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## Regulation of Insulin Secretion in Relation to Nitric Oxide, Carbon Monoxide and Acid alpha-Glucoside Hydrolase Activities

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# Nitric oxide, islet acid glucan-1,4- $\alpha$ -glucosidase activity and nutrient-stimulated insulin secretion

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## Abstract

The mechanism of nutrient-evoked insulin release is clearly complex. One part of that mechanism is postulated to be the activation of the glycogenolytic enzyme acid glucan-1,4- $\alpha$ -glucosidase. As nitric oxide (NO) has been found to be a potent inhibitor of glucose-stimulated insulin secretion, we have now investigated a possible influence of exogenous NO and inhibition of endogenous NO production on islet acid glucan-1,4- $\alpha$ -glucosidase activity in relation to insulin release stimulated by glucose and L-arginine. In isolated islets, NO derived from the intracellular NO donor hydroxylamine inhibited the activation of acid glucan-1,4- $\alpha$ -glucosidase and its isoform acid  $\alpha$ -glucosidase in parallel with inhibition of glucose-stimulated insulin release. In comparison, other lysosomal enzymes were largely unaffected. Similarly, the spontaneous NO donor sodium nitroprusside, as well as NO

gas, when added to islet homogenates, suppressed the activities of these acid  $\alpha$ -glucosidehydrolases and, to a lesser extent, the activities of other lysosomal enzymes. Finally, in the presence of the NO synthase inhibitor  $N^G$ -nitro-L-arginine methyl ester, insulin release from isolated islets stimulated by glucose or L-arginine was markedly potentiated in parallel with an accompanying increase in the activities of acid glucan-1,4- $\alpha$ -glucosidase and acid  $\alpha$ -glucosidase. Other lysosomal enzymes and neutral  $\alpha$ -glucosidase were not influenced. We propose that an important inhibitory effect of NO on the insulin secretory processes stimulated by glucose and L-arginine is exerted via inactivation of islet acid glucan-1,4- $\alpha$ -glucosidase, a putative key enzyme in nutrient-stimulated insulin release.

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## Introduction

We and others have previously presented histochemical, immunocytochemical and biochemical evidence, for the occurrence of a constitutive  $Ca^{2+}$ /calmodulin-dependent nitric oxide synthase (cNOS) activity in mouse and rat islet endocrine cells (Panagiotidis *et al.* 1992, 1994, Schmidt *et al.* 1992, Corbett *et al.* 1993, Salehi *et al.* 1996, Henningsson & Lundquist 1998, Åkesson *et al.* 1999, Alm *et al.* 1999, Henningsson *et al.* 1999). Further, in a series of combined *in vitro* and *in vivo* experiments in the mouse we observed that selective inhibition of islet NOS activity markedly enhanced insulin release stimulated by glucose or arginine (Panagiotidis *et al.* 1992, 1994, 1995, Åkesson *et al.* 1996, 1999, Salehi *et al.* 1996, 1998c,d, Henningsson & Lundquist 1998, Henningsson *et al.* 1999). In accordance with this, the NO donors sydnonimine (SIN)-1 and hydroxylamine inhibited glucose- and arginine-stimulated insulin secretion (Panagiotidis *et al.* 1994, 1995, Salehi *et al.* 1996, 1998d, Henningsson & Lundquist 1998). Thus NO evolution by islet cNOS activity seems to serve as a negative modulator of nutrient-stimulated insulin release.

Similar results have also been reported with regard to isolated rat islets (Cunningham *et al.* 1994, Sjöholm 1996) and the isolated perfused rat pancreas (Gross *et al.* 1995), although some earlier data, mostly obtained from  $\beta$ -cell lines, suggested that islet NO production either enhanced or had no effect on nutrient-stimulated insulin secretion (Laychock *et al.* 1991, Jones *et al.* 1992, Schmidt *et al.* 1992).

