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Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet β cells modulates insulin secretion in rats

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Abstract

Capsaicin-sensitive afferent neurons including transient receptor potential vanilloid subfamily 1, TRPV1, and neurohormonal peptides participate in the physiological regulation of pancreatic endocrine. However, the direct effect of capsaicin on insulin secretion remains unknown. Our present study showed that TRPV1 is expressed in islet β cells as well as in neurons in rat pancreas, and also in rat β cell lines, RIN and INS1. Capsaicin (10^{-11}–10^{-9} M) dose-dependently increased insulin secretion from RIN cells, and this effect was inhibited by either a TRPV1 inhibitor capsazepine or EDTA. Systemic capsaicin (10 mg/kg, s.c.) increased plasma insulin level 1 h after the treatment. We demonstrated for the first time that TRPV1 is functionally expressed in rat islet β cells and plays a role in insulin secretion as a calcium channel. This study may account for the influences of capsaicin on the food intake and energy consumption as well as on the pathophysiological regulation of pancreatic endocrine.

Keywords: Capsaicin; Vanilloid receptor; Rat islet β cell; Insulin secretion; Capsazepine

Capsaicin, the main ingredient of hot chili peppers, which is used as an important spice for enhancing the palatability of food, selectively activates the peripheral terminal of sensory C and Aδ fibers, generally referred to as polymodal nociceptors, and evokes a sensation of tingling and burning pain [1,2]. Transient receptor potential vanilloid subfamily 1 (TRPV1), known as vanilloid receptor 1 (VR1), a receptor responsible for capsaicin action, has been cloned by Caterina et al. [3] from rat dorsal root ganglia and recognized as a common molecular target for protons and noxious heat (>43 °C) as well as vanilloid compounds [3,4]. Several vanilloid receptor homologues have been also cloned and demonstrated the presence in not only neural but also non-neural cells [5].

Capsaicin-sensitive afferent neurons including neurohormonal peptides such as calcitonin gene-related peptide (CGRP), substance P (SP), and neurokinin A (NK-A) participate in the physiological regulation of pancreatic endocrine and exocrine secretions. However, since capsaicin effects on islet β cells and on sensory nerves are confused, the effect of capsaicin on insulin secretion from pancreatic islets is still controversy. Systemic capsaicin enhances plasma insulin level after glucose challenge in dog [6]. Neonatal de-afferentiation by

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capsaicin treatment increases glucose tolerance without the changes of the secretory response of insulin in mice and in the isolated islets, suggesting that capsaicin-sensitive afferents exert inhibitory influences on insulin secretion [7]. CGRP has a stimulatory effect at 10^{-10} and 10^{-9} M, but an inhibitory effect at 10^{-8} M, on insulin secretion in the isolated perfused porcine pancreas, the latter effect through the enhanced somatostatin release [8]. In vitro experiments on rat isolated pancreatic islets exposed to capsaicin (0.25 and 2.5 μM) for 4 days showed no effect on β-cell function including the basal and glucose-stimulated insulin release [9]. CGRP has been shown to inhibit insulin release from individual rat β cells in culture [10] and to inhibit carbachol-induced insulin release in mice [11]. Capsaicin infusion stimulated SP and NK-A, both increased release of insulin, glucagon, and exocrine secretion from the isolated perfused porcine pancreas via NK-1 receptor [12]. However, the direct effect of capsaicin on insulin secretion remains unknown. Furthermore, the presence and localization of TRPV1 in the pancreas has not been studied and the involvement of TRPV1 in pancreatic physiological regulation is also limited [13], although the roles of capsaicin-sensitive afferent nerves are well studied. We have found that TRPV1 is present in islet endocrine cells as well as in neurons in rat pancreas [14]. Taken together, these findings led us to speculate that the effect of capsaicin on insulin secretion would be due to the activation of TRPV1 not only in capsaicin-sensitive afferent neurons but also in islet β cells, since endocrine cells share various neuronal transmitter, peptides, and receptors with neurons. Several papers have shown the presence of TRPV1 in other peripheral cells, such as a human bronchial epithelial cell line [15], skin epidermal keratinocytes [16,17], and urinary bladder epithelial cells [18] and additionally rat gastric epithelial cell line (RGM-1) [19].

