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L-Histidine sensing by calcium sensing receptor inhibits voltage-dependent calcium channel activity and insulin secretion in β -cells

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Abstract

Aims—Our goal was to test the hypothesis that the histidine-induced activation of calcium sensing receptor (CaR) can regulate calcium channel activity of L-type voltage dependent calcium channel (VDCC) due to increased spatial interaction between CaR and VDCC in β -cells and thus modulate glucose-induced insulin secretion.

Main methods—Rat insulinoma (RINr1046-38) insulin-producing β -cells were cultured in RPMI-1640 medium on 25 mm diameter glass coverslips in six-well culture plates in a 5% CO₂ incubator at 37°C. The intracellular calcium concentration, [Ca²⁺]_i, was determined by ratio fluorescence microscopy using Fura-2AM. The spatial interactions between CaR and L-type VDCC in β -cells were measured by immunofluorescence confocal microscopy using a Nikon C1 laser scanning confocal microscope. The insulin release was determined by enzyme-linked immunosorbent assay (ELISA).

Key findings—The additions of increasing concentrations of L-histidine along with 10 mM glucose resulted in 57% decrease in [Ca²⁺]_i. The confocal fluorescence imaging data showed 5.59 to 8.62-fold increase in colocalization correlation coefficient between CaR and VDCC in β -cells exposed to L-histidine thereby indicating increased membrane delimited spatial interactions between these two membrane proteins. The insulin ELISA data showed 54% decrease in 1st phase of glucose-induced insulin secretion in β -cells exposed to increasing concentrations of L-histidine.

Significance—L-histidine-induced increased spatial interaction of CaR with VDCC can inhibit calcium channel activity of VDCC and consequently regulate glucose-induced insulin secretion by β -cells. The L-type VDCC could therefore be potential therapeutic target in diabetes.

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Keywords

Calcium sensing receptor; colocalization correlation coefficient; L-histidine; insulin secretion; intracellular calcium; L-type voltage dependent calcium channel

Introduction

In β -cells, the membrane depolarization caused by glucose metabolism, induces rapid conformational changes in L-type voltage dependent calcium channels (VDCC) that switches VDCC from a Ca^{2+} -impermeable to a highly permeable Ca^{2+} -pore that in turn allows extracellular calcium to enter the cytoplasm (Catterall 2000; Islam 2010). The Ca^{2+} entry through VDCC directly induces trafficking of secretory granules and triggers insulin secretion (Islam 2010; Yang and Berggren 2005). The disorder of β -cell VDCC has been observed in diabetic patients and also in diabetic animal models (Yang and Berggren 2005). The dysregulation of β -cell VDCC can lead to β -cell dysfunction and β -cell death in both type 1 and type 2 diabetes (Yang and Berggren 2005).

