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Nitric oxide (NO) — Production and regulation of insulin secretion in islets of freely fed and fasted mice

Dag Eckersten [a], Ragnar Henningsson [b,*]

[a] Department of Nephrology and Transplantation Skane University Hospital, Sweden
[b] Department of Anesthesiology, Central Hospital of Karlstad, 65185 Karlstad, Sweden

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ABSTRACT

Production of nitric oxide through the action of nitric oxide synthase (NOS) has been detected in the islets of Langerhans. The inducible isoform of NOS (iNOS) is induced by cytokines and might contribute to the development of type-1 diabetes, while the constitutive isoform (cNOS) is thought to be implicated in the physiological regulation of insulin secretion. In the present study we have detected and quantified islet cNOS- and iNOS-derived NO production concomitant with measuring its influence on insulin secretion in the presence of different secretagogues: glucose, L-arginine, L-leucine and α-ketoisocaproic acid (KIC) both during fasting and freely fed conditions. In intact islets from freely fed mice both cNOS- and iNOS-activity was greatly increased by glucose (20 mmol/l). Fasting induced islet iNOS activity at both physiological (7 mmol/l) and high (20 mmol/l) glucose concentrations. NOS blockade increased insulin secretion both during freely fed conditions and after fasting. L-arginine stimulated islet cNOS activity and did not affect islet iNOS activity. L-leucine or KIC, known to enter the TCA cycle without affecting glycolysis, did not affect either islet cNOS- or iNOS activity. Accordingly, insulin secretion stimulated by L-leucine or KIC was unaffected by addition of L-NAME both during feeding and fasting. We conclude that both high glucose concentrations and fasting increase islet total NO production (mostly iNOS derived) which inhibit insulin secretion. The insulin secretagogues L-leucine and KIC, which do not affect glycolysis, do not interfere with the islet NO–NOS system.

1. Introduction

Since the discovery of nitric oxide (NO) as the endothelial derived relaxing factor (EDRF) more than three decades ago [1,2], its role as an ubiquitous messenger molecule is unquestionable [3]. The secretion of insulin is regulated by a complex chain of events evoked in response to metabolic, nervous and hormonal factors. The major insulin secretagogue, glucose, is known to initiate insulin release by closure of ATP-sensitive K^+ channels, with subsequent depolarization and opening of voltage-dependent Ca^{2+} channels leading to increased intracellular Ca^{2+} and subsequent exocytosis of insulin [4,5]. This secretory process is modulated by activation of different types of second messengers [5]. After discovering [6–8] that endocrine pancreas harbors the constitutive isoform of nitric oxide synthase (cNOS), L-arginine-derived nitric oxide (NO) was assigned a role as a novel modulator of insulin release stimulated by glucose and the NO-precursor L-arginine [6,8,9]. cNOS-derived NO is an important messenger molecule in various cell types e.g. endothelial cells and neurons [2], whereas NO produced by e.g. macrophages upon cytokine activation of the Ca^{2+}/calmodulin independent isoform of NOS (iNOS) is cytotoxic and implicated in macrophage-induced β-cell destruction and thus insulin-dependent diabetes mellitus (IDDM) [10,11].

In addition to glucose also amino acids and some of their α-ketoacid counterparts stimulate insulin secretion [12]. α-ketoisocaproate (KIC), the transamination product of leucine, has been shown to induce insulin secretion as well as leucine itself, though partly through different not fully understood mechanisms [13,14]. KIC has been shown to stimulate insulin both at low and high glucose concentrations, whereas leucine only stimulates insulin secretion during low glucose concentrations [15].

The purpose of this study was to detect and quantify cNOS- and iNOS-derived NO production within mouse islets of Langerhans concomitant with measuring its influence on insulin secretion in the presence of different secretagogues: glucose, L-arginine, L-leucine and α-ketoisocaproic acid (KIC). We have repeatedly shown that cNOS-derived NO is a negative modulator of glucose-stimulated insulin secretion. In this study we have also investigated the effect of fasting upon islet NO production. The concept "starvation diabetes" is well known and studies have shown negative effects of starvation upon glucose-stimulated insulin secretion, as well as decreased insulin release in response to other fuel agonists [16]. The influence of starvation upon the islet NOS system has to our knowledge never been studied.

