H$_2$O$_2$-induced Ca$^{2+}$ influx and its inhibition by N-(p-amylcinnamoyl)anthranilic acid in the β-cells: involvement of TRPM2 channels

Muhammad R. Bari $^a$, Sanian Akbar $^a$, Mohamed Eweida $^a$, Frank J.P. Kühn $^b$, Amanda Jabin Gustafsson $^a$, Andreas Lückhoff $^b$, Md. Shahidul Islam $^{a,c,*}$

$^a$ Karolinska Institutet, Department of Clinical Sciences and Education, Research Centre, Stockholm South Hospital, Stockholm, Sweden
$^b$ Institute of Physiology, Medical Faculty, University hospital RWTH, Aachen, Germany
$^c$ Uppsala University Hospital, AR division, Uppsala, Sweden

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Abstract

Type 2 melastatin-related transient receptor potential channel (TRPM2), a member of the melastatin-related TRP (transient receptor potential) subfamily is a Ca$^{2+}$-permeable channel activated by hydrogen peroxide (H$_2$O$_2$). We have investigated the role of TRPM2 channels in mediating the H$_2$O$_2$-induced increase in the cytoplasmic free Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) in insulin-secreting cells. In fura-2 loaded INS-1E cells, a widely used model of β-cells, and in human β-cells, H$_2$O$_2$ increased [Ca$^{2+}$]$_i$, in the presence of 3 mM glucose, by inducing Ca$^{2+}$ influx across the plasma membrane. H$_2$O$_2$-induced Ca$^{2+}$ influx was not blocked by nimodipine, a blocker of the L-type voltage-gated Ca$^{2+}$ channels nor by 2-aminoethoxydiphenyl borate, a blocker of several TRP channels and store-operated channels, but it was completely blocked by N-(p-amylcinnamoyl)anthranilic acid (ACA), a potent inhibitor of TRPM2. Adenosine diphosphate ribose, a specific activator of TRPM2 channel and H$_2$O$_2$, induced inward cation currents that were blocked by ACA. Western blot using antibodies directed to the epitopes on the N-terminal and on the C-terminal parts of TRPM2 identified the full length TRP M2 (TRPM2-L), and the C-terminally truncated TRPM2 (TRPM2-S) in human islets. We conclude that functional TRPM2 channels mediate H$_2$O$_2$-induced Ca$^{2+}$ entry in β-cells, a process potently inhibited by ACA.

Keywords: calcium influx • TRPM2 • TRP channels • insulin-secreting cells • microfluorometry • N-(p-amylcinnamoyl)anthranilic acid • calcium signalling

Introduction

The type 2 melastatin-related transient receptor potential channel (TRPM2; formerly called TRPC7 and LTRPC2) is a non-specific cation channel permeable to Na$^+$, K$^+$ and albeit weakly to Ca$^{2+}$ [1]. In addition to the full-length TRPM2 (called TRPM2-L), a short form of TRPM2 (called TRPM2-S), where the four C-terminal trans-membrane domains and the putative pore-forming domain are deleted, have been described. TRPM2 is activated by hydrogen peroxide (H$_2$O$_2$), a model substance used as a paradigm of oxidative stress. TRPM2 channels are, thus, thought to be sensors for oxidative stress. Reactive oxygen species and oxidative stress have been implicated in the pathogenesis of diabetes. A number of studies have demonstrated that H$_2$O$_2$ induces β-cell death [2, 3]. There is evidence suggesting that TRPM2 mRNA is expressed in human islets [4]. TRPM2 current has been studied mostly in rat insulinoma RIN-5F, and Cambridge rat insulinoma G1 (CRI-G1) cells [5, 6]. In these cells, it has been demonstrated that TRPM2 channels are involved in insulin secretion [5]. Moreover, it has been shown that H$_2$O$_2$-induced death of insulinoma cells is prevented by antisense TRPM2 [7]. However, insulinoma cells of type RIN-5F and CRI-G1 are highly undifferentiated, poorly glucose responsive and thus, show limitations as models of β-cells. A better
model of β-cells is INS-1E cells. These are highly differentiated rat insulinoma cells that are currently widely used in experimental diabetes research [8]. It is not known whether INS-1E cells express functional TRPM2 channels. Moreover, TRPM2 proteins have not yet been demonstrated in human β-cells. The main aims of this study were to: (i) investigate the role of the TRPM2 channels in H₂O₂-induced [Ca²⁺]ᵢ increase in INS-1E cells; (ii) to test N-(p-amylcinnamoyl)anthranilic acid (ACA) as an inhibitor of TRPM2 channels in these cells and (iii) to identify the TRPM2 proteins in human islets.