We have also shown that another free radical,  $H_2O_2$ , generated either by increased monoamine oxidase activity (Lundquist *et al.* 1991, Panagiotidis *et al.* 1993) or by the ' $H_2O_2$ -donor' tert-butylhydroperoxide (Panagiotidis *et al.* 1994, Åkesson & Lundquist 1999) has an inhibitory effect on glucose-stimulated insulin secretion. These data suggested to us that low levels of free radicals such as NO and  $H_2O_2$  might negatively modulate the insulin secretory processes through effects linked to oxidative or S-nitrosylation mechanisms either by disturbing the balance of the reduced glutathion/oxidized glutathion ratio (GSH/GSSG), which has long been known to be important for glucose-stimulated insulin release (Hellman *et al.* 1974, Ammon & Mark 1985), and/or by direct

nitrosylation of important thiol groups leading to inactivation of regulatory enzyme proteins or ion channels (Stamler *et al.* 1992). In this context our attention was drawn to an earlier observation that one of several signals in the complex cascade of nutrient-stimulated insulin release is transduced through activation of islet acid glucan-1,4- $\alpha$ -glucosidase (Lundquist 1985, 1986, Lundquist & Panagiotidis 1992), a lysosomal glycogenolytic enzyme belonging to the  $\alpha$ -glucoside hydrolase family (Pazur & Kleppe 1962, Smith *et al.* 1968). This enzyme preferentially cleaves  $\alpha$ -1,4-linked glucose polymers and might thus attack certain pools of islet vacuolar glycogen to produce high compartmentalized concentration of free glucose, which, in turn, could act as a regulatory transducer (cybernetic, metabolic or osmotic) in the multifactorial process of insulin release (Lundquist *et al.* 1996, Salehi *et al.* 1998b, 1999). Isoforms of acid  $\alpha$ -glucoside hydrolases are known to contain several cysteine residues (Barrett 1972) and are thus possible targets for thiol inactivation processes. Therefore the aim of the present investigation was to study the influence of NO on insulin-releasing processes in relation to possible effects on islet acid  $\alpha$ -glucoside hydrolase activities.

## Materials and Methods

### Animals

Female mice of the Naval Marine Research Institute (NMRI) strain (B&K, Sollentuna, Sweden), weighing 25–30 g, with a standard pellet diet (B&K) and water available *ad libitum* were used throughout the study. The animal experiments were approved by the local animal welfare committee (Lund, Sweden), and were in accordance with the international standard recommended by NIH.

### Drugs and chemicals

Collagenase (CLS 4) was obtained from Worthington Biochemicals (Freehold, NJ, USA). L-Arginine, hydroxylamine,  $N^G$ -nitro-L-arginine methylester (L-NAME) and methylumbelliferyl-coupled substrates were purchased from Sigma Chem. Co. (St Louis, MO, USA). BSA was from ICN Biomedicals (High Wycombe, Bucks, UK) and sodium nitroprusside (SNP) was from Merck AG (Darmstadt, Germany). All other chemicals were from British Drug Houses Ltd (Poole, Dorset, UK) or Merck AG (Darmstadt, Germany). The radioimmunoassay kits for insulin determination were obtained from Diagnostica (Falkenberg, Sweden).