* Corresponding author. Tel.: +46 54617434; fax: +46 54616099.
E-mail address: Ragnar.Henningsson@liv.se (R. Henningsson).

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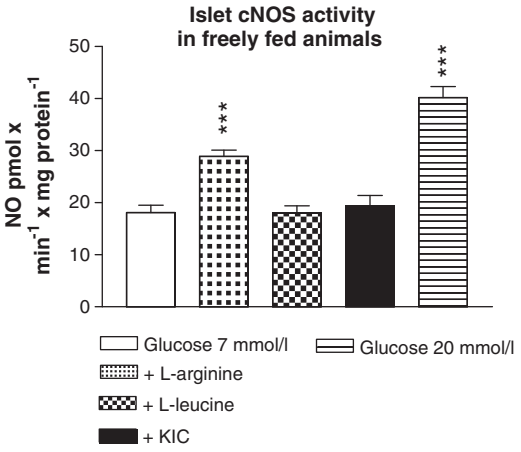

Fig. 1. Islet constitutive nitric oxide synthase (cNOS) activity in islets from freely fed mice in the presence of different secretagogues: glucose 7 mmol/l; glucose 7 mmol/l + L-arginine 10 mmol/l; glucose 7 mmol/l + L-leucine 10 mmol/L; glucose 7 mmol/l + KIC 10 mmol/l or glucose 20 mmol/l. NO production was measured as L-citrulline formation (pmol × min^{-1} × mg $protein^{-1}$). Values are means ± SEM for 8–12 batches of islets taken from 2 to 3 mice in each group. Asterisks (*) indicate probability level of random difference compared with Glucose 7 mmol/l (control). ***$p<0.001$.

2. Materials and methods

2.1. Animals

Female mice of the NMRI strain (B&K, Sollentuna, Sweden) weighing 25–30 g were used in all studies. They were fed a standard pellet diet (B&K) and tap water ad libitum. The experiments were approved by the Ethical Committee for Animal Research at Lund University.

2.2. Drugs and chemicals

Collagenase (CLS4) was obtained from Worthington Biochemicals (Freehold, NJ). L-NAME, 7-nitroindazole, L-arginine, L-leucine and KIC were from Sigma (St. Louis, MO). BSA was from ICN Biochemicals (High Wycombe, UK). All other chemicals were from Merck (Darmstadt, Germany). The RIA kits for insulin determination were obtained from Diagnostika (Falkenberg, Sweden).

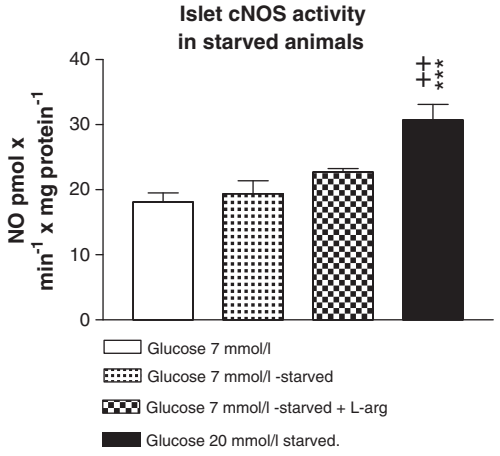

Fig. 2. Islet constitutive nitric oxide synthase (cNOS) activity in islets from 24 h starved mice (and freely fed control) in the presence of different secretagogues: glucose 7 mmol/l; glucose 7 mmol/l + L-arginine 10 mmol/l and glucose 20 mmol/l. NO production was measured as L-citrulline formation (pmol × min^{-1} × mg $protein^{-1}$). Values are means ± SEM for 6–8 batches of islets taken from 2 to 3 mice in each group. Asterisks (*) indicate probability level of random difference compared with glucose 7 mmol/l (freely fed control). *** $p<0.001$ and asterisks ($^{++}$) indicate probability level of random difference compared with Glucose 7 mmol/l (starved control). $^{++}p<0.01$.