The present study was undertaken to investigate the expression of TRPV1 in rat pancreas and rat β cell lines and examine the effect of capsaicin on insulin secretion in vitro and in vivo. We demonstrated that TRPV1 is functionally expressed and modulates insulin secretion in rat islet β cells.

Materials and methods

Chemicals and animals. Capsaicin, capsazepine, EDTA, RPMI-1640, and all other chemicals were obtained from Sigma Chemical (St. Louis, MO, USA). Capsaicin and capsazepine were dissolved with dimethyl sulfoxide (DMSO) for stock solution and diluted with saline. 0.1% DMSO in saline was used as vehicle.

All studies were approved by the Keio University Animal Research Committee and all animals were handled according to the institutional guidelines (Keio University, Kyoto Pharmaceutical University, and Tokyo Medical and Dental University). Male Sprague–Dawley rats weighing 200–250 g (Saitama Experimental Animals Supply, Saitama, Japan) were used in the present study and some rats were fasted overnight, but had free access to water.

Cells and cell culture. Rat insulinoma cell lines RINm5F [20] and INS1 [21] cells were provided from Dr. Kuroda (Department of Pathology, Tokyo Medical University, Tokyo, Japan) and from Dr. Fujita (Institute of Advanced Medical Research, Keio University, Tokyo, Japan), respectively. RIN and INS1 were cultured in RPMI-1640 medium containing 2 mg/ml (11.1 mM) glucose and 2.5 mM L-glutamine, supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 IU/ml streptomycin.

Immunofluorescence. Three rats were euthanized by the terminal exsanguinations under sodium pentobarbital anesthesia (35 mg/kg, i.p.). Pancreata and Th7–Th13 dorsal root ganglia (DRGs) were removed and used for immunofluorescence, Western blot analysis, and reverse transcription-polymerase chain reaction studies as described below.

Small cut samples of pancreata and DRGs were fixed with Zamboni's fixative, subsequently cryoprotected with 10%, 15%, and 20% sucrose in phosphate-buffered saline, pH 7.4 (PBS), mounted in O.C.T. compound (Miles, Elkhart, IN) at −20 °C and cut on a cryostat (Leica Microsystems, Wetzlar, Germany) at 8 μm thickness. Immunofluorescence staining was performed as previously described [22], using primary anti-TRPV1 antibody (Ab); goat polyclonal Ab against the C-terminus (C-Ab, 1:100, sc-8671, Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit polyclonal Ab against the N-terminus (N-Ab, 1:10,000, PA1-747, Affinity BioReagents, Golden, CO), followed by rhodamine-conjugated donkey anti-goat or rabbit IgG Ab (1:1000, Chemicon International, Temecula, CA). The sections were observed under a fluorescence microscope (Nikon, Tokyo, Japan) and the images were captured and recorded using a charge-coupled device color video camera and image software (C5810 Image System, Hamamatsu Photonics, Hamamatsu, Japan).

