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Tetracaine stimulates extracellular Ca^{2+}-independent insulin release

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Abstract

The effect of the local anesthetic, tetracaine, on ^{45}Ca efflux, cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$ and insulin secretion in pancreatic B-cells was studied. At a physiological level of $[Ca^{2+}]_o$, tetracaine (0.1–5 mM) dose-dependently inhibited insulin secretion induced by 22 mM glucose. Paradoxically, at the same glucose concentration but in the absence of external Ca^{2+}, tetracaine dose-dependently increased insulin secretion. At a low glucose level (2.8 mM) tetracaine failed to affect secretion, either in the presence or absence of external Ca^{2+}. At high (22 mM) or low (2.8 mM) glucose, $[Ca^{2+}]_i$ was increased by tetracaine in a dose-dependent manner. Tetracaine (2 mM) also increased the ^{45}Ca efflux from isolated islets. This effect was of the same magnitude at both low and high glucose concentrations, and was independent of the presence of extracellular Ca^{2+}. Finally, tetracaine increased ^{45}Ca efflux from islets perifused in the presence of thapsigargin. In conclusion, our data indicate that tetracaine releases Ca^{2+} from a thapsigargin-insensitive store in pancreatic B-cells. Under suitable experimental conditions, insulin release can be elicited by a $[Ca^{2+}]_o$-independent pathway. The existence of a ryanodine-like Ca^{2+} channel in pancreatic B-cells is proposed.

Keywords: Pancreatic B-cell; Insulin secretion; $[Ca^{2+}]_i$; Tetracaine

1. Introduction

The nature of pancreatic B-cell glucose-sensing is based on two well-defined but not exclusive pathways that couple biochemical and electrical events to insulin release. The first pathway involves the inhibition of K^+ efflux through K_{ATP} channels, which is modulated by glucose metabolism and leads to cell membrane depolarization. The second involves a rise in intracellular Ca^{2+} concentration, as a consequence of membrane depolarization and Ca^{2+} influx through L-type Ca^{2+} channels (Prentki and Matschinsky, 1987). However, in some experimental conditions, these two events can not account for the triggering of insulin secretion. When the membrane potential of B-cells is voltage-clamped with depolarizing agents, e.g., combining glibenclamide or diazoxide with a high $[K^+]_o$ (Gembal et al., 1992; Sato et al., 1992), glucose is still able to stimulate exocytosis. A K_{ATP}-independent hypothesis has therefore been proposed to explain the increased insulin release when the membrane potential is not altered by the presence of glucose (Aizawa et al., 1994). A Ca^{2+}-independent pathway for insulin secretion has also been observed when protein kinase C and protein kinase A, which modulate B-cell function, are stimulated (Komatsu et al., 1995).

The release of Ca^{2+} from intracellular stores seems to play a minor role in B-cell stimulation. Although glucose has a stimulating effect on phosphoinositide (PI) hydrolysis (Rasmussen et al., 1995), the main insulinotropic effect of IP_3 generation is related to cholinergic activation (Wollheim and Biden, 1986; Bordin et al., 1995; Boschero et al., 1995). However, in mouse pancreatic B-cells, IP_3-dependent Ca^{2+} mobilization accounts for about 30% of the total Ca^{2+} sequestered into intracellular stores (Nilsson et al., 1987), indicating that B-cells possess both IP_3-sensitive and IP_3-insensitive intracellular Ca^{2+} pools.

In most excitable cells, the main mechanisms of Ca^{2+} signaling are mediated by the activation of a specific intracellular channel, namely the ryanodine receptor (RyR) (Clapham, 1995; Pozzan et al., 1994). A variety of pharmacological agents that interact with RyR have been used to study biochemical and functional aspects of intracellular Ca^{2+} release (Coronado et al., 1994). Indeed, modulators of RyR such as caffeine and local anesthetics are known to induce Ca^{2+} mobilization and insulin secretion.

In the present study, we used the local anesthetic tetracaine to explore the participation of intracellular Ca^{2+} release in the stimulus-secretion coupling. We observed

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that tetracaine produces a marked increase in the $[Ca^{2+}]_i$, as a result of Ca^{2+} mobilization from an IP_3-insensitive, non-mitochondrial Ca^{2+} store. Furthermore, we found that a tetracaine-sensitive Ca^{2+} pool modulates glucose-induced insulin secretion.

