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Low doses of hydrogen peroxide impair glucose-stimulated insulin secretion via inhibition of glucose metabolism and intracellular calcium oscillations

Eduardo Rebelato*, Fernando Abdulkader, Rui Curi, Angelo R. Carpinelli

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP 05508-000, Brazil

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Abstract

The inhibitory effect of hydrogen peroxide (H_2O_2) on glucose-stimulated insulin secretion was previously reported. However, the precise mechanism involved was not systematically investigated. In this study, the effects of low concentrations of H_2O_2 (5-10 μmol/L) on glucose metabolism, intracellular calcium ($[Ca^{2+}]_i$) oscillations, and dynamic insulin secretion in rat pancreatic islets were investigated. Low concentrations of H_2O_2 impaired insulin secretion in the presence of high glucose levels (16.7 mmol/L). This phenomenon was observed already after 2 minutes of exposure to H_2O_2. Glucose oxidation and the amplitude of $[Ca^{2+}]_i$ oscillations were dose-dependently suppressed by H_2O_2. These findings indicate that low concentrations of H_2O_2 reduce insulin secretion in the presence of high glucose levels via inhibition of glucose metabolism and consequent impairment in $[Ca^{2+}]_i$ handling.

1. Introduction

Chronically elevated glucose levels are known to cause cytotoxic effects possibly through an excessive formation of reactive oxygen species [1,2] that, associated with low activities of classic antioxidant enzymes in pancreatic islets [3], promotes oxidative stress and dysfunction of insulin-secreting cells. Furthermore, oxidative stress caused by hydrogen peroxide (H_2O_2) formation during amyloid aggregation has been related to the development of type 2 diabetes mellitus [4]. Hydrogen peroxide was reported to inhibit the activities of glyceraldehyde-3-phosphate-dehydrogenase (glycolytic pathway) [5] and aconitase (Krebs cycle) [6]. Thus, uncontrolled reactive oxygen species formation can overcome the intracellular redox balance with possible consequences to glucose metabolism. The impairment of β-cell metabolism by exposure to H_2O_2 was first suggested by Nakazaki et al (1995) [7]. This was confirmed by experiments in insulin-secreting cell lines that showed decreased intracellular levels of adenosine triphosphate (ATP) and inhibition of insulin secretion by high H_2O_2 concentrations [8]. Hydrogen peroxide exposure leads to a marked elevation in intracellular calcium ($[Ca^{2+}]_i$) concentration, presumably due to lipid peroxidation [9] or opening of calcium channels such as TRPM2, whose activity has been related to H_2O_2-induced β-cell death [10]. Sustained rises in $[Ca^{2+}]_i$ are closely associated to glucose-stimulated insulin secretion (GSIS); but in fact, oscillations of $[Ca^{2+}]_i$ better represent this process [11]. The above-mentioned effects of H_2O_2 were observed at pharmacologic concentrations [12], and there is no systematic report on the events of stimulus-secretion coupling involved in the impairment of β-cell function induced by H_2O_2. We show herein that low concentrations of H_2O_2 (5-10 μmol/L), close to physiologic levels [12], reduce insulin secretion through inhibition of glucose metabolism and $[Ca^{2+}]_i$ oscillations in rat pancreatic islets.

Conflict of interest: There is no conflict of interest with regard to any of the authors of this paper.

Animal institutional approval: This study was approved by the Animal Experimentation Committee of the Institute of Biomedical Sciences, University of Sao Paulo, and followed the institutional guidelines for laboratory animal care.

* Corresponding author. Tel.: +55 11 3091 7246; fax: +55 11 3091 7285.
E-mail address: rebelato@icb.usp.br (E. Rebelato).

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2. Materials and methods

2.1. Animals

Female albino rats (150-200 g) were obtained from the Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil. The animals were kept in groups of 5 at 23°C in a room with a light-dark cycle of 12:12 hours (lights on at 7:00 AM), having free access to food and water. This study was approved by the Animal Experimentation Committee of the Institute of Biomedical Sciences, University of Sao Paulo, and followed the institutional guidelines for laboratory animal care. The pancreatic islets were isolated by collagenase digestion [13].

