Glucose induces an acute increase of superoxide dismutase activity in incubated rat pancreatic islets

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Glucose induces an acute increase of superoxide dismutase activity in incubated rat pancreatic islets. Am. J. Physiol. 276 (Cell Physiol. 45): C507–C510, 1999.—The activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSP) in isolated rat pancreatic islets exposed to high glucose concentration for a short period of time (60 min) was determined. High glucose concentration (16.7 mM) did not significantly alter catalase activity. GSP activity was increased by glucose at 5.6 mM, remaining elevated at higher concentrations up to 16.7 mM. However, the activity of SOD increased with glucose concentration, and this increment was closely correlated with the rate of insulin secretion (r = 0.96). High potassium (30 mM) did not increase SOD activity, suggesting that the increase in intracellular ionic calcium concentration does not stimulate this enzyme activity. α-Ketoisocaproic acid and pyruvate, which are metabolized through the TCA cycle, did not increase SOD activity, indicating that the stimulation of SOD activity might be triggered by a factor produced through glycosis or the pentose phosphate pathway.

antioxidant enzymes; glycemia; pancreatic islets; insulin secretion

INSULIN SECRETION by pancreatic β-cells is mainly induced by circulating glucose (16). The glucose metabolism of the β-cells causes several cellular alterations that result in an increase in intracellular ionic calcium concentration following insulin granule extrusion. These alterations are 1) closure of the ATP-sensitive potassium channels, resulting in β-cell membrane depolarization (17), and the opening of the voltage-sensitive calcium channels (22); 2) activation of phospholipase C, phospholipase A₂, and adenyl cyclase, which leads to the formation of substances that also increase the intracellular ionic calcium concentration (e.g., inositol trisphosphate) and/or potentiate the response of the exocytotic apparatus to ionic calcium (e.g., protein kinase C, protein kinase A, arachidonic acid, cAMP) (for reviews see Refs. 1 and 11).

In several tissues, under physiological conditions, glucose oxidation results in the generation of hydrogen peroxide and oxygen free radicals such as the hydroxyl radical (30). Reactive oxygen species are known to interact with nucleic acids, proteins, and the lipid bilayer of cell membranes, leading to cell damage (13). Pancreatic islets present low activity of antioxidant enzymes (14, 18), being highly susceptible to cellular damage. Cytokines produced from macrophages and lymphocytes infiltrating pancreatic islets in type 1 (insulin-dependent) diabetes may induce β-cell damage by an increase in the production of oxygen free radicals in the islet cells (5, 18, 25). The damage of islet β-cells induced by the production of oxygen free radicals results from lipid peroxidation (21) and the consequent production of aldehydes (24).

It has been demonstrated that a high glucose concentration causes overexpression of Cu/Zn superoxide dismutase (Cu/Zn-SOD), catalase, and glutathione peroxidase (GSP) in cultured human endothelial cells (7). However, the incubation of isolated rat pancreatic islets with high glucose concentration (30 mM) for 48 h (chronic exposure) does not show significant changes in activity and expression of SOD (the first cellular defense against free radicals), catalase, and GSP (26). These observations refer to a prolonged exposure to high glucose concentration, which does not usually occur under physiological conditions.

Pancreatic β-cells are constantly subjected to transient increases in glycemia, returning to normal levels after a short period of time. Therefore, the purpose of this study was to investigate the activity of the antioxidant enzymes (SOD, catalase, and GSP) in isolated rat pancreatic islets exposed to high glucose concentrations for a short period of time (60 min).

MATERIALS AND METHODS

Animals. Female albino rats weighing 150–200 g (45–60 days old) were obtained from the Institute of Biomedical Sciences, USP, São Paulo, Brazil. The animals were kept in groups of five at 23°C in a room with a light-dark cycle of 12:12 h (lights on from 7:00 AM). Chemicals and enzymes. Collagenase, cytochrome c, glutathione (reduced form), α-ketoisocaproic acid, β-NADPH, purine, t-butyl-hydroperoxide, and xanthine oxidase were purchased from Sigma Chemical (St. Louis, MO); glutathione reductase and pyruvate were from Boehringer Mannheim; and sodium azide was from BDH Chemicals (Poole, England, UK).
Islet isolation and incubation. Rat pancreatic islets were isolated as described by Lacy and Kostianovsky (15). This method was slightly modified from our previous publications (6, 10). Batches of 150 islets were incubated in 0.5 ml of Krebs-Henseleit buffer (in mM: 139 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 124 Cl⁻, and 24 HCO₃⁻) at 37°C for 60 min in the absence (control) and in the presence of 5.6, 8.3, 11.1, 14, or 16.7 mM glucose. A similar experiment was performed in the presence of pyruvate (1 mM), α-ketoglutarate (10 mM), and high potassium concentration (30 mM). After incubation, the medium was removed and the islets were washed three times with phosphate buffer (10 mM sodium phosphate at pH 7.4). Preliminary experiments established that this procedure does not cause detectable loss of SOD activity. The islets were then suspended in 150 µl phosphate buffer and homogenized by sonication. The activity of total SOD, catalase, and GSP was then determined.