2. Materials and methods

2.1. Islets and B-cell isolation

Islets from fed adult female Wistar rats were isolated by collagenase digestion. To prepare B-cells, islets were dispersed into cells in Ca^{2+}-free saline in the presence of 0.5 mM EGTA followed by preincubation for 30 min in the same medium. Cell viability as assessed by Trypan blue exclusion was about 95%. The cell culture was maintained at 37°C for 2 to 4 days in RPMI-1640 medium with 2 mM glutamine, 10% fetal calf serum, 10 mM glucose, penicillin (100 IU/ml) and streptomycin (100 μg/ml) in an atmosphere of 5% CO_2. The medium was renewed every 48 h. After 2 days the cells were firmly attached to glass coverlips.

2.2. Medium

The medium used in all experiments was a Hepes-bicarbonate buffer containing (in mM): 100 NaCl, 5 KCl, 2.56 $CaCl_2$, 20 Na-Hepes (pH 7.4), 0.5% bovine albumin and different concentrations of glucose and tetracaine (see Results). Ca^{2+}-deprived medium, used in some perifusion experiments, contained 0.5 mM EGTA. The medium was equilibrated with a mixture of 95%O_2 and 5%CO_2.

2.3. Insulin secretion

For insulin secretion, groups of five islets were first preincubated at 37°C in 0.75 ml of the Hepes-buffer containing 5.6 mM glucose. This medium was then replaced with fresh buffer and the islets further incubated for 1 h under various experimental conditions. The insulin content in the supernatant of each sample and the insulin extracted from the islets at the end of the incubation period, were measured by radioimmunoassay as previously described (Scott et al., 1981) using rat insulin as a standard. Insulin release was expressed as a percentage of the total islet insulin content.

2.4. $[Ca^{2+}]_i$ measurements

$[Ca^{2+}]_i$ measurements were performed as described elsewhere (Rojas et al., 1994) using indo-1 as a cytoplasmic Ca^{2+} indicator. Briefly, isolated B-cells attached to glass coverslips were loaded with indo-1/AM by incubating them for 1–2 h at room temperature in a Na-Hepes bicarbonate medium containing 2 μM indo-1/AM and pluronic acid (0.02%). The coverslips were then transferred to a perifusion chamber and the cells were continuously perifused with identical medium free of indo-1 and pluronic acid. Different concentrations of tetracaine were applied using the same perifusion system. Changes in cytosolic Ca^{2+} were measured by a micro-fluorimetric technique using an excitation wavelength of 355 nm. The resulting fluorescence (F) at 410 and 485 nm was measured continuously. A computer program calculated the fluorescence ratio ($R = F_{410}/F_{485}$), which was converted to $[Ca^{2+}]_i$ using a calibration curve for Ca^{2+}.

2.5. ^{45}Ca measurements

^{45}Ca efflux from perifused islets was performed as previously described (Herchuelz and Malaisse, 1980). Briefly, groups of 100 islets were labeled with $^{45}CaCl_2$ (20 μCi/ml) for 90 min. The islets were then washed four times with a radioisotope-free medium and transferred to a small chamber in which they were perifused for 80 min with medium containing different concentrations of glucose, Ca^{2+}, and tetracaine. ^{45}Ca efflux was expressed as the fractional outflow rate (percentage of islet content per min).

2.6. Data analysis

The data are presented as the means ± S.E. of n experiments. The statistical significance of the differences between means was assessed by analysis of variance followed by Dunnett's test when several experimental groups were compared with the control group. When only two groups were involved, Students' t-test was used. Differences were considered significant at $P < 0.05$.

3. Results

In the presence of physiological $[Ca^{2+}]_o$ and 22 mM glucose, insulin release was dose dependently reduced by tetracaine (0.1–5 mM; solid bars in Fig. 1A). The secretory response was completely blocked at 5 mM of the anesthetic ($P < 0.01$) whereas 50% inhibition was observed at 0.5 mM tetracaine ($P < 0.01$), as previously observed (Freinkel et al., 1975). In contrast, at the same glucose concentration but in the absence of extracellular Ca^{2+}, tetracaine evoked a dose-dependent increase in insulin release (Fig. 1B, solid bars). A two-fold increase above basal was observed at 1 mM tetracaine ($P < 0.05$). Maximal insulin secretion was achieved at 5 mM tetracaine, and was approx. 9-times greater than the basal secretion (4.44 ± 0.68% and 0.48 ± 0.03% of islet insulin content, respectively; $P < 0.01$). No alterations in the secretory response were observed when the medium contained a low glucose concentration (2.8 mM), either in the presence or in the absence of extracellular Ca^{2+} (open bars in Fig. 1).