### Experimental procedure

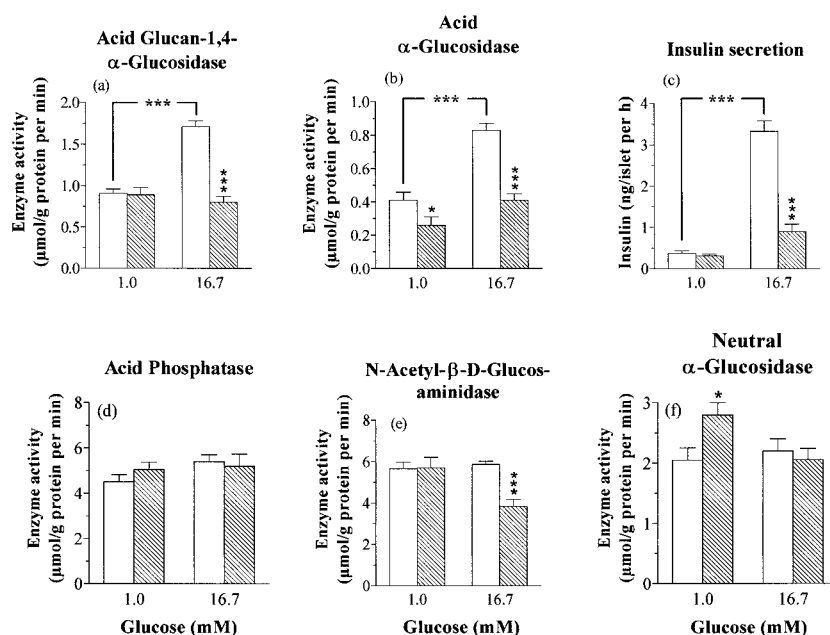
Isolation of pancreatic islets from freely fed mice was accomplished by retrograde injection of a collagenase

solution via the bile-pancreatic duct (Gotoh *et al.* 1985). The freshly isolated islets were preincubated for 30 min at 37 °C in Krebs–Ringer bicarbonate (KRB) buffer pH 7.4, supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), 0.1% BSA and 1 mM glucose. Each incubation vial contained 30 islets in 1.5 ml buffer solution and was gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> to obtain constant pH and oxygenation. After preincubation, the buffer was changed to a medium containing 1 mM, 7 mM or 16.7 mM glucose with or without test substances, and the islets were incubated for 120 min. All incubations were performed at 37 °C in an incubation box (30 cycles/min). Immediately after incubation, an aliquot of the medium was removed for assay of insulin. The islets were then thoroughly washed in glucose-free KRB buffer and collected in 200  $\mu$ l ice-cold acetate–EDTA buffer (1.1 mM EDTA and 5 mM sodium acetate pH 5.0) and thereafter stored at –20 °C. After sonification, islet homogenates were analyzed for lysosomal enzyme activities. To test possible direct effects of NO on the different enzyme activities, aliquots of islet homogenates were either incubated with SNP (300  $\mu$ M) for 60 min at 37 °C in acetate buffer (50 mM; pH 4.0) or directly gassed with helium followed by NO until saturation. The procedures for determination of acid phosphatase (pH 4.5), acid  $\alpha$ -glucosidase (pH 4.0), N-acetyl- $\beta$ -D-glucosaminidase (pH 5.0) and neutral  $\alpha$ -glucosidase (pH 6.5) with methylumbelliferyl-coupled substrates have been described previously (Lundquist 1985). Islet glucan-1,4- $\alpha$ -glucosidase activity with glycogen as substrate was determined at pH 4.0 as described in detail elsewhere (Lundquist 1971, 1985). Protein was analysed according to the method of Lowry *et al.* (1951). Insulin was determined with a radioimmunoassay (Heding 1966). Probability levels of random differences were determined by Student's unpaired *t*-test or analysis of variance followed by Tukey–Kramer's multiple comparisons test where applicable.

## Results

### *Influence of the intracellular NO donor hydroxylamine on glucose-stimulated insulin release and islet lysosomal enzyme activities in intact islets*

To test whether NO might influence islet  $\alpha$ -glucoside hydrolase activity, isolated islets were incubated in conditions of low or high glucose in the absence and presence of the intracellular NO donor hydroxylamine. Figure 1 shows the influence of 300  $\mu$ M hydroxylamine on islet lysosomal enzyme activities and insulin secretion both at a low, basal concentration of glucose (1 mM) and at a high concentration of glucose (16.7 mM). High glucose induced a marked increase in insulin release (Fig. 1c). At low glucose a modest inhibition was recorded for acid  $\alpha$ -glucosidase (Fig. 1b), and a slight increase for neutral



