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Mechanisms involved in the effect of nitric oxide synthase inhibition on L-arginine-induced insulin secretion

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1 A constitutive nitric oxide synthase (NOSc) pathway negatively controls L-arginine-stimulated insulin release by pancreatic β cells. We investigated the effect of glucose on this mechanism and whether it could be accounted for by nitric oxide production.

2 NOSc was inhibited by N^{ω}-nitro-L-arginine methyl ester (L-NAME), and sodium nitroprusside (SNP) was used as a palliative NO donor to test whether the effects of L-NAME resulted from decreased NO production.

3 In the rat isolated perfused pancreas, L-NAME (5 mM) strongly potentiated L-arginine (5 mM)-induced insulin secretion at 5 mM glucose, but L-arginine and L-NAME exerted only additive effects at 8.3 mM glucose. At 11 mM glucose, L-NAME significantly inhibited L-arginine-induced insulin secretion. Similar data were obtained in rat isolated islets.

4 At high concentrations (3 and 300 μM), SNP increased the potentiation of arginine-induced insulin output by L-NAME, but not at lower concentrations (3 or 30 nM).

5 L-Arginine (5 mM) and L-ornithine (5 mM) in the presence of 5 mM glucose induced monophasic β cell responses which were both significantly reduced by SNP at 3 nM but not at 30 nM; in contrast, the L-ornithine effect was significantly increased by SNP at 3 μM.

6 Simultaneous treatment with L-ornithine and L-arginine provoked a biphasic insulin response.

7 At 5 mM glucose, L-NAME (5 mM) did not affect the L-ornithine secretory effect, but the amino acid strongly potentiated the alteration by L-NAME of L-arginine-induced insulin secretion.

8 L-Citrulline (5 mM) significantly reduced the second phase of the insulin response to L-NAME (5 mM) + L-arginine (5 mM) and to L-NAME + L-arginine + SNP 3 μM.

9 The intermediate in NO biosynthesis, N^{G}-hydroxy-L-arginine (150–300 μM) strongly counteracted the potentiation by L-NAME of the secretory effect of L-arginine at 5 mM glucose.

10 We conclude that the potentiation of L-arginine-induced insulin secretion resulting from the blockade of NOSc activity in the presence of a basal glucose concentration (1) is strongly modulated by higher glucose concentrations, (2) is not due to decreased NO production but (3) is probably accounted for by decreased levels of N^{G}-hydroxy-L-arginine or L-citrulline, resulting in the attenuation of an inhibitory effect on arginase activity.

Keywords: Insulin secretion; glucose; L-arginine; nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester; L-citrulline; N^{G}-hydroxy-L-arginine; sodium nitroprusside

Introduction

Previous *in vitro* studies performed in our laboratory in rats (Gross *et al*., 1992; 1993; 1994; 1995) as well as by others in mice (Panagiotidis *et al*., 1992; 1995) have suggested that nitric oxide (NO) plays an important negative control on pancreatic β cell function. So, with respect to glucose-induced insulin secretion, we showed in the rat isolated perfused pancreas, that N^{ω}-nitro-L-arginine methyl ester (L-NAME), a potent competitive inhibitor of constitutive nitric oxide synthase (NOSc) activity, was able at increasing concentrations, to convert progressively the biphasic pattern of insulin response to glucose into a significantly greater monophasic one (Gross *et al*., 1993; 1995). This suggests that endogenously produced NO is implicated in both the pattern and the magnitude of the β cell response to the sugar. However, sodium nitroprusside (SNP), a chemical NO donor, was able to only partly correct L-NAME-induced alterations and this in the high micromolar range (Gross *et al*., 1994; 1995).

As for L-arginine-induced insulin secretion, in the presence of a physiological but non-stimulating glucose concentration, L-NAME potentiated the moderate monophasic response to the amino acid into a very strong biphasic response. Since the secretory effect of L-arginine depends on and increases with the underlying glucose concentration (Levin *et al*., 1972), we first investigated whether L-NAME potentiation of the β cell response to this amino acid could also be modulated by changes in glucose concentration. Secondly, we were interested to determine if this potentiation, resulting from NOSc blockade, might be accounted for by decreased NO production or by alternative but related mechanisms and especially changes in arginase activity (the second metabolic pathway of L-arginine). Indeed, (i) L-NAME, inactive *per se* on arginase activity (Robertson *et al*., 1993), was expected to depress the production of NO and L-citrulline (Chen & Mehta, 1996) as well as N^{G}-hydroxy-L-arginine, and (ii) L-citrulline (Hrabak *et al*., 1994) and N^{G}-hydroxy-L-arginine (Daghigh *et al*., 1994; Boucher *et al*., 1994) are known to be inhibitors of arginase activity.

