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Interaction of the islet nitric oxide system with L-arginine-induced secretion of insulin and glucagon in mice

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1 Several recent *in vitro* studies have suggested that production of nitric oxide (NO) from the islet NO system may have an important regulatory influence on the secretion of insulin and glucagon. In the present paper we have investigated, mainly with an *in vivo* approach, the influence and specificity of the NO synthase (NOS) blocker N^G-nitro-L-arginine methyl ester (L-NAME) on L-arginine-induced secretion of insulin and glucagon.

2 In freely fed mice, L-NAME pretreatment (1.2 mmol kg^{-1}) influenced the dynamics of insulin and glucagon release following an equimolar dose of L-arginine, the specific substrate for NOS activity, in that the NOS inhibitor enhanced the insulin response but suppressed the glucagon responses. This was reflected in a large decrease in the plasma glucose levels of the L-NAME pretreated animals.

3 L-NAME pretreatment did not influence the insulin and glucagon secretory responses to the L-arginine-enantiomer D-arginine, which cannot serve as a substrate for NOS activity.

4 Replacing L-NAME pretreatment by pretreatment with D-arginine or L-arginine itself, which both carry the same cationic charge and are devoid of NOS inhibitory properties, did not mimic the effects of L-NAME on L-arginine-induced hormone release.

5 Fasting the animals for 24 h totally abolished the L-NAME-induced potentiation of L-arginine stimulated insulin release suggesting that the sensitivity of the β-cell secretory machinery to NO-production is greatly changed in the fasting state. However, the L-NAME-induced suppression of L-arginine stimulated glucagon release was unaffected by starvation.

6 In isolated islets from freely fed mice, L-arginine (5 mM) stimulated insulin release was greatly enhanced and glucagon release markedly suppressed by the presence of the NOS inhibitor L-NAME in the incubation medium. These effects were abolished in isolated islets taken from 24 h fasted mice.

7 Our present results, which showed that the NOS inhibitor L-NAME markedly enhances insulin release but suppresses glucagon release induced by L-arginine in the intact animal, give strong support to our previous hypothesis that the islet NO system is a negative modulator of insulin secretion and a positive modulator of glucagon secretion. Additionally, we observed that the importance of the β-cell NO-production for secretory mechanisms, as evaluated by the effect of L-NAME on L-arginine-induced insulin release, was greatly changed after starvation, an effect less prominent with regard to glucagon release.

Keywords: Insulin secretion; glucagon secretion; L-arginine; D-arginine; nitric oxide synthase inhibition; N^G-nitro-L-arginine methyl ester; starvation

Introduction

The nitric oxide (NO) system has been identified as an important mechanism implicated in the regulation of cellular function and communication in many tissues (Moncada *et al.*, 1991). NO is derived from the metabolism of L-arginine to L-citrulline through the action of the enzyme NO synthase (NOS) (Moncada *et al.*, 1991). Several isoforms of NOS have been described among which are both constitutive Ca^{2+}/calmodulin dependent and inducible Ca^{2+}/calmodulin independent forms (Moncada *et al.*, 1991; Förstermann *et al.*, 1991; Knowles & Moncada, 1994).

There is now good histochemical and immunohistochemical evidence for the presence of both a constitutive NOS (cNOS) and an inducible NOS (iNOS) in the islets of Langerhans (Panagiotidis *et al.*, 1992a; 1994a; Schmidt *et al.*, 1992; Corbett *et al.*, 1993). This discovery may have important implications for the regulation of islet hormone release and hence for the physiology and pathophysiology of diabetes mellitus. Thus the iNOS has been shown to be involved in mechanisms responsible for β-cell destruction (Sandler *et al.*, 1991; Corbett *et al.*, 1993), whereas the functional significance of islet cNOS has been debated (Jansson & Sandler 1991; Laychock *et al.*, 1991; Jones *et al.*, 1992; Panagiotidis *et al.*, 1992a; 1994a; 1995; Schmidt *et al.*, 1992). Results from previous investigations in rat isolated islets or rat perfused pancreas are conflicting showing that NOS inhibition decreased (Laychock *et al.*, 1991; Schmidt *et al.*, 1992), did not influence (Jones *et al.*, 1992) or increased (Gross *et al.*, 1995) insulin release. However, NOS inhibition in mouse isolated islets increased insulin release and inhibited glucagon release (Panagiotidis *et al.*, 1992a; 1994a; 1995). The reasons for these discrepancies are presently not known, but it should be emphasized in this context that in *in vitro* experiments, with both rat and mouse islets, the presence of various NO donors always inhibited insulin release and enhanced glucagon release (Antoine *et al.*, 1993; Cunningham *et al.*, 1994; Panagiotidis *et al.*, 1994a; 1995; Gross *et al.*, 1995) suggesting that NO is mainly a negative modulator of insulin release and a positive modulator of glucagon release.

Thus most studies on the importance of cNOS for insulin secretory mechanisms have so far focused on various pharmacological manipulations of islet cNOS in different *in vitro* systems (Laychock *et al.*, 1991; Jones *et al.*, 1992; Panagiotidis *et al.*, 1992a; 1994a; 1995; Gross *et al.*, 1995). Therefore, the present investigation deals with a mainly *in vivo* approach to further elucidate the influence of the islet NO system on insulin and glucagon secretion induced by L-arginine in both freely fed and fasted mice. Additionally some *in vitro* experiments with islets taken from both fed and fasted mice were performed.

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Methods

Animals

Female mice of the NMRI strain (B & K, Sollentuna, Sweden), weighing 25–30 g, were used throughout the experiments. They were given a standard pellet diet (Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum* before and during the experiments or fasted for 24 h where indicated. Fasted animals received tap water *ad libitum*.