**Figure 1** Effect of hydroxylamine on islet activities of different lysosomal enzymes, neutral  $\alpha$ -glucosidase and insulin secretion. Islets were incubated for 2 h in the absence (open columns) or presence (cross-hatched columns) of 300  $\mu$ M hydroxylamine, at a glucose concentration of 1 mM (the two columns to the left) or 16.7 mM (the two columns to the right). (a) Acid glucan-1,4- $\alpha$ -glucosidase; (b) acid  $\alpha$ -glucosidase; (c) insulin secretion; (d) acid phosphatase; (e) *N*-acetyl- $\beta$ -D-glucosaminidase; (f) neutral  $\alpha$ -glucosidase. Enzyme activities are expressed as  $\mu$ mol glucose (acid glucan-1,4- $\alpha$ -glucosidase) or 4-methylumbelliferone liberated/g protein per min. Insulin secretion is expressed as ng insulin released/islet per h. Values are means  $\pm$  S.E.M. for 8–10 batches of islets in each group; data from four independent experiments. \* $P < 0.05$ , \*\*\* $P < 0.001$  compared with no hydroxylamine, or for comparison indicated.

$\alpha$ -glucosidase (an enzyme attributed to the endoplasmic reticulum) (Fig. 1f), whereas acid phosphatase, *N*-acetyl- $\beta$ -D-glucosaminidase, acid glucan-1,4- $\alpha$ -glucosidase and basal insulin release were unaffected at 1 mM glucose (Fig. 1a,c-e). At high glucose (16.7 mM), hydroxylamine markedly inhibited glucose-stimulated insulin release (Fig. 1c) and strongly suppressed the activities of acid glucan-1,4- $\alpha$ -glucosidase ( $-55\%$ ) (Fig. 1a) and acid  $\alpha$ -glucosidase ( $-50\%$ ) (Fig. 1b). *N*-Acetyl- $\beta$ -D-glucosaminidase was modestly inhibited whereas acid phosphatase and neutral  $\alpha$ -glucosidase activities were unaffected by the NO donor (Fig. 1d-f).

#### *Influence of direct addition of SNP or exogenous NO gas on lysosomal enzyme activities in islet homogenates*

Table 1 shows the effect of the spontaneous NO donor SNP and of exogenous NO gas on lysosomal enzyme activities in islet homogenates. Both acid and neutral  $\alpha$ -glucoside hydrolases in islet homogenates exposed to SNP during an incubation time of 60 min at pH 4.0 lost approximately 20–45% of their enzyme activity. Acid phosphatase decreased by 25%, whereas *N*-acetyl- $\beta$ -D-

glucosaminidase activity was virtually unaffected. Addition of NO gas in aqueous solution (NO saturated) to islet homogenates induced a much stronger inhibition of the acid  $\alpha$ -glucoside hydrolase activities than SNP. In contrast, the effect of NO on acid phosphatase and neutral  $\alpha$ -glucosidase was not different from that of SNP. However, *N*-acetyl- $\beta$ -D-glucosaminidase activity was somewhat reduced by NO.

#### *Influence of the NOS inhibitor L-NAME on islet lysosomal enzyme activities and glucose-stimulated insulin release in intact islets*

The next series of experiments was undertaken to test the influence of NOS inhibition on lysosomal enzyme activities in relation to glucose-stimulated insulin secretion in intact islets. Such an approach, using 5 mM of the NOS-inhibitor L-NAME, suppresses the endogenous evolution of cNOS-derived NO (Åkesson *et al.* 1999). Isolated islets were therefore incubated at 1 mM or 16.7 mM glucose in the absence and presence of L-NAME (5 mM). Figure 2a and b show that the activities of the acid  $\alpha$ -glucoside hydrolases were unaffected at 1 mM glucose

**Table 1** Influence of SNP or exogenous NO on islet lysosomal enzyme activities and neutral  $\alpha$ -glucosidase in islet homogenates. SNP (300  $\mu$ M) was added to crude islet homogenates and the mixture was incubated for 60 min at 37 °C or, in another set of experiments, the islet homogenates were gassed with helium followed by NO until saturation. Controls were run in parallel. The SNP experiments were conducted at pH 4.0 (50 mM acetate buffer). Results are expressed as percentage controls, means  $\pm$  S.E.M. of five to eight observations in each group