The activities and distribution of VDCC within the β -cell are regulated by various molecular complexes such as Ca^{2+} /calmodulin, GPCRs such as calcium sensing receptor (CaR), protein kinases, and inositol phosphates (Brown et al. 1993; Hofer and Brown 2003; Yang and Berggren 2005). CaR is a 123 kDa seven transmembrane extracellular Ca^{2+} sensing protein that resides within caveolin-rich membrane domains and belongs to family C of GPCRs (Hofer and Brown 2003). CaR is activated by Ca^{2+} , divalent and trivalent cations, polycations, amino acids, and polyamines (Hofer and Brown 2003). The CaR has been detected in various tissues including pancreatic α and β -cells (Brown et al. 1993; Cheng et al. 2004; Gray et al. 2006; Hofer and Brown 2003; Justinich et al. 2008; Leech and Habener 1998; Raczy et al. 2002; Rasschaert and Malaisse 1999; Smajilovi and Tfelt-Hansen 2007; Squires et al. 2000; Vizard et al. 2008). In addition to sensing Ca^{2+} , Mg^{2+} , multivalent cations, ionic strength, pH, CaR has been shown to be allosterically activated by various amino acids with particular selectivity towards natural L-amino acids (Conigrave and Brown 2006; Conigrave et al. 2000; Lee et al. 2007). Although the precise role of L-amino acids in β -cells is not fully understood, the interaction of CaR with L-amino acids could affect intracellular Ca^{2+} mobilization and consequently affect the insulin secretion by the β -cells. CaR may also participate in β -cell replication and differentiation and thus regulate nutrient-induced insulin secretion (Leech and Habener 1998). The C-terminus of the CaR has been shown to interact with and inactivate two inwardly rectifying K channels, Kir4.1 and Kir4.2 in the kidney that are expressed in the distal nephron as well as in other tissues (Huang et al. 2007). The molecular mechanisms whereby the activation of GPCRs such as CaR regulating calcium channels such as VDCC activity have not been investigated. Therefore, in order to have a better understanding of L-amino acids-induced molecular interactions between CaR and VDCC and its influence on calcium channel activity and glucose-induced insulin secretion, we have carried out confocal fluorescence measurements on insulin-producing β -cells exposed to varying concentrations of L-histidine and also determined changes in the intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$) and insulin secretion. The results are described and discussed in the light of alterations in colocalization correlation coefficient of these two membrane proteins.

Materials and methods

Cell culture

RINr 1046-38 (abbreviated as RIN, a rat insulinoma cell line), insulin-producing β -cells, were kindly provided by Dr. Bruce Chertow (previously at the Veterans Administration

Medical Center, Huntington, WV). RINr 1046-38 insulin producing β -cells have been derived from radiation-induced insulinoma in rats (Chick et al. 1977; Efrat et al. 1988). RIN cells were grown on 25 mm diameter glass coverslips in six-well culture plates in a 5% CO₂ incubator at 37°C. The cell culture medium was RPMI-1640 supplemented with 10% (w/v) fetal bovine serum, 100 units/ml of penicillin, and 100 μ g/ml of streptomycin.

[Ca²⁺]_i measurements—The [Ca²⁺]_i in RIN cells was determined by measuring the kinetics of glucose-induced changes in [Ca²⁺]_i in RIN cells loaded with 5 μ M Fura-2AM at 37°C in a microincubation chamber, on a Nikon inverted fluorescence microscope (Nikon Corporation, Tokyo, Japan) equipped for fluorescent digital ratiometric imaging using Metafluor imaging software (Universal Imaging Corp., Westchester, PA). Briefly, the 25 mm diameter coverslip containing a monolayer of RIN cells was incubated with 5 μ M Fura-2AM and 0.05% (w/v) Pluronic F-127, a non-ionic detergent that facilitates dye loading into the RIN cells by dispersing the hydrophobic Fura-2AM, for 30 min at 37°C followed by thrice washing with Hanks' balanced salt solution (HBSS) and incubating further for 10 to 15 min in HBSS. The cells were successively excited at 340 and 380 nm wavelengths and the fluorescence emitted at 510 nm was measured. The addition of various concentration of L-histidine was carried out by adding directly a small aliquot of stock solution of L-histidine into the incubation medium containing HBSS and 10 mM glucose during the course of [Ca²⁺]_i measurements as indicated in the Figures (see Results). The equation provided by Grynkiewicz et al (1985) was used to calculate [Ca²⁺]_i. At the end of each experiment the *in situ* calibration of [Ca²⁺]_i was performed. All images were corrected for the background emission.