2.2. Reagents

Type V collagenase, bovine serum albumin, and phenylethylamine were purchased from Sigma (St Louis, MO). [U-^{14}C]-Glucose, ^{125}I-insulin, and biodegradable scintillation liquid were obtained from Amersham (Little Chalfont, Bucks, United Kingdom). Insulin antibody was a gift from Dr Leclercq-Meyer, Université Libre de Bruxelles, Belgium. Hydrogen peroxide was obtained from Merck (Darmstadt, Germany). Fluo-4-AM was purchased from Invitrogen (Eugene, OR). D-Glucose and salts for buffer preparation were obtained from Labsynth (Diadema, Brazil).

2.3. Dynamic insulin secretion

Batches of 100 islets were transferred to a perifusion chamber. Perifusion was performed at 37°C with Krebs-Henseleit buffer (in millimoles per liter: 139 Na^+, 5 K^+, 1 Ca^{2+}, 1 Mg^{2+}, 124 Cl^-, 24 HCO_3^-; pH 7.4) containing albumin (0.2%) and different glucose concentrations at a constant flow rate of 1 mL/min. Perifusion medium was collected at 1- or 2-minute intervals starting at the 31st minute. The collected perifusate was frozen and assayed for insulin by radioimmunoassay. The first 30 minutes of perifusion were performed in the presence of 2.8 mmol/L glucose. The changes in glucose concentration and the additions of H_2O_2 are indicated in the figures.

2.4. Measurement of [U-^{14}C]-glucose oxidation

Groups of 225 islets were incubated in 2.4 mL of Krebs-Henseleit buffer with albumin (0.2%), at 37 °C, for 60 minutes in glass vials containing a filter paper and 400 μL phenylethylamine, diluted 1:1 vol/vol in methanol, in a separate compartment. The incubation buffer contained 3.7 kBq/mL [U-^{14}C]-glucose for 2.8 mmol/L glucose and 22.2 kBq/mL [U-^{14}C]-glucose for 16.7 mmol/L glucose in the absence or presence of H_2O_2 (5, 10, 20, or 200 μmol/L). The incubation was stopped by adding 400 μL HCl (10 mol/L), and the vials were shaken for an additional 60-minute period. The filter paper with phenylethylamine was transferred to a plastic tube with 1.8 mL of biodegradable scintillation liquid, and the $^{14}CO_2$ adsorbed was measured in a scintillation counter (Beckman Instruments, Fullerton, CA).

2.5. Intracellular calcium measurements

Freshly isolated rat islets were loaded with fluo-4-AM (2.5 μmol/L) in RPMI-1640 medium for 3 hours at room temperature. Islets were allowed to adhere to poly-L-lysine–pretreated glass coverslips mounted inside a heated chamber (37°C) on the stage of an inverted confocal microscope (LSM510 Axiovert 100M; Carl Zeiss, Jena, Germany). The preparation was then continuously perifused with Krebs-Henseleit buffer containing 2.8 mmol/L glucose (initial, 3 minutes), followed by 16.7 mmol/L glucose (37 minutes) with or without H_2O_2 (5 or 10 μmol/L) during the last 30 minutes of perifusion. Islets were excited at 488 nm, and emission was collected through a 505 to 550 nm band-pass filter [14]. Images were collected at 2-second intervals. Increases in $[Ca^{2+}]_i$ are displayed as upward deflections. Individual β-cells were selected as regions of interest that responded to high glucose with a rise in $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$ responses were determined by normalization to the highest fluorescence peak after baseline subtraction using Origin 7.0 software (OriginLab, Northampton, MA). The maximum $[Ca^{2+}]_i$ spike amplitude of single β-cells over 2-minute intervals was averaged during the last 30 minutes of recording.

