A THAPSIGARGIN-SENSITIVE CATIONIC CURRENT LEADS TO MEMBRANE DEPOLARIZATION, CALCIUM ENTRY AND INSULIN SECRETION IN RAT PANCREATIC BETA-CELLS

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ABSTRACT

Glucose-induced insulin secretion by pancreatic β-cells depends on membrane depolarization and $[Ca^{2+}]_i$ increase. We correlated voltage- and current-clamp recordings, $[Ca^{2+}]_i$ measurements and insulin reverse hemolytic plaque assay to analyze the activity of a thapsigargin-sensitive cationic channel that can be important for membrane depolarization in single rat pancreatic β-cells.

We demonstrate the presence of a thapsigargin-sensitive cationic current which is mainly carried by Na$. Moreover, in basal glucose concentration (5.6 mM), thapsigargin depolarizes the plasmatic membrane producing electrical activity and increases $[Ca^{2+}]_i$. The later is prevented by nifedipine, indicating that Ca$^{2+}$ enters the cell through L-type Ca$^{2+}$ channels, which are activated by membrane depolarization.

Thapsigargin also increased insulin secretion by increasing the percentage of cells secreting insulin and amplifying hormone secretion by individual β-cells. Nifedipine blocked the increase completely in 5.6 mM glucose and partially in 15.6 mM glucose. We conclude that thapsigargin potentiates a cationic current that depolarizes the cell membrane. This in turn, increases Ca$^{2+}$ entry through L-type Ca$^{2+}$-channels promoting insulin secretion.

KEYWORDS

Non-selective cationic channels, calcium channels, stimulus-secretion coupling
INTRODUCTION

GLUCOSE-INDUCED INSULIN SECRETION by pancreatic β cells depends on the rise of intracellular Ca\(^{2+}\) \([\text{Ca}\^{2+}]_i\). In non-stimulating glucose concentrations (below 6.0 mM), the membrane potential is at resting level. When glucose concentration increases, β-cell membrane exhibits an oscillating electrical activity. First, a slow membrane depolarization is observed, this is followed by a fast depolarization phase to a plateau level, on which bursts of action potentials are superimposed and finally the membrane repolarizes. The time that the membrane spends firing in the depolarized plateau state is determined by extracellular glucose concentration (17).

It is well accepted that when the glucose concentration raises, the ATP/ADP ratio increases as a result of glucose metabolism, leading to the closure of K\(_{\text{ATP}}\) channels (1). This leads to a slow membrane depolarization via non-selective cationic channels, resulting in the subsequent activation of voltage-sensitive Na\(^+\) and Ca\(^{2+}\) channels (22). Ca\(^{2+}\) entry through L-type channels is a determining factor for insulin secretion (11).

The slow depolarization that follows K\(_{\text{ATP}}\) channel closure is an important event that is not fully understood. It has been suggested that it is due to sodium entrance through a yet unidentified voltage-independent cationic channel, since a study shows that this depolarization is eliminated by the removal of extracellular Na\(^+\) (3). Alternatively, it has been shown that maneuvers that deplete intracellular calcium stores, such as the application of the microsomal Ca\(^{2+}\)-ATPase inhibitor thapsigargin (TG), or activation of G-protein-coupled receptors associated to the inositol triphosphate (IP\(_3\)) cascade also result in the activation of non-selective cationic currents in a wide variety of cells, including the murine insulinoma βTC3-neo cells (18). This current referred to as Ca\(^{2+}\)-release-activated non-selective current (I\(_{\text{CRAN}}\)) is activated also by the non-selective cationic current activator, maitotoxin.
It has been shown that maitotoxin induces cell membrane depolarization, as a result of increased Na\(^+\) permeability in mouse β-cells, resembling the effects of high glucose concentrations on cell membrane potential (23).

In order to explore cationic channels that could be important for membrane depolarization in rat pancreatic β-cells, we analyzed the early effects of TG on: 1) cationic currents, 2) electrical activity, 3) intracellular calcium concentration and 4) insulin secretion.