Immunofluorescence labeling—To determine the spatial interactions between CaR and L-type VDCC, β -cells were exposed to 0, 5, 10, and 15 mM L-histidine for 15 min at 37°C, the β -cells were then fixed with Bouin solution, washed thrice with phosphate buffer saline (PBS) followed by dehydration with 50%, 70%, 95 %, and finally 100% ethanol in PBS. The cells were rehydrated by using 100%, 95%, 70%, 50%, and finally 0% ethanol and then permeabilized with 0.2% Triton X-100. To block non-specific binding of antibodies, the RIN cells were first blocked with the blocking buffer containing 2% bovine serum albumin in PBS followed by blocking with 5% normal goat serum in blocking buffer. The RIN cells were then treated with (i) polyclonal antibody against CaR (Santa Cruz Biotechnology, Inc, USA) and (ii) antibody (anti-Cav1.2) raised against L-type VDCC, Cav1.2 (α_1 c) (Alomone Labs Ltd, Israel). The cells were then treated with Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 546 donkey anti-goat IgG fluorescent secondary antibodies (Invitrogen, Molecular Probes, Eugene, OR, USA) and the coverslips were mounted onto the glass slide. The specificities of the antibodies were checked by using peptides against CaR and L-type VDCC antibodies. By using these peptides, we did not find any fluorescence signal using confocal fluorescence microscopy.

Confocal fluorescence microscopy—The confocal fluorescence images were scanned on a Nikon TE2000U inverted fluorescence microscope equipped with a Nikon D-Eclipse C1 laser scanning confocal microscope system (Nikon Corp., USA). The z-series scanning were done at every 1 μ m up to a z-depth of 10 μ m by using a Nikon 40 $\times$ 1.30 NA DIC H/ N2 Plan Fluor oil immersion objective. The built-in Nikon EZ-C1 software and Metamorph 6 software (Universal Imaging Corp., USA) were used for confocal image acquisition and analyses.

Confocal image analysis and calculation of colocalization correlation coefficient of VDCC with CaR—The 3-D confocal images obtained and processed as mentioned above by using Nikon EZ-C1 software were imported into Metamorph 6

(Universal Imaging Inc., PA) software for further analysis. In order to determine the colocalization of VDCC with CaR, the confocal image was color thresholded for green (VDCC) and red (CaR) channels together. The colocalization correlation coefficient were measured by using Correlation Plot plug-in present in the Metamorph 6 software (Costes et al. 2004; Manders et al. 1993). The correlation coefficient (r) of the data is defined as:

$$r = \sum xy / NS_x S_y$$

where

r = correlation coefficient,

xy = product of deviation scores,

N = sample size,

S_x = standard deviation of X (intensities in first image), and

S_y = standard deviation of Y (intensities in second image).

The range of values of the correlation coefficient is -1.0 to $+1.0$. A value of 1.0 shows that the data are perfectly correlated with one another. This will only happen if the two images are identical. A correlation coefficient of -1.0 is observed when there is an inverse relationship between intensities in the two images.

Glucose-induced insulin release—After 30 minutes of incubation of RIN cells with the indicated concentrations of L-histidine directly added to the cell culture medium, the cell culture medium was removed and the cells were washed twice with D-PBS buffer containing calcium and magnesium but no glucose. The cells were then incubated with D-PBS with 5.5 mM glucose for 1 hr at 37°C and the supernatant were collected. The cells were then incubated with D-PBS containing 20 mM glucose at 37°C for 10 and 30 minute and the supernatant were collected for the insulin assay by ELISA as described below. The cells were then lysed with NP40 Cell Lysis RIPA Buffer containing 25 mM Tris-HCl pH7.5, 150 mM NaCl, 1% NP-40, 1 mM EDTA pH 8.0, 1 mM PMSF, 1 mM Na_3VO_4 , and 1 X Protease Inhibitor Cocktail-P2714. The total protein concentrations were determined by using BCA protocol using BCA Protein Assay kit (Thermo Scientific, 23227).