2.6. Statistical analysis

Results are presented as means ± SEM. Statistical analysis was performed by analysis of variance and Tukey's multiple comparison post test. Differences were considered significant for $P < .05$.

3. Results

3.1. Effect of H_2O_2 on dynamic insulin secretion

The effect of a high concentration of H_2O_2 (200 μmol/L) on the dynamic insulin secretion was evaluated in 2.8 and 16.7 mmol/L glucose. In both glucose concentrations, insulin secretion was reduced (Fig. 1A). The stimulation of insulin secretion by the rise in glucose from 2.8 to 16.7 mmol/L (GSIS) was completely abolished. This concentration of H_2O_2 may have a toxic effect on pancreatic islets that would explain the absence of GSIS under this condition. We then investigated the effect of lower H_2O_2 concentrations (5, 10, and 20 μmol/L), which are closer to the extracellular concentrations of H_2O_2 associated with its signaling functions [12]. Short period (2 minutes) of intermittent exposure to low concentrations of H_2O_2 attenuated the increment in insulin secretion induced by 16.7 mmol/L, but no effect was observed at 2.8 mmol/L glucose (Fig. 1B). To determine if this inhibitory effect of H_2O_2 on insulin secretion at 16.7 mmol/L glucose is transient and dependent on the concentration of H_2O_2, we next performed perifusion experiments in which H_2O_2 (5 and 10 μmol/L) was applied concomitantly to the step in glucose concentration from 2.8 to 16.7 mmol/L. The exposure of islets to H_2O_2 dose-