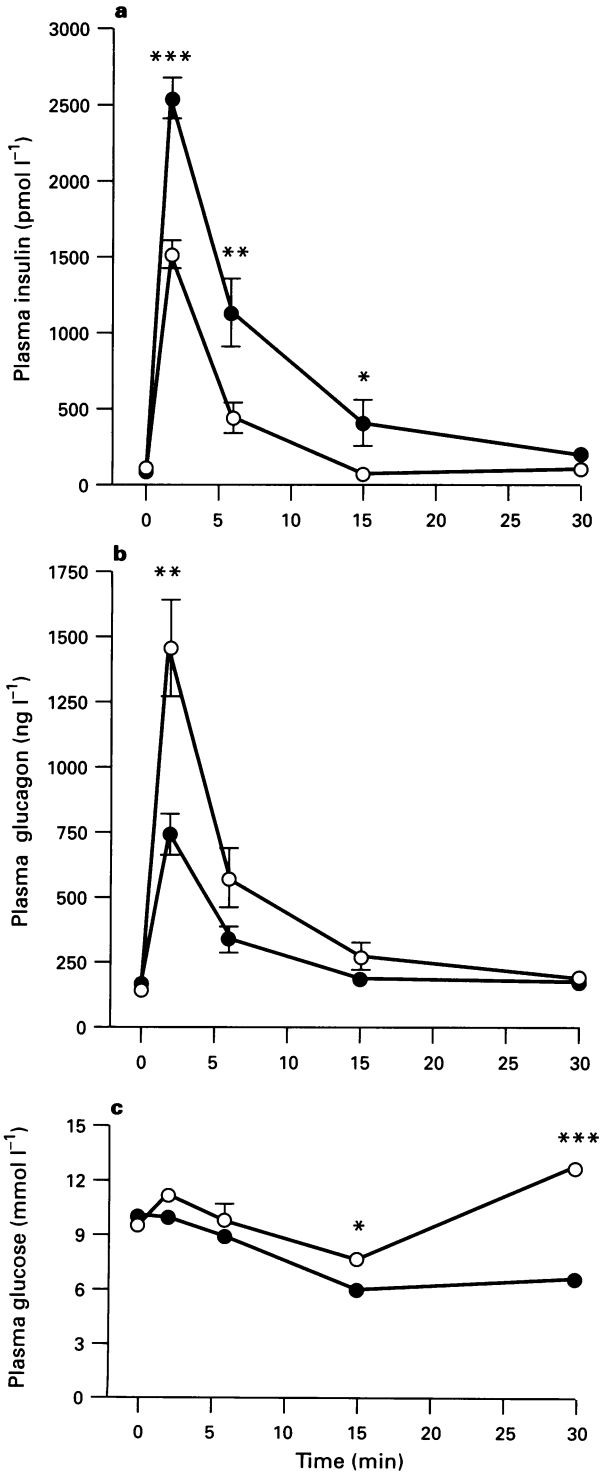

Figure 1 Effect of i.v. pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME) (1.2 mmol kg^{-1}) (●) or saline (○) on the plasma concentrations of insulin, glucagon and glucose after an i.v. injection of an approximately half-maximal dose of L-arginine (1.2 mmol kg^{-1}). L-NAME or saline was given 15 s before L-arginine. Values are mean ± s.e.mean (vertical lines) from 7 animals in each group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Experimental protocol

In vivo *studies*. L-arginine, D-arginine and N^G-nitro-L-arginine methyl ester (L-NAME) were dissolved in 0.9% NaCl. The agents were injected i.v. (volume load, 10 μl g^{-1} mouse). Pretreatment with L-NAME, D-arginine or saline (0.9% NaCl) was performed with an i.v. injection 15 s before the injection of L or D-arginine. Basal controls received saline. Blood sampling was performed as described previously (Rerup & Lundquist, 1966). Repeated experiments in our laboratory have shown that the peak plasma levels of insulin and glucagon following a rapid i.v. injection of L-arginine are achieved at approximately 2 min (unpublished). The concentration of insulin and glucagon in plasma were determined by radioimmunoassay (Heding, 1966; Ahrén & Lundquist, 1982; Panagiotidis *et al.*, 1992b). Plasma glucose concentrations were determined enzymatically (Bruss & Black, 1978).

In vitro *studies*. Preparation of isolated pancreatic islets from the mouse was performed by retrograde injection of a collagenase solution via the bile-pancreatic duct (Gotoh *et al.*, 1985). For each experiment 150–250 islets from 2–4 mice were isolated and pooled. The freshly isolated islets were preincubated at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 10 mM HEPES, 0.1% bovine serum albumin and 7 mM glucose. Each incubation vial was gassed with 95% O_2 and 5% CO_2 to obtain constant pH and oxygenation. After preincubation the buffer was changed to a medium supplemented with test agents and the islets (8 islets per 1.5 ml medium in each incubation vial) were incubated for 60 min. All incubations were performed at +37°C in an incubation box (30 cycles per min). Each experiment comprised 4–6 vials for each test group. Immediately after incubation aliquots of the medium were removed and frozen for subsequent assay of insulin and glucagon (Heding, 1966; Panagiotidis *et al.*, 1992b).

Drugs and chemicals

Collagenase (CLS-4) was purchased from Worthington Biochem., Corp., Freehold, NJ, U.S.A. L- and D-arginine, the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), as well as hydroxylamine were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Bovine serum albumin was from INC Biomedicals Ltd., High Wycombe, U.K. All other drugs and chemicals were from British Drug Houses Ltd., Poole, U.K. or Merck AG, Darmstadt, Germany. The radioimmunoassay kits for insulin determinations were obtained from Novo Nordisk Ltd., Bagsværd, Denmark and those for glucagon determinations from Eurodiagnostics Ltd., Malmö, Sweden. The antiserum used in the glucagon assay was highly selective against pancreatic glucagon.