Methods

Adult male Wistar rats (Iffa Credo, Lyon, France) were used in this study. They received a standard pellet diet (U.A.R., Epinay-sur-Orge, France), and had free access to tap water; they weighed 340–370 g at the moment of the experiments. After

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sodium pentobarbitone anaesthesia, the pancreas was totally isolated from all neighbouring tissues according to the techniques of Loubatières *et al*. (1969), then transferred into a thermostated (37.5°C) plastic chamber and perfused through its own arterial system. The perfusion medium, administered with a peristaltic pump via an open circuit, was a Krebs Ringer Bicarbonate buffer containing 2 g l^{-1} bovine serum albumin (fraction V, Sigma, St-Louis Mo, France); it had the following ionic composition (mM): NaCl 108, KH_2PO_4 1.19, KCl 4.74, $CaCl_2$ 2.54, $MgSO_4$ $7H_2O$ 1.19, $NaHCO_3$ 18. A mixture of 95% O_2, 5% CO_2 was continuously bubbled through the buffer to provide an adequate oxygen supply and to keep the pH close to 7.35; glucose and drugs were added according to the experimental protocols. The perfusion pressure (35–45 cmH_2O), measured with a water manometer was maintained constant; it was selected to obtain a pancreatic flow rate of 2.5 ml min^{-1}. A pressure limiter allowed part of the medium not accepted by the organ to return to the original reservoir. So, any change in pancreatic vascular bed resistance induced by drugs could be detected by measuring pancreatic outflow with graduated test tubes. For each experiment a 30 min adaptation was allowed before the first sampling. All samples were immediately frozen until insulin assay.

Islets of Langerhans were isolated from fed rats by collagenase digestion (Lacy & Kostianovsky, 1967) and preincubated during 60 min in Krebs Ringer Bicarbonate buffer containing 2.8 mM glucose. In experiments with L-NAME, the inhibitor was added during the last 20 min before addition of the stimuli. Batches of 3 islets were then incubated for 30 min in tubes containing 1 ml buffer supplemented with glucose and drugs at appropriate concentrations. Aliquots of 200 μl were immediately frozen until insulin assay.

Insulin concentrations in pancreatic effluent and islet incubation media were determined by a radioimmunological method (Herbert *et al*., 1965) with Novo rat insulin as standard; the sensitivity of the assay was 0.1 ng ml^{-1}. Insulin outputs were calculated by multiplying the hormone concentration (ng ml^{-1}) in the effluent by the flow rate (ml min^{-1}).

N^{ω}-nitro-L-arginine methyl ester hydrochloride, L-arginine hydrochloride, L-ornithine, L-citrulline N^{G}-hydroxy-L-arginine acetate salt were from the Sigma Chemical Company.

Kinetic data are expressed in ng min^{-1} and insulin release from isolated islets in ng per islet 30 min. They appear on figures as means ± s.e.mean. In the text and figures, insulin outputs are also given as mean integrated data; for this we calculated the areas under the curves (AUC) for different periods of treatments and values, unless otherwise stated, were divided by the number of minutes. Both kinetics and integrated data were submitted to analysis of variance followed by the multiple comparison test of Newman-Keuls.

Results

Effect of increasing glucose concentrations on L-NAME-induced alteration of L-arginine-induced insulin secretion in rat isolated pancreas

In the presence of 5 mM glucose (Figure 1a) basal insulin output ranged around 1 ng min^{-1}. L-NAME-induced a progressive slight increase to about 4 ng min^{-1} after the 35 min treatment. The transient (5 min) insulin response to L-arginine alone peaked at 39.7 ± 4.5 ng min^{-1} after 1 min. In contrast, superimposition of L-arginine on L-NAME resulted into a very strong potentiation of L-arginine effect, which led to a sustained biphasic response. Mean integrated data for the period corresponding to the 20 min of L-arginine treatment averaged 4.8 ± 0.4, 3.9 ± 0.1 and 63.8 ± 2.8 ng min^{-1} for L-arginine, L-NAME and both drugs when combined, respectively (Figure 1d).

In the presence of 8.3 mM glucose, basal insulin levels were higher as a result of this slightly stimulating glucose concentration (Figure 1b); they ranged between 14.2 ± 1.8 and 16.3 ± 2.5 ng min^{-1}. During the first 5 min with L-NAME alone, insulin secretion increased, then stabilized at about 3 fold higher values ($P<0.001$), and rapidly returned to baseline levels upon cessation of infusion. L-Arginine alone again induced a transient stimulation of insulin secretion which peaked (134.0 ± 8.6 ng min^{-1}) at the 2nd min. In the combined treatment a greater increase occurred with a mean integrated AUC of 96.6 ± 4.1 ng min^{-1} corresponding roughly to the sum of those recorded with L-NAME (46.0 ± 5.6 ng min^{-1}) and L-arginine (42.5 ± 4.0 ng min^{-1}) alone.