We demonstrate a direct correlation between the activation of a TG-sensitive cationic channel, cell depolarization, changes in [Ca\(^{2+}\)], and insulin secretion in pancreatic β-cells.

**MATERIAL AND METHODS**

*Materials.* Reagents were obtained from the following sources: collagenase type IV from Worthington (Freehold, NJ); guinea-pig insulin antiserum from Biogenesis (Sandown, NH); bovine serum albumin, Hanks’ balanced salt solution (HBSS), chromium chloride, staphylococcal protein A, HEPES, trypsin, trypan blue, nystatin and poly-L-lysine from Sigma (St. Louis, MO); tissue culture dishes (Corning); Spinner-Eagles’ salt solution from Microlab (Mexico D.F., Mexico); fetal bovine serum from Equitech-Bio (Ingram, Tx); guinea-pig complement, RPMI-1640 salts, and penicillin-streptomycin-amphotericin B solution from Life Technologies (Grand Island, NY); thapsigargin from Alomone Labs (Jerusalem, Israel); fluo-4-AM from Molecular Probes (Eugene, OR).

*Pancreatic β-cell culture.* Animal care was performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH No. 85-23, reviewed 1985). Young adult male Wistar rats (250-280 g) were obtained from the local animal facility, maintained in a 14 h light (0600-2000)/10 h dark cycle, and allowed free access to standard laboratory rat diet.
and tap water. Animals were anesthetized with sodium pentobarbital (40 mg/kg), and after pancreas
dissection were killed by cervical dislocation.

Single pancreatic β-cells were obtained by collagenase digestion and islet dissociation by
mechanical disruption in calcium-free medium, as described previously (15). Single β-cells were
platted at low density (10000 cells/cm²) on glass coverslips previously coated with poli-L-lysine
and cultured for 24 to 72 h, in RPMI-1640 supplemented with 200 U/ml penicillin G, 200 mg/ml
streptomycin, 0.5 mg/ml amphotericin B, and 10 % of fetal bovine serum (FBS).

**Electrophysiological Recordings and Analysis.** Whole-cell voltage-clamp recordings (2)
were performed at 20-22° C using an Axopatch 200B amplifier (Axon Instruments, Foster City,
CA). Patch electrodes were pulled from Kimax-51 capillary tubes (Kimble Glass Inc., Vineland,
NJ) and had a tip resistance of 1.5 to 3 MΩ. Electrode tips were coated with Sylgard (Dow Corning,
Midland, MI).

The pulse protocol used for the analysis of the currents consisted of applying depolarizing
test pulses from –120 to +60 mV in 20 mV steps for 250 ms, from a holding potential of –80 mV.
After recording control currents, thapsigargin (200 nM) was added with the aid of a Picospritzer
(General Valve, NY).

The whole-cell conductance of the TG-activated current was calculated using equation 1:
\[ g = \frac{I_2 - I_1}{V_2 - V_1} \]
where \( I_2 \) is the current measured at -100 mV \( (V_2) \) and \( I_1 \) is the current measured at the holding
potential at -80 mV \( (V_1) \).

**Current clamp experiments.** The perforated mode of the patch clamp technique was utilized
in all measurements. An EPC9 amplifier was used. Data acquisition was controlled by the Pulse
connected to the bath solution via a 100 mM KCl agar bridge. Nystatin was dissolved in methanol
and added to the pipette solution at a final concentration of 100 µg/ml.
Current clamp experiments were performed as previously described (20). Briefly, the patch pipette was filled with nystatin solution and once a GΩ seal was formed, cell capacitative artifacts were monitored in response to a 1 mV voltage step until electrical access was gained. After this, the amplifier mode was switched from voltage-clamp to current-clamp and the experiments were conducted as described in the legend of figure 3.

Recoding solutions. The ionic composition of the external solutions is summarized in Table 1.

**Internal Solution (mM)**

115 CsAsp, 10 CsCl, 5 CsF, 2.5 EGTA, mM HEPES, 10, pH 7.2, 290-295 mOsm. For perforated patch experiments the CsAsp was replaced by 115 KAsp.