Statistics

Levels of significance between sets of data were assessed by Student's unpaired *t* test or analysis of variance followed by Tukey-Kramer's multiple comparisons test where appropriate.

Results

In vivo *dynamics of insulin and glucagon release following stimulation by L-arginine after pretreatment with saline or L-NAME*

Groups of mice were pretreated with an i.v. injection of the NO synthase inhibitor, L-NAME (1.2 mmol kg^{-1}). After 15 s the animals were injected i.v. with L-arginine (1.2 mmol kg^{-1})

and the dynamics of the insulin, glucagon and glucose responses were compared with the responses in an appropriate control group of animals pretreated with 0.9% NaCl. Figure 1 shows that the L-arginine-induced insulin response was enhanced, whereas the glucagon response was suppressed after pretreatment with L-NAME. This increase in insulin release and decrease in glucagon release was reflected in a marked depression of the plasma glucose levels in animals pretreated with the NO synthase inhibitor (Figure 1).

Specificity of NO synthase inhibition on L-arginine stimulated hormone release in vivo

The next series of experiments was designed to study the influence of the different arginine analogues D-arginine and L-NAME on D-arginine or L-arginine-induced insulin and glucagon response *in vivo*. Table 1 (groups A–E) shows the effect of pretreatment with an i.v. injection of L-NAME (1.2 mmol kg^{-1}) or D-arginine (1.2 mmol kg^{-1}) on the D-arginine-induced hormone response. It is seen that both L-NAME and D-arginine induced a modest enhancement of basal plasma insulin levels (from approximately 150 pmol l^{-1} to 300 pmol l^{-1}) at 2 min after injection. Animals pretreated with L-NAME or D-arginine 15 s before D-arginine administration displayed plasma levels of approximately 450 pmol l^{-1}, showing that L-NAME had no potentiating effect on D-arginine-induced insulin response. In fact, the insulin response to L-NAME + D-arginine was approximately the same as the sum of the responses to L-NAME itself and D-arginine itself. With regard to the glucagon response, L-NAME did not influence either basal or D-arginine-induced glucagon secretion. However, D-arginine + D-arginine, induced a slightly increased glucagon response compared with saline + D-arginine. Table 1 (groups F–J) shows the effect of L-arginine (1.2 mmol kg^{-1}) on the insulin and glucagon responses following pretreatment with either saline or 1.2 mmol kg^{-1} of D-arginine, L-arginine or L-NAME. The effect of saline + 2.4 mmol kg^{-1} (double dose) of L-arginine is also shown; 1.2 mmol kg^{-1} of L-arginine has previously been shown to induce an approximately half-maximal effect on insulin release (Lundquist, 1986). In the present series of experiments, L-arginine induced an increase of insulin release of approximately +900 pmol (group F). Thus, L-arginine was much more potent than D-arginine (+150 pmol; group A). Further, it was seen that pretreatment with the NOS inhibitor L-NAME greatly potentiated the insulin response to L-arginine (from 1038 pmol l^{-1} to 3100 pmol l^{-1}). Pretreatment with D-arginine had no significant effect on L-arginine-induced insulin release, whereas pretreatment with L-arginine followed by another L-arginine injection as well as pretreatment with saline followed by a double dose of L-arginine induced insulin responses which were far below the 3100 pmol l^{-1} brought about by L-NAME + L-arginine. As expected, L-NAME suppressed L-arginine-induced glucagon release. Pretreatment with D-arginine or L-arginine had no appreciable effect on the glucagon response to L-arginine. Plasma glucose levels were not influenced within the time period studied (2 min) (as previously shown Figure 1).

Influence of fasting on the in vivo *effect of L-NAME on the L-arginine-induced insulin and glucagon response*

Figure 2 shows a comparison of the effect of L-NAME on L-arginine stimulated insulin and glucagon response in freely fed and 24 h fasted mice. Figure 2a and b (freely fed mice) show the increase in basal and L-arginine-induced insulin secretion (a) and decrease in L-arginine-induced glucagon secretion (b) following pretreatment with the NOS inhibitor. Basal glucagon levels were not influenced by L-NAME itself (Figure 2b). In contrast, Figure 2c and d show that in 24 h fasted mice the effect of L-NAME on basal and L-arginine stimulated insulin release is abolished (c). L-Arginine-induced glucagon response, however, was still suppressed by L-NAME in fasted mice (d). Figure 2 also shows that fasting greatly reduced the insulin response to L-arginine (compare a and c in Figure 2). The glucagon response to L-arginine, however, was of the same magnitude in freely fed and fasted mice (compare b and d in Figure 2). As expected, basal plasma levels of insulin were greatly reduced in 24 h fasted animals (compare a and c in Figure 2).

Influence of fasting on the effect of L-NAME on insulin and glucagon secretion from isolated islets

Figure 3 shows a comparison of the effects of the NO synthase inhibitor L-NAME on L-arginine induced insulin and glucagon secretion from islets isolated from either freely fed (Figure 3a and b) or 24 h fasted (Figure 3c and d) mice. Figure 3a and b show that L-arginine (5 mM) enhanced basal (7 mM glucose), insulin (a) and glucagon (b) release from fed islets. Further, in fed islets L-NAME (5 mM) potentiated L-arginine (5 mM) stimulated insulin release (a) but suppressed L-arginine-induced glucagon release (b). L-NAME itself had no effect. However, in fasted islets (Figures 3c and d) L-NAME did not display any effect on either insulin release (c) or glucagon release (d) stimulated by L-arginine. L-Arginine itself increased glucagon release (d) but had no significant effect on insulin release (c) in fasted islets.