In the presence of 11 mM glucose (Figure 1c), basal insulin outputs were elevated (61.8 ± 5.5 to 66.5 ± 10.2 ng min^{-1}) at time 45 min. L-NAME again induced a marked, sustained and reversible increase in insulin secretion. L-arginine in contrast to the transient effect at 5 and 8.3 mM glucose, provoked this time a marked biphasic insulin release with a mean AUC of 317.8 ± 22.0 ng min^{-1} which decreased to 211.0 ± 16.3 ng min^{-1} ($P<0.01$) in the presence of L-NAME.

Effect of increasing concentrations of glucose on L-NAME-induced alteration of L-arginine-induced insulin secretion in isolated islets

L-NAME and L-arginine were tested at 10 mM in the presence of three different glucose concentrations (Figure 2). At 2.8 mM glucose, L-arginine raised insulin release, expressed in ng per islet 30 min, to 0.7 ± 0.1 vs 0.4 ± 0.1 ($P<0.01$) in controls. No significant change was observed with L-NAME alone (0.7 ± 0.2 ng, NS); in contrast, for the combined treatment a greater increase to 1.8 ± 0.3 ($P<0.001$ vs control, L-arginine and L-NAME alone groups) occurred. The increment achieved represents twice the sum of the values recorded with each drug alone. At 8.3 mM glucose, L-NAME did not alter the L-arginine stimulating effect. In the presence of 16.7 mM glucose, the inhibitor completely prevented the L-arginine stimulating effect; insulin release was 2.9 ± 0.6 vs 5.1 ± 0.8 ng per islet 30 min with L-arginine alone ($P<0.01$).

Effect of SNP on the alteration of L-arginine-induced insulin secretion provoked by L-NAME

To follow the development and the kinetics of the effect of NOSc blockade, L-NAME was, in this and all the following experimental sets, given simultaneously with L-arginine. In these conditions the monophasic response to L-arginine alone was converted into a biphasic response (see Figure 5a), but the magnitude of the first phase was not significantly affected. Mean integrated responses were 4.8 ± 0.5 vs 17.7 ± 2.1 ng min^{-1} (Figure 3) in the absence and presence of L-NAME, respectively ($P<0.001$). SNP at 3 nM induced a slight reduction (14.5 ± 1.2 ng min^{-1}; NS) which disappeared at 30 nM (16.3 ± 2.0 ng min^{-1}); increasing the SNP concentration to 3 and 300 μM induced even a further increase in insulin release to 36.1 ± 2.6 and 43.0 ± 5.5 ng min^{-1}, respectively ($P<0.001$ vs L-NAME + L-arginine group).

Effect of SNP on L-arginine and L-ornithine-induced insulin secretion

SNP at 3 nM significantly reduced the effect of both L-arginine and L-ornithine from 95.6 ± 9.7 and 73.8 ± 8.1 ng 20 min to 63.2 ± 9.0 ($P<0.05$) and 49.2 ± 5.8 ng 20 min ($P<0.05$), respectively (Table 1). These effects could no longer be observed with higher SNP concentrations in the 30 nM to 300 μM range. The only significant observation was a stimulating effect of 3 μM SNP on L-ornithine-induced insulin secretion.

Effect of simultaneous infusion of L-arginine and L-ornithine

Each amino acid, given alone, induced a monophasic β cell response (Figure 4a); when infused together they provoked a

