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BRIEF REPORT

Islet NADPH oxidase activity is modulated unevenly by different secretagogues

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Abstract NADPH oxidase expression and activity have been measured in pancreatic islets under normal conditions, but its potential modulatory role upon insulin secretion remains unclear. We have currently studied NADPH oxidase activity in islets isolated from normal rats as well as the effect of its inhibition upon insulin secretion stimulated by different secretagogues. Glucose, arginine, fatty acids and KCl increased islet NADPH oxidase activity in a stimulus-dependent manner. DPI inhibited such increase in different proportions and affected unevenly insulin secretion, namely, it decreased the effect of high glucose, increased that of oleic acid and KCl, without changing the one induced by palmitic acid. Our data provide evidence that the contribution of NADPH activity to reactive oxygen species production in normal rat islets as well as its effect upon insulin secretion is uneven and highly stimulus-dependent.

Keywords Insulin secretion · Pancreas · Fatty acids · Islet cells · Reactive oxygen species

Introduction

It is accepted that a low and transient level of reactive oxygen species (ROS) may act as mediator for different biological responses [1, 2], including insulin secretion induced by various secretagogues [3, 4].

Two different sources of ROS have been described for pancreatic β-cells: (a) the mitochondrial oxidative phosphorylation that generates the reactive superoxide ($\cdot O_2^-$) anion as a by-product; under normal circumstances, the anion is rapidly converted to H_2O_2 by superoxide dismutase but, if there is an increased flow of oxidable substrates or of intracellular Ca^{2+} or a decreased ADP concentration, the $\cdot O_2^-$ production augments; and (b) the activation of a phagocyte-like NADPH oxidase (NOX2) as well as NOX1, NOX4, NOXO1 and NOXA1—expressed in pancreatic islets—also produce $\cdot O_2^-$, probably through a PKC-dependent activation [5, 6]. However, neither the relative contribution of each of these two systems to islet ROS production under normal conditions, nor their possible modulatory role upon insulin secretion elicited by different secretagogues have been fully established so far.

In an attempt to answer these questions, we have studied and compared NADPH oxidase activity and the effect of its inhibition upon insulin secretion in islets isolated from normal rats and challenged with glucose, arginine, fatty palmitic and oleic acids and KCl.

Results

NADPH oxidase activity

Glucose (16.6 mmol/l) and palmitic and oleic acids increased significantly NADPH oxidase activity above the levels measured in the presence of 3.3 mmol/l glucose (Fig. 1a). This effect was not observed with arginine and 20 mmol/l KCl. Palmitate induced the highest increase in peroxide production (412%), while 16.6 mmol/l glucose

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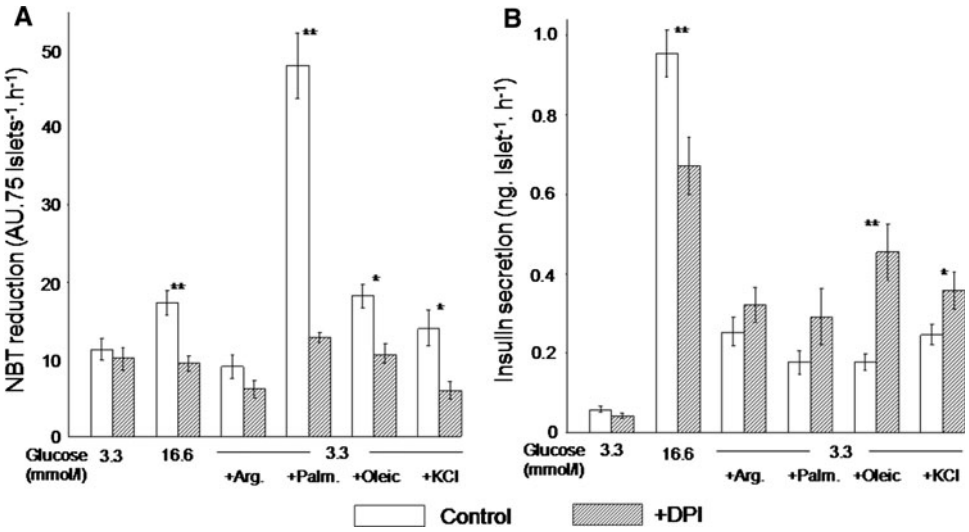

Fig. 1 **a** Nitro blue tetrazolium (NBT) reduction by islets incubated with 3.3 and 16.6 mmol/l glucose, 10 mmol/l arginine, 0.15 mmol/l, palmitic or oleic acids and 20 mmol/l KCl in the presence of 3.3 mmol/l glucose, with our without 10 ± mol/l diphenylene iodonium (DPI). Results are means ± SEM in arbitrary units (AU), $n = 7$. ** $P < 0.01$; * $P < 0.05$. **b** Insulin secreted by isolated islets incubated under the same experimental conditions as in A. Results are means ± SEM in ng islet^{-1} h^{-1}, $n = 20$

and oleic acid accounted for a 52 and 60% increase, respectively. Whereas, diphenylene iodonium (DPI, 10 μmol/l) added to the media from the beginning of the incubation significantly decreased the NADPH oxidase activity induced by 16.6 mmol/l glucose (45%), palmitic (73%) and oleic (41%) acids and KCl (57%), it did not affect significantly the effect of 3.3 mmol/l glucose or arginine.