Materials and methods

Materials

Fura-2 AM was from Invitrogen (Stockholm, Sweden). H₂O₂ (30% [W/W]), adenosine diphosphate ribose, nimodipine, 2-aminooxydiethylphosphoryl borate (2-APB) were from Sigma. ACA was from Calbiochem (Stockholm, Sweden). INS-1E cells were from C. B. Wollheim, Geneva, Switzerland.

Cell culture

We used a highly differentiated rat insulinoma cell line INS-1E cells (S5 clone) [8, 9]. Glucose-stimulated insulin secretion in these cells is similar to that reported previously [8, 9]. The cells were cultured in RPMI-1640 medium, supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 500 μM 2-mercaptoethanol (2-ME), 2.5% foetal bovine serum, 50 μg/ml penicillin and 50 μg/ml streptomycin.

Isolation and culture of human islets

Human islets were provided by the Cell Isolation and Transplantation Centre at the University of Geneva School of Medicine. Use of islets for in vitro experiments was approved by the local ethical committee. Islets from three donors were used for experiments. Islets were purified by an automated procedure using a continuous digestion-filtration device [10, 11]. Islets were dispersed into single cells by repeated pipetting after automated procedure using a continuous digestion-filtration device [10, 11]. Islets were dispersed into single cells by repeated pipetting after digestion in 0.025% trypsin in Ca²⁺- and Mg²⁺-free HBSS. Dispersed cells were cultured on glass cover slips for measurement of [Ca²⁺]ᵢ.

Measurement of [Ca²⁺]ᵢ

Cells cultured on cover slips were incubated in RPMI 1640 containing 0.1% bovine serum albumin (BSA) and 1 μM fura-2 AM for 35 min. The cover slips were then left for 10 min. at room temperature (RT) in a solution containing (in mM) 140 NaCl, 3.6 KCl, 0.5 NaH₂PO₄, 0.5 MgSO₄, 1.5 CaCl₂, 10 HEPES, 3 glucose and 0.1% BSA (pH 7.4). Nominal Ca²⁺ was replaced by 150 mM N-methyl-D-glucamine (NMDG⁺) and pH was titrated with HCl. The pipette solution contained (in mM) 145 Cs-glutamate, 8 NaCl, 2 MgCl₂, 1 Cs-EGTA, 0.88 CaCl₂, 10 HEPES, pH adjusted by CsOH to 7.2. This solution contained 1 μM free Ca²⁺. If not otherwise stated cells were held at a potential of ~60 mV at 22°C. The current–voltage (I–V) relations were obtained during voltage ramps from −90 to +60 mV applied for 400 ms.

Recording of TRPM2 current

Cells were analysed with the patch-clamp technique in the whole-cell mode, using an EPC 9 patch-clamp amplifier equipped with a personal computer with Pulse and X chart software (HEKA, Lampercht, Germany). The extracellular solution contained (in mM) 140 NaCl, 1.2 MgCl₂, 1.2 CaCl₂, 5 KCl, 10 HEPES, pH adjusted by NaOH to 7.4. For Na⁺-free solutions, NaCl was replaced by 150 mM N-methyl-D-glucamine (NMDG⁺) and pH was titrated with HCl. The pipette solution contained (in mM) 145 Cs-glutamate, 8 NaCl, 2 MgCl₂, 1 Cs-EGTA, 0.88 CaCl₂, 10 HEPES, pH adjusted by CsOH to 7.2. This solution contained 1 μM free Ca²⁺. If not otherwise stated cells were held at a potential of ~60 mV at 22°C. The current–voltage (I–V) relations were obtained during voltage ramps from −90 to +60 mV applied for 400 ms.