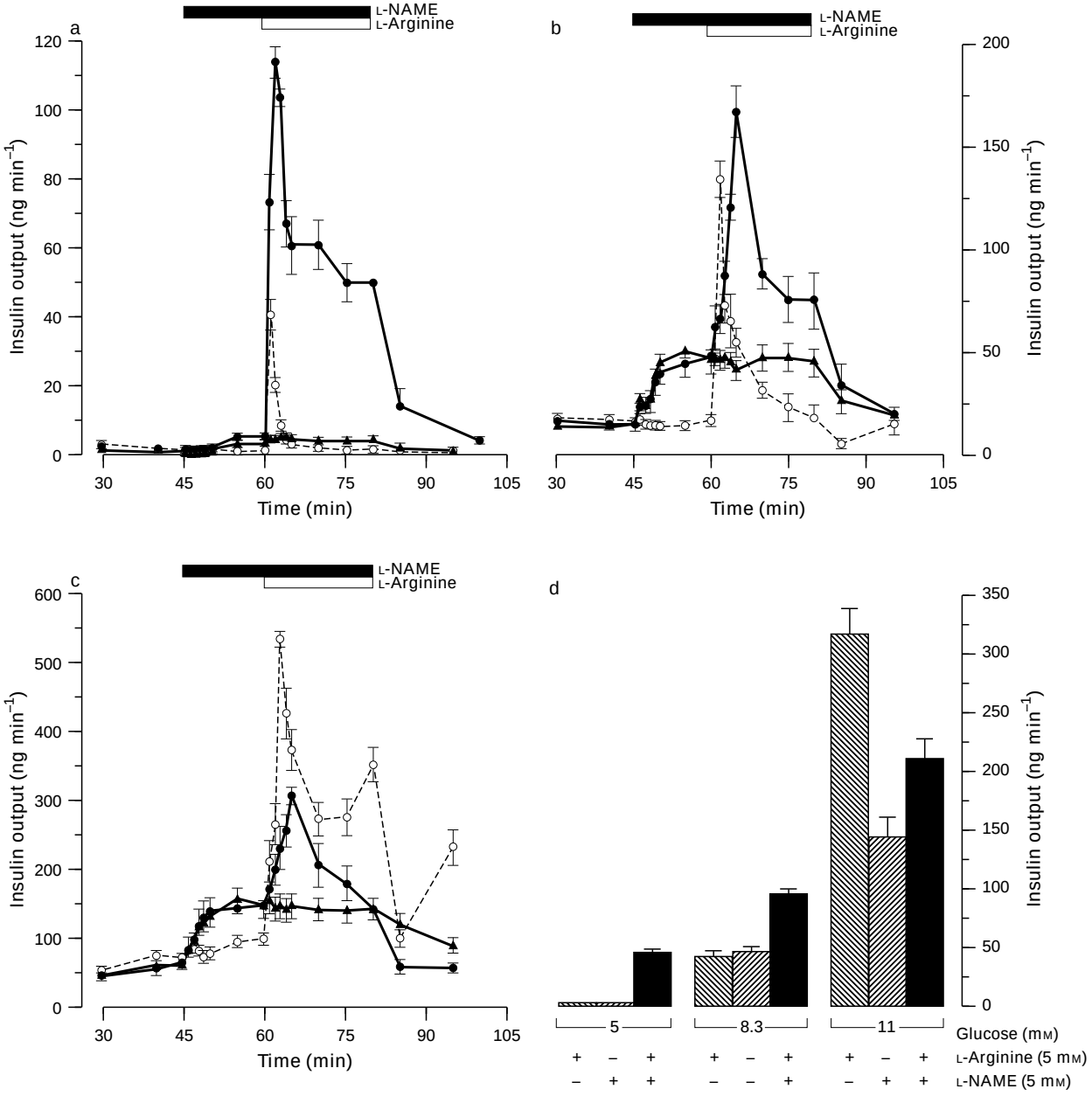

Figure 1 (a, b, c) Effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME 5 mM) on insulin secretion induced by L-arginine (5 mM) in the rat isolated perfused pancreas; glucose was present in the perfusion medium at 5, 8.3 and 11 mM respectively. Traces show arginine alone (○), L-NAME alone (▲) and L-NAME + L-arginine (●). Points are means and vertical lines s.e.mean of 5–8 experiments. L-NAME and L-arginine were present for the periods shown by the horizontal bars. (d) The integrated insulin responses from the experiments depicted in (a), (b) and (c).

clear biphasic insulin response. L-Ornithine and L-arginine produced a synergistic effect during the last 15 min of infusion; indeed the corresponding integrated insulin response achieved (88.9 ± 10.0 ng 15 min) was 2 to 3 times higher than the sum of L-arginine (22.2 ± 5.1 ng 15 min) and L-ornithine (12.6 ± 1.9 ng 15 min) effects when given alone.

Effect of L-ornithine on L-NAME-induced potentiation of insulin response to L-arginine

Unlike L-arginine, L-NAME was unable to alter the monophasic insulin response to L-ornithine (Figure 4b). However, L-ornithine strongly potentiated the effect of L-NAME + L-arginine; integrated insulin output reached 676.4 ± 54.7 ng 20 min ($P < 0.001$ vs 259.5 ± 23.9 in the absence of L-ornithine and 106.7 ± 29.6 ng 20 min in the L-NAME + L-ornithine group).

Effect of L-citrulline on L-NAME-induced alteration of insulin response to L-arginine

In the presence of L-citrulline (5 mM), the second phase of the L-arginine + L-NAME-induced insulin response was decreased by more than 50% (Figure 5a); integrated insulin output reached 113.8 ± 7.5 ng 15 min ($P < 0.001$ vs 259.5 ± 23.9 in the absence of L-citrulline).