	Control	SNP	Control	NO
Acid glucan-1,4- $\alpha$ -glucosidase	100 $\pm$ 3.1	83.7 $\pm$ 3.5**	100 $\pm$ 4.4	39.7 $\pm$ 6.9***
Acid $\alpha$ -glucosidase	100 $\pm$ 1.5	54.4 $\pm$ 3.3***	100 $\pm$ 4.0	40.9 $\pm$ 2.8***
Neutral $\alpha$ -glucosidase	100 $\pm$ 1.1	59.0 $\pm$ 4.5***	100 $\pm$ 3.0	61.6 $\pm$ 1.9***
Acid phosphatase	100 $\pm$ 2.6	73.0 $\pm$ 1.5***	100 $\pm$ 3.0	69.2 $\pm$ 3.2***
N-acetyl- $\beta$ -D-glucosaminidase	100 $\pm$ 0.6	97.0 $\pm$ 0.9	100 $\pm$ 1.5	64.2 $\pm$ 2.7***

\*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared with respective control.

but markedly and significantly increased, as was insulin release (Fig. 2c), at 16.7 mM glucose, in the presence of L-NAME. Other enzyme activities (Fig. 2d–f) were not affected.

#### *Influence of the NOS-inhibitor L-NAME on islet lysosomal enzyme activities and L-arginine-stimulated insulin release in intact islets*

As the NO precursor L-arginine is, in addition, a potent insulin secretagogue, we performed a series of experiments in which we measured islet lysosomal enzyme activities in relation to L-arginine-stimulated insulin release in the absence and presence of L-NAME. We have recently shown (Henningsson & Lundquist 1998) that L-arginine has a dual action on insulin release: stimulation (major effect) through cationic and metabolic properties (Henquin 1992), and a concomitant inhibition (minor effect) through its ability to produce NO. Figure 2a–c show that L-arginine itself induced an increase of the activities of islet acid glucan-1,4- $\alpha$ -glucosidase and acid  $\alpha$ -glucosidase in parallel with an increased insulin release. The activities of neutral  $\alpha$ -glucosidase or other lysosomal enzymes such as acid phosphatase and N-acetyl- $\beta$ -D-glucosaminidase were not affected by L-arginine (Fig. 2d–f). Figure 2a–c also show that NOS-inhibition by L-NAME in the presence of L-arginine induced a marked increase in both acid  $\alpha$ -glucosidase activities and insulin release. Basal insulin release at 7 mM glucose was not influenced by L-NAME, neither were enzyme activities measured (Fig. 2a–f).

## Discussion

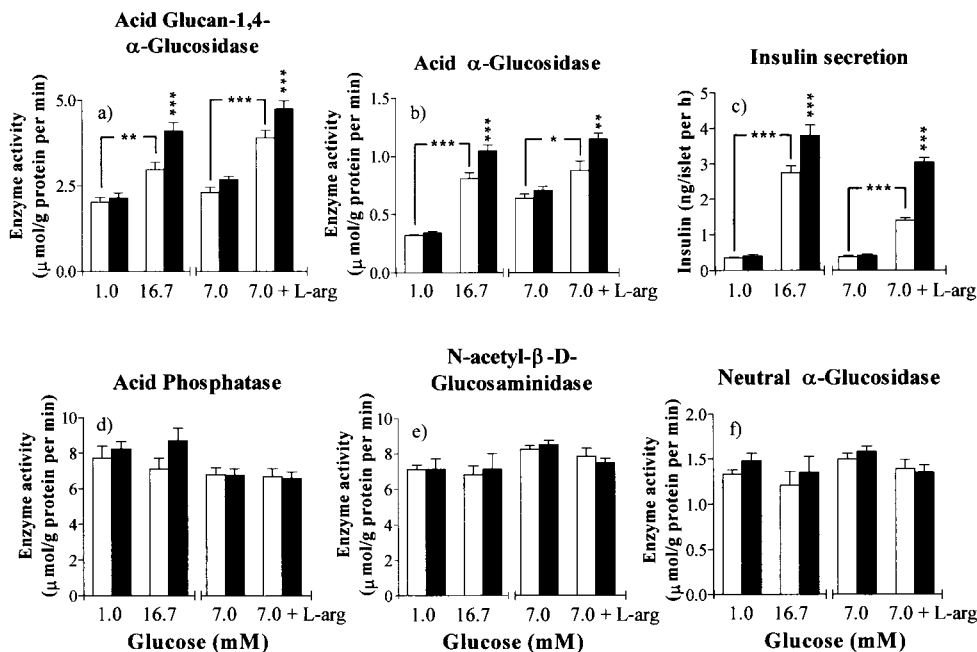
The results of the present study show that the intracellular NO donor hydroxylamine inhibits the lysosomal acid glucan-1,4- $\alpha$ -glucosidase activity in parallel with a suppression of glucose-stimulated insulin release from isolated islets and that the NOS-inhibitor L-NAME brought about