Western blot analysis of human islets

Human islets were homogenized in an ice-cold buffer consisting of 150 mM NaCl, 20 mM Tris, pH 7.5, 1% NP40, 1 mM ethylenediaminetetraacetic acid and protease inhibitors. The homogenate was centrifuged at 20,000 rpm for 30 min. at 4°C. The supernatant containing the membrane proteins was collected, and protein concentration was measured by Bio-Rad protein assay kit (Biorad, Sundbyberg, Sweden). A total of 90 μg of protein was fractionated by 10% SDS-polyacrylamide gel electrophoresis and transferred to PVDF membrane. The membranes were blocked by 5% non-fat milk overnight at 4°C, in Tris-buffered saline with Tween-20 (TBS-T). This step was followed by overnight incubation at 4°C with the primary TRPM2 antibody (dilution 1:300). The primary antibodies were: (i) an affinity-purified rabbit polyclonal IgG directed against an epitope on the N-terminal part of TRPM2 (anti-TRPM2-N) (BL 970. Cat. no. A300–414A, Bethyl Laboratories, Inc.). The PVDF membranes were then washed with TBS-T buffer, and incubated with goat anti-rabbit IgG conjugated to horseradish-peroxidase (1:10,000) for 1 h at RT. Membranes were washed and the immunoreactive bands were detected by enhanced chemiluminescence method and exposure to x-rays film. Antibody specificity was tested by using a blocking peptide (TRPM2 blocking peptide BP969, catalogue no. BP300–413A, Bethyl Laboratories, Inc.).

Statistical analysis

Data are displayed as mean ± S.E.M., n indicating the number of independent experiments. Student’s t-test (unpaired) was used for testing statistical significance and P < 0.05 was accepted as statistically significant.
Results

H$_2$O$_2$ increased [Ca$^{2+}$]$_i$ by inducing Ca$^{2+}$ entry through TRPM2 channels

INS-1E cells were cultured in the presence of 2-ME. During the measurement of [Ca$^{2+}$]$_i$, 2-ME was omitted from the medium. To limit damage to the cells during the experiments, the duration of the experiments was kept as short as possible. To be able to detect relatively small changes in [Ca$^{2+}$]$_i$, we loaded cells with low concentration of fura-2, because the chelating action of high concentrations of fura-2 makes detection of small [Ca$^{2+}$] changes difficult [13]. We first established the concentrations of H$_2$O$_2$ that could increase [Ca$^{2+}$]$_i$ reproducibly, without causing major damage to the cells. As shown in Fig. 1A, after the application of H$_2$O$_2$ (200 µM), [Ca$^{2+}$]$_i$ increased to a plateau in ~2–3 min., the magnitude of maximal increase being 29 ± 3 nM (n = 16). All [Ca$^{2+}$] changes were confirmed to be true [Ca$^{2+}$]$_i$ changes, by examining the respective F340 and F380 traces, which moved in opposite directions with change of [Ca$^{2+}$]$_i$. No [Ca$^{2+}$] change was observed for a period of up to 10 min. in cells where H$_2$O$_2$ was not applied (Fig. 1C). After the washout of H$_2$O$_2$, [Ca$^{2+}$]$_i$ returned to the baseline indicating that there was no major drift in the baseline (Fig. 1A). A return of [Ca$^{2+}$]$_i$ to the basal level also suggested that the cells were not severely damaged by exposure to 200 µM H$_2$O$_2$. 50 µM H$_2$O$_2$ increased [Ca$^{2+}$]$_i$, the magnitude of which was comparable to that obtained with 200 µM H$_2$O$_2$ (Fig. 1E). However, [Ca$^{2+}$]$_i$ increase by 50 µM H$_2$O$_2$ was more variable compared to that obtained by 200 µM H$_2$O$_2$ (Fig. 1E). [Ca$^{2+}$]$_i$ increases by higher concentrations of H$_2$O$_2$ (e.g. 500 µM and 1 mM) were not completely reversible on washout which suggested persistent activation of the Ca$^{2+}$-entry pathways (Fig. 1B and D). [Ca$^{2+}$]$_i$ response to a given concentration of H$_2$O$_2$ (i.e. 200 µM or 500 µM) varied, but on the average, the magnitude of [Ca$^{2+}$]$_i$ increase obtained by 200 µM H$_2$O$_2$ was similar to that obtained by 500 µM H$_2$O$_2$ (Fig. 1E). For these reasons, we used either 200 µM or 500 µM of H$_2$O$_2$ in subsequent experiments. As shown in Fig. 1F and G, H$_2$O$_2$ (200 µM and 500 µM) increased [Ca$^{2+}$]$_i$ in INS-1E cells and human β-cells. When Ca$^{2+}$ was omitted from the extracellular medium, [Ca$^{2+}$]$_i$ response to 200 µM H$_2$O$_2$ was abolished (Fig. 2B and C). In these experiments, cells were exposed to nominally Ca$^{2+}$-free medium for 1 min. before addition of H$_2$O$_2$. In separate experiments, we established that exposure of cells to nominally Ca$^{2+}$-free medium for such short period, did not deplete the ER Ca$^{2+}$ store, because carbacbol increased [Ca$^{2+}$]$_i$ by releasing Ca$^{2+}$ from the ER under such conditions (data not shown). The maximal [Ca$^{2+}$]$_i$ change in the Ca$^{2+}$-containing and the Ca$^{2+}$-free medium were 39 ± 2 nM and 5 ± 10 nM, respectively (P = 0.01, n = 6). In Fig. 2D, [Ca$^{2+}$]$_i$ was first raised by 500 µM H$_2$O$_2$ in the presence of Ca$^{2+}$-containing extracellular medium. When [Ca$^{2+}$]$_i$ increased to a plateau, the medium was switched to the nominally Ca$^{2+}$-free medium. This resulted in the return of [Ca$^{2+}$]$_i$ to the basal level, indicating that the [Ca$^{2+}$]$_i$ increase by H$_2$O$_2$ was due to the entry of Ca$^{2+}$ across the plasma membrane (Fig. 2D, c.f. Fig. 1B).