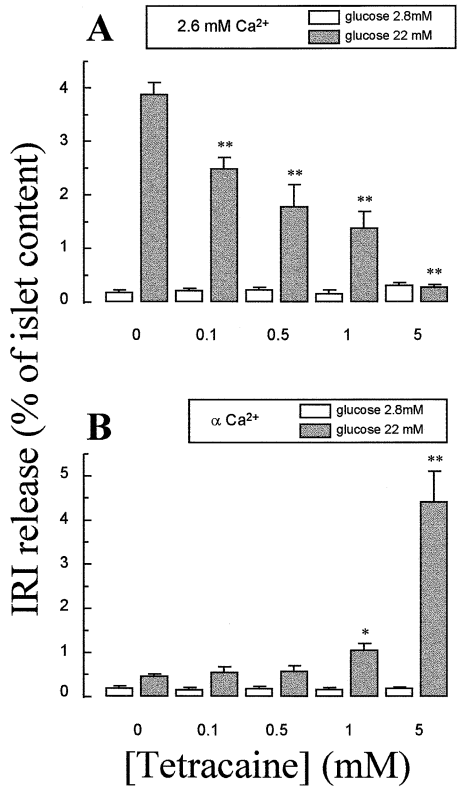

Fig. 1. Effect of tetracaine on insulin secretion. Prior to the application of tetracaine, groups of five islets each were preincubated for 45 min at 37°C in a Hepes-bicarbonate medium containing 5.6 mM glucose. This preincubation medium was then replaced with Hepes-bicarbonate containing 2.8 mM or 22 mM glucose and increasing concentrations (0–5 mM) of tetracaine. (A) Experiments carried out in the presence of 2.6 mM Ca^{2+}. (B) Experiments performed in the absence of Ca^{2+} (α Ca^{2+}). Columns represent the cumulative (1 h) insulin secretion, expressed as a percentage of the total islet content. Values are means ± S.E. of 7–19 experiments. * $P < 0.05$; ** $P < 0.01$.

Previous observations that local anesthetics alter Ca^{2+} permeability in different cell types, including B-cells (Norlund and Sehlin, 1985), provided evidence that tetracaine could affect insulin secretion by altering Ca^{2+} handling in the islet. To verify this hypothesis, we performed further experiments using two different techniques in order to measure Ca^{2+} movements under experimental conditions similar to those above. In the first, we used a microfluorimetry technique to measure changes in $[Ca^{2+}]_i$ induced by tetracaine in isolated B-cells. Fig. 2 shows the net increase in $[Ca^{2+}]_i$ relative to the baseline values in the absence of tetracaine. The values attained before the addition of the anesthetic were 98.5 ± 6.1 nM and 137.8 ± 19 nM for low (Fig. 2A) and high (Fig. 2B) glucose concentrations, respectively. At both low and high glucose levels, tetracaine elicited rapid elevations in $[Ca^{2+}]_i$ which were clearly not dependent on the glucose concentration. The dose-dependent rises in $[Ca^{2+}]_i$ were rapidly reverted when tetracaine was removed from the medium. The net increases in $[Ca^{2+}]_i$ at both glucose concentrations were 30, 90, 180 and 880 nM, respectively, for 0.1, 0.5, 1 and 5 mM tetracaine.

We also studied the effects of tetracaine on ^{45}Ca fluxes from perifused islets. In agreement with the $[Ca^{2+}]_i$ results, the pattern of ^{45}Ca efflux was identical at both levels of glucose (2.8 mM and 22 mM; Fig. 3A). In Ca^{2+}-deprived medium, tetracaine also increased the efflux of Ca^{2+} (Fig. 3B). Inspite of the difference in resting ^{45}Ca efflux rates, maximal efflux values in the presence of tetracaine and with either 2.8 mM or 22 mM glucose were essentially the same. Together, the $[Ca^{2+}]_i$ and ^{45}Ca measurements indicate that tetracaine mobilizes Ca^{2+} from intracellular stores(s) in a glucose-independent pathway.

