A) Expression of TRPA1 mRNA in STC-1 cells and in mouse duodenal mucosa. PCR product was confirmed with sequencing. On the left PCR product of STC-1 cells; marker; on the right PCR product from mouse duodenal mucosa. (B) Expression of TRPA1 mRNA in human duodenum. Lanes from the left: negative control, human duodenum; molecular weight marker; STC-1 cells.

3.2. AITC increases intracellular calcium in STC-1 cells

As TRPA1 are known to be calcium permeable non-selective cation channels we monitored the effect of AITC on the intracellular free calcium concentration ($[Ca^{2+}]_i$) in STC-1 cells using fura-2-AM. AITC (100 μM) caused an increase in $[Ca^{2+}]_i$ indicating the presence of functional TRPA1 channels in STC-1 cells (Fig. 2A). The rise in $[Ca^{2+}]_i$ was significantly

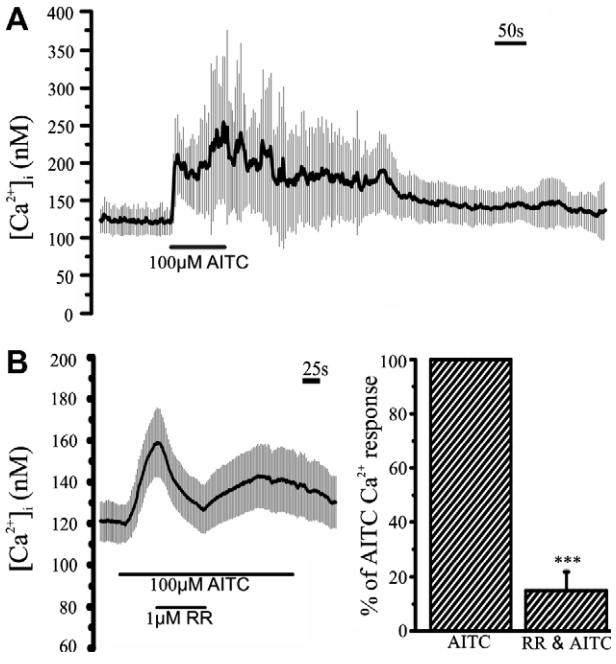

Fig. 2. (A) TRPA1 agonist AITC evoked a rise in $[Ca^{2+}]_i$ in STC-1 cells (153 ± 14 nM, $n = 75$). Trace represents the averaged ± S.D. of six cells. (B) In the *left panel*, averaged ± S.D. calcium traces of 18 cells showing ruthenium red's effect on TRPA1 evoked $[Ca^{2+}]_i$ response. Where indicated the cells were challenged with 100 μM AITC and 1 μM ruthenium red (RR). In the *right panel*, the average S.E.M. of three experiments measured under similar conditions as in left panel.

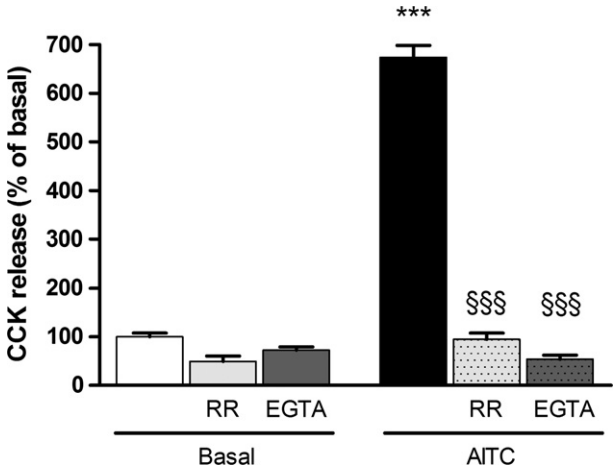

Fig. 3. AITC (100 μM) stimulates CCK release from STC-1 cells and the stimulation is blocked by 10 μM ruthenium red (RR) or by EGTA. $^{***}P < 0.001$ basal vs. AITC; $^{§§§}P < 0.001$ AITC vs. AITC + RR or AITC + EGTA. Values are expressed as percentage of basal secretion. Each experiment was repeated at least twice, $n = 8–16$ per treatment.

blocked by 1 μM TRPA1 antagonist ruthenium red (RR) (Fig. 2B).