[Ca$^{2+}$]$_i$ increase by H$_2$O$_2$ (500 µM) was not inhibited by nimodipine (5 µM), a blocker of the L-type voltage-gated Ca$^{2+}$ channels (Fig. 3A and B). The maximal [Ca$^{2+}$]$_i$ increase by H$_2$O$_2$ in the control group and in the nimodipine group were 27 ± 5 and 25 ± 6 nM, respectively (P = 0.63 n = 15) (Fig. 3C). 2-APB (50 µM), a blocker of several TRP channels and some store-operated Ca$^{2+}$ channels, also did not inhibit H$_2$O$_2$-induced Ca$^{2+}$ entry (Fig. 3D and E).
The magnitudes of \([Ca^{2+}]_{i}\) increases in the control group and in the 2-APB group were 13 ± 2 and 14 ± 1 nM, respectively \((P = 0.75, n = 10)\) (Fig. 3F). Flufenamic acid and econazole are two inhibitors of TRPM2 [14, 15]. We found that both flufenamic acid and econazole increased \([Ca^{2+}]_{i}\) by themselves and were thus, not suitable for use in further experiments (data not shown). Instead, we tested the effect of ACA, a potent blocker of TRPM2 [16]. In separate experiments, we demonstrated that ACA (20 μM) itself did not increase \([Ca^{2+}]_{i}\) in INS-1E cells (Fig. 3G). As shown in Fig. 3H (trace a), H2O2 (200 μM) induced a typical increase of \([Ca^{2+}]_{i}\) in the control cells. When H2O2 was applied in the presence of ACA (20 μM), there was no increase of \([Ca^{2+}]_{i}\) (trace b). Instead, on the average, \([Ca^{2+}]_{i}\) decreased in the ACA-treated cells, despite continued presence of H2O2. The maximal changes of \([Ca^{2+}]_{i}\) by H2O2 in the absence of, and in the presence of ACA were 23 ± 1 and −9 ± 6 nM, respectively \((P = 0.01, n = 6)\) (Fig 3I). These observations indicated that the H2O2-induced \([Ca^{2+}]_{i}\) entry was due to the activation of the TRPM2 channels.

