

L-Arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide

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Abstract

The mechanism of L-arginine stimulation of glucose-induced insulin secretion from mouse pancreatic islets was studied. At 16.7 mmol/l glucose, L-arginine (10 mmol/l) potentiated both phases 1 and 2 of glucose-induced insulin secretion. This potentiation of glucose-induced insulin secretion was mimicked by the membrane depolarizing agents tetraethylammonium (TEA, 20 mmol/l) and K⁺ (60 mmol/l), which at 16.7 mmol/l glucose obliterated L-arginine (10 mmol/l) modulation of insulin secretion. Thus L-arginine may potentiate glucose-induced insulin secretion by stimulation of membrane depolarization. At 3.3 mmol/l glucose, L-arginine (10 mmol/l) failed to stimulate insulin secretion. In accordance with membrane depolarization by the electrogenic transport of L-arginine, however, L-arginine (10 mmol/l) stimulation of insulin secretion was enabled by the K⁺ channel inhibitor TEA (20 mmol/l), which potentiates membrane depolarization by L-arginine. Furthermore, L-arginine (10 mmol/l) stimulation of insulin secretion was permitted by forskolin (10 μmol/l) or tetradecanoylphorbol 13-acetate (0.16 μmol/l), which, by activation of protein kinases A and C respectively sensitize the exocytotic machinery to L-arginine-induced Ca²⁺ influx. Thus glucose may sensitize L-arginine stimulation of insulin secretion by potentiation of membrane depolarization and by activation of protein kinase A or protein kinase C. Finally, L-arginine stimulation of glucose-induced insulin secretion was mimicked by N^G-nitro-L-arginine methyl ester (10 mmol/l), which stimulates membrane depolarization but inhibits nitric oxide synthase, suggesting that L-arginine-derived nitric oxide neither inhibits nor stimulates insulin secretion. In conclusion, it is suggested that L-arginine potentiation of glucose-induced insulin secretion occurs independently of nitric oxide, but is mediated by membrane depolarization, which stimulates insulin secretion through protein kinase A- and C-sensitive mechanisms.

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Introduction

The mechanism of L-arginine stimulation of insulin secretion is not fully clarified. Previous data from several laboratories have suggested that potentiation of glucose-induced insulin secretion by L-arginine is mediated by β-cell membrane depolarization resulting from the electrogenic transport of the cationic amino acid (1–5). Thus L-arginine has been shown to depolarize the plasma membrane in a way which is potentiated by glucose (4), and to stimulate Ca²⁺ influx through voltage-sensitive Ca²⁺ channels (4–6).

Other data have, however, questioned membrane depolarization as the sole mechanism in L-arginine stimulation of insulin secretion from pancreatic β-cells. Thus sizeable L-arginine stimulation of insulin secretion persists in rat islets depolarized by high K⁺ (3), suggesting that L-arginine in addition to stimulation of Ca²⁺ influx may stimulate insulin secretion by other mechanisms (3, 7).

L-Arginine-induced glucagon release is also a Ca²⁺-dependent mechanism (8) that results from membrane depolarization (9) and the rise of cytoplasmic Ca²⁺ (10) in α-cells. Activation of protein kinase C also participates in the effects of L-arginine on glucagon secretion (11–13), but it is not known whether it also contributes to L-arginine-induced insulin secretion.

Yet other data have focused on the possible significance of nitric oxide (NO) in L-arginine stimulation of insulin secretion. Thus L-arginine-derived NO has been suggested not to be involved in L-arginine stimulation of insulin secretion (6, 14, 15), to enhance L-arginine stimulation of insulin secretion (16, 17), or to inhibit L-arginine stimulation of insulin secretion from pancreatic β-cells (18–20). In accordance with a possible significance of NO in L-arginine stimulation of insulin secretion, islets have been found to express the constitutive, Ca²⁺/calmodulin-dependent nitric oxide synthase (cNOS), which may exhibit glucose-sensitive NO synthesis in the presence of L-arginine (16, 20).

However, a possible inhibitory role of L-arginine-derived NO in insulin secretion relies largely on the observations that cationic cNOS inhibitors like N^G-nitro-L-arginine methyl ester (L-NAME), which *per se* may have a capacity to stimulate membrane depolarization and insulin secretion, increases L-arginine stimulation of insulin secretion (18–20).

The aim of the present study was, therefore, threefold: to assess the possible significance of membrane depolarization, to evaluate the possible significance of protein kinase C and to judge the possible importance of NO in L-arginine potentiation of glucose-induced insulin secretion.

It is demonstrated that L-arginine stimulates insulin secretion through membrane depolarization, and that activation of both protein kinase A and protein kinase C may accentuate the stimulatory effect of membrane depolarization in glucose-induced insulin secretion. These processes occur independently of NO formation.