Enzyme assays. The activities of SOD, catalase, and GSP were measured by spectrophotometric assays. The extraction medium for the measurement of the enzyme activities was 10 mM sodium phosphate buffer at pH 7.4. Total SOD activity was measured by inhibition of the cytochrome c reduction rate induced by superoxide anions, monitored at 550 nm at 25°C, utilizing the xanthine/xanthine oxidase system as the source of O₂. SOD competes for superoxide and decreases the reduction rate of cytochrome c (12). One unit of SOD is defined as that amount of enzyme that inhibits by 50% the rate of cytochrome c reduction, under specified conditions. The activity of Mn-SOD was determined after adding cyanide to the assay medium. The procedure used for catalase and GSP assays was similar to those reported by Beutler (2) and Wendel (29), respectively. Catalase activity was determined by measuring the consumption of hydrogen peroxide at 230 nm and 30°C. The activity of GSP was measured by following the rate of oxidation of the reduced form of glutathione. The formation of oxidized glutathione was monitored by a decrease in the concentration of NADPH, measured at 340 nm and 37°C, caused by the addition of glutathione reductase to the medium. The enzyme assays were performed in a Gilford (Response model) spectrophotometer. A similar procedure has been previously used by Pereira et al. (20) and Pithon-Curi et al. (20a).

Expression of the results. The activities of the enzymes are expressed as micromoles per minute per milligram of protein. The rates of insulin release are presented as microunits per 60 min per islet.

Protein determination. Protein from the islet homogenates was measured by the method of Bradford (4), using BSA as standard.

Statistical analysis. Results are presented as means ± SE from 8 pools of 150 islets each. ANOVA was employed to indicate significant effects of glucose. The level of significance was set for P < 0.05.

RESULTS AND DISCUSSION

A short time exposure of the pancreatic islets to high glucose concentration (16.7 mM) did not significantly alter catalase activity (data not shown). In contrast, GSP activity (in µmol · min⁻¹ · mg protein⁻¹) was increased (2.7-fold) by glucose even at 5.6 mM concentration (Fig. 1) and was 1.64 ± 0.08 in the absence and 4.37 ± 0.17 in the presence of 5.6 mM glucose (means ± SE of 8 determinations). This effect remained unchanged in the presence of 8.3 mM (4.66 ± 0.17) and 16.7 mM (4.75 ± 0.21) glucose. These findings support the proposition that the activity of hydrogen peroxide-consuming enzymes is not efficiently influenced by glucose concentrations. In fact, inactivation of hydrogen peroxide has been proposed to be of critical importance for the removal of reactive oxygen species in insulin-producing cells (26).

High glucose concentrations have been reported to produce cellular stress in vascular endothelial cells and to increase SOD expression (7). However, exposure of pancreatic islets to 30 mM glucose for 48 h does not show significant alteration in Cu/Zn-SOD and Mn-SOD activities, abundance of the mRNA of the enzymes, and the corresponding protein contents (26). In the present study, SOD activity in 1-h-incubated pancreatic islets increased with the increase in glucose concentration.
The increment of this enzyme activity was also closely correlated with the rate of insulin secretion \( r = 0.96 \); Fig. 2). To elucidate whether insulin stimulates Cu/Zn-SOD or Mn-SOD activity, the measurement of total SOD activity was performed in the presence of cyanide (2 mM) (20). The addition of cyanide (an inhibitor of Cu/Zn-SOD activity) altered the increase in total SOD activity caused by high glucose concentrations (data not shown). Therefore, glucose does not acutely affect Mn-SOD activity, as has been reported to occur in pancreatic islets incubated for 90 min in the presence of interleukin-1β (3).

The observations above led us to postulate that the activity of Cu/Zn-SOD is acutely stimulated by glucose. This may function as an important mechanism to prevent the toxic effects of high glucose concentrations. However, when a high glucose concentration is maintained for a prolonged period of time (such as 48 h), this regulation is somehow lost (26), which might favor the occurrence of glucocytotoxicity. In fact, it has been postulated that SOD counteracts islet vascular alterations in low-dose streptozotocin-treated mice (19) and plays an important role in longevity and degenerative diseases (28). Whether the findings presented herein are linked to the observations of Vinci et al. (27) remains to be established. These authors reported that Cu (II) is able to prevent the interleukin-1β inhibition of glucose-induced insulin release and glucose oxidation in isolated pancreatic islets.

An important question remains: What is the mechanism for the effect of glucose on SOD activity? Induction of insulin secretion by high potassium concentration (30 mM) instead of glucose did not increase SOD activity \( 221.0 \pm 7.9 \mu mol \cdot min \cdot mg \cdot protein \) in islets exposed to high potassium concentration vs. \( 240.0 \pm 7.0 \) for the control group; means \( \pm SE \) of 4 experiments). This observation supports the proposition that neither the increase in intracellular ionized calcium concentration nor the events involved in the process of exocytosis of the insulin granules seem to stimulate this enzyme activity.

It is well known that Cu/Zn-SOD activity is found in the cytosol, whereas Mn-SOD activity is restricted to mitochondria (20). Therefore, the authors believe that the stimulation of SOD activity might occur by a cytosolic factor produced through glycolysis or the pentose phosphate pathway. In fact, α-ketoisocaproic acid (10 mM) and pyruvate (1 mM), which are metabolized through the TCA cycle in the mitochondria leading to insulin release (23), did not increase the SOD activity compared with control (incubation in the absence of substrates; 216.0 \pm 6.0 \mu mol \cdot min \cdot mg \cdot protein \) for α-ketoisocaproate, 140.0 \pm 1.0 for pyruvate, and 240.0 \pm 7.0 for the control groups; means \( \pm SE \) of 4 experiments). In contrast to glucose, pyruvate caused a significant reduction (41.7%) of the SOD activity. As also reported by others (9), pyruvate is able to react with hydrogen peroxide and may exert protective effects against cellular damages caused by the oxygen reactive species. Therefore, further studies are needed to elucidate this important point.

In conclusion, the findings presented support the proposition that glucose acutely stimulates Cu/Zn-SOD activity in pancreatic islets. This may make up an important physiological mechanism to protect the islets against oxygen toxicity due to the periodic exposure to high glucose concentration that occurs after feeding episodes.

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