3.3. AITC stimulates CCK release from STC-1 cells

STC-1 cells were stimulated with 100 μM AITC for 20 min and CCK release measured with CCK-RIA. As shown in Fig. 3, AITC significantly stimulated CCK secretion 6.7-fold compared to basal levels. This stimulation was completely blocked by the TRPA1 channel blocker ruthenium red (10 μM). To evaluate the contribution of extracellular calcium in AITC stimulated CCK release, we chelated extracellular calcium by 2 mM EGTA. In the presence of EGTA, AITC did not stimulate CCK release indicating that AITC induced CCK release is dependent on the influx of extracellular calcium (Fig. 3).

3.4. Blockade of L-type voltage-gated calcium channels

Previously, activation of L-type calcium channels by depolarization of the membrane potential has been shown to be involved in CCK release from STC-1 cells [19]. STC-1 cells were stimulated with 100 μM AITC in the presence of L-type voltage-gated calcium channel blocker 10 μM nifedipine and 5 μM nimodipine. AITC stimulated CCK release was reduced by 36% in the presence of the nifedipine or nimodipine ($P < 0,001$) (Fig. 4) suggesting that the primary calcium influx and CCK release in response to AITC is mediated via TRPA1 channels.

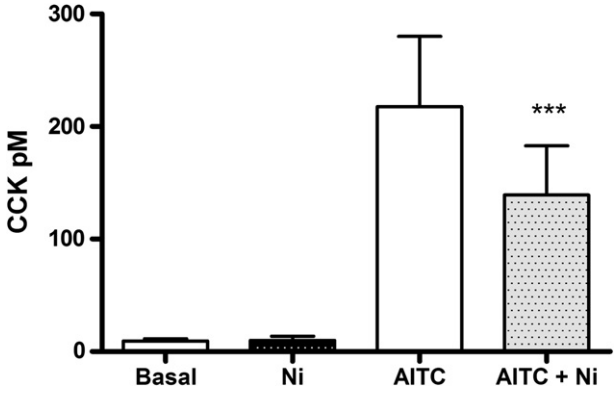

Fig. 4. Blocking L-type VGCCs with nifedipine (10 μM) or with nimodipine (5 μM) reduced the AITC-induced CCK secretion by 36%. Data from experiments with nifedipine and nimodipine are combined; $n = 12$ per treatment ($^{***}P < 0.001$ AITC vs. AITC + Ni).

4. Discussion

We demonstrate in the present study that TRPA1 channels are expressed in the human and mouse duodenal mucosa and in the mouse intestinal neuroendocrine STC-1 cell line indicating that these channels might accomplish so far unknown responses in our body. Just recently, Stokes et al. found TRPA1 protein by immunostaining in human intestinal mucosa [11] yet no physiological function of TRPA1 in small intestine has been described to date.

AITC present in mustard oils as well as in wasabi (a japanese horseradish), is a membrane permeable electrophilic compound which activates TRPA1 [20,21]. Stimulation of STC-1 cells with AITC lead to a robust increase in intracellular calcium levels and stimulation of CCK release. CCK secretion from STC-1 cells and from native intestinal endocrine I-cells is tightly coupled to increased intracellular calcium levels since food derived secretagogues and luminal CCK releasing peptides stimulate CCK secretion via influx of calcium. AITC stimulated CCK release was dependent on extracellular calcium since the response was completely blocked with EGTA and ruthenium red [3]. Similarly, also AITC induced contraction of rat urinary bladder is dependent on extracellular calcium since the response was totally abolished in the absence of calcium and presence of EGTA [10].

In STC-1 cells typical stimuli causing depolarization or increase in cAMP levels activate voltage-gated calcium channels leading to calcium influx and CCK secretion [22]. The L-type voltage-gated calcium channel blockers nimodipine and nifedipine have been previously shown to totally block the L-type voltage-gated channels activated by various stimuli in STC-1 cells in the same or even lower concentrations that used in this study [18,19,23,24]. Blocking the L-type voltage-gated calcium channels only partially (by 36%) reduced the AITC induced CCK release suggesting that voltage-gated calcium channels contribute to some extent to the calcium influx in response to AITC stimulation, yet the major proportion of the increased calcium levels is due to calcium influx via TRPA1.