Table 1 Effect of pretreatment with saline, N^G-nitro-L-arginine methyl ester (L-NAME), D-arginine (D-Arg) or L-arginine (L-Arg) on the basal (saline), D-arginine or L-arginine stimulated insulin and glucagon response *in vivo*

Group	*Pretreatment*	*Treatment*	*Plasma insulin* (pmol l^{-1})	P-*value insulin vs. control*	*Plasma glucagon* (ng l^{-1})	P-*value glucagon vs control*
A	Saline	Saline	156±37.2	–	233±40	–
B	L-NAME	Saline	312±52.8	<0.05	198±30	NS
C	Saline	D-Arg	276±31.2	<0.05	470±49	<0.01
D	L-NAME	D-Arg	474±39	<0.01	476±55	<0.01
E	D-Arg	D-Arg	438±60	<0.01	663±67	<0.001
F	Saline	L-Arg	1038±54	<0.001	1181±116	<0.001
G	D-Arg	L-Arg	1332±156	<0.001	1142±131	<0.001
H	L-Arg	L-Arg	1710±264	<0.001	1331±84	<0.001
I	Saline	L-Arg (2.4)	2148±330	<0.001	1600±203	<0.001
J	L-NAME	L-Arg	3102±234	<0.001	803±105	<0.001

All agents were given i.v. in a dose of 1.2 mmol kg^{-1} except for group I where the dose of L-arginine was 2.4 mmol kg^{-1}. Pretreatment was given 15 s before treatment. Blood sampling was performed at 2 min (peak levels of insulin and glucagon response) following injection of the different treatment agents. Values are mean±s.e.mean from 8–15 animals in each group. *P*-values *vs* basal controls (saline + saline) are denoted in the table. Significant differences between other treatment groups are denoted below; Plasma insulin: C *vs* D, $P<0.01$; C *vs* E, $P<0.05$; F *vs* H, $P<0.01$; F *vs* I, $P<0.01$; H *vs* J, $P<0.01$; I *vs* J, $P<0.05$. Plasma glucagon: C *vs* E, $P<0.05$; H *vs* J, $P<0.01$; I *vs* J, $P<0.01$.

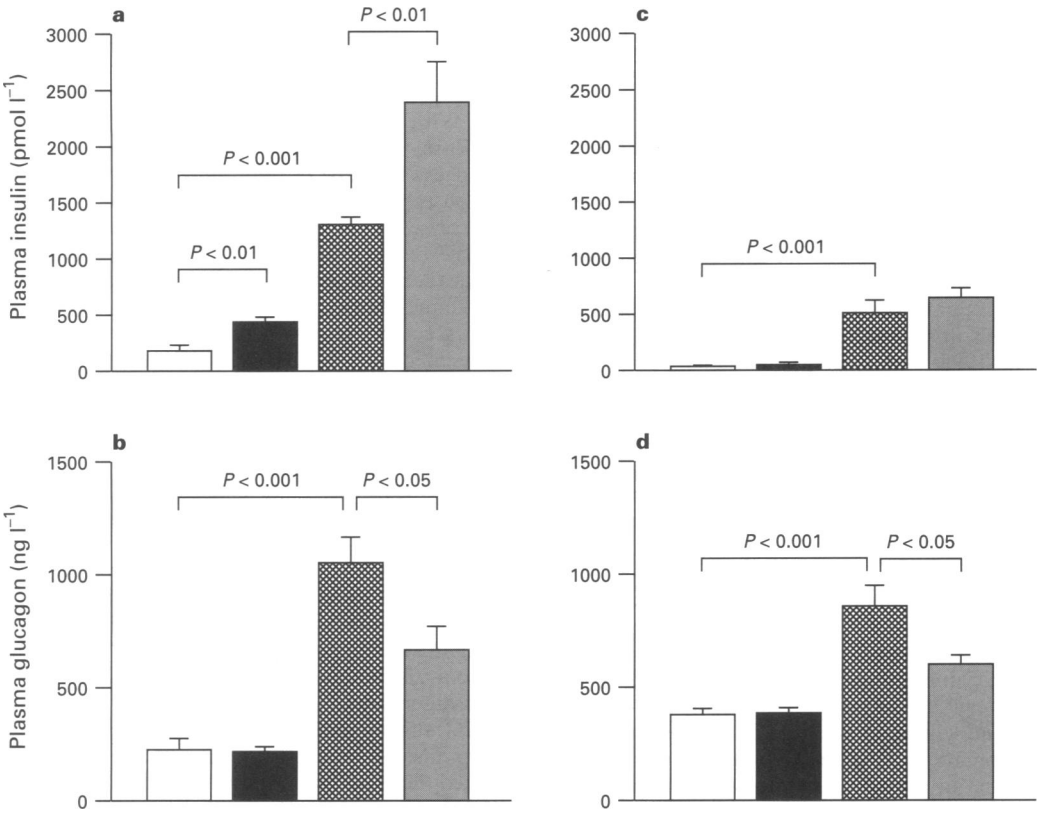

Figure 2 Effect of pretreatment (15 s) with N^G-nitro-L-arginine methyl ester (L-NAME) (1.2 mmol kg^{-1}) or saline on basal or L-arginine (1.2 mmol kg^{-1}) stimulated insulin and glucagon secretion in freely fed (a and b) and 24 h fasted (c and d) mice. Data from animals injected with saline + saline (open columns), L-NAME + saline (solid columns), saline + L-arginine (cross-hatched columns), and L-NAME + L-arginine (stippled columns) are shown. Values are mean ± s.e.mean for 5–10 animals in each group.