Western blot analysis. For Western blot analysis, pancreas and DRGs were homogenized in ice-cold 50 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA and 1% vol/vol protease inhibitor cocktail (Sigma). Then, the homogenized samples were centrifuged at 100,000*g* for 1 h at 4 °C. The supernatant was removed and the pellet was resuspended in the homogenized buffer. The protein concentration of the suspension was determined using a BCA protein assay kit (Pierce). The samples (1 μg/lane for DRGs and 10 μg for pancreas) were then electrophoresed on 10% SDS–polyacrylamide gels and transferred electrophoretically to PVDF membrane (Hybond-P, Amersham International, Little Chalfont, UK). The membrane was incubated with anti-TRPV1 C-Ab (1:500) and treated with horseradish peroxidase-conjugated donkey anti-goat IgG (1:1000, Chemicon International, Temecula, CA). The immune complexes were visualized by the reaction with a DAB staining kit.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA from RIN and INS1 cells (1×10^5 cell), rat pancreas, and DRGs was extracted using a total RNA isolation kit (RNeasy Protect Mini Kit, Qiagen, Valencia, CA). Furthermore, islets were isolated from pancreas in the RNA stabilization reagent (RNAlater, Qiagen) by handpicked method under a Zeiss stereomicroscope followed by total RNA extract. Total RNA was reverse transcribed with a first strand cDNA synthesis kit (iScript cDNA Synthesis Kit, Bio-Rad). The PCR primers of rat TRPV1 were sense (5′-TCATGGGTGAGACCG TCAACAAG-3′) and antisense (5′-TGGCTTAAGGGATCCCGTAT AAT-3′), giving rise to a 428 bp PCR product as previously described [19]. The PCR primers of rat GAPDH, generating a 310 bp PCR product (sense: 5′-GAACGGGAAGCTCACTGGCATGGC-3′, antisense: 5′-TGAGGTCCACCACCCTGTTGCTG-3′), were used as an internal control [23]. For TRPV1, an aliquot of the RT reaction product served as a template in 30 cycles with 30 s of denaturation at 95 °C and 1 min of extension at 68 °C using the Advantage 2 polymerase mixture (Clontech, Palo Alto, CA) on a thermal cycler (TaKaRa TP-240, Ohtsu, Shiga, Japan) or in 40 cycles with 30 s of denaturation at 95 °C, 30 s of annealing at 62 °C, and 30 s of extension at 72 °C using a PCR kit (iQ Supermix, Bio-Rad) on a thermal cycler

(iCycler iQ, Bio-Rad). PCR for GAPDH was performed in 25 cycles with annealing at 66 °C. A portion of the PCR mixture was electrophoresed in 1.8% agarose gel in TAE buffer (40 mM Tris, 2 mM EDTA, and 20 mM acetic acid, pH 8.1), and the gel was stained with ethidium bromide and photographed.

Insulin secretion. In in vitro insulin secretion study, RIN cells were used to examine the effect of capsaicin, since RT-PCR showed the abundant expression of TRPV1 in RIN cells rather than INS1 cells. RIN cells were cultured in RPMI1640 medium containing 2 mg/ml glucose at 1×10^5 in a six-well flat-bottomed culture plate (FALCON, Becton–Dickinson Labware, Bedford, MA) for 48 h and used for the experiments after reaching confluence. The culture medium was aspirated, and the cells were treated with 1 ml fresh culture medium containing capsaicin (10^{-11}–10^{-6} M) or vehicle for 1 h, with or without capsazepine (10^{-7} M) or EDTA (10^{-7} M). Then, the medium was collected and stored at −20 °C. The secreted insulin into the medium was measured with Insulin ELISA kit U-type (range 25–1500 pg/ml) or R-type (range 0.5–10 ng/ml) (Shibayagi, Gunma, Japan) according to the manufacturer's protocol. Cell viability or cell damage due to the exposure to capsaicin was eliminated with propidium iodide (1 μM, Molecular Probes, Eugene, OR) staining, which stains the damaged cell nucleus, at the end of experiments as previously described [24].

To examine the in vivo effect of capsaicin on insulin secretion, non-neurotoxic, gastro-protective dose of capsaicin (10 mg/kg) [25] was subcutaneously administered to the overnight-fasted rats. Under sodium pentobarbital anesthesia (35 mg/kg), blood was taken from abdominal aorta 1 h after capsaicin treatment. Plasma insulin level was measured with Insulin ELISA kit R-type as described above.

Statistics. All data are expressed as means ± SEM. Comparisons between two groups in the in vivo insulin secretion study were made by an unpaired *t* test. Comparisons between the groups in cell culture study were made by one-way ANOVA followed by Fischer's least significant difference test. *p* value less than 0.05 was considered as significant.