Effect of L-citrulline on SNP-induced increase of the insulin response to L-NAME + L-arginine infusion

In the presence of SNP (3 μM), the second phase of insulin release induced by L-NAME + L-arginine was strongly potentiated to 632.4 ± 45.8 ng 15 min ($P < 0.001$ vs 259.5 ± 23.9 ng 15 min in the absence of SNP) (Figure 5b). Addition of L-citrulline (5 mM) markedly reduced the effect of

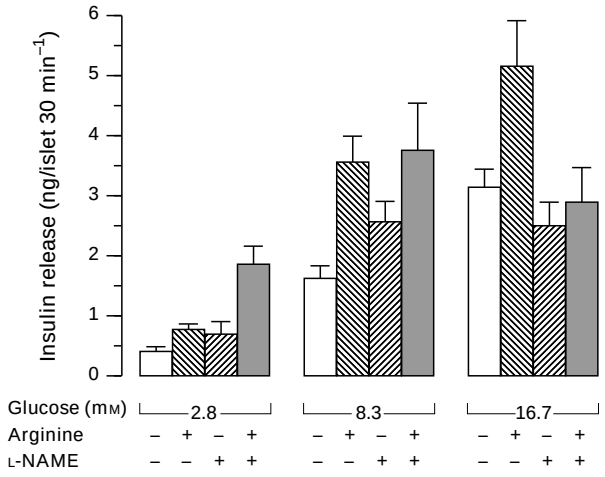

Figure 2 Effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10 mM) on insulin secretion induced by L-arginine (10 mM) in rat isolated islets of Langerhans. Values are means and vertical lines s.e.mean from 6–8 experiments; each experiment is the mean of 3 batches of 3 islets incubated in the same conditions. The media contained glucose as indicated on the figure. L-NAME was present in the preincubation media 20 min just before and during the incubation with arginine.

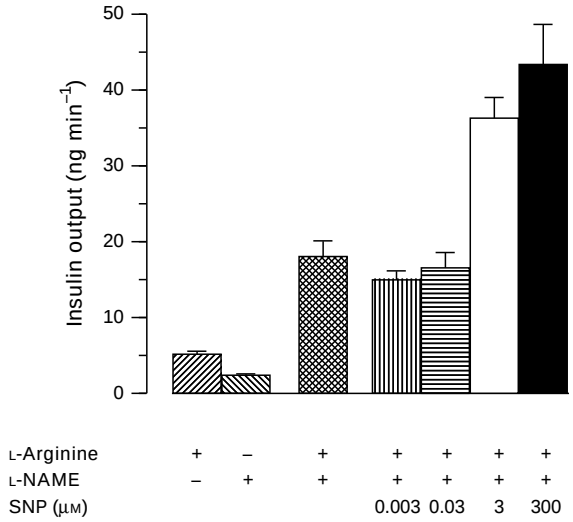

Figure 3 Effect of sodium nitroprusside (SNP) on the potentiation of L-arginine (5 mM) induced insulin secretion by N^{ω}-nitro-L-arginine methyl ester (L-NAME 5 mM) in the rat isolated perfused pancreas. The perfusion medium contained glucose (5 mM) throughout and SNP was present as indicated on the figure. Columns represent mean integrated insulin responses. Values are means ± s.e.mean of 5–8 experiments.

Table 1 Effect of sodium nitroprusside (SNP) on arginine- and ornithine-induced insulin secretion

	SNP (μM)				
	0	*0.003*	*0.03*	*3*	*300*
Arginine 5 mM	95.6 ± 9.7	63.2* ± 9.0	96.3 ± 18.2	83.0 ± 6.1	111.4 ± 9.6
Ornithine 5 mM	73.8 ± 8.1	49.2* ± 5.8	59.4 ± 6.5	103.0* ± 10.5	83.3 ± 9.2

Values represent integrated insulin responses for the 20 min of pancreas perfusion; they are expressed in ng 20 min and are given as means ± s.e.mean of 6–7 experiments. *$P<0.05$ versus the corresponding value in the absence of SNP.

SNP to 305.2 ± 36.8 ng 15 min (NS vs value recorded in the L-NAME + L-arginine group).

Effect of N^{G}-hydroxy-L-arginine on the L-NAME-induced potentiation of the L-arginine secretory effect

The intermediate in NO biosynthesis, N^{G}-hydroxy-L-arginine, when infused at 150 and 300 μM, provoked a marked dose-related decrease in the second phase of L-NAME + L-arginine-induced insulin response to, respectively, 113.5 ± 17.8 and 61.5 ± 11.6 ng 15 min ($P<0.001$ vs 259.5 ± 23.9 in the absence of N^{G}-hydroxy-L-arginine) (Figure 5c).