a parallel increase in acid glucan-1,4- $\alpha$ -glucosidase activity and insulin release stimulated both by glucose and by L-arginine. Hence the present data add favorably to the earlier hypothesis (Lundquist 1975) that one of the multiple signals involved in nutrient-stimulated insulin release might be conveyed via activation of this glycogenolytic, glucose-producing enzyme, the intimate mechanisms of action of which in the secretory process remain unclear. It should be noted that we have previously provided good direct evidence for the involvement of the enzyme in the stimulus–secretion coupling by showing that a series of chemically different, selective  $\alpha$ -glucoside hydrolase inhibitors such as the pseudotetrasaccharide acarbose, the deoxynojirimycin derivatives miglitol and emiglitate, and the indolizine-alkaloid castanospermine dose-dependently and markedly inhibit acid glucan-1,4- $\alpha$ -glucosidase activity in parallel with a dose-dependent suppression of glucose-stimulated insulin release (Salehi & Lundquist 1993a,b, Salehi *et al.* 1995, 1998a, 1999, Lundquist *et al.* 1996).

#### *Effects of NO donors*

The present findings suggest to us that NO induced inactivation of the acid  $\alpha$ -glucoside hydrolases might contribute to the inhibitory action of NO on nutrient-stimulated insulin release. It should be noted that NO might have multiple effects on the stimulus–secretion coupling of the  $\beta$ -cell. Opening of ATP-sensitive K<sup>+</sup> channels (K<sub>ATP</sub> channels) (Tsuura *et al.* 1994, Antoine *et al.* 1996), binding to iron–sulfur enzymes (e.g. the Krebs cycle enzyme aconitase) (Welsh & Sandler 1992, Corbett & McDaniel 1994) or S-nitrosylation of various plasma membrane or cytosolic regulator proteins containing critical thiol groups (Panagiotidis *et al.* 1992, 1994, 1995, Salehi *et al.* 1996, Henningsson & Lundquist 1998, Åkesson *et al.* 1999) have been suggested as possible NO affected targets in suppressing the stimulus–secretion coupling of nutrient-stimulated insulin release. Now we add another possible NO target, the acid glucan-1,4- $\alpha$ -





**Figure 2** Effect of L-NAME on islet activities of different lysosomal enzymes and insulin secretion at low and high glucose and in the presence of L-arginine. Isolated islets were incubated for 2 h in the absence (open columns) or presence (black columns) of 5 mM L-NAME at 1 mM or 16.7 mM glucose (the four columns to the left) and 7 mM glucose or 7 mM glucose + 10 mM L-arginine (the four columns to the right). (a) Acid glucan-1,4- $\alpha$ -glucosidase; (b) acid  $\alpha$ -glucosidase; (c) insulin secretion; (d) acid phosphatase; (e) N-acetyl- $\beta$ -D-glucosaminidase; (f) neutral  $\alpha$ -glucosidase. Enzyme activities are expressed as  $\mu$ mol glucose (acid glucan-1,4- $\alpha$ -glucosidase) or 4-methylumbelliferone liberated/g protein per min. Insulin release is expressed as ng insulin release/islet per h. Values are means  $\pm$  S.E.M. for 8–10 batches of islets in each group; data from four independent experiments at 1 mM or 16.7 mM glucose and four independent experiments at 7 mM glucose with or without L-arginine. \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001 compared with no L-NAME, or for comparison indicated.