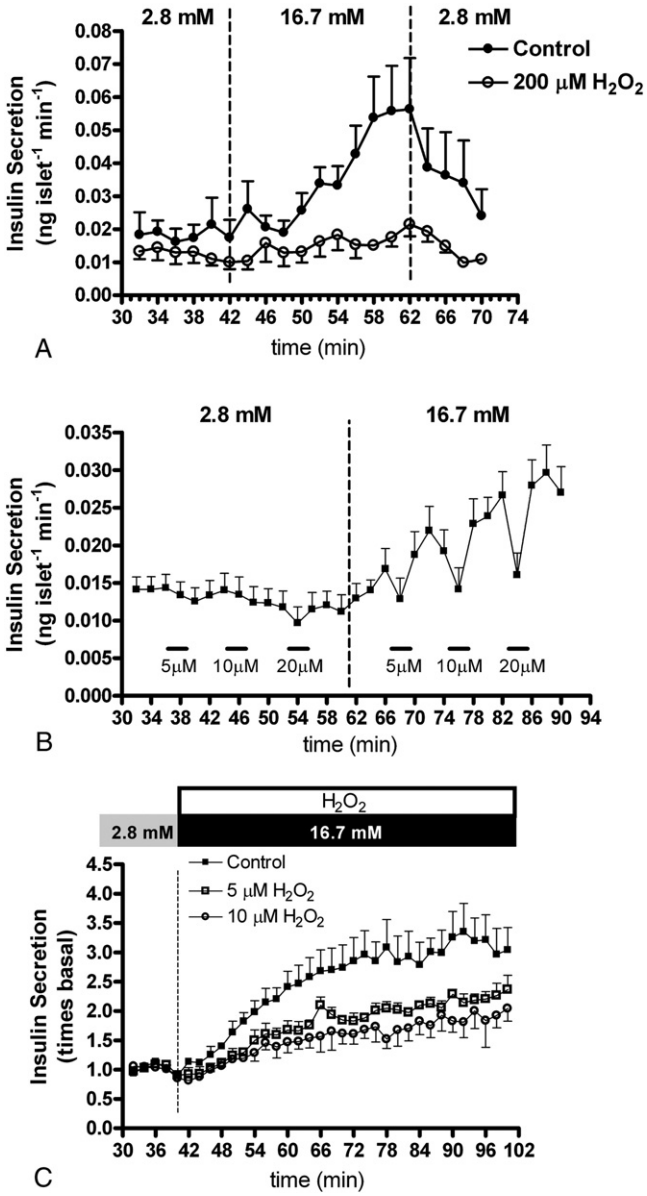

Fig. 1. Inhibition of dynamic insulin secretion by H_2O_2. The concentration of glucose in the perifusion medium was changed between 2.8 and 16.7 mmol/L as indicated. A, Perifusion of islets in the absence or presence of H_2O_2 (200 μmol/L) throughout the whole experiment (n = 6). B, Short-term infusion (2 minutes) of increasing concentrations of H_2O_2 (at 5, 10, and 20 μmol/L) during the islet perifusion is indicated in bars (n = 6). C, Perifusion of islets at 2.8 mmol/L glucose in the absence of H_2O_2 followed by 16.7 mmol/L glucose stimulus in the absence or presence of 5 or 10 μmol/L H_2O_2 (n = 4). The results are expressed as mean ± SEM.

dependently decreased insulin secretion induced by 16.7 mmol/L glucose (Fig. 1C). This inhibitory effect of H_2O_2 on GSIS lasted throughout the exposure to H_2O_2 (Fig. 1C).

3.2. Effect of H_2O_2 on glucose oxidation

The involvement of glucose metabolism in the effect of H_2O_2 on insulin secretion was evaluated in incubations of pancreatic islets in the presence of H_2O_2. The addition of 200 μmol/L H_2O_2 markedly reduced [U-^{14}C]-glucose oxidation at 2.8 (66%) and 16.7 mmol/L (77%) glucose (Fig. 2A). Lower concentrations of H_2O_2 (5, 10, and 20 μmol/L) dose-dependently decreased [U-^{14}C]-glucose oxidation (by 26%, 46%, and 58%, respectively) in the presence of 16.7 mmol/L glucose (Fig. 2B).

3.3. Effect of H_2O_2 on $[Ca^{2+}]_i$ oscillations

The effect of low concentrations of H_2O_2 on glucose-stimulated $[Ca^{2+}]_i$ oscillations was also studied. Hydrogen peroxide addition (5 and 10 μmol/L) time-dependently suppressed glucose-stimulated $[Ca^{2+}]_i$ oscillations (Fig. 3A). The inhibitory effect of H_2O_2 on maximum $[Ca^{2+}]_i$ spike amplitude was also concentration dependent (Fig. 3B, C).

4. Discussion

The findings presented herein show the inhibitory effect of low concentrations of H_2O_2 (at 5 and 10 μmol/L) on GSIS by hampering metabolic and $[Ca^{2+}]_i$-coupled mechanisms.