Discussion

Two major findings arose from our study: firstly, glucose strongly affected the control exerted by NOS activity on L-

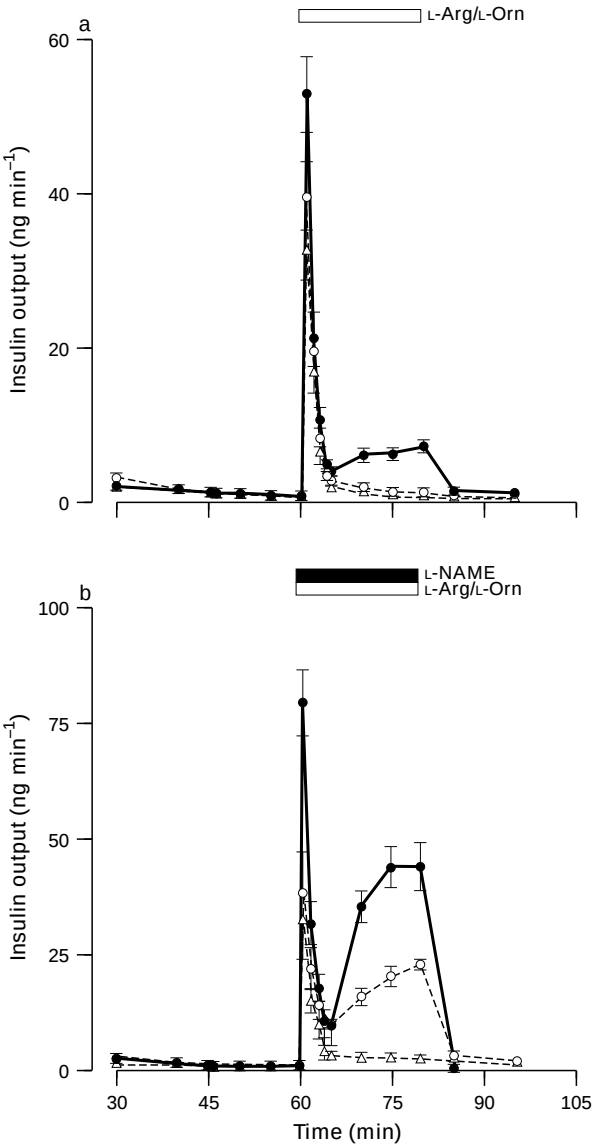

Figure 4 (a, b) Effect of L-ornithine (5 mM) on insulin secretion induced by L-arginine (5 mM) and L-arginine + L-NAME (5 mM), respectively. (a) Shows effect of (△) L-ornithine alone, (○) L-arginine alone and (●) L-ornithine + L-arginine. (b) The effect of (△) L-NAME + L-ornithine, (○) L-NAME + L-arginine and (●) L-NAME + L-arginine + L-ornithine. Values are mean and vertical lines s.e.mean of 5–8 experiments. The perfusion medium contained glucose (5 mM) throughout. Secretagogues and L-NAME were present for the periods shown by the horizontal bars. L-arg: L-arginine; L-Orn: L-ornithine.

arginine-induced insulin secretion; secondly, the potentiation of L-arginine-induced insulin secretion by blockade of NOSc activity under basal conditions, does not primarily result from a decrease of NO production. It may result from that of the two other co-products of the enzyme activity: L-citrulline and/or N^G-hydroxy-L-arginine, an important intermediate in NO generation.