Quantitative measurement of insulin released by using ultrasensitive ELISA—The insulin (rat) ultrasensitive EIA kit was obtained from ALPCO Diagnostics (Salem, NH, USA). The glucose-induced insulin release in the supernatants obtained from β -cells as described above was carried out by using 25 μL of the supernatant and following the enzyme-linked immunosorbent assay (ELISA) assay protocol as described by the manufacturer of this insulin ELISA kit (ALPCO). A calibration curve was constructed from the insulin standards provided by the manufacturer. The absorbance of the 96-well culture plate ELISA was read at 450 nm and a reference wavelength of 640 nm in a Bio-Tek Synergy HT 96 well plate reader using KC4 junior software.

Data analysis—The data are expressed as means $\pm$ standard error of mean. The statistical significance of difference between various treatments were calculated by using analysis of variance (ANOVA) and considered significant when $P < 0.05$.

Results

L-histidine induced changes in the $[\text{Ca}^{2+}]_i$ of RIN cells

As shown in Fig. 1, the addition of 10 mM glucose alone to RIN cells resulted in increase in $[\text{Ca}^{2+}]_i$ from a basal value of 94.45 ± 16 nM ($n=8$) to a value of 332 ± 35 nM ($n=8$) thereby

producing a change in $[Ca^{2+}]_i$, ($\Delta[Ca^{2+}]_i$), of 238 ± 19 nM (also see Table 1). The results also suggest that the β -cells used in the present study do indeed respond to glucose. However, the addition of 5 mM, 10 mM, and 15 mM L-histidine along with 10 mM glucose produced $\Delta[Ca^{2+}]_i$ of 136 ± 14 nM ($n = 27$), 124 ± 8 nM ($n = 21$), and 104 ± 9 nM ($n = 21$) respectively. This data suggest that the addition of 5 mM, 10 mM, and 15 mM L-histidine along with 10 mM glucose caused 43%, 48%, and 57% decrease in the $\Delta[Ca^{2+}]_i$ of RIN cells respectively as compared to the control without L-histidine.

L-histidine-induced interaction between VDCC and CaR

To determine the spatial interactions between CaR and L-type VDCC, β -cells were exposed to 0, 5, 10, and 15 mM L-histidine for 15 min at 37°C, the β -cells were then fixed with Bouin solution and immunolabeled as described above. The confocal fluorescence images presented in Fig. 2 showing VDCC (left panel) and CaR (middle panel) as well as the merged images (right panel) indicate that the addition of L-histidine to the RIN cells resulted in increase in colocalization of VDCC with CaR. In order to quantify the degree of colocalization of CaR with VDCC, the 3-D confocal fluorescence imaging data was further analyzed by calculating colocalization correlation coefficient (see Materials and methods section for details). While in control RIN cells, the correlation coefficient was 0.0624 ± 0.0135 whereas in L-histidine-treated RIN cells at 5, 10, and 15 mM histidine the correlation coefficient increased to 0.4437 ± 0.0893 , 0.5385 ± 0.1638 , and 0.3493 ± 0.0975 ($n = 3$) respectively. This data suggest that the addition of 5, 10, and 15 mM histidine to RIN cells resulted in 7.11, 8.62, and 5.59-fold increase in the colocalization correlation coefficient thereby indicating significant increases in interaction between VDCC and CaR ($P < 0.05$). It is noteworthy to point out that the theoretical range of values of correlation coefficient is -1.0 to $+1.0$ wherein a value of 1.0 shows the data are perfectly correlated with one another that only happens when two images are identical or the two proteins are completely colocalized, and a value of -1.00 indicates an inverse relationship between pixel intensities in the two images thereby suggesting no colocalization between two proteins. The correlation plots showing CaR pixel intensities (on Y-axis) and VDCC (on X-axis) are shown for control and L-histidine-treated RIN cells in Fig. 3. These correlation plots are visual demonstration, in terms of pixel intensities, of colocalization between CaR (Y-axis) and VDCC (X-axis) in control, and in 5, 10, and 15 mM L-histidine-treated RIN cells (Fig. 3). It is evident from these plots of colocalization correlation in terms of pixel intensities of CaR and VDCC that the presence of L-histidine did induce increased spatial interactions between CaR and VDCC.