2.3. Assay of islet NOS

Preparation of isolated pancreatic islets from the mouse was performed by retrograde injection of a collagenase solution via the bile-pancreatic duct [17]. Islets were then isolated and handpicked under a stereomicroscope at room temperature. The freshly isolated islets were then thoroughly washed and collected in ice-cold buffer (200 islets in 840 µl buffer) containing 20 mmol/l HEPES, 0.5 mmol/l EDTA and 1 mM DL-dithiothreitol, pH 7.2 and immediately frozen at −20 °C. On the day of assay, the islets were sonicated on ice, and the buffer solution containing the islets was supplemented to also contain 0.45 mmol/l $CaCl_2$, 2 mmol/l NADPH, 25 U calmodulin, and 0.2 mmol/l L-arginine, 0.2 mmol/l L-leucine or 0.2 mmol/l KIC in a total volume of 1 ml. iNOS activity was measured under similar conditions as cNOS activity, except that $CaCl_2$ and calmodulin were omitted. The buffer composition is essentially the same as previously described for assay of NOS in brain tissue using radiolabeled L-arginine [18]. The homogenate was then incubated at 37 °C under constant air bubbling, 1.0 ml/min, for 3 h. It was ascertained that under these conditions, the reaction velocity was linear for at least 6 h. Aliquots of the incubated homogenate (200 µl) were then passed through a 1 ml Ampred CBA cation-exchange column for HPLC analysis. The amount of L-citrulline formed was measured in a Hitachi F 1000 fluorescence spectrophotometer (Merck) as previously described [7]. NO and citrulline are produced in equimolar concentrations. The method has been described in detail earlier [7,19], the only difference being that the incubation was now performed at 37 °C instead of at room temperature [7]. Protein

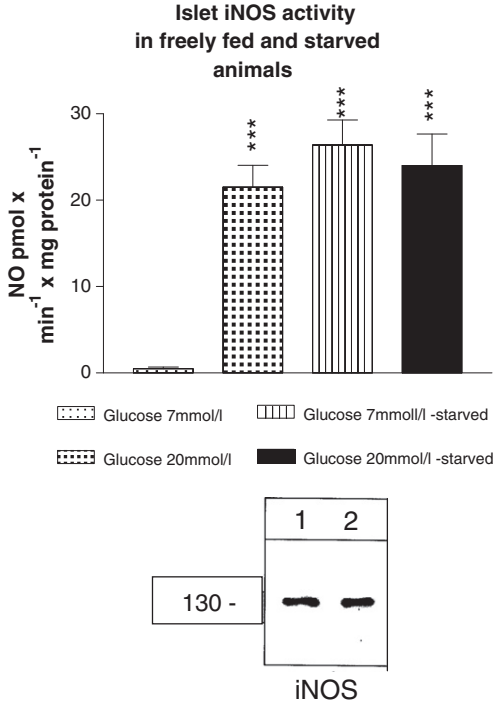

Fig. 3. Islet inducible nitric oxide (iNOS) activity in islets from freely fed or 24 h starved mice in the presence of glucose 7 mmol/l or glucose 20 mmol/l. NO production was measured as L-citrulline formation (pmol × min^{-1} × mg $protein^{-1}$). Values are means ± SEM for 8–12 batches of islets taken from 2 to 3 mice in each group. Asterisks (*) indicate probability level of random difference compared with glucose 7 mmol/l (control). ***$p<0.001$. Western blots from incubations with iNOS antibody in islet tissue. Lane 1: islet tissue from islets of freely fed mice incubated with 20 mmol/l glucose. Lane 2: loading control. At least four different experiments have been performed to ensure reproducibility. Compare with Fig. 4.