glucosidase which, in contrast to the aforementioned regulatory proteins, is located in the lysosomal-vacuolar system of the  $\beta$ -cell (Lundquist 1972b). In this context it is important to note that cNOS is reportedly a cytosolic enzyme in most tissues (Knowles & Moncada 1994), although recent data (Lajoix *et al.* 1999) suggest that cNOS in rat islet  $\beta$ -cells is mainly localized to insulin secretory granules. As NO is known to be a highly reactive molecule, both the intracellular site and the amount of the evolved NO might be decisive for its cellular effects. Hence, the NO produced by cNOS activity might first affect the insulin secretory granules and then various cytosolic, lysosomal, mitochondrial and plasma membrane targets.

In contrast to most other NO donors such as SNP (Feelisch 1991), hydroxylamine delivers its NO by the intracellular route, after having been metabolized by catalase (DeMaster *et al.* 1989). The intracellular route of NO production in this context is important, because several previous studies have shown that a deranged balance of the intracellular reduced/oxidized thiol groups (especially the GSH/GSSG system) will induce an impair-

ment of glucose-stimulated insulin release (Hellman *et al.* 1974, Ammon & Mark 1985). In contrast, agents affecting thiol groups facing the outside of the plasma membrane might stimulate insulin release (Hellman *et al.* 1973, Ammon *et al.* 1984), and thus different NO donors delivering NO directly into the extracellular milieu, such as SNP, will not mimic intracellular NO production in an appropriate way. Hence we consider hydroxylamine the NO donor of choice when studying intracellular effects of NO on insulin release in intact islets. In the present investigation we also used SNP, for comparative purposes, as SNP in aqueous solution is known to deliver NO in a spontaneous way and thus we could study its effects directly on the enzyme activities in islet homogenates. In this context however, it should be noted that SNP reportedly might have additional effects unrelated to NO formation (Feelisch 1991).

An interesting observation with regard to the effect of NO on the different  $\alpha$ -glucoside hydrolase activities in intact islets was the fact that the glycogen-preferring isoform – the acid glucan-1,4- $\alpha$ -glucosidase (Pazur & Kleppe 1962, Abdullah *et al.* 1963) – was unaffected by

hydroxylamine in the presence of a low substimulatory concentration of glucose (1 mM), but was strongly inhibited at a high stimulatory concentration of glucose (16.7 mM). This observation raised the question of whether a specific glucose activation of acid glucan-1,4- $\alpha$ -glucosidase in intact islets unmasks critical cysteine residues at the active site of the enzyme molecule to render them accessible to the action of NO. In this context it is worth mentioning that we have previously shown that an acute i.v. injection of glucose increases the 'free' in relation to the 'total' activity of  $\beta$ -cell acid glucan-1,4- $\alpha$ -glucosidase activity (lysosomal 'labilization'), whereas the activity of the classical lysosomal enzyme  $\beta$ -glucuronidase was unaffected (Lundquist 1985). Hence, as in other tissues (Novikoff *et al.* 1975), there exists a certain heterogeneity among lysosomes/lysosomal enzyme activities in the  $\beta$ -cell. Consequently it is not inconceivable that different isoforms of acid  $\alpha$ -glucoside hydrolase enzymes might exist in different lysosome populations – for example, the acid glucan-1,4- $\alpha$ -glucosidase activity might be localized to specialized lysosomes, 'crinosomes' (Lundquist 1972c), involved in glucose-induced stimulus–secretion coupling, whereas the  $\alpha$ -glucosidases might be localized in a population of lysosomes containing a majority of classical lysosomal enzymes.