Insulin secretion

Insulin secretion was significantly increased above baseline levels (3.3 mmol glucose) by 16.6 mmol/l glucose, arginine, palmitic and oleic acids, and KCl (Fig. 1b). Addition of 10 μmol/l DPI to the incubation media produced different effects on insulin secretion; it decreased significantly the response of 16.6 mmol/l glucose, increased that elicited by oleic acid and KCl, did not show a significant increase in the release induced by palmitic acid, and did not affect significantly the one elicited by 3.3 mmol/l glucose or arginine.

Discussion

Herein we show that NADPH oxidase activity in islet cells increased significantly when challenged by some but not all of the insulin secretagogues tested. We did not find any correlation between the magnitude of the secretagogues' effect upon the enzyme activity and insulin secretion, i.e., the highest enzyme activity was triggered by palmitate, while glucose 16.6 mmol/l induced the highest release of insulin. These data suggest that in normal islets and in vitro conditions: (1) as previously demonstrated [7], NADPH oxidase contributes to islet ROS production, being the magnitude of this effect stimulus-dependent, and (2) the enzyme activity and the consequent superoxide production would play a modulatory role upon insulin secretion that varies according to the stimulus used.

Since the nitro blue tetrazolium (NTB) technique employed estimates NADPH oxidase activity according to the superoxide production, we can thus conclude that while the enzyme is the main responsible for such production under the stimulus of palmitic acid, its role is less important in the case of KCl, 16.6 mmol/l glucose and oleic acid, being almost negligible with arginine and 3.3 mmol/l glucose. Since we used identical concentrations of palmitic and oleic acid in the medium, the difference recorded in their stimulation upon NADPH activity would be related to some specific rather than a concentration-dependent effect.

Regarding the possible modulatory role of NADPH oxidase upon insulin secretion, our data show that the effective inhibition of NADPH oxidase activity decreased significantly the secretion of insulin elicited by 16.6 mmol/l glucose, increased the one triggered by oleic acid and KCl, but did not affect significantly that of palmitic acid, arginine and 3.3 mmol/l glucose. We could therefore assume that in the case of high glucose, the ROS produced participates as a positive mediator for the stimulatory effect of the hexose, while it would play the opposite effect in the case of insulin release in response to either oleic acid or KCl. Further, ROS production under our conditions would not apparently play any significant role upon insulin release stimulated by low glucose, arginine and palmitic acid. Our experimental design does not provide an objective evidence to explain this uneven behaviour. Although we found that the glucose-stimulated ROS generation may be important for the acute regulation of insulin secretion, we should consider that at higher levels and acting for long periods, ROS may hamper mitochondrial energy

metabolism and exert negative effects upon both insulin secretion and β-cell mass [8].

It could be argued that DPI is not a totally specific inhibitor of NADPH oxidase, but the literature about the issue is mixed. While Weir et al. have reported that it exerts an inhibitory effect on mitochondrial complex I of the electron transport system [9], Morgan et al. found similar blunting effects upon NADPH oxidase-dependent ROS production of either DPI or an antisense oligonucleotide for $p47^{PHOX}$ [10].

In brief, our data provide evidence that the contribution of NADPH activity to ROS production in normal rat islets as well as its effect upon insulin secretion is uneven and highly stimulus-dependent.

Materials and methods

Albumin free from fatty palmitic and oleic acids, NBT and DPI were obtained from Sigma-Aldrich Chemical (St Louis, Mo, USA). The complex albumin-palmitic or oleic acid was prepared according to Spector et al. [11].

Normal male adult Wistar rats (150–200 g) were housed in rooms with controlled temperature, a 12-h light/12-h dark cycle and fed a commercial diet and tap water ad libitum. The animals were killed by cervical dislocation removing the pancreas to isolate islets by collagenase digestion [12].

Superoxide generation was detected by NBT assay [13], as described by Oliveira et al. [6]. Isolated islets (75 islets/375 µl KRB with 1% BSA) were incubated for 2 h with 0.2% NBT (37°C, 95% O_2 to 5% CO_2) in the presence of 3.3 and 16.6 mmol/l glucose, and 3.3 mmol/l glucose plus arginine (10 mmol/l), albumin-palmitic or oleic acid complexes (0.15 mmol/l), and KCl (20 mmol/l), with or without 10 µmol/l DPI (NADPH oxidase inhibitor). To avoid changes in osmolarity of the media, when we tested the effect of 20 mmol/l KCl, the NaCl concentration was correspondingly decreased. At the end of the incubation period, the islets were sonicated in 100 µl 50% acetic acid to dissolve the NBT-reduced formazan; thereafter, the absorbance of each sample was determined at 620 nm.

In order to study insulin secretion, 5 islets were incubated in 600 µl KRB previously gassed (95% O_2 to 5% CO_2) at 37°C under the conditions described above but without the addition of 0.2% NBT; insulin was measured in aliquots of the medium by radioimmunoassay using a rat insulin standard [14].

Statistical analyses were performed using ANOVA and Student's t test.

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