Glucose-induced insulin release

Basal rates—The control and L-histidine-treated RIN cells were exposed to 5.5 mM glucose for 1 hr at 37°C to determine the basal rate of insulin secretion from these β -cells. The data as shown in Fig. 4 indicates while in control β -cells the basal rate of insulin secretion was 36.86 ± 10.43 ($n = 3$) pg insulin released/mg protein/min whereas in L-histidine treated β -cells the basal rates of insulin secretion were 62.63 ± 28.50 , 48.13 ± 13.24 , and 42.39 ± 15.75 ($n = 3$) pg insulin released/mg protein/min in β -cells treated with 5 mM, 10 mM, and 15 mM L-histidine respectively (Fig. 4). The statistical analysis by ANOVA resulted in a P value > 0.05 thereby indicating that treatment of β -cells with increasing concentration of L-histidine does not significantly affect the basal rate of insulin secretion from the β -cells.

Insulin secretion at 20 mM glucose—After the determination of the basal rates of insulin secretion, the β -cells were exposed to 20 mM glucose for 10 min at 37°C and the rates of 1st phase of insulin secretion were measured by ELISA as described above. The data as shown in Fig. 4 indicates while in control β -cells the rate of 20 mM glucose-induced

insulin secretion was 489.15 ± 30.74 ($n=3$) pg insulin released/mg protein/min whereas in L-histidine treated β -cells the 20 mM glucose-induced insulin secretion rates were 333.57 ± 29.03 , 225.08 ± 52.44 , and 240.05 ± 43.58 ($n=3$) pg insulin released/mg protein/min in β -cells pretreated with 5 mM, 10 mM, and 15 mM L-histidine respectively (Fig. 4). The statistical analysis by ANOVA resulted in a P value of < 0.05 thereby indicating that pretreatment of β -cells with increasing concentration of L-histidine significantly decreased the 20 mM glucose-induced rate of insulin secretion from the β -cells.

2nd Phase of insulin secretion: We also measured the 20 mM glucose-induced secretion after 30 min of addition of 20 mM glucose to the control and L-histidine pretreated β -cell in order to determine the second-phase of insulin secretion. However, we did not find statistically significant changes in the second phase of insulin secretion rates (data not shown).

Discussion

The β -cell uptake and metabolism of glucose lead to closure of ATP-sensitive K^+ channels, depolarization of the plasma membrane and subsequently influx of Ca^{2+} (see Fig. 1) through VDCCs followed by insulin secretion (see Fig. 4). The Ca^{2+} signal provided by this influx of Ca^{2+} is accompanied by release of Ca^{2+} from the endoplasmic reticulum. The subsequent increase in the $[Ca^{2+}]_i$ as shown in Fig. 1 is an important determinant for insulin granule exocytosis as shown in Fig. 4. The increase in $[Ca^{2+}]_i$ triggers direct interactions between exocytotic proteins situated in the insulin-containing granule membrane and those localized in the plasma membrane that initiates the fusion of insulin-containing granules with the plasma membrane, *i.e.*, insulin exocytosis (Islam 2010; Yang and Berggren 2005). The regulation of activity of VDCC in β -cell is crucial to provide an optimal concentration of Ca^{2+} in the β -cells for the processes such as exocytosis of insulin-containing granule. The dysfunctions in such regulations of VDCC can result in type 1 and type 2 diabetes. The Ca^{2+} -influx through β -cell's VDCC can also mediate activation of mitogen-activated protein kinases p42 and p44 signaling (Benes et al. 1998; Gomez et al. 2002). Ca^{2+} signals in β -cells can activate the transcription factor nuclear factor kappa B (NF- κ B) involved in the regulation of cell cycle and apoptosis (Bernal-Mizrachi et al. 2002; Parkash 2008; Parkash et al. 2004).