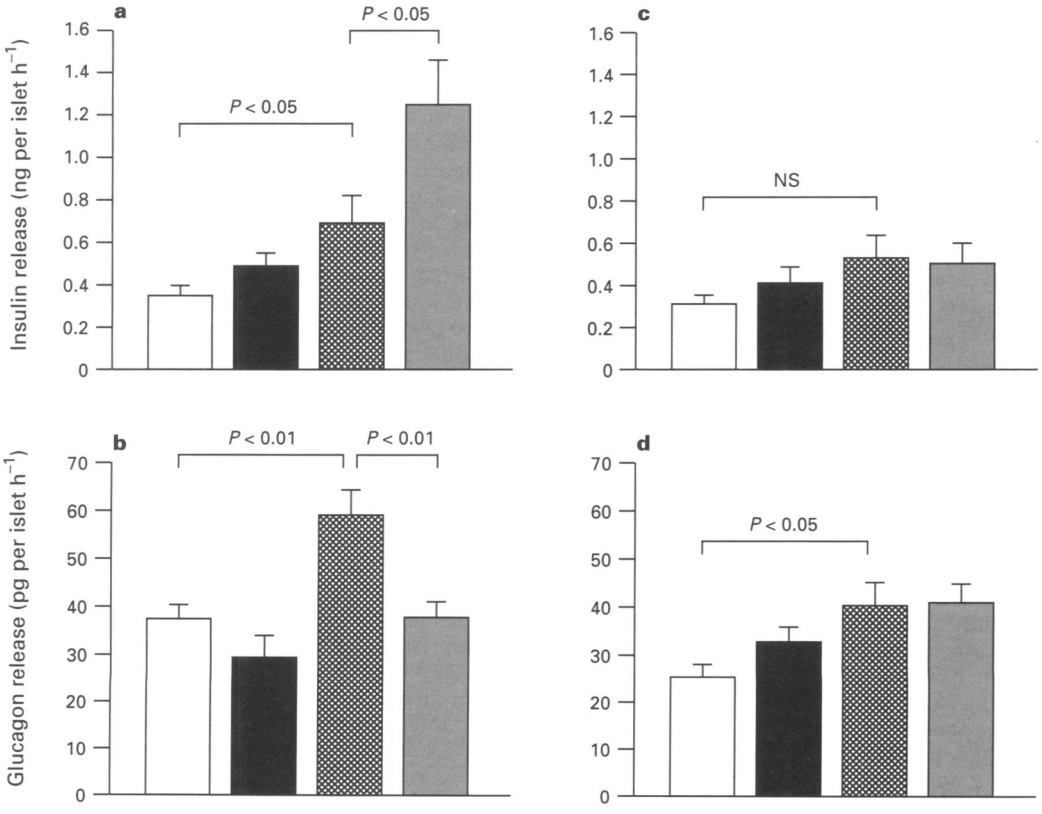

Figure 3 Effect of L-arginine (5 mM) (cross-hatched columns) and N^G-nitro-L-arginine methyl ester (L-NAME) (5 mM) + L-arginine (stippled columns) as well as L-NAME itself (solid columns) in the presence of 7 mM glucose on insulin and glucagon secretion from isolated islets taken from freely fed (a and b) and 24 h fasted (c and d) animals. Basal controls (7 mM glucose) are denoted by open columns. Values are mean ± s.e.mean from 10–14 incubation vials containing 8 islets each. The incubation period was 60 min. Each experiment comprised 4–6 incubation vials for each of the 4 test groups.

Discussion

Previous data from our laboratory (Lundquist *et al.*, 1991; Panagiotidis *et al.*, 1992a; 1993a, b, c; 1994a, b; 1995) have suggested that low levels of free radicals such as NO and H_2O_2 produced by the islet itself may serve as important physiological modulators of the insulin secretory processes. With regard to NO, we were the first to propose that NO, derived from islet cNOS activity, was a negative modulator of insulin secretion (Panagiotidis *et al.*, 1992a) and a positive modulator of glucagon secretion (Panagiotidis *et al.*, 1994a). Although previous *in vitro* data (Laychock *et al.*, 1991; Jones *et al.*, 1992; Schmidt *et al.*, 1992) have suggested that NO either promoted or did not influence insulin secretory processes, we (Panagiotidis *et al.*, 1994a; 1995) and others (Cunningham *et al.*, 1994; Gross *et al.*, 1995) have consistently found that the main effect of NO is to suppress insulin release both from isolated islets and from the perfused pancreas.