**TRPM2 current in INS-1E cells**

In patch-clamp experiments 0.6 mM ADP ribose was dialysed into the cells through the patch pipette. The pipette solution also contained 1 μM Ca^{2+} to facilitate development of ADP ribose-dependent current [17]. Figure 4A shows rapid development of inward currents of −1 nA after establishment of the whole-cell configuration. The inward currents were minimized when extracellular Na^{+}...
In the absence of ADP ribose, no such current developed, even if cells were infused with pipette solution containing 1 mM Ca²⁺ (see first 150 sec. in the recording of Fig. 4B). After extracellular application of H₂O₂ (~10 mM), inward currents developed gradually and the currents were immediately suppressed when the bath solution contained NMDG⁺ (Fig. 4B). The currents could be repeatedly restored by reperfusion with standard bath solution, even when the solution did not contain H₂O₂ (Fig. 4B). After external application of ACA (50 µM), the current gradually declined to almost basal levels. After washout of ACA, currents were partially restored demonstrating that the ACA effect was reversible (Fig. 4B). The corresponding I–V relation is shown in Fig. 4C. The H₂O₂-evoked currents showed a reversal potential close to 0 mV and inward currents were minimized in the presence of NMDG⁺, which is characteristic for a non-selective cation current (NSCC) like TRPM2.

TRPM2 proteins in human islets

We performed Western blotting with membrane preparations from human islets. The blot was probed with anti-TRPM2-N and anti-TRPM2-C antibodies. The immunogen for anti-TRPM2-N was the peptide ILKELSKEEDETDSEEMLA, which represents the amino acids 658–677 of human TRPM2 encoded within exon 13. The immunogen for anti-TRPM2-C was the peptide KAAEEPDAEPRKKTPEEGDS, which represents amino acids 1216–1237 of human TRPM2 encoded within exon 25. In Western blotting of human islets ~171 kD bands representing TRPM2-L were detected by both anti-TRPM2-N (Fig. 5A) and anti-TRPM2-C antibodies (Fig. 5A1). As expected, a ~95 kD band representing the TRPM2-S was detected by the anti-TRPM2-N antibody (Fig. 5A) but not by the anti-TRPM2-C antibody (Fig. 5A1). In control experiments we found that the ~75 kD bands were non-specific ones, because they were detected even when the membranes were treated with the corresponding blocking peptides.
Discussion

Effects of H$_2$O$_2$ on [Ca$^{2+}$]$_i$ changes in INS-1E cells have been reported before [2]. However, it remained unknown whether H$_2$O$_2$ can trigger Ca$^{2+}$ influx in these cells, and in that case, what could be the identity of the Ca$^{2+}$ influx pathways. The emergence of TRPM2 as a H$_2$O$_2$-sensitive channel prompted us to examine if TRPM2 could be a link between H$_2$O$_2$ and [Ca$^{2+}$]$_i$ increase in INS-1E cells. We demonstrate that a short (~5 min.) exposure of H$_2$O$_2$ (50–500 μM) to INS-1E cells, increased [Ca$^{2+}$]$_i$ solely by inducing Ca$^{2+}$ entry across the plasma membrane. This was evident from the observation that no [Ca$^{2+}$]$_i$ increase by H$_2$O$_2$ was observed when Ca$^{2+}$ was omitted from the extracellular medium. H$_2$O$_2$ activates TRPM2 by acting on the cytoplasmic side of the channel [18]. Externally applied H$_2$O$_2$ does not freely pass through the plasma membrane [19]. For this reason, and because of the presence of catalase in the cytoplasm, the effective cytoplasmic concentration of H$_2$O$_2$ that increased [Ca$^{2+}$]$_i$ in our experiments, is likely to be lower. The high [Ca$^{2+}$]$_i$ increase caused by 1 mM H$_2$O$_2$ is due to multiple non-specific mechanisms including the release of Ca$^{2+}$ from the mitochondria and the ER, as has been described before [20, 21]. In our study, H$_2$O$_2$-activated Ca$^{2+}$ entry was not blocked by nimodipine, indicating that the L-type voltage-gated Ca$^{2+}$ channels did not mediate the Ca$^{2+}$ entry. Moreover, 2-APB did not block the Ca$^{2+}$ entry suggesting a lack of involvement of some of the store-operated Ca$^{2+}$ channels or the inositol 1,4,5 trisphosphate receptor [22]. As an inhibitor of TRPM2-channel, the role of 2-APB remains controversial [23, 24]. On the other hand, H$_2$O$_2$-induced Ca$^{2+}$ entry was completely blocked by ACA, a potent blocker of TRPM2 channel [16]. In fact, in ACA-treated cells there was, on the average, a small decrease in [Ca$^{2+}$]$_i$ suggesting some basal activity of TRPM2 channels at 37°C. ACA, however, is not entirely specific for TRPM2, because it also blocks TRPM8 and TRPC6 [16]. Nevertheless, Ca$^{2+}$ entry that is induced by H$_2$O$_2$ and ADP ribose, and is blocked by ACA is most likely to be mediated through the TRPM2 channels.