The protein-protein interactions that occur in cellular milieu provide the foundation for the formation of complex molecular architecture and networks that in turn constitute various cellular signaling pathways (Yang and Berggren 2005). By using fluorescence microscopy along with deconvolution analysis it has been shown that the expressed CaV1.3 subunit-enhanced green fluorescent protein (GFP) and enhanced blue fluorescent protein-syntaxin 1 were targeted to and colocalized in the β -cell plasma membrane (Benes et al. 1998; Yang and Berggren 2005). The confocal fluorescence microscopy results presented in Fig. 2 in the present study show that VDCC interacts with CaR in β -cell exposed to increasing L-histidine concentrations. This observation was further strengthened by the 5.59 to 8.62-fold increase in colocalization correlation coefficient as indicated above in β -cells treated with L-histidine as opposed to the control β -cells. The correlation plots (see Fig. 3) showing interaction between pixels representing CaR on Y-axis and pixels representing VDCC on X-axis is another important visual demonstration of such L-histidine-induced CaR-VDCC interactions in insulin producing β -cells. It is highly likely that such increased spatial interactions between L-type VDCC and CaR attenuates the 1st phase of insulin secretion (see Fig. 4) *i.e.*, the exocytosis of insulin-granule localized in the plasma membrane that initiates the fusion of insulin-containing granules with the plasma membrane, *i.e.*, insulin exocytosis. This attenuation of 1st phase of insulin secretion upon treatment of β -cells with increasing concentrations of L-histidine (Fig. 4) can be directly correlated to the attenuation of glucose-induced changes in the intracellular concentration ($\Delta[Ca^{2+}]_i$) in β -cells treated with

increasing concentrations of L-histidine (see Fig. 1 and Table 1) because the increase in the $[Ca^{2+}]_i$ is an important determinant for insulin granule exocytosis. We suggest that allosteric activation of CaR by L-histidine affects the intracellular calcium mobilization and consequently affect insulin secretion by β -cells (Conigrave and Brown 2006; Conigrave et al. 2000; Lee et al. 2007).

Leech and Habener (1998) have shown that the amino acid L-His potentiates glucose-induced insulin secretion and have proposed that this effect is mediated, at least in part, by increased activation of the CaR. The complex effects of L-histidine on glucose-induced insulin secretion may reflect the coupling of the CaR to heterotrimeric G proteins such as Gq/11, G_s , and $G_{i/o}$ that can produce stimulatory and inhibitory effects on insulin secretion by β -cells (Hofer and Brown 2003; Yang and Berggren 2005). Nevertheless, the exact role of CaR in the regulation of insulin secretion is not fully understood (Malaisse et al. 1999; Rasschaert and Malaisse 1999; Squires et al. 2000; Straub et al. 2000). By using a relatively specific CaR agonist, the phenylalkylamine R-467, Straub et al. (2000) have shown a potentiation of glucose-induced insulin secretion from mouse islets and β HC9 insulinoma cells. The parathyroid CaR expressed in HEK-293 cells activates phospholipase C (PLC) signaling and inhibits cyclic AMP (cAMP) production induced by G_s coupled receptors (Brown and MacLeod 2001).