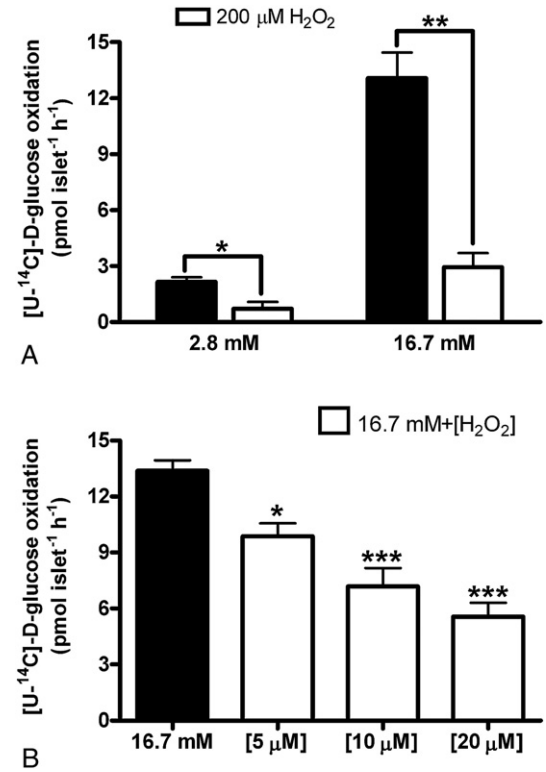

Fig. 2. Inhibition of [U-^{14}C]-glucose oxidation by H_2O_2. [U-^{14}C]-Glucose oxidation was measured in islets incubated for 60 minutes (A) at 2.8 and/or 16.7 mmol/L glucose in the absence or presence of 200 μmol/L H_2O_2 (n = 4) or (B) increasing concentrations of H_2O_2 (5, 10, and 20 μmol/L) at 16.7 mmol/L glucose (n = 6). The results are expressed as mean ± SEM. *P < .05, **P < .01, and ***P < .001 due to treatment with H_2O_2.

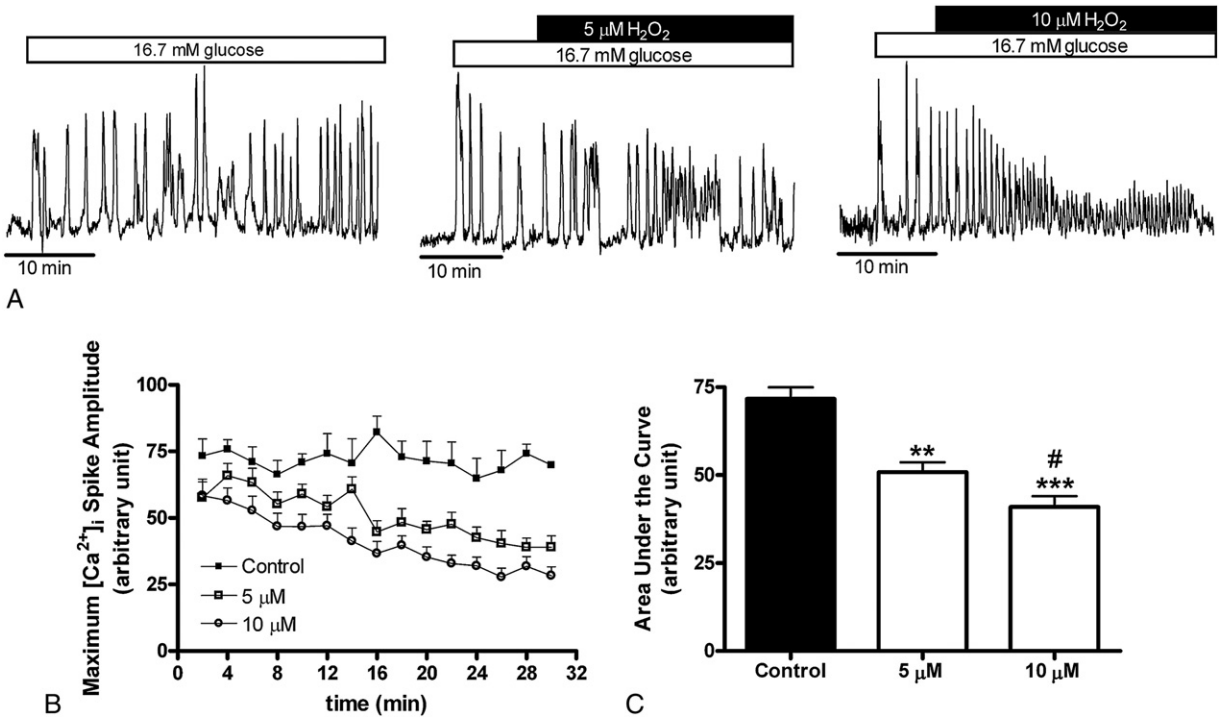

Fig. 3. Reduction of $[Ca^{2+}]_i$ oscillation amplitude by H_2O_2. A, Representative recordings of $[Ca^{2+}]_i$ response in β-cells to 2.8 and 16.7 mmol/L glucose without (left panel) and with 5 μmol/L (central panel) and 10 μmol/L (right panel) H_2O_2. B, The maximum $[Ca^{2+}]_i$ spike amplitude over 2-minute intervals during the last 30 minutes of recording with 16.7 mmol/L glucose stimulation in the absence (black squares) or in the presence of 5 (white squares) or 10 μmol/L (white circles) H_2O_2 (n = 50 cells from 5 independent experiments). C, Area under the curves from panel B. The results are expressed as mean ± SEM. $^{**}P < .01$ and $^{***}P < .001$ vs control; $^{\#}P < .05$ vs 5 μmol/L H_2O_2.