ACA has generally been used as an inhibitor of phospholipase A$_2$ (PLA$_2$), which plays an important role in mediating insulin secretion and [Ca$^{2+}$] oscillations in β-cell [25–29]. However, in our experiments, activation of TRPM2 by H$_2$O$_2$ did not involve activation of PLA$_2$. This is evident from the observation that H$_2$O$_2$ activated TRPM2 current, even when cells were internally dialysed and the recordings were performed at 22°C (Fig. 4B). In fact, it is known that H$_2$O$_2$ does not activate PLA$_2$, rather it inhibits the enzyme [30]. Given that ACA is now established as a potent blocker of TRPM2, caution is needed in interpreting previous reports where ACA was used as the sole inhibitor of PLA$_2$. Another inhibitor of PLA$_2$, namely AACOCF$_3$ does not inhibit TRPM2 [16]. However, AACOCF$_3$ was not suitable for use in our experiments, because it is oxidized by H$_2$O$_2$.

Consistent with H$_2$O$_2$-induced [Ca$^{2+}$]$_i$ increase, we recorded NSCC induced by H$_2$O$_2$ and ADP ribose in INS-1E cells. The NSCC was carried mainly by Na$^+$ in the inward direction. Such Na$^+$ currents were minimized by NMDG$^+$, in spite of the presence of 1.2 mM Ca$^{2+}$ in the external solution. In a previous study, we demonstrated that very small Ca$^{2+}$ currents could be detected when external Na$^+$ was replaced by NMDG$^+$, even when the external Ca$^{2+}$ concentration was raised to 10 mM [18]. The contribution of Ca$^{2+}$ to the overall currents was not resolved in the experiments shown in Fig. 4. It is known from previous studies that Ca$^{2+}$ is only weakly permeable through TRPM2 channel, the permeability ratio pCa:pNa being ~0.6–0.7 [31]. However, the permeability of Ca$^{2+}$ measured in the presence of high extracellular Ca$^{2+}$ can lead to an underestimation of Ca$^{2+}$ fluxes under physiological conditions [32]. Functionally, the importance of the Ca$^{2+}$ fluxes through the TRPM2 channels is evident from the increase in [Ca$^{2+}$]$_i$ detected by fura-2 after activation of TRPM2 by H$_2$O$_2$. In patch-clamp experiments, we also used 0.6 mM ADP ribose together with 1 μM free Ca$^{2+}$. This protocol allowed rapid development of a sizable current after attaining the whole-cell configuration (Fig. 4A). In contrast, the development of currents after extracellular application of H$_2$O$_2$, even in the presence of intracellular Ca$^{2+}$, was delayed. Such delay may be due to the fact that H$_2$O$_2$ does not gate TRPM2 directly, rather it may act by increasing the concentration of intracellular ADP ribose [33, 34]. The activation of TRPM2 currents by H$_2$O$_2$ was long lasting and persisted even after wash out of H$_2$O$_2$ an observation consistent with previous reports [18, 31]. After substitution of the NMDG$^+$-containing bath solution (which suppressed inward currents) with normal bath solution (which supported inward currents) the inward currents increased immediately to the maximal level. This indicated that the channel activation persisted after the initial exposure to H$_2$O$_2$. In contrast, the release from ACA-block showed much slower kinetics, an observation consistent with a decrease in the open probability of the channel by ACA [16]. Thus, we have demonstrated an inward current that is activated by ADP-ribose (Fig. 4A) and by H$_2$O$_2$ (Fig. 4B) and is inhibited by ACA. This pharmacological profile establishes that the current is mediated through the TRPM2 channels.