In accordance with our previous *in vitro* studies, we have now shown, in a series of complementary *in vivo* experiments, that the L-arginine induced insulin response is increased and the glucagon response decreased after pretreatment of the animals with the NOS inhibitor, L-NAME. In order to avoid, as far as possible, any indirect systemic effects of L-NAME, we administered the drug i.v. 15 s before the i.v. injection of L-arginine. As can be seen from our first series of experiments (Figure 1), the NOS inhibiting effect was very rapid in onset and manifested already at the peak level (2 min) of L-arginine-induced insulin and glucagon release. The enhanced insulin response in combination with the suppressed glucagon response was reflected in a marked and long-lasting depression of the plasma glucose levels in the L-NAME-pretreated animals. L-Arginine is believed to exert at least part of its hormone releasing action as a consequence of its depolarizing cationic charge (Jansson & Sandler, 1991; Panagiotidis *et al.*, 1995; Gross *et al.*, 1995). Since the L-arginine analogue L-NAME has similar cationic properties to L-arginine itself, the question arose as to whether this cationic charge of L-NAME, and not its specific NOS inhibiting effect, could contribute to the L-NAME-potentiating action on L-arginine-induced insulin release. This was tested by performing a series of experiments with the L-arginine enantiomer D-arginine, which carries the same cationic charge as L-arginine and L-NAME but is not a substrate for cNOS activity (Moncada *et al.*, 1991). The data obtained in these experiments showed that both L-NAME and D-arginine alone, at the same dose level as L-arginine (1.2 mmol kg^{-1}), induced a slight increase in basal insulin levels. Further, pretreatment with either L-NAME or D-arginine had the same additive effect on D-arginine-induced insulin release. Thus, L-NAME did not potentiate D-arginine-induced insulin release at all, suggesting that the large potentiating effect of L-NAME on L-arginine-induced insulin release was mainly a result of its NOS-inhibitory property. Moreover, replacing L-NAME pretreatment with an equimolar dose of either D-arginine or L-arginine brought about an increase in the L-arginine-induced insulin response that was approximately 4–5 times lower than after NOS inhibition by L-NAME-pretreatment. Thus, pretreatment with D-arginine or L-arginine could not reproduce the effect of L-NAME on the response of the β-cell to the subsequent injection of L-arginine. The present data thus suggest that NO is a negative modulator of insulin release. An inhibitory action of NO on insulin releasing mechanisms has previously been shown in isolated islets and perfused pancreas by the use of NO donors such as hydroxylamine, sydnonimine-1 (SIN-1) and sodium nitroprusside (SNP) (Antoine *et al.*, 1993; Cunningham *et al.*, 1994; Pangiotidis *et al.*, 1994a, 1995; Gross *et al.*, 1995). The effect of SNP could be related to activation of ATP-sensitive K^+ channels (Antoine *et al.*, 1993). With regard to the effects of NOS inhibitors, however, the possibility remains that NOS inhibition by L-NAME is not the sole explanation for the increased insulin secretion. Thus, it is not inconceivable that NOS normally uses up part of the available L-arginine so that it cannot directly enhance insulin release (e.g. by the ornithine-glutamate metabolic pathway) and that L-NAME inhibits this alternative 'NOS-blockade' of L-arginine. The slight increase in basal insulin levels observed after injection of L-NAME itself is possibly explained by its charge-related action.

With regard to the influence of cNOS activity on glucagon release, available *in vitro* data (Panagiotidis *et al.*, 1994a) suggest that cNOS inhibition by L-NAME inhibits glucagon releases and that the intracellular NO donor hydroxylamine increases it. The present *in vivo* data are in accordance with these results, suggesting that NO is a positive modulator of glucagon release. Pretreatment with L-NAME inhibited the L-arginine-induced glucagon response, whereas it had no effect on glucagon release stimulated by D-arginine suggesting that the effect of L-NAME is NOS-specific. Further, pretreatment with D-arginine or L-arginine, instead of L-NAME, did not suppress the subsequent glucagon response to L-arginine, suggesting again that the inhibitory effect of L-NAME is NOS-specific and also that pretreatment with D-arginine or L-arginine, carrying the same charge as L-NAME, does not affect the stimulating action of L-arginine on glucagon release. In fact, the dose of L-arginine selected, 1.2 mmol kg^{-1}, which is half maximal with regard to insulin release (Lundquist, 1986) seemed to have a near-maximal effect on glucagon release (Table 1). Further, it should be noted that, in contrast to the slightly stimulating effects of both L-NAME and D-arginine on basal insulin levels, L-NAME had no effect on basal plasma glucagon levels, whereas D-arginine was a stimulator. These data suggest different modes of action of L-NAME and D-arginine on glucagon release and argue against a charge-related depolarizing and Ca^{2+} influx promoting effect being common to both. It should be recalled that not only insulin release but also glucagon release is initiated by depolarization and subsequent influx of Ca^{2+} (Lundquist *et al.*, 1976; Hellman *et al.*, 1994). However, there is a possibility that a charge-induced depolarization by L-NAME on the glucagon-releasing cell is at the same time antagonized by its NOS-inhibitory property and thus neither stimulation nor inhibition is seen.

The last part of the present study deals with the influence of fasting on L-arginine-induced insulin and glucagon release in relation to NOS inhibition both *in vivo* and *in vitro*. Fasting is known to induce a state of poor responsiveness to different insulin secretagogues, among which are both glucose (the most important insulin releaser) and L-arginine (Hedeskov 1980; Lundquist & Lövdahl 1983, Lundquist 1986). The nature of this deficient responsiveness of the fasting β-cell is still unclear and probably very complex. Hence the question arose as to whether a perturbed production of islet NO could be of decisive importance in this context. In accordance with previous *in vivo* results in mice (Lundquist, 1986), the L-arginine-induced insulin release was reduced by approximately 50% in fasted mice as compared to freely fed animals. Similar results were obtained with L-arginine-induced insulin release from isolated islets. In fact, 5 mM L-arginine did not induce a significant insulin release in islets from fasted mice. In contrast to the highly significant L-NAME-induced potentiation of L-arginine stimulated insulin release observed in freely fed animals and islets, the NOS inhibitor had no effect at all on L-arginine-induced insulin release either in fasted animals or in fasted islets. These data suggest that the sensitivity of the secretory machinery to NO is greatly changed during fasting either by a suppression of the cNOS enzyme activity itself and/or, more likely, as a consequence of an insufficient supply of indispensable cofactors for cNOS activity such as Ca^{2+} and NADPH, which, following suppression of the blood glucose levels, are known to decrease in the β-cell during fasting (Hedeskov, 1980). The *in vivo* data from freely fed and fasted mice, respectively, also strongly argue against the possibility that the L-NAME-induced suppression of L-arginine-stimulated glucagon release could simply be a consequence of the enhanced insulin release, insulin being a potent inhibitor of glucagon release (Maruyama *et al.*, 1984). As seen in Figure 2, the suppressive effect of L-NAME on L-arginine-induced glucagon

release in fasted mice is of the same magnitude as in freely fed mice, yet there is no L-NAME-induced potentiation of L-arginine stimulated insulin release in the fasted mice. A similar conclusion could be inferred from the data in Figure 1 showing that L-NAME-induced suppression of L-arginine-induced glucagon release appears as early (2 min) as the increase in insulin release.