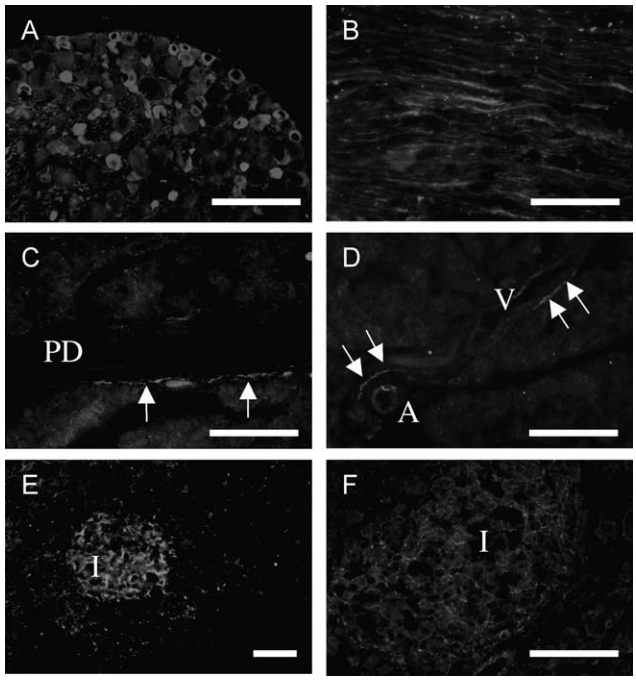

Fig. 1. Immunofluorescence for TRPV1 in rat dorsal root ganglion (DRG) and pancreas. Rat DRG and pancreas were immunostained with anti-TRPV1 C-terminus antibody (A–E) or N-terminus antibody (F). TRPV1 localized in small neuronal cells in DRG (A) and in varicose neuronal fibers in the dorsal root (B). In the pancreas, TRPV1 was recognized in the varicose nerve fibers around the pancreatic duct (C) and the vessels (D). Note the TRPV1 expression in the endocrine cells, located in the core portion of pancreatic islet (E). Immunostaining using N-Ab clearly demonstrated the membrane localization of TRPV1 in the islet endocrine cells (F). Internal bars = 100 μm. I, islet; PD, pancreatic duct; A, arteriole; V, venule.

Results

Expression of TRPV1 in rat pancreas and in rat β cell lines

As a positive control, DRGs were immunostained with anti-TRPV1 C-Ab. TRPV1-like immunoreactivity (TRPV1-LI) was detected in small DRG neurons and varicose neuronal fibers (Figs. 1A and B), consistent with the previous studies [3,4]. In the pancreas, TRPV1 was also expressed in the varicose nerve fibers around the pancreatic duct (Fig. 1C) and the vessels (Fig. 1D), showing the TRPV1-positive afferent innervations in the pancreas. Furthermore, TRPV1-LI was observed in the pancreatic islets, mainly in the endocrine cells localized in the core portion of islets (Fig. 1E), corresponding to insulin secreting β cells. Immunostaining using another Ab, anti-TRPV1 N-Ab, clearly demonstrated the membrane localization of TRPV1-LI in the islet endocrine cells (Fig. 1F).

In Western blot analysis, the expression of TRPV1 protein (~95 kDa) was detected in the rat pancreas as well as in the DRGs (Fig. 2A). Since it was difficult to get large amount of protein from DRGs, 1 μg protein from the DRGs was loaded, whereas 10 μg from the pancreas, resulting in the less expression of TRPV1 in the DRGs than in the pancreas. The distinct expression of TRPV1 mRNA was detected in the whole rat pancreas and the isolated islets as well as in the DRGs by RT-PCR using specific rat TRPV1 primers (Fig. 2B). TRPV1 mRNA level was higher in DRGs and the isolated islets than in the whole pancreas. We also confirmed the expression of TRPV1 mRNA in rat β cell lines. RT-PCR demonstrated the expression of TRPV1 mRNA in RIN and INS1 cells as well as rat DRGs, although the amount expressed was lower in INS1 than in RIN and it was less than in DRGs (Fig. 2C).