To further understand the mechanism of action of tetracaine on Ca^{2+} release, we performed ^{45}Ca efflux measurements in the presence of thapsigargin. This drug is a specific blocker of the Ca^{2+}-ATPase pump and is generally used to promote depletion of the IP_3-sensitive Ca^{2+} store in the endoplasmic reticulum (ER) (Thastrup et al., 1990). Fig. 4 shows that thapsigargin did not alter the

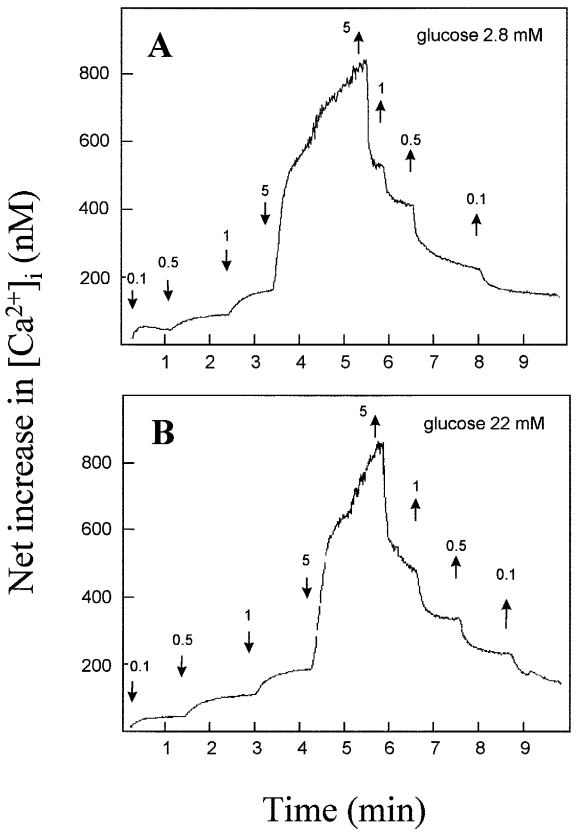

Fig. 2. Effect of tetracaine on $[Ca^{2+}]_i$. Isolated B-cells, cultured for 2–4 days, were prelabeled with the intracellular Ca^{2+} marker indo-1/AM for 1 h after which the cells were extensively washed and treated with increasing concentrations (0–5 mM) of tetracaine. The experiments were performed with medium containing 2.6 mM Ca^{2+} and 2.8 mM glucose (A) or 22 mM glucose (B). The arrows indicate either the addition (↓) or removal (↑) of tetracaine in order to achieve the concentration indicated above the arrows. The traces are representative of at least three experiments.