CCK is released from enteroendocrine I-cells in the mucosa of duodenum and proximal jejunum in the intestinal phase of digestion in response to fatty acids and proteins in the intestinal lumen [25] and other duodenal peptides [26]. CCK activates the CCK1 receptors in the afferent fibers of the vagus nerve which in addition to the elevated plasma CCK levels mediates the digestive effects of CCK by stimulating exocrine pancreatic secretion and gallbladder contraction. CCK prolongs gastric emptying time and has an inhibitory effect on food intake. Our findings showing the mucosal expression of TRPA1 channels and CCK release upon activation with mustard oil derived AITC tempt to speculate that spices in our daily food could stimulate digestive actions. Recently, functional TRPA1 expression was demonstrated in native mouse

jejunum, ileum and colon and AITC stimulated contraction of isolated proximal and distal colon and to a lesser extent also jejunum and ileum [27]. Consumption of spicy food has been shown to increase thermogenesis via activation of sympathetic nervous system and some indications of increased satiety in humans are also reported [28].

In conclusion, we show that TRPA1 channels are expressed in human and mouse duodenal mucosa and that activation of TRPA1 stimulates CCK release in intestinal neuroendocrine STC-1 cells. Our study suggests that ingestion of TRPA1 activators as part of our daily meal (e.g. in the form of wasabi or garlic) may stimulate CCK secretion from the intestine and thus improve the digestive functions.

Acknowledgements: We thank Riitta Kauppinen for excellent technical assistance. The work was supported in part by grants from the Academy of Finland (to K.H.H. and to K.Å.) and from Sigrid Juselius Foundation (to K.Å.) and Northern Savo Cultural Foundation (to A.K.P.).

References

[1] Story, G.M., Peier, A.M., Reeve, A.J., Eid, S.R., Mosbacher, J., Hricik, T.R., Earley, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W., McIntyre, P., Jegla, T., Bevan, S. and Patapoutian, A. (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112, 819–829.

[2] Bandell, M., Story, G.M., Hwang, S.W., Viswanath, V., Eid, S.R., Petrus, M.J., Earley, T.J. and Patapoutian, A. (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41, 849–857.

[3] Jordt, S.E., Bautista, D.M., Chuang, H.H., McKemy, D.D., Zygmunt, P.M., Hogestatt, E.D., Meng, I.D. and Julius, D. (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427, 260–265.

[4] Bautista, D.M., Movahed, P., Hinman, A., Axelsson, H.E., Sterner, O., Hogestatt, E.D., Julius, D., Jordt, S.E. and Zygmunt, P.M. (2005) Pungent products from garlic activate the sensory ion channel TRPA1. Proc. Natl. Acad. Sci. USA 102, 12248–12252.

[5] Bautista, D.M., Jordt, S.E., Nikai, T., Tsuruda, P.R., Read, A.J., Poblete, J., Yamoah, E.N., Basbaum, A.I. and Julius, D. (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124, 1269–1282.

[6] Obata, K., Katsura, H., Mizushima, T., Yamanaka, H., Kobayashi, K., Dai, Y., Fukuoka, T., Tokunaga, A., Tominaga, M. and Noguchi, K. (2005) TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. J. Clin. Invest. 115, 2393–2401.

[7] Corey, D.P., Garcia-Anoveros, J., Holt, J.R., Kwan, K.Y., Lin, S.Y., Vollrath, M.A., Amalfitano, A., Cheung, E.L., Derfler, B.H., Duggan, A., Geleoc, G.S., Gray, P.A., Hoffman, M.P., Rehm, H.L., Tamasauskas, D. and Zhang, D.S. (2004) TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432, 723–730.

[8] Kindt, K.S., Viswanath, V., Macpherson, L., Quast, K., Hu, H., Patapoutian, A. and Schafer, W.R. (2007) *Caenorhabditis elegans* TRPA-1 functions in mechanosensation. Nat. Neurosci. 10, 568–577.

[9] Nagata, K., Duggan, A., Kumar, G. and Garcia-Anoveros, J. (2005) Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J. Neurosci. 25, 4052–4061.

[10] Andrade, E.L., Ferreira, J., Andre, E. and Calixto, J.B. (2006) Contractile mechanisms coupled to TRPA1 receptor activation in rat urinary bladder. Biochem. Pharmacol. 72, 104–114.

[11] Stokes, A., Wakano, C., Koblan-Huberson, M., Adra, C.N., Fleig, A. and Turner, H. (2006) TRPA1 is a substrate for de-ubiquitination by the tumor suppressor CYLD. Cell. Signal. 18, 1584–1594.