Effect of capsaicin on insulin secretion in RIN cells

The effect of capsaicin on insulin secretion was examined in RIN cells, since RT-PCR study showed the higher expression of TRPV1 in RIN cells than in INS1 cells and our preliminary data showed that the lower basal secretion of insulin was observed in INS1 cells (data not shown). Furthermore, the insulin secretion from RIN cells was optimal at 1 h after the treatment. Capsaicin dose-dependently increased the insulin secretion into the medium compared with vehicle control in the range from 10^{-11} to 10^{-9} M, whereas the higher

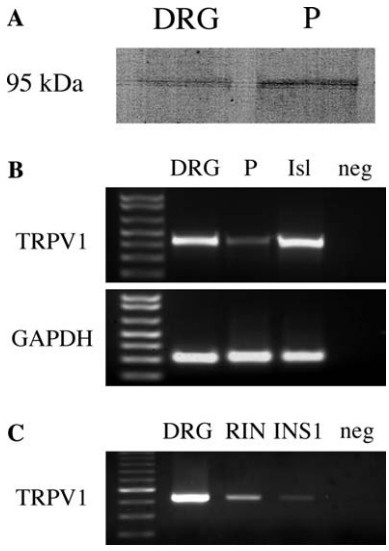

Fig. 2. Western blot and RT-PCR analysis for TRPV1 in rat pancreas and rat β cell lines. (A) Western blot. The homogenated samples of rat dorsal root ganglion (DRG, 1 μg/lane) and pancreas (P, 10 μg/lane) were applied. The expression of TRPV1 protein (around 95 kDa) was detected in pancreas as well as in DRG using anti-TRPV1 C-terminus antibody. (B) RT-PCR for TRPV1 in rat DRG, whole pancreas (P), and isolated islets (Isl). The expression of TRPV1 mRNA was detected in whole pancreas and isolated islets as well as in DRG. Note the higher expression of TRPV1 in the isolated islets than that in whole pancreas. GAPDH expression was confirmed in all RNA samples by RT-PCR. Neg: water only instead of DNA template. (C) RT-PCR for TRPV1 in rat β cell lines, RIN and INS1 cells. Both cell lines expressed TRPV1 mRNA, whereas the expression is higher in RIN than in INS1 cells. Neg: water only instead of DNA template.

concentration of capsaicin (10^{-8}–10^{-6} M) had less effect on insulin secretion (Fig. 3A). We confirmed that the cytotoxic effect of capsaicin (10^{-8}–10^{-6} M) was not observed by propidium iodide staining at the end of the experiments (data not shown), suggesting that less effect of higher concentration of capsaicin on insulin secretion was not due to the cell injury, consistent with the earlier study in which capsaicin (10^{-9}–10^{-6} M) alone has no effect on the cell viability of TRPV1-expressing rat gastric epithelial cell line (RGM-1) [19]. The increased insulin secretion by capsaicin 10^{-9} M was inhibited with capsazepine 10^{-7} M (Fig. 3B), showing that capsaicin effect was specific to the activation of TRPV1 on RIN cells. Furthermore, EDTA 10^{-7} M abolished the capsaicin-induced insulin secretion and further declined the insulin secretion below the baseline, suggesting that the activation of TRPV1 by capsaicin stimulated the Ca^{2+} entry into the cells and that the basal insulin secretion was also regulated with extracellular Ca^{2+}, consistent with the involvement of L-type voltage-dependent Ca^{2+} channel in the insulin secretion [26].

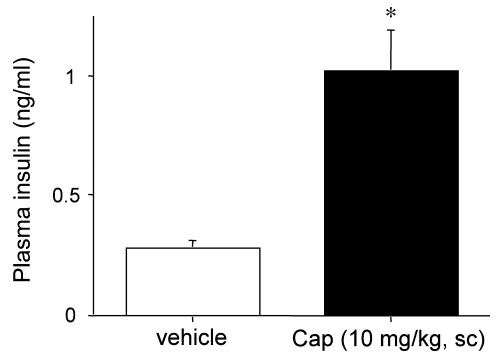

Fig. 4. In vivo effect of capsaicin (Cap) on insulin secretion in the fasted rats. Plasma insulin level was measured 1 h after vehicle or capsaicin (10 mg/kg, s.c.) treatment in the overnight fasted rats. Each datum is expressed as mean ± SEM ($n = 4$). *$p < 0.05$ vs. vehicle.