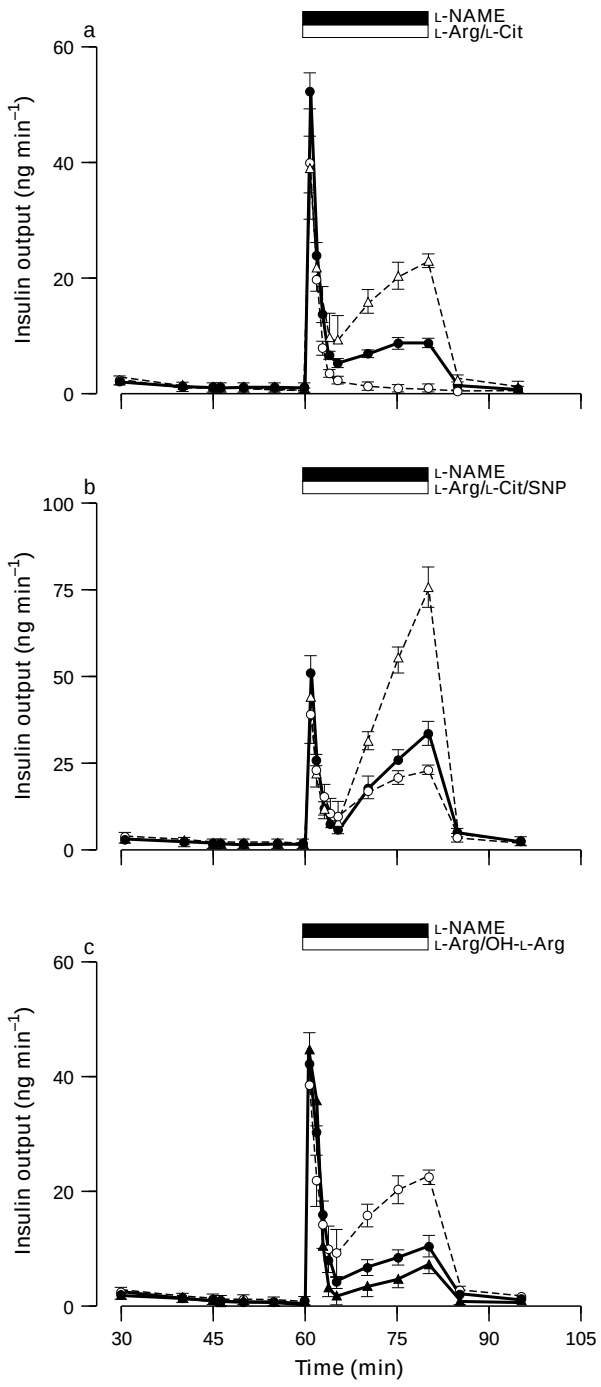

Figure 5 (a, b) Effect of L-citrulline (5 mM) on insulin secretion induced by L-arginine (5 mM) + N^{ω}-nitro-L-arginine (L-NAME, 5 mM) and L-arginine + L-NAME + sodium nitroprusside (SNP, 3 μM), respectively. (a) Effect of: (○) arginine alone, (△) L-arginine + L-NAME, (●) L-arginine + L-NAME + L-citrulline. (b) Effect of: (○) L-arginine + L-NAME, (△) L-arginine + L-NAME + SNP, (●) L-arginine + L-NAME + SNP + L-citrulline. (c) Effect of N^G-hydroxy-L-arginine at 150 μM (●) and 300 μM (▲) on insulin secretion induced by L-arginine + L-NAME (○). In (a,b and c) the perfusion medium contained glucose (5 mM) throughout. Values are means and vertical lines s.e.mean of 3–6 experiments. L-NAME, L-ornithine, L-citrulline, SNP and N^G-hydroxy-L-arginine were present in the perfusion medium as indicated by the horizontal bars. L-Orn: L-ornithine; L-Cit: L-citrulline; L-Arg: L-arginine; OH-L-Arg: N^G-hydroxy-L-arginine.

The insulinotropic effect of L-arginine is well known to increase with higher glucose concentrations (Levin *et al*., 1972); this prompted us to study if the control exerted by NOSc activity could likewise be modulated by increasing glucose concentrations. First, it must be mentioned that in the absence of L-arginine, the modest increase in basal insulin release induced by the NOSc inhibitor L-NAME at low glucose (5 mM), increases at higher (8.3 mM) and unphysiological (11 mM) concentrations of the sugar; this confirms that NOSc activity plays an important negative control on glucose-induced insulin secretion. This is consistent with the ability of the sugar, within seconds upon exposure to β cells (Pralong *et al*., 1990), to increase intracellular levels of NADPH and Ca^{2+}, two important co-factors of NOSc activity (Moncada *et al*., 1989). Surprisingly, the strong potentiation by L-NAME of L-arginine-induced insulin secretion, observed at low basal glucose in the isolated perfused pancreas, is blunted at 8.3 mM and even reversed into an inhibitory effect at high (11 mM) glucose. A similar trend was also obtained in isolated islets, although upon a wider range of glucose concentrations, possibly due to a difference in glucose sensitivity between the two *in vitro* preparations. Taken together, our data show that glucose is able to relieve progressively the strong inhibitory tone exerted by NOSc activity on the insulinotropic effect of L-arginine. In our experiments performed at high glucose, L-NAME even disclosed a possible stimulating effect on NOSc activity in L-arginine-induced insulin secretion, as previously suggested by other authors (Laychock *et al*., 1991; Schmidt *et al*., 1992). However, the high glucose concentration required questions the physiological significance of such an observation. The mechanism involved in the glucose modulating effect remains to be determined; it might tentatively be related to the greater secretory effect of L-arginine in the presence of increasing concentrations of the sugar.

Conflicting results have been obtained with regard to the effect of NO on insulin secretion. SNP (Gross *et al*., 1995) as well as other NO donors (Panagiotidis *et al*., 1995) have been shown to inhibit glucose-induced insulin secretion and to provoke hyperpolarization of the membrane from mouse pancreatic β cells (Krippeit-Drews *et al*., 1995).