Amino acids have been shown to increase the affinity of the CaR for Ca^{2+} and L-histidine was found to be the most potent at increasing CaR activity (Conigrave et al. 2000). However in insulin producing β -cells, several multiple receptor-mediated effects may affect the glucose-induced insulin secretion in the presence of amino acids (Bolea et al. 1997). Although the signaling pathways activated by the CaR in β -cells are not known, the negative data has argued against a role for G_s , Gq/11, G_i , and G_o (Malaisse et al. 1999; Squires et al. 2000). Coexpression of the CaR and the GLP-1R in HEK-293 cells showed that elevated extracellular Ca^{2+} produces a PTX-sensitive inhibition of GLP-1-induced cAMP accumulation, consistent with CaR coupling to G_i (Leech and Habener 1998). Ca^{2+} and amino acids can stimulate different patterns of $[Ca^{2+}]_i$ oscillation when the CaR is expressed in HEK-293 cells, and it was suggested that this difference in activity might reflect the ability of the CaR to discriminate between different agonists (Young and Rozengurt 2002). Ca^{2+} and L-histidine might differentially regulate coupling of the CaR to Gq/11 and $G_{i/o}$. This difference between Ca^{2+} and L-histidine effects on cAMP production might be important for a physiological role of the CaR in islets where $[Ca^{2+}]_o$ is not known to substantially change but amino acids levels do increase after a normal meal. Recently, Mamillapalli et al. (2008) have shown that while CaR coupled to $G\alpha_i$ in normal mammary epithelial cells (MMECs), but in MCF-7 breast cancer cells CaR coupled to $G\alpha_s$. Their data suggest that malignant transformation of breast epithelial cells can lead to a switch in G-protein coupling that reverses the normal suppression of PTHrP by calcium. Exposure of MMECs to increasing concentrations of extracellular calcium or to calcimimetics inhibited PTHrP gene expression and decreased the secretion of PTHrP into the media (Ardehsirpour et al. 2006; VanHouten et al. 2004). In contrast, activation of the CaR in Comma-D or MCF-7 cells stimulated PTHrP gene expression and increased the secretion of PTHrP into the media. Mamillapalli et al. (2008) have proposed that these opposing effects of the CaR can be correlated to a change in its G-protein coupling such that while in MMECs, the receptor couples to $G\alpha_i$ whereas in Comma-D cells and in MCF-7 cells it switches to $G\alpha_s$.

The interaction between G proteins and VDCC that results in inhibition of VDCC is one of the well-characterized mechanisms of VDCC regulation and it is voltage-dependent and membrane delimited. In addition to forming Ca^{2+} -conducting pores in the plasma membrane, the VDCC subunits also interact with many other proteins to form complex molecular networks. The VDCC is a very important constituent of Ca^{2+} handling molecular

network and it plays critical roles in the spatio-temporal regulation of $[Ca^{2+}]_i$ in the β -cell (Berggren and Larsson 1994). The glucose metabolism that causes influx of Ca^{2+} also results in the activation Ca^{2+} handling molecular networks in the β -cell leading to generation of complex $[Ca^{2+}]_i$ signals (Efanova et al. 1998; Islam 2010; Juntti-Berggren et al. 1993). In normal physiological conditions, the proper regulation of $[Ca^{2+}]_i$ by various constituents of Ca^{2+} handling networks maintain β -cell growth, insulin secretion, cell proliferation, and differentiation. However, in pathophysiological situations, the changes in the intracellular and/or extracellular environment can lead to dysregulation of $[Ca^{2+}]_i$ due to altered functioning of the Ca^{2+} handling molecular networks which can cause β -cell death (Juntti-Berggren 1993; Yang and Berggren 2005). In addition to direct regulation of VDCC by G proteins, the activation of CaR by L-histidine can initiate a number of intracellular signaling pathways (Isaev et al. 2004; Strock and Diverse-Pierluissi 2004). The C-terminus of CaR is involved in positively cooperative response to Ca^{2+} (Gamma and Breitwieser 1998) and binding to a scaffold protein, filamin-A (Hjalm et al. 2001). Several CaR-interacting proteins such as filamin, potassium channels, signalling proteins, chaperone and trafficking proteins may lead to the regulatory functioning of CaR (Gamma and Breitwieser 1998; Hjalm et al. 2001; Huang et al. 2007; Lourdel et al. 2002). The CaR has four domains that are exposed to the intracellular space and that are available to interact with other proteins, three intracellular loops that connect transmembrane (TM) domains and the C-terminus. The intracellular loops could interact with G proteins as well as other proteins including proteins involved in signaling (Strock and Diverse-Pierluissi 2004). The C-terminus of the CaR has been shown to interact with and inactivate two inwardly rectifying K channels, Kir4.1 and Kir4.2 in the kidney that are expressed in the distal nephron as well as other tissues (Huang et al. 2007; Lourdel et al. 2002). The findings described in the present study suggest that CaR indeed interact with VDCC spatially. The interaction of CaR with VDCC in tissues could allow control over channel activity and consequently the insulin secretion (see Fig. 4) by the β -cells via direct CaR-VDCC contact.