Effect of capsaicin on insulin secretion in the fasted rats

To test the systemic and acute effect of non-neurotoxic dose of capsaicin on insulin secretion in rats, capsaicin (10 mg/kg, s.c.) was administered to the overnight-fasted rats and plasma insulin level was measured 1 h after the treatment. Compared with vehicle control, capsaicin treatment significantly increased the plasma insulin level (Fig. 4).

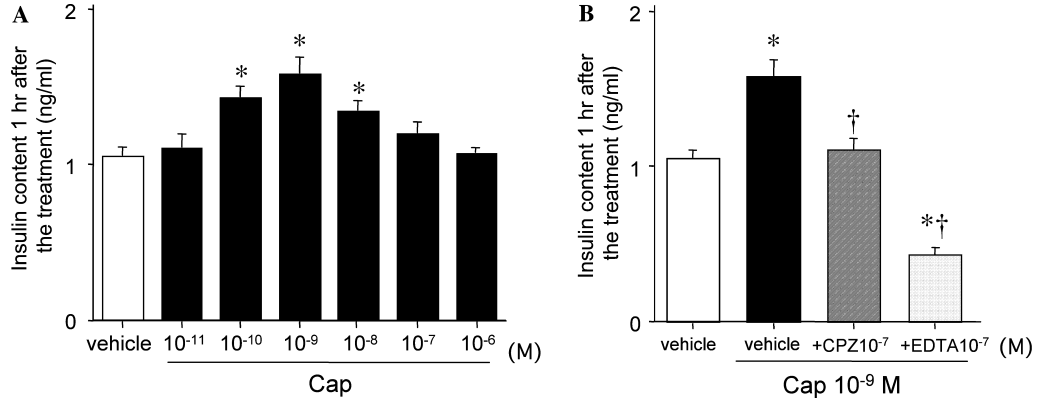

Fig. 3. Effect of capsaicin (Cap) on insulin secretion in RIN cells. (A) Dose–response to capsaicin. The cells (1×10^5) were treated with 1 ml culture medium including either capsaicin (10^{-11}–10^{-6} M) or vehicle for 1 h, and the secreted insulin in the medium was measured. Each datum is expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs. vehicle. (B) The increased insulin secretion by capsaicin 10^{-9} M was inhibited by capsazepine (CPZ 10^{-7} M) and further diminished by EDTA (10^{-7} M). Each datum is expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs. vehicle, †$p < 0.05$ vs. Cap + vehicle.

Discussion

In the present study, we demonstrated the expressions of TRPV1 in islet endocrine cells as well as in neuronal fibers in rat pancreas and also in rat β cell lines, RIN and INS1. We further determined that capsaicin stimulated insulin secretion from RIN cells, and this effect of capsaicin was inhibited by either a TRPV1 antagonist capsazepine or EDTA. These findings strongly suggest that TRPV1 expressed in islet β cells modulates insulin secretion as a Ca^{2+} channel. The presence of TRPV1 has been established in the dorsal root, trigeminal, nodose ganglia [27], and brain [28,29] in addition to the originally reported dorsal root ganglia [3]. Subsequently, several vanilloid receptor variants and homologues have been recognized in not only neural but also various non-neural cells, such as bronchial epithelial cells [15], keratinocytes in epidermis [16,17], and urinary bladder epithelial cells [18], and additionally in gastric epithelial cell lines RGM-1 [19]. These observations suggest that TRPV1 and its homologues are more broadly distributed than previously thought. In the present study, we provided for the first time the direct evidence for the existence of TRPV1 in rat pancreas, especially in islet β cells, and in RIN and INS1 cells, the rat insulinoma cell lines.