Surprisingly, the L-NAME-induced inhibition of L-arginine stimulated glucagon release was not affected by the fasting state *in vivo*, whereas no effect of L-NAME was observed in fasted islets *in vitro*. One possible explanation might be that, in the fasting *in vivo* situation, the glucagon releasing cells, which are situated in the islet periphery, are significantly influenced by NOS-containing nerves running along the islet border (Panagiotidis *et al.*, 1992a). The effect of these nerves is abolished in the isolated islet system and thus no effect of L-NAME is seen *in vitro*. Another explanation goes back to our previous observation, that the NO donor hydroxylamine could induce glucagon release at concentrations 10–100 times lower than those required for inhibition of insulin release (Panagiotidis *et al.*, 1994a; 1995). Thus the NO-modulated hormone releasing system in the glucagon releasing cell might be much more sensitive to NO than that of the insulin producing β-cell. If this sensitivity is reduced by the combined effects of fasting plus isolation of islets no influence of L-NAME is detectable. The intimate details of these mechanisms await further studies. In this context it should be noted that there is general agreement that a cNOS activity resides in the insulin producing β-cells (Panagiotidis *et al.*, 1992a; 1994a; Schmidt *et al.*, 1992; Corbett *et al.*, 1993; Bouwens & Klöppel, 1994), whereas there are conflicting reports as to whether cNOS activity is also located in the glucagon releasing cells (Corbett *et al.*, 1993; Bouwens & Klöppel, 1994).

In conclusion, in a large series of *in vivo* experiments we have shown that pretreatment of mice with the NOS inhibitor L-NAME greatly enhances L-arginine-induced insulin release, whereas the glucagon response is suppressed. These effects seem to be the result of a specific L-NAME-induced blockade of NO production, since they could not be reproduced by replacing L-NAME with analogues such as D-arginine or L-arginine itself, which both carry the same cationic charge as L-NAME and are devoid of NOS inhibitory properties. Moreover, hormone release stimulated by D-arginine, which, in contrast to L-arginine, is not a substrate for NOS activity, was not influenced by L-NAME pretreatment. Finally, we observed that a starvation period of 24 h almost totally abolished the L-NAME-induced effect on L-arginine stimulated insulin release both *in vivo* and *in vitro*, suggesting that the influence of the β-cell NO production on the secretory machinery is greatly changed in the fasting state. NO-dependent glucagon release, however, was abolished in fasted islets but unaffected in the fasted animal suggesting a higher sensitivity and/or an additional source of NO production influencing the glucagon releasing cell. The present results give strong support to our previous hypothesis (Panagiotidis *et al.*, 1992a; 1994a; 1995) that islet production of NO is of physiological importance as a negative modulator of insulin release and a positive modulator of glucagon release.

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References

AHREN, B. & LUNDQUIST, I. (1982). Glucagon immunoreactivity in plasma from normal and dystrophic mice. *Diabetologia*, **22**, 258–263.

ANTOINE, M.-H., HERMANN, M., HERCHUELZ, A. & LEBRUN, P. (1993). Sodium nitroprusside inhibits glucose-induced insulin release by activating ATP-sensitive K^+ channels. *Biochim. Biophys. Acta*, **1175**, 293–301.

BOUWENS, L. & KLÖPPEL, G. (1994). Cytochemical localization of NADPH-diaphorase in the four types of pancreatic islet cell. *Histochemistry*, **101**, 209–214.

BRUSS, M.L. & BLACK, A.L. (1978). Enzymatic microdetermination of glycogen. *Anal. Biochem.*, **84**, 309–312.

CORBETT, J.A., WANG, J.L., MISKO, T.P., ZHAO, W., HICKEY, W.F. & MCDANIEL, M.L. (1993). Nitric oxide mediates IL-1β-induced islet dysfunction and destruction: Prevention by dexamethasone. *Autoimmunity*, **15**, 145–153.

CUNNINGHAM, J.M., MABELY, J.G., DELANEY, C.A. & GREEN, I.C. (1994). The effect of nitric oxide donors on insulin secretion, cyclic GMP and cyclic AMP in rat islets of Langerhans and the insulin-secreting cell lines HIT-T15 and RINm5F. *Mol. Cell. Endocrinol.* **102**, 23–29.

FÖRSTERMAN, U., SCHMIDT, H.H.H.W., POLLOCK, J.S., SHENG, H., MITCHELL, J.A., WARNER, T.D., NAKANE, M. & MURAD, F. (1991). Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem. Pharmacol.*, **42**, 1849–1857.

GOTOH, M., MAKI, T., KIYOIZUMI, T., SATOMI, S. & MONACO, A.P. (1985). An improved method for isolation of mouse pancreatic islets. *Transplantation*, **40**, 437–438.

GROSS, R., ROYE, M., MANTEGHETTI, M., HILLAIRE-BUYS, D. & RIBES, G. (1995). Alterations of insulin response to different β cell secretagogues and pancreatic vascular resistance induced by N^{ω}-nitro-L-arginine methyl ester. *Br. J. Pharmacol.*, **116**, 1965–1972.