Confocal Calcium Measurements. Changes in cytosolic calcium in pancreatic β-cells were monitored using the fluorescent calcium indicator fluo-4-AM as previously described (21). Briefly, cells were placed on glass coverslips and allowed to attach to the glass surface for 24 h in RPMI with 10% BFS. The cells were then loaded with 5 μM fluo-4-AM diluted in RPMI media and incubated for 30 min at 37°C with 5% CO₂. Following incubation, cells were washed once with low-glucose physiological solution (containing in mM: 120 NaCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 4.75 KCl, 5.6 glucose, 20 HEPES and 0.05% BSA). Calcium measurements were carried out using a BioRad MRC 1024 confocal microscope. The excitation wavelength was 488 nm and emission was collected at 535 nm. An increment in the intensity of fluorescence indicated an increase in cytosolic calcium. The average of 3 images was recorded every 3 s. All drugs were applied to the dish with a micropipette. In some experiments, cells were incubated for 5 min at room temperature with nifedipine (5 μM) prior to the addition of TG. Cell fluorescence was subtracted from background and autofluorescence.
Reverse hemolytic plaque assay (RHPA). To identify insulin-secreting cells and measure insulin secretion by single cells we used a RHPA (16) as described previously (15). Briefly, after 48 h in culture, cells were challenged for 1 h in HBSS containing 5.6 or 15.6 mM glucose, in control conditions or with TG (200 nM), in the presence of an insulin antiserum (1:20 in HBSS), and further incubated for 30 min with guinea pig complement. Insulin released during the incubation time was revealed by the presence of hemolytic plaques surrounding insulin-secreting cells. The size of the plaques was measured by projecting the image on a monitor attached to a video camera and Nikon Axiophot inverted microscope, with the aid of the JAVA video analysis software (Version 1.40; Jandel Scientific, Corte Madera, CA).

Plaque size was expressed as area; cells that formed plaques were counted, and the results were expressed as the percentage of insulin secreting cells. All experiments were performed by duplicate, and at least 100 cells were counted per experimental condition. The overall secretory activity of β-cells under a given experimental condition was expressed as a secretion index, calculated by multiplying the average plaque area by the percentage of plaque-forming cells.

Statistical Analysis. Significant differences between data were evaluated by analysis of variance (ANOVA), followed by Fisher’s multiple range test, using the Number Cruncher statistical system (NCSS 4.2, Dr. Jerry L. Hintze, Kaysville, UT). All results are expressed as mean ± SEM.

RESULTS

TG increases a cationic current in β –cells. We used whole-cell configuration of the patch-clamp technique to measure the effects of TG on membrane currents in single rat β-cells. Figure 1A illustrates representative families of current traces recorded in response to different voltage pulses from a holding potential of -80 mV, before (1) and after exposure to 200 nM TG (2). The effects of
TG developed immediately after application of the reagent and were not reversible following its withdrawal (data not shown). Figure 1B shows the mean ± SEM current to voltage (I-V) relationships obtained in 4 different experiments. The reversal potential of the current in control conditions is around 0 mV and does not significantly changes with TG application.

The slope conductances were calculated according to equation 1 (see Material and methods section). Assuming a capacitance of 8.4 pF, slope conductance was 20 pS/pF for control cells and 33 pS/pF for TG-treated β-cells. These results demonstrate that TG increases a small cationic current (I_{cat}).

We then studied the ionic selectivity of I_{cat} by replacing Na^+ with NMDG or removing Ca^{2+} from the external solution. I-V curves obtained in each condition are illustrated in figure 2. As shown in Figure 2 A, I_{cat} decreased when Na^+ was replaced with NMDG in the external solution (Table 1, 2), compared to control conditions (figure 1B). The current measured at -100 mV (figure 2D) decreased nearly by 65 % compared to control conditions (Table 1, 1).

In contrast, in Ca^{2+} free solution (Table 1, 3) the current magnitude decreased, but changes are not statistically significant (Figure 2B and D) compared to control conditions (Fig 1B; Table 1, 1).