TRPV1 acts as a non-selective cation channel with high permeability for divalent cations, especially Ca^{2+} [3], followed by neurotransmitter release such as CGRP from afferent nerves. Insulin release from β cell is also triggered by Ca^{2+} influx through a L-type voltage-dependent channel activated by intracellular ATP and cAMP. The mechanism by which glucose induces insulin secretion from β cells is known as that glucose stimulation increases the ATP concentration, which closes the K_{ATP} channels, depolarizing the cell membrane and opening L-type voltage-dependent Ca^{2+} channel, allowing Ca^{2+} influx, and triggering exocytosis of insulin granules [26]. These findings and our result that capsaicin-induced insulin release was abolished by extracellular EDTA suggest that capsaicin activates TRPV1 and Ca^{2+} influx followed by the insulin release. Another similarity between TRPV1 activation/sensitization and insulin release is that both are modulated with protein kinase C (PKC)-α [30,31] and cAMP [32,33], also suggesting the coupling regulation of insulin secretion with TRPV1 sensitization.

It is unclear why high concentration of capsaicin (10^{-8}–10^{-6} M) had less effect on insulin secretion. Since the cell injury was not induced by capsaicin (10^{-8}–10^{-6} M) in the present study and in the previous study using RGM-1 [19], the cytotoxic effect of capsaicin may not be involved. It is possible that the de-sensitization of TRPV1 was induced by high concentration of capsaicin; however, capsaicin (0.25 or 2.5 μM) treatment for 4 days has no effect on β cell function including insulin release [7]. Another possibility is that higher capsaicin induces the secondary inhibitory effect on insulin secretion, as the inhibitory effect of high concentration of CGRP on insulin secretion via somatostatin release [10].

Capsaicin is the main ingredient of hot chili peppers, used as an important spice for enhancing the palatability of food. In the present study, systemic capsaicin treatment increases plasma insulin level, consistent with previous studies [6,34]. Kawada et al. [34] show capsaicin (6 mg/kg, i.p.) immediately transiently increases oxygen consumption and respiratory quotient followed by the parallel increases of serum glucose, free fatty acid, and immunoreactive insulin (IRI), through, in part, an indirect β-adrenergic action, resulting in the enhancement of energy metabolism in rats. Our result from in vivo capsaicin effect may involve, in part, the direct stimulation of insulin secretion from islet β cells in addition to the indirect neuronal reflex or hormonal response. These findings also suggest that capsaicin effects on food intake and energy consumption may involve the enhanced insulin secretion by its direct action on pancreatic islet β cells. Furthermore, the different spatial distributions of TRPV1 in islet β cells as well as afferent nerves lead us to speculate that the effects of capsaicin on insulin secretion are biphasic; early fast response (afferents) and prolonged late response (β cells); might be involved in the mechanism by which the capsaicin treatment has the hypoglycemic effect in dogs [35].

The endogenous ligand in pancreas for TRPV1 is still unknown. Since TRPV1 responds to capsaicin, proton, and noxious heat by increasing the intracellular Ca^{2+} concentration [3,4], and the inflammatory mediator such as bradykinin [36] or ATP [37] modulates TRPV1 sensitivity, the inflammatory state in pancreas such as pancreatitis may directly affect the insulin secretion from β cells. Furthermore, a recent paper shows that ethanol directly activates TRPV1 and potentiates its response to capsaicin [38], suggesting the clinical relevance to alcoholic pancreatitis.

In conclusion, the present study demonstrated that TRPV1 is functionally expressed in rat islet β cells and plays a role in insulin secretion. TRPV1 activated with capsaicin acts as a calcium channel to release insulin. This study may account for the influences of capsaicin on the food intake and energy consumption as well as on the pathophysiological regulation of pancreatic endocrine secretion. Modulation of insulin secretion with TRPV1 sensitization may be related with the pathogenesis in chronic pancreatitis [13] or chronic ethanol consumption [14,38] involving the abnormal response to serum glucose. Also, TRPV1 would be a new therapeutic target for type 2 diabetes mellitus where some β-cell function remains [35], since dietary capsaicin has no effect on the serum parameters in rat model of type 1 diabetes mellitus induced by streptozotocin [39].

Acknowledgments

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