[12] Abello, J., Ye, F., Bosshard, A., Bernard, C., Cuber, J.C. and Chayvialle, J.A. (1994) Stimulation of glucagon-like peptide-1 secretion by muscarinic agonist in a murine intestinal endocrine cell line. Endocrinology 134, 2011–2017.

[13] Kieffer, T.J., Huang, Z., McIntosh, C.H., Buchan, A.M., Brown, J.C. and Pederson, R.A. (1995) Gastric inhibitory polypeptide release from a tumor-derived cell line. Am. J. Physiol. 269, E316–E322.

[14] Glassmeier, G., Herzig, K.H., Hopfner, M., Lemmer, K., Jansen, A. and Scherubl, H. (1998) Expression of functional GABAA receptors in cholecystokinin-secreting gut neuroendocrine murine STC-1 cells. J. Physiol. 510 (Pt 3), 805–814.

[15] Jansen, A., Hoepfner, M., Herzig, K.H., Riecken, E.O. and Scherubl, H. (2000) GABA(C) receptors in neuroendocrine gut cells: a new GABA-binding site in the gut. Pflugers Arch. 441, 294–300.

[16] Rindi, G., Grant, S.G., Yiangou, Y., Ghatei, M.A., Bloom, S.R., Bautch, V.L., Solcia, E. and Polak, J.M. (1990) Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression. Am. J. Pathol. 136, 1349–1363.

[17] Larsson, K.P., Akerman, K.E., Magga, J., Uotila, S., Kukkonen, J.P., Nasman, J. and Herzig, K.H. (2003) The STC-1 cells express functional orexin-A receptors coupled to CCK release. Biochem. Biophys. Res. Commun. 309, 209–216.

[18] Chen, M.C., Wu, S.V., Reeve Jr., J.R. and Rozengurt, E. (2006) Bitter stimuli induce Ca^{2+} signaling and CCK release in enteroendocrine STC-1 cells: role of L-type voltage-sensitive Ca^{2+} channels. Am. J. Physiol. Cell. Physiol. 291, C726–C739.

[19] Mangel, A.W., Scott, L. and Liddle, R.A. (1996) Depolarization-stimulated cholecystokinin secretion is mediated by L-type calcium channels in STC-1 cells. Am. J. Physiol. 270, G287–G290.

[20] Hinman, A., Chuang, H.H., Bautista, D.M. and Julius, D. (2006) TRP channel activation by reversible covalent modification. Proc. Natl. Acad. Sci. USA 103, 19564–19568.

[21] Macpherson, L.J., Dubin, A.E., Evans, M.J., Marr, F., Schultz, P.G., Cravatt, B.F. and Patapoutian, A. (2007) Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature 445, 541–545.

[22] Basavappa, S., Liddle, R.A. and Mangel, A.W. (1994) Characterization of ATP-sensitive potassium channels in intestinal, cholecystokinin-secreting cells. Biochem. Biophys. Res. Commun. 204, 855–860.

[23] Yoshida, H., Tsunoda, Y. and Owyang, C. (1999) Diazepam-binding inhibitor33–50 elicits Ca^{2+} oscillation and CCK secretion in STC-1 cells via L-type Ca^{2+} channels. Am. J. Physiol. 276, G694–G702.

[24] Wang, Y., Prpic, V., Green, G.M., Reeve Jr., J.R. and Liddle, R.A. (2002) Luminal CCK-releasing factor stimulates CCK release from human intestinal endocrine and STC-1 cells. Am. J. Physiol. Gastrointest. Liver Physiol. 282, G16–G22.

[25] Herzig, K.H., Bardocz, S., Grant, G., Nustede, R., Folsch, U.R. and Pusztai, A. (1997) Red kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. Gut 41, 333–338.

[26] Herzig, K.H. (1998) Cholecystokinin- and secretin-releasing peptides in the intestine—a new regulatory interendocrine mechanism in the gastrointestinal tract. Regul. Peptides 73, 89–94.

[27] Penuelas, A., Tashima, K., Tsuchiya, S., Matsumoto, K., Nakamura, T., Horie, S. and Yano, S. (2007) Contractile effect of TRPA1 receptor agonists in the isolated mouse intestine. Eur. J. Pharmacol. 576, 143–150.

[28] Westerterp-Plantenga, M.S., Smeets, A. and Lejeune, M.P. (2005) Sensory and gastrointestinal satiety effects of capsaicin on food intake. Int. J. Obes. (Lond.) 29, 682–688.