When both cations were omitted from the external media (Table 1, 4), the currents were minimized (figure 2C and D). Under these conditions, TG-sensitive I_{cat} was reduced by 82 % compared to the current recorded in the external solution with Na^+ and Ca^{2+} (Fig 1B; Table 1,1). These results indicate that the principal charge carrier of I_{cat} is Na^+, with a minimal contribution of Ca^{2+} ions.

_TG increases electrical activity in β-cells._ We studied the effects of TG on single β-cell electrical activity in 5.6 mM glucose with the current-clamp perforated patch-clamp technique. Figure 3A shows that in control conditions cells were polarized, around -70 mV and electrically silent. Few seconds after TG application the membrane slowly depolarized to a plateau level where action
potentials were superimposed. Figure 3B shows the mean firing frequency of 14 different cells in the presence of 5.6 mM glucose indicating that TG induced depolarization and electrical activity even at this low glucose concentration; when the $K_{ATP}$ is presumably active.

**TG increases intracellular calcium in β-cells.** In order to evaluate the effect of TG on $[Ca^{2+}]$, in 5.6 mM glucose we measured single β-cells fluorescence with confocal microscopy. Figure 4A shows that TG increased $[Ca^{2+}]$, in the cells, which remained elevated throughout the recording period. In contrast, in the presence of nifedipine, calcium signals were transient (figure 4B). This result indicates that the sustained phase of the $[Ca^{2+}]$, increment is the result of calcium influx through L-type Ca$^{2+}$ channels. Moreover, when $[Ca^{2+}]$, was measured in a Ca$^{2+}$ free-solution, the sustained phase of the calcium signal induced by TG decreased by nearly 92 % (figure 4C), consistently with the notion of the sustained phase is the result of L-type Ca$^{2+}$ channels activity.

**TG increases insulin secretion in different glucose concentrations.** In control experiments, when single β-cells are incubated for one hour in the presence of an insulin antiserum with a stimulatory glucose concentration of 15.6 mM, they secreted 2.5-fold more insulin than in the basal glucose in 5.6 mM glucose concentration (figure 5). In these conditions, TG increased individual insulin secretion, by 100 % in both glucose concentrations (table 2). TG also increased the percentage of β-cells that secreted insulin (plaque-forming cells) by 18 % and 34 %, in basal and stimulatory glucose concentrations, respectively. Consequently, TG increases total insulin secretion, given by the insulin secretion index (see Methods), by 2.5-fold in both glucose concentrations with respect to controls (figure 5 and table 2). The cellular mechanism that explains this increment involves the amplification of hormone secretion by individual cells and an increase in the percentage of insulin-secreting cells.
We have previously shown that the L-type channel blocker nifedipine does not affect basal insulin secretion (19). In this study, no difference was found when cells were incubated simultaneously with TG and nifedipine in 5.6 mM glucose (figure 5 and table 2).

We have previously observed that in 15.6 mM glucose, nifedipine decreases insulin secretion index by nearly 60 % (19). In the present study, we have reproduced this result and observed that TG and nifedipine together, decreased insulin secretion index by 41% compared to control cells in 15.6 mM glucose.

In order to estimate the percentage of insulin secretion that is not dependent on L-type channel activation, we subtracted the insulin secretion index in the presence of nifedipine from the value obtained with TG and nifedipine together, and this result was expressed as a percentage of the control index in 15.6 mM glucose. When TG is present, nearly 23 % of the secretion in this high glucose concentration is not dependent on L-type calcium channel activation.

Finally, we found in three different experiments that the insulin secretion index in the complete absence of glucose is 176 ± 64 and 398 ± 100 in control conditions and with TG, respectively (p< 0.05). In the absence of glucose in the external solution, only a small percentage of cells secreted enough insulin as to be detected by the RHPA, 21 and 26 % in control and TG, respectively. Compared to the basal glucose concentration (5.6 mM), individual cells secreted nearly 0.5 times less insulin in 0 glucose. However, in the later condition TG increased insulin secretion by 126 %, indicating that TG induced increase is not dependent on glucose presence in the extracellular medium.
DISCUSSION

In this study we demonstrate that TG potentiates a cationic current ($I_{\text{cat}}$) already present in single β-cells under resting conditions. $I_{\text{cat}}$ is mainly carried by Na$^+$ with a small contribution of Ca$^{2+}$. This current depolarizes the membrane, which leads to Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels and increased insulin secretion by single rat β-cells.