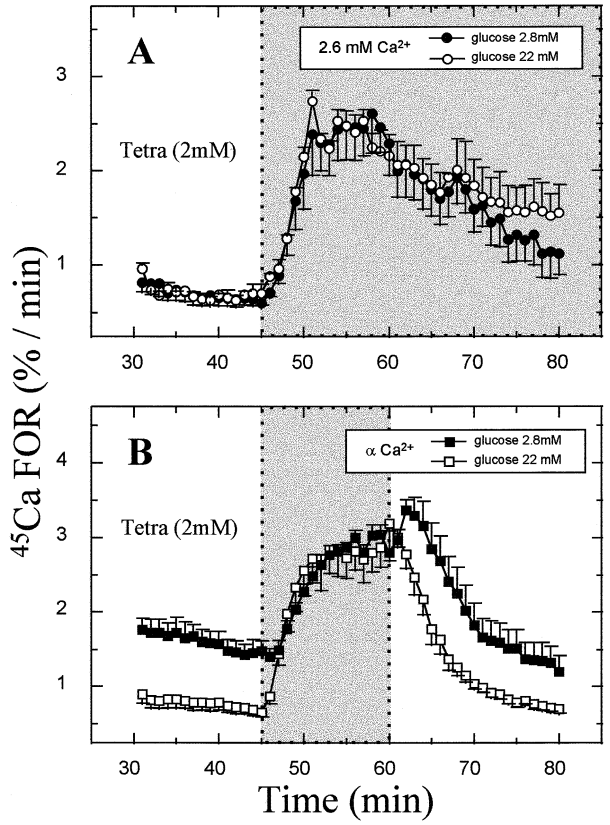

Fig. 3. Effects of tetracaine on the ^{45}Ca fractional outflow rate. Groups of 100 islets each were prelabeled with $^{45}CaCl_2$ for 90 min. The islets were then washed four times with non-radioactive medium, placed in a small chamber and perifused for 80 min in the presence of 2.6 mM Ca^{2+} (A) or in the absence of the cation (B). Glucose 2.8 or 22 mM was present throughout the perifusion period. Tetracaine (2 mM) was present during the period indicated by the shadowed areas. Each point is the mean ± SE of four experiments.

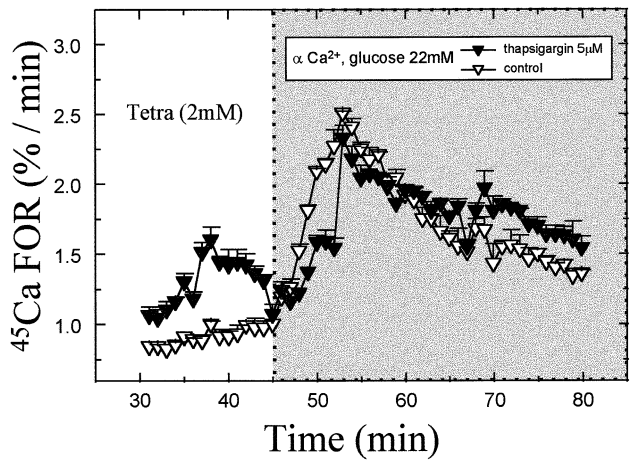

Fig. 4. Effect of thapsigargin (5 μM) on tetracaine-induced increase in ^{45}Ca efflux. Groups of 100 islets, prelabeled with $^{45}CaCl_2$, were perifused for 80 min with a Ca^{2+}-deprived medium containing 22 mM glucose. Tetracaine (2 mM) was introduced at the 45th min and maintained until the end of perifusion period (shaded area), either in the presence (open triangles) or the absence (solid triangles) of thapsigargin (5 μM). The latter was introduced at min 30th and maintained until the end of the experiments. Each point is the mean ± S.E. of four experiments.

amount of ^{45}Ca released by tetracaine. The initial increase in ^{45}Ca efflux (30th to 45th min) from thapsigargin-treated islets reflects the Ca^{2+} leak from ER (solid triangles in Fig. 4). When tetracaine (2 mM) was added to the solution (45th min), the ^{45}Ca efflux increased significantly attaining essentially the same level, irrespective of whether the thapsigargin-sensitive Ca^{2+} reservoir had previously been depleted or not. The small differences in the effluxes with or without thapsigargin after the 45th min indicate different time responses. Integrating the data to eliminate the differences in time delays showed that the areas under the curves were identical and corresponded to 25% of the initial ^{45}Ca content. These results rule out the possibility of tetracaine acting by the same mechanism as thapsigargin.

4. Discussion

Early studies reported opposite effects of tetracaine on glucose-induced insulin secretion. Freinkel et al. (1975) demonstrated that glucose-induced insulin secretion was inhibited when isolated rat islets were incubated with tetracaine. On the other hand, dynamic insulin secretion experiments using rat (El Motal et al., 1987) or mouse (Norlund and Sehlin, 1983) islets showed that tetracaine potentiated the secretory response induced by glucose. In our hands, tetracaine inhibited the secretory response at a physiological $[Ca^{2+}]_o$, and stimulated insulin release when islets were incubated in a Ca^{2+}-deprived medium. One possible explanation for these apparently paradoxical effects could be that B-cells regulate $[Ca^{2+}]_i$ within a very narrow range (Rojas et al., 1994). Since tetracaine elicited a very high increase in the $[Ca^{2+}]_i$, a rise in this cation above a critical level could result in a decrease in the effect of the sugar by collapsing the secretory machinery. In parathyroid cells (Nygren et al., 1987) and pancreatic B-cells (Hellman et al., 1994), desensitization of the secretory mechanism by continuous exposure of the cells to high Ca^{2+} has been observed and is accompanied by a reduction in protein phosphorylation (Jones et al., 1992). However, we can not discard the possibility that, in addition to desensitization by exposure to high Ca^{2+}, the inhibition of insulin secretion observed in the presence of high glucose and a physiological $[Ca^{2+}]_o$ could be associated to an intra-islet regulatory mechanism involving the participation of glucagon and somatostatin (for a review, see Marks and Morgan, 1994). This could partially explain the discrepancy between our results (obtained using static incubations) and those based on dynamic analysis (El Motal et al., 1987; Norlund and Sehlin, 1983). In the present study, we have demonstrated that by stimulating intracellular Ca^{2+} release with tetracaine, it is possible to induce insulin secretion in a $[Ca^{2+}]_o$-independent manner. As reported for the K_{ATP}-independent pathway (Gembal et al., 1993), the stimulatory effect of tetracaine involves a glucose-dependent mechanism. Considering that the effect of the anesthetic in inducing insulin release was only