Furthermore, by Western blotting, we have demonstrated for the first time, the presence of TRPM2 proteins in human islets. By using anti-TRPM2-N and anti-TRPM2-C antibodies, we identified two isoforms of TRPM2 in these cells [35]. The anti-TRPM2-N antibody detected not only the full length TRPM2-L, but also the C-terminally truncated TRPM2-S, in human islets. TRPM2-S isoform appeared as a ~95 kDa band detected by the anti-TRPM2-N, and not by the anti-TRPM2-C. TRPM2-S itself does not form a channel but instead it acts as a dominant negative of TRPM2-L [35]. Thus, human β-cells have not only the H$_2$O$_2$-sensitive isoform but also the protective isoform of TRPM2. The relative abundance of these isoforms may determine the extent of H$_2$O$_2$-induced TRPM2-mediated Ca$^{2+}$ influx [36].

Previous studies have examined the effects of H$_2$O$_2$ on insulin secretion, [Ca$^{2+}$]$_i$ changes, membrane potential changes, glucose metabolism, mitochondrial metabolism and β-cell death [2, 20, 21, 37]. It is evident from these reports that exposure of β-cells to relatively high concentration of H$_2$O$_2$ for prolonged period inhibits metabolism, leading to opening of the KATP channels, hyperpolarization of membrane potential and inhibition of insulin secretion.
More recently, Pi et al. demonstrated that 1–4 μM H2O2 induces insulin release from INS-1 cells suggesting that H2O2 may act as a signal for insulin secretion [3]. These investigators used a clone of INS-1 cells which is different from ours. The authors did not report whether 1–4 μM H2O2 increased [Ca2+]i in their clone of INS-1 cells. Maechler et al. demonstrated that the threshold concentration of H2O2 for insulin secretion from INS-1E cells was 200 μM, a concentration that invariably increased [Ca2+]i in our experiments [2]. However, in our patch-clamp experiments, we used 10 mM H2O2 to obtain a large current that displays a fingerprint of current properties of TRPM2 channel. Even when TRPM2 current was activated by 10 mM H2O2, the current could be completely blocked by ACA. TRPM2 is a temperature sensitive channel and its regulation by temperature has been studied by Togashi et al. [5]. Whereas, our microfluorometry experiments were performed at 37°C, the patch-clamp experiments were done at 22°C for technical reasons. It may be mentioned that patch-clamp experiments are performed under conditions that are not strictly physiological, and it is often necessary to use 10 mM H2O2 for inducing TRPM2 currents in native cells when experiments are performed at 22°C [18, 37]. However, in transfected cells where TRPM2 is overexpressed, a sizable current can often be detected by micromolar H2O2 [7].

In summary, we have demonstrated that in INS-1E cells, H2O2 (50–500 μM) applied for a short period (5–10 min.) increased [Ca2+]i by triggering Ca2+ entry through the TRPM2 channels.

Consistent with this, H2O2- and ADP ribose-activated TRPM2 current was detected in INS-1E cells. By Western blotting, two major isoforms of TRPM2 were identified in human islets. It has been demonstrated that glucose increases production of H2O2 in INS-1(832/13) cells [3]. However, we demonstrate that concentrations of H2O2 that could be relevant from signalling point of view (e.g. 1–4 μM) fail to activate TRPM2 channel. Nevertheless, given the known roles of TRPM2 channels in redox- and cytokine-mediated cell death, these channels may be of relevance in the pathogenesis of type 1 and type 2 diabetes, where β-cell damage contributes to the disease process. Inhibitors of TRPM2 channels are thus, of potential interest as therapeutic agents.

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