Several studies have shown the involvement of $K_{\text{ATP}}$ and voltage-dependent channels in the electrical activity of β-cells, however few studies account for the importance of cationic channels in this process. These channels play an important role because they participate in the slow depolarization phase that precedes the fast depolarization and the plateau potential, where action potential firing is observed. Without the presence of this cationic channels, blockade of $K_{\text{ATP}}$ channels would not result in membrane depolarization.

In the first experiments we used TG as a tool to deplete intracellular [Ca$^{2+}$]$i$ stores and observed that TG depolarized the membrane. In this study, we focused in determining the immediate effects of TG on β-cells. We found that TG increases the magnitude of a cationic current ($I_{\text{cat}}$) already present in β-cells. $I_{\text{cat}}$ increment induces membrane depolarization and the concomitant activation of voltage-gated Na$^+$ and Ca$^{2+}$ channels, resulting in action potential firing and calcium influx through nifedipine-sensitive, L-type channels. The final consequence of the activation of $I_{\text{cat}}$ is a marked increment in insulin secretion even at basal glucose concentrations (where $K_{\text{ATP}}$ channels are presumably active).

Other studies have shown the presence of similar cationic currents, mainly permeable to Na$^+$ that are modulated by the muscarinic agonist carbachol, in mouse pancreatic β-cells and in the insulinoma cell line HIT-T15 (13). Moreover, a similar cationic current activated by maitotoxin (MTX) has been described in mouse pancreatic β-cells (23).

On the other hand, other Na$^+$ currents activated by maitotoxin and by depletion of intracellular Ca$^{2+}$ stores, referred to as Ca$^{2+}$-release-activated non-selective current ($I_{\text{CRAN}}$), have
been described in mouse β cells (18). The depletion of Ca\textsuperscript{2+} stores may also induce a sustained, voltage-independent Ca\textsuperscript{2+} entry (10, 14), which enhances glucose-induced electrical activity (23, 12) in β-cells.

We also demonstrate that TG induces a slow depolarization and increases electrical activity, maintaining high [Ca\textsuperscript{2+}],. Moreover, when nifedipine was added, Ca\textsuperscript{2+} signals decreased even in the continuous presence of TG (figure 4B), indicating the importance of L-type Ca\textsuperscript{2+} channel activity for the sustained influx of calcium evoked by TG. It is unlikely that TG effects on electrical activity and insulin secretion would be produced by the direct activation of L-type Ca\textsuperscript{2+} channels because it has been shown that TG does not directly affect L-type Ca\textsuperscript{2+} currents in β-cells (9).

It has been previously shown that exposure of islets to TG in 10 mM glucose resulted in increased action potential firing of β-cells (6). However, in this stimulating glucose concentration, TG effect was considered unlikely to be due to the activation of a depolarizing cationic conductance.

A Ca\textsuperscript{2+}-dependent, non-selective cation current, activated by glucagon-like peptide 1 (GLP-1), has been observed in HIT, mouse and human β-cells (7). The GLP\textsubscript{1}- activated channel is active in the presence of low glucose, which suggests that it could play a role in membrane depolarization (8). Like I\textsubscript{CRAN}, this current is activated by MTX and blocked by SKF96365, but whether or not these currents are identical remains to be determined.