#### Effects of NOS-inhibition

In accordance with a negative modulation of acid glucan-1,4- $\alpha$ -glucosidase activity and glucose-stimulated insulin release induced by the NO donors, a positive modulation of these parameters was found after suppressing endogenous NO production with the NOS-inhibitor L-NAME. No effect by L-NAME was observed at basal (1 mM or 7 mM) glucose either on enzyme activity or on insulin release, in keeping with previous results showing that basal insulin release is little affected by the acid glucan-1,4- $\alpha$ -glucosidase activity (Lundquist 1972a, 1985, Salehi *et al.* 1995, Lundquist *et al.* 1996, Salehi & Lundquist 1996) and that there is little endogenous NO production from islets incubated in conditions of low glucose (Åkesson *et al.* 1999, Henningsson *et al.* 1999). The relative increase in acid glucan-1,4- $\alpha$ -glucosidase activity in the NOS-inhibited, glucose-stimulated islets again suggests that glucose stimulation of acid glucan-1,4- $\alpha$ -glucosidase activity may unmask critical thiol groups (cysteine residues) at the active site of the enzyme molecule, which in turn might make them accessible for NO-induced S-nitrosylation. As glucose does indeed stimulate islet NO production (Henningsson *et al.* 1999), full expression of glucose-stimulated activation of acid glucan-1,4- $\alpha$ -glucosidase and, consequently, glucose-stimulated insulin release, cannot be achieved unless NO production is suppressed. Similarly, insulin release stimulated by the NO precursor L-arginine was markedly increased by the NO inhibitor L-NAME in parallel with an increased activity of

the acid  $\alpha$ -glucoside hydrolases. However, in this case the increase in acid glucan-1,4- $\alpha$ -glucosidase was less pronounced than with glucose. This is probably explained by the fact that the insulin releasing effect of L-arginine is not only nutrient-stimulated but is due in major part to its cationic property (Henquin 1992, Henningsson & Lundquist 1998).

As previously mentioned, the acid glucan-1,4- $\alpha$ -glucosidase is most probably not the sole target for the action of NO to suppress glucose-stimulated insulin release. Thus NO, produced in large amounts by interleukin-1 $\beta$ -induced iNOS activation, has been reported to suppress aconitase activity (Welsh & Sandler 1992) and the NO donor hydroxylamine is reportedly able to open the K<sub>ATP</sub> channels (Antoine *et al.* 1996) and to suppress phosphofructokinase activity (Tsuura *et al.* 1994). However, recent observations in our laboratory (Henningsson & Lundquist 1998, Åkesson & Lundquist 1999) have shown that the major effect of hydroxylamine-derived NO is elicited independently of membrane depolarization events, and thus probably exerted at more distal steps in nutrient-induced stimulus–secretion coupling of the  $\beta$ -cell, for example on the lysosome–acid glucan-1,4- $\alpha$ -glucosidase system.

#### Conclusion

We show here that NO derived from the NO donors hydroxylamine and SNP has significant inhibitory effects on the activities of the lysosomal enzymes acid glucan-1,4- $\alpha$ -glucosidase and acid  $\alpha$ -glucosidase, and that NOS-inhibition by L-NAME markedly increases these enzyme activities in islets stimulated by glucose or by L-arginine. As an increase (NO donor) or a decrease (NOS inhibition) in intracellular NO production is accompanied by inhibition (NO donor) or potentiation (NOS inhibition) of insulin release stimulated by glucose or L-arginine, we propose that one of the multiple effects of NO on the insulin secretory process is exerted via inhibition of the activity of the acid glucan-1,4- $\alpha$ -glucosidase, a putative key enzyme in nutrient-stimulated insulin release. This inhibitory action by NO is conceivably accomplished through S-nitrosylation of critical thiol groups in the active site of the enzyme molecule, although further investigations are needed to elucidate its intimate mechanisms of action in the stimulus–secretion coupling of the insulin secretory process.

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