observed in the presence of stimulatory glucose concentrations we speculate that a tetracaine-sensitive Ca^{2+} store can play a role in the mechanism of insulin secretion under physiological conditions.

Local anesthetics may interact with multiple cellular sites, including membrane receptors and ionic channels (reviewed by Butterworth and Strichartz, 1990). Our study has shown that tetracaine evoked the release of Ca^{2+} from an IP_3-insensitive intracellular pool. This conclusion is based on the observation that tetracaine increases $[Ca^{2+}]_i$ regardless of the presence or absence of extracellular Ca^{2+}, and that thapsigargin does not affect the mechanism of intracellular Ca^{2+} release stimulated by tetracaine. We also observed that tetracaine-induced Ca^{2+} release was not altered by addition of the metabolic poison sodium azide (data not shown), indicating that the $[Ca^{2+}]_i$ increase is not due to mitochondrial Ca^{2+} leakage.

In most excitable cells, two well-defined mechanisms for internal Ca^{2+} release are present. The first of these is represented by the IP_3 pathway and the second by the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism. CICR is a well-known process, first described in skeletal muscle, whereby an increase in $[Ca^{2+}]_i$ causes further release of Ca^{2+} by acting on a specific receptor-operated channel known as the ryanodine receptor (RyR). Many drugs, including ryanodine, caffeine, sulphydryl reagents, and local anesthetics have been used to study the activity of RyR (Coronado et al., 1994). Although RyR has not yet been identified in B-cells, several lines of evidence suggest that ryanodine-like channels are linked to islet function. The modulation of an IP_3-insensitive intracellular Ca^{2+} store by thimerosal (Islam et al., 1992), as well as by the combination of ryanodine and caffeine (Chen et al., 1996) is strong evidence for the existence of RyR in insulin-secreting cells. The demonstration of a voltage-sensitive mechanism for Ca^{2+} extrusion (Roe et al., 1994) also suggests the activation of ryanodine-like channels. In addition, the controversial endogenous RyR agonist cADP-ribose was reported to cause Ca^{2+} release and to stimulate insulin secretion from pancreatic B-cells (Takasawa et al., 1993).

Perhaps the most intriguing observation made in the present study is that tetracaine elicited a dose-dependent increase in $[Ca^{2+}]_i$, in all of experimental conditions used. This seems to contradict recent observation showing that the local anesthetic procaine inhibits caffeine-induced Ca^{2+} release (Chen et al., 1996). However, local anesthetics are known to have complex effects on cellular function which depend on experimental conditions such as pH, ATP and Mg^{2+} concentrations as well as the cell type. It is noteworthy that tetracaine, but not procaine, evoked a dose-dependent Ca^{2+} release in rat myotubes (Jaimovich and Rojas, 1994). In this context, our data clearly demonstrate the presence of a tetracaine-sensitive mechanism for intracellular Ca^{2+} release in B-cells which may involve a channel functionally related to RyR.

In summary, we have demonstrated that insulin secretion can be elicited by a $[Ca^{2+}]_o$-independent pathway which triggers Ca^{2+} release from a specific intracellular Ca^{2+} store. Our results also suggest the participation of a ryanodine-like Ca^{2+} channel in intracellular Ca^{2+} handling during glucose-induced B-cell stimulation.

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