We have previously described that insulin secretion by single β-cells in 15.6 mM glucose is nearly 2.5-fold higher than in 5.6 mM glucose (4). In this study, we observed that acute TG treatment increases insulin secretion in both glucose concentrations. This effect is reflected in two measured parameters:

a) Amplification of insulin secretion by individual cells, because TG increases the plaque area which is proportional to the amount of hormone secreted by the cell, by nearly 100 % in both glucose concentrations.
b) The recruitment of previously silent cells which in control conditions do not secrete a detectable amount of insulin, because in the presence of TG the percentage of insulin-secreting cells increases by 18\% and 34\%, in 5.6 mM and 15.6 mM glucose, respectively.

TG also increases insulin secretion in the absence of glucose in the extracellular medium by 126\% compared to the control. This increment is very similar to that previously observed in single β-cells treated with carbachol in 0 glucose (5).

TG may use different mechanisms to increase insulin secretion. For example, TG could transiently increase $[Ca^{2+}]_i$ by depleting intracellular Ca\(^{2+}\) stores, however our results suggest that the principal mechanism involved is an increase in the cationic current that leads to membrane depolarization and Ca\(^{2+}\) entry through L-type channels. In fact, the effect of TG on insulin secretion in 5.6 mM glucose is completely abolished by nifedipine, while in 15.6 mM glucose it is only partially inhibited.

We conclude that in basal glucose concentration (5.6 mM), depletion of intracellular Ca\(^{2+}\) stores by TG application increases the activity of a cationic current ($I_{\text{cat}}$). The mechanism leading to $I_{\text{cat}}$ activation after depletion of internal Ca\(^{2+}\) stores remains to be identified.

$I_{\text{cat}}$ carried mostly by Na\(^+\), depolarizes the plasma membrane which results in the subsequent activation of voltage-sensitive Na\(^+\) and Ca\(^{2+}\) channels; Ca\(^{2+}\) entry, and as a direct consequence of these actions, insulin secretion (figure 6).

The mechanism leading to cationic channel modulation in normal β-cells remains to be established. However, the properties of the cationic current described here resemble those previously found in the literature for non-selective cationic channels activated upon depletion of intracellular Ca\(^{2+}\) stores (18, 10, 14) and more interestingly, to carbachol-induced cationic currents (13). Since carbachol application would result in inositol triphosphate (IP\(_3\)) production and release of Ca\(^{2+}\) from internal stores, it is probable that TG could promote similar effects on $[Ca^{2+}]_i$, mimicking the effects of carbachol. This could be a plausible mechanism for the modulation of $I_{\text{cat}}$ to control insulin secretion.
FOOTNOTES

1 The following abbreviations were used: TG, thapsigargin; [Ca\(^{2+}\)]\(_i\), intracellular Ca\(^{2+}\) concentration; KATP, ATP-sensitive potassium channel; I\(_{\text{CRAN}}\), Ca\(^{2+}\)-release-activated non-selective current; ER, endoplasmic reticulum; RHPA, reverse hemolytic plaque assay; NMDG\(^+\), N-methyl-D-glucamine; I\(_\text{cat}\), cationic current modulated by TG; NSCC, non-selective cationic channels; GLP1, glucagon-like peptide 1; IP\(_3\), inositol triphosphate; MTX, maitotoxin.
ACKNOWLEDGMENTS

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GRANTS

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REFERENCES


FIGURE LEGENDS

**Fig. 1.** TG activates an inwardly rectifying current in beta cells. Panel A illustrates representative current families elicited by voltage steps from a holding potential of -80 mV (see Experimental procedures section). (1) Cells under control conditions and (2) immediately after 200 nM TG. B, Mean I-V relationships obtained from 9 cells from 4 different experiments, control conditions (solid circles) and after 200 nM TG (open circles).

**Fig. 2.** The TG-induced current is carried mainly by sodium ions. A, Mean I-V relationships obtained in a Na⁺-free solution (table 1, 2) under control conditions (solid circles) and after 200 nM TG (open circles). B, Mean I-V relationships obtained with the Ca²⁺-free solution (Table 1,3). C, Mean I -V relationships obtained the extracellular solution Na⁺- and Ca²⁺-free (Table 1,4). D, Mean peak current obtained at – 100 mV for the conditions explained above. Notice the small current obtained with 10 mM Ca²⁺ in the absence of extracellular Na⁺. In all cases measurements were obtained from at least 20 different cells from 4 different experiments.