Conclusions

In this study we have shown that treatment of β -cells with L-histidine resulted in (i) increased spatial interaction between CaR and VDCC, (ii) attenuation of glucose-induced increase in $[Ca^{2+}]_i$, ($\Delta[Ca^{2+}]_i$) and consequently (iii) decrease in the 1st phase of glucose-induced insulin secretion by the β -cells. Such increased spatial interaction between CaR and VDCC due to activation of CaR by L-histidine inhibited the L-type VDCC channel activity resulting in attenuation of $\Delta[Ca^{2+}]_i$ and therefore attenuation of 1st phase of insulin secretion i.e., the exocytosis of insulin-granule localized in the plasma membrane that initiates the fusion of insulin-containing granules with the plasma membrane, *i.e.*, insulin exocytosis. In normal physiological conditions, the proper regulation of $[Ca^{2+}]_i$ by various constituents of Ca^{2+} handling networks maintain β -cell growth, proliferation, and differentiation whereas in pathophysiological situations, the changes in the intracellular and/or extracellular environment can lead to dysregulation of $[Ca^{2+}]_i$.

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Fig. 1.

L-histidine attenuated the glucose-induced increase in $[Ca^{2+}]_i$ in β -cells. Kinetics of glucose-induced increase in $[Ca^{2+}]_i$ in insulin producing RIN cells in presence of various concentrations of L-histidine. The Fura-2 loaded RIN cells were exposed to 10 mM glucose in presence of 5, 10, and 15 mM L-histidine and the kinetics of increase in $[Ca^{2+}]_i$ was determined by using Fura-2 ratio fluorescence microscopy as described in Materials and methods.

Fig. 2.

L-histidine-induced increase in colocalization of VDCC with CaR in β -cells. The insulin producing RIN cells were treated with 0 mM (A), 5 mM (B), 10 mM (C) and 15 mM (D) L-histidine. The confocal fluorescence images of immunofluorescent labeled β -cells show increased spatial interaction between VDCC (left panel) and CaR (middle panel) as indicated in the merged images (right panel).

Fig. 3.

Increased spatial interactions between CaR and VDCC in L-histidine-treated β -cells. The correlation plot showing CaR pixels on Y-axis (Red) and VDCC pixels on X-axis (Green) in control and L-histidine treated RIN cells indicating increased spatial interactions between these two membrane proteins in L-histidine treated RIN cells.

Fig. 4.

L-histidine attenuated the 1st phase of glucose-induced insulin secretion in β -cells. The rate of basal insulin secretion determined at 5.5 mM glucose was not affected significantly upon treatment of RIN cells with L-histidine. The data indicate statistically no significant difference between the control and L-histidine treated RIN cells in the rate of basal insulin secretion ($P > 0.05$; $n = 3$). However, L-histidine significantly attenuated the 20 mM glucose-induced 1st phase of insulin-granule exocytotic activity of RIN cells ($P < 0.05$; $n = 3$).

Table 1

L-histidine attenuated the glucose-induced increase in the $[Ca^{2+}]_i$, ($\Delta[Ca^{2+}]_i$), in β -cells

Sample	$\Delta[Ca^{2+}]_i$, nM	% Decrease
Control	238 ± 19 (n =8)	0
5 mM L-histidine	136 ± 14 (n =27)	43
10 mM L-histidine	124 ± 8 (n = 21)	48
15 mM L-histidine	104 ± 9 (n =21)	57