Hydrogen peroxide inhibited glucose oxidation and $[Ca^{2+}]_i$ oscillation response to glucose, impairing GSIS.

Nakazaki et al (1995) [7] were the first authors to report the effect of H_2O_2 on plasma membrane ion currents in pancreatic β-cells, using the patch-clamp technique. Exposure of these cells to H_2O_2 ($\geq$30 μmol/L) increased the activity of ATP-sensitive potassium channels in perforated whole-cell patch-clamp configuration, which was abolished in the presence of ATP. Action currents observed during perifusion with 11.1 mmol/L glucose were also suppressed [7]. The inhibitory effect of H_2O_2 on GSIS was attributed to the suppression of glucose metabolism and confirmed by the decrease in intracellular ATP concentration [8]. We also observed that high concentration of H_2O_2 (200 μmol/L) markedly suppressed both glucose oxidation and GSIS. These inhibitory effects of high levels of H_2O_2 were probably a consequence of oxidative stress. Accordingly, previous reports showed that exposure to 100 μmol/L H_2O_2 markedly increases the permeability of plasma membrane to Ca^{2+} [9,10].

Despite the information above, H_2O_2 plays an important role as a signaling molecule [15], being also reported to prevent apoptosis due to induction of thioredoxin-1 expression [16]. The maximum H_2O_2 levels that are intracellularly generated range from 0.5 to 0.7 μmol/L, when the extracellular concentrations are 10 times higher [12]. To achieve this intracellular condition, 5 and 10 μmol/L H_2O_2 were applied in dynamic insulin secretion experiments, resulting in suppression of GSIS. This effect was observed already after 2 minutes of exposure in the presence of high glucose levels only, indicating that this inhibitory effect involves the GSIS mechanisms in a fast and recoverable manner. Moreover, this inhibition persisted throughout the H_2O_2 exposure period, being similar to the direct inhibition of glucose oxidation by H_2O_2. In fact, H_2O_2 has been reported to inhibit the activity of glyceraldehyde-3-phosphate-dehydrogenase [5] and aconitase [6].

Contrary to previous reports on total islet $[Ca^{2+}]_i$ increase induced by high concentration (100 μmol/L) of H_2O_2 at low glucose levels [9], low H_2O_2 concentrations decreased the amplitude of $[Ca^{2+}]_i$ oscillations induced by high glucose. Thus, the proposed increase in total islet $[Ca^{+2}]_i$ probably reflected a toxic effect of high H_2O_2 concentrations [12]. Conversely, we report herein that the impairment of GSIS by low H_2O_2 is linked to suppression of the cell ability to handle calcium. It is interesting to note that the reduction in calcium spike amplitude is mirrored by the dose-dependent inhibition of glucose oxidation.

Low H_2O_2 levels have been reported to enhance insulin secretion at low glucose concentration [17]. On the other hand, Martens et al (2005) [18] observed that the intracellular H_2O_2 content is suppressed by glucose metabolism in pancreatic β-cells. Thus, the negative effect of H_2O_2 addition on insulin secretion appears to be mainly related to the impairment of glucose metabolism. In this sense, during

GSIS, an increase in H_2O_2 may interfere with the glucose-dependent intracellular redox control [18,19], suppressing glycolytic flux and GSIS.

Our findings show that low levels of H_2O_2 reduce GSIS via inhibition of glucose metabolism and $[Ca^{2+}]_i$ handling. The inhibitory pathway described herein may be implicated in the loss of pancreatic β-cell function caused by oxidative stress during the development of type 2 diabetes mellitus.

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