**Fig. 3.** TG depolarizes beta cells. A, current-clamp representative experiment of a single β cell measured with the perforated patch-clamp technique (see Experimental procedures section). At time zero, (indicated by the vertical arrow) 200 nM TG was applied, resulting in a slow depolarization which produced cell action potential firing. B, Average firing frequency before and after TG addition. Values measured from 14 independent cells obtained from 3 different experiments.

**Fig. 4.** Effect of nifedipine on the TG-induced calcium increment. Single beta cells cultured for 48 h were platted on glass coverslips and loaded with 5 µM Fluo-4-AM as indicated in Experimental
procedures section. Each panel illustrates a representative single cell (upper panels in A and B) obtained at time points indicated by the vertical arrows. A, cells exposed to 200 nM TG under control conditions and B, cells exposed to 200 nM TG in the continuous presence of 5 µM nifedipine. Each solid line shown in the lower panels from A and B illustrates the fluorescence increment (ΔF/ΔFo) in response to TG from a single cell. C, mean ± SD of the fluorescence increment measured at the time point illustrated by the last arrow on A and B. Fluorescence increments induced by the addition of 200 nM TG under control conditions (+Ca²⁺), in the presence of 5 mM EGTA in the bath (Ca²⁺-free) and in the presence of normal extracellular Ca²⁺ and 5 µM nifedipine (NIF). Measurements obtained from at least 20 different cells from 4 independent experiments.

Fig. 5. Effect of TG on insulin secretion by single β cell. Insulin secretion by single β cells was measured with the RHPA (see Experimental procedures section). The overall secretory activity of β cells under a given experimental condition is expressed as a secretion index. C, represents control cells; TG, cells exposed to thapsigargin (200 nM); NIF, cells exposed to nifedipine (5 µM). Data are the mean ± SEM of four different experiments by duplicate. Symbols denote statistically significant differences (*) with respect to control cells in 5.6 mM glucose, (†) with respect to TG treated cells in each glucose concentration, (‡) with respect to control cells in 15.6 mM glucose, (§) with respect to cells exposed to nifedipine to 15.6 mM glucose; p < 0.05.

Fig. 6. Proposed model for TG effect on membrane depolarization and insulin secretion. In basal glucose concentration (5.6 mM), depletion of intracellular Ca²⁺ stores by TG application increases the activity of Icat. The mechanism leading to Icat activation after depletion of internal Ca²⁺ stores remains to be identified (question mark). This current, depolarizes the plasma membrane which results in the subsequent activation of voltage-sensitive Na⁺ and Ca²⁺ channels, Ca²⁺ entry, and insulin secretion by pancreatic β-cells.
<table>
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<th>NaCl</th>
<th>KCl</th>
<th>CaCl₂</th>
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<td>(4) WCR</td>
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<td>(5) Current Current clamp and Confocal microscopy</td>
<td>120</td>
<td>4.75</td>
<td>1.8</td>
<td>-</td>
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<td>5.6</td>
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<td>1.2 KH₂PO₄ 1.2 Mg₂SO₄ 0.05% BSA</td>
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pH 7.35, 290-295 mOsm/kg. TTX in nM concentration
Table 2. Insulin secretion by single β-cells in response to a one-hour incubation with TG in different extracellular glucose concentrations.

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<th>Condition</th>
<th>Glucose (mM)</th>
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<td>15.6</td>
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(*) Denotes significance level with respect to the control in 5.6 mM glucose; p<0.01 (†) Denotes significance level with respect to the control in 15.6 mM glucose, in both groups; p<0.01, (n = 4 experiments by duplicate). + n = 3 experiments by duplicate. Data are the mean ± SEM.
Figure 1

A

B
Figure 3

A

B
Figure 4

A

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C

\[ \frac{\Delta F}{\Delta F_0} \]

\begin{tabular}{c c c}
+Ca^{2+} & -Ca^{2+} & NIF \\
30 & 5 & 10 \\
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Figure 6