HEDESKOV, C.J. (1980). Mechanism of glucose-induced insulin secretion. *Physiol. Rev.*, **60**, 442–509.

HEDING, L. (1966). A simplified insulin radioimmunoassay method. In *Labelled Proteins in Tracer Studies*, ed. Donato, L., Milhaud, G. & Sirchis, J. pp. 345–350. Brussels: Euratom.

HELLMAN, B., GYLFE, E., BERGSTEN, P., GRAPENGIESSER, E., LUND, P.E., BERTS, A., DRYSELIUS, S., TENGHOLM, A., LIU, Y.J., EBERHARDSON, M. & CHOW, R.H. (1994). The role of Ca^{2+} in the release of pancreatic islet hormones. *Diabète Metab.*, **20**, 123–131.

JANSSON, L. & SANDLER, S. (1991). The nitric oxide synthase inhibitor N^G-nitro L-arginine stimulates pancreatic islet insulin release in vitro, but not in the perfused pancreas. *Endocrinology*, **128**, 3081–3085.

JONES, P.M., PERSAUD, S.J., BJAALAND, T., PEARSON, J.D. & HOWELL, S.L. (1992). Nitric oxide is not involved in the initiation of insulin secretion from rat islets of Langerhans. *Diabetologia*, **35**, 1020–1027.

KNOWLES, R.G. & MONCADA, S. (1994). Nitric oxide synthases in mammals. *Biochem. J.*, **298**, 249–258.

LAYCHOCK, R.G., MODICA, M.E. & CAVANAUGH, C.T. (1991). L-Arginine stimulates cyclic guanosine 3′,5′-monophosphate formation in rat islets of Langerhans and RINm5F insulinoma cells: evidence for L-arginine: nitric oxide synthase. *Endocrinology*, **129**, 3043–3052.

LUNDQUIST, I. (1986). Islet amyloglucosidase activity: some characteristics, and its relation to insulin secretion stimulated by various secretagogues. *Diabetes Res.*, **3**, 31–41.

LUNDQUIST, I., FANSKA, R. & GRODSKY, G.M. (1976). Direct calcium-stimulated release of glucagon from the isolated perfused rat pancreas and the effect of chemical sympathectomy. *Endocrinology*, **98**, 815–818.

LUNDQUIST, I. & LÖVDAHL, R. (1983). Effect of fasting on islet lysosomal enzyme activities and the in vivo insulin response to different secretagogues. *Horm. Metabol. Res.*, **15**, 11–14.

LUNDQUIST, I., PANAGIOTIDIS, G. & STENSTRÖM, A. (1991). Effect of L-DOPA administration on islet monoamine oxidase activity and glucose-induced insulin release in the mouse. *Pancreas*, **6**, 522–527.

MARUYAMA, H., HISATOMI, A., ORCI, L., GRODSKY, G.M. & UNGER, R.H. (1984). Insulin within islets is a physiologic glucagon release inhibitor. *J. Clin. Invest.*, **74**, 2296–2299.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43,** 109–142.

PANAGIOTIDIS, G., ÅKESSON, B., ALM, P. & LUNDQUIST, I. (1994a). The nitric oxide system in the endocrine pancreas induces differential effects on the secretion of insulin and glucagon. *Endocrine*, **2,** 787–792.

PANAGIOTIDIS, G., ÅKESSON, B., RYDELL, E.L. & LUNDQUIST, I. (1995). Influence of nitric oxide synthase inhibition, nitric oxide and hydroperoxide on insulin release induced by various secretagogues. *Br. J. Pharmacol.*, **114,** 289–296.

PANAGIOTIDIS, G., LINDSTRÖM, P., STENSTRÖM, A. & LUNDQUIST, I. (1993a). Glucose modulation of islet monoamine oxidase activity in lean and obese hyperglycemic mice. *Metabolism*, **42,** 1398–1404.

PANAGIOTIDIS, G., SALEHI, A.A., WESTERMARK, P. & LUNDQUIST, I. (1992b). Homologous islet amyloid polypeptide: effects on plasma levels of glucagon, insulin and glucose in the mouse. *Diabetes Res. Clin. Pract.*, **18,** 167–171.

PANAGIOTIDIS, G., STENSTRÖM, A. & LUNDQUIST, I. (1993b). Influence of β-adenoceptor stimulation and glucose on islet monoamine oxidase activity and insulin secretory response in the mouse. *Pancreas*, **8,** 368–374.

PANAGIOTIDIS, G., STENSTRÖM, A. & LUNDQUIST, I. (1993c). Effects of adrenergic and cholinergic stimulation on islet monoamine oxidase activity and insulin secretion in the mouse. *Eur. J. Pharmacol.*, **233,** 285–290.

PANAGIOTIDIS, G., STENSTRÖM, A. & LUNDQUIST, I. (1994b). In vivo action of cyclic AMP modulating secretagogues on islet monoamine oxidase activity and insulin release. *Endocrine*, **2,** 571–576.

RERUP, C. & LUNDQUIST, I. (1966). Evaluation of a new technique of multiple serial sampling. *Acta Endocrinol.*, **52,** 357–367.

SANDLER, S., EIZIRIK, D.L., SVENSSON, C., STRANDELL, E., WELSH, M. & WELSH, N. (1991). Biochemical and molecular actions of interleukin-1 on pancreatic β-cells. *Autoimmunity*, **10,** 241–253.

SCHMIDT, H.H.H.W., WARNER, T.D., ISHII, K., SHENG, H. & MURAD, F. (1992). Insulin secretion from pancreatic β cells caused by L-arginine-derived nitrogen oxides. *Science*, **255,** 721–723.

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