Conversely, in studies with aqueous NO solutions, a stimulating effect has also been shown as a result of Ca^{2+} release from mitochondria in the insulin secreting β cell line INS1 (Laffranchi *et al*., 1995) or from endoplasmic reticulum in rat pancreatic β cells (Willmott *et al*., 1995). These discrepant data might result from differences in experimental conditions, especially the nature of β cell secretagogues used and the concentrations of NO donors tested. Indeed, it appears from our data that SNP is able to inhibit L-arginine- and L-ornithine-induced insulin secretion at nanomolar concentrations, consistent with the low NO levels produced by the constitutive form of NOS (Moncada *et al*., 1991), but not at higher micromolar concentrations, which appear to stimulate the L-ornithine secretory effect. Taken together with the L-NAME effect in the unstimulated condition, these observations further confirm that basal constitutive NO production can be responsible for an inhibitory tone on β cell function. However, our results also provide strong evidence that a decrease in NO production cannot account for the potentiation of L-arginine secretory effect resulting from NOSc blockade. Indeed, if SNP as previously found (Gross *et al*., 1995), did suppress the increase in vascular resistance induced by L-NAME and reversed it into a dilator effect in the micromolar range (which validates SNP as an efficient substitutive NO donor to compensate for decreased endogenous production), the NO donor, at nanomolar concentrations, did not significantly affect L-NAME potentiation of L-arginine-induced insulin secretion; in addition, at higher concentrations SNP further accentuated the L-NAME effect. The failure of SNP to correct L-NAME-induced

alterations of β cell response to L-arginine can be related to observations made by others. In hepatocytes, induction of the inducible NOS decreased glucose output and urea production (Stadler *et al*., 1995); this could be prevented by L-NAME and overcome by high concentrations of L-arginine. However, NO donors were only able to reproduce the decrease in glucose output; they were ineffective in reducing urea production suggesting that NO is not responsible for all the effects resulting from changes in NOS activity. Due to the tight analogy of the β cell functional response to L-arginine and L-ornithine (Blachier *et al*., 1989), we were interested in the second metabolic pathway of L-arginine, i.e. the production by arginase of urea and L-ornithine. These latter compounds represent the major metabolites generated from L-arginine at a rate found to 'yield the highest metabolic flow encountered so far in the study of islet metabolism' (Malaisse *et al*., 1989). Our data show that in basal glucose conditions, L-arginine and L-ornithine provoke, when infused alone, a monophasic insulin response; however, when given together the two amino acids induce a biphasic response, which in a way mimics the effect of NOSc blockade on L-arginine-induced insulin secretion. Therefore we assume that an increased L-ornithine formation from L-arginine could be the factor underlying the second phase of the L-arginine effect in the presence of L-NAME. In fact, the reverse situation has already been demonstrated in murine macrophages by Granger *et al*. (1990), who showed that NOS activity decreases the relative flux of L-arginine through the arginase pathway. Our assumption is further supported (i) by the inability of L-NAME to potentiate L-ornithine effect and (ii) by the remarkable capability of L-ornithine to strengthen L-NAME potentiation of L-arginine-induced insulin secretion. Finally and more definitely, studies with L-citrulline (in the millimolar range) and N^G-hydroxy-L-arginine (in the micromolar range) provide very strong evidence that an increase of the arginase pathway accounts for the L-NAME potentiating effect. Indeed, in addition to NO, the blockade of NOSc also depresses the formation of L-citrulline (co-produced with NO) and the metabolic intermediate N^G-hydroxy-L-arginine, which have been shown to be respectively weak (Hrabak *et al*., 1994) and potent (Daghigh *et al*., 1994; Boucher *et al*., 1994) inhibitors of liver and macrophage arginase; moreover, N^G-hydroxy-L-arginine has recently been shown to inhibit arginase in the RINm5F insulin secreting cell line (Cunningham *et al*., 1996). The marked reduction, by both metabolites, of the potentiation of L-arginine-induced insulin secretion by L-NAME strongly suggests that this potentiation results, to a large extent, from the decreased production of L-citrulline and especially N^G-hydroxy-L-arginine, and the consequent attenuation of an inhibitory tone exerted by these compounds on arginase activity under normal conditions.

In conclusion, the inhibitory effect exerted by NOSc activity of L-arginine-induced insulin secretion is strongly modulated by glucose; the sugar at higher concentrations reverses the effect of the blockade of the enzyme observed under basal conditions. A major and original finding of this study is also that L-NAME-induced potentiation of L-arginine secretory effect, unlike vascular constrictor effects, does not result from a decreased NO production, but very likely from an imbalance between NOSc and arginase activities.

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