Materials and methods

Materials

Crude bacterial collagenase was obtained from Boehringer, Mannheim, Germany. Human serum albumin was from Behringwerke AG, Marburg, Germany. [¹²⁵I]Insulin and guinea-pig anti-insulin serum were from Novo Nordisk A/S, Bagsvaerd, Denmark. [U-¹⁴C]Glucose (230–350 mCi/mmol) was from Amersham International, Amersham, Bucks, UK; N^G-nitro-L-arginine methyl ester (L-NAME) was from Calbiochem, San Diego, CA, USA; L-arginine was from BDH, Poole, Dorset, UK; tetraethylammonium chloride (TEA), 12-*O*-tetradecanoylphorbol 13-acetate (TPA), forskolin and diazoxide were from Sigma Chemical Co., St Louis, MO, USA. All other chemicals were of analytical grade.

Preparation and culture of islets

Islets were prepared by collagenase digestion of the pancreas of male albino mice (NMRI) (approx. 18–22 g body weight) fed *ad libitum* on a standard laboratory diet. Islets were kept in tissue culture for 22–24 h in TCM 199 medium (1.26 mmol/l Ca²⁺, 5.5 mmol/l glucose) supplemented with 10% (v/v) newborn calf serum (Gibco, Paisley, Strathclyde, UK), 20 mmol/l HEPES, 5 mmol/l NaHCO₃, 100 units penicillin/ml, and 100 µg streptomycin/ml.

Insulin release

Insulin release from islets was determined by perfusion in a noncirculating system with beads of 0.25 ml Bio-Gel P2 (Bio-Rad Laboratories, Rockville Center, NY, USA) as a supporting medium, as described previously (21). Twenty-five islets per chamber were perfused at 37°C at a flow rate of 0.26 ml/min. The effluent

medium was collected for periods of 5 or 10 min. Insulin was determined by radioimmunoassay. The rate of insulin release was expressed as ng/min/100 islets.

Glucose oxidation

Glucose oxidation (formation of ¹⁴CO₂ from [U-¹⁴C]glucose) was determined as described previously (22).

Statistics

Results are given as means ± S.E.M. for *n* experiments. Statistical evaluation of the data was made by ANOVA, followed by the Newman-Keuls test for multiple comparisons; not significant, *P* > 0.05.

Results

Effects of L-arginine at 16.7 mmol/l glucose

At 16.7 mmol/l glucose, L-arginine (10 mmol/l) potentiated both phase 1 (45–50 min) and phase 2 (50–105 min) glucose-induced insulin secretion (Fig. 1a). This ability of L-arginine to potentiate both phases of glucose-induced

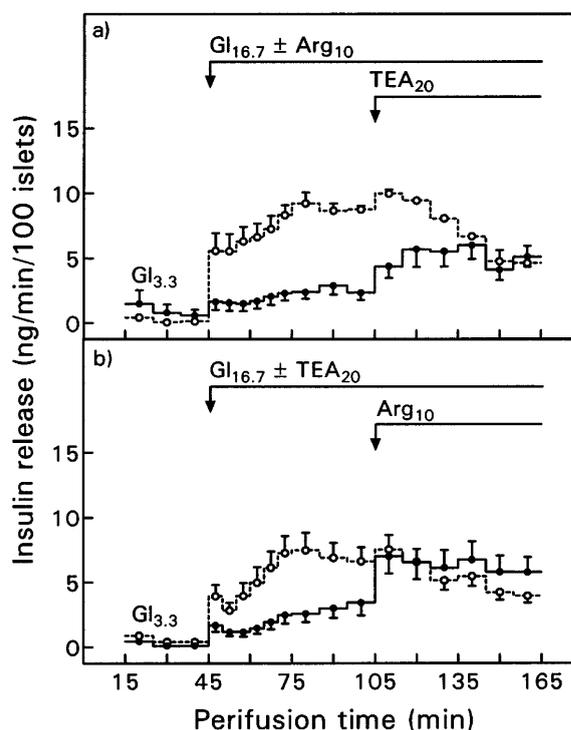


Figure 1 Effects of L-arginine and TEA on glucose-induced insulin secretion. (a) After 45 min at 3.3 mmol/l glucose (Gl_{3.3}), islets were perfused at 16.7 mmol/l glucose (Gl_{16.7}) with (○) or without (●) 10 mmol/l L-arginine (Arg₁₀). At the time point indicated both media were supplemented with 20 mmol/l TEA (TEA₂₀). (b) Islets were perfused at 16.7 mmol/l glucose with (○) or without (●) TEA (20 mmol/l). Later 10 mmol/l L-arginine (Arg₁₀) was added to both media as indicated. Results are means ± S.E.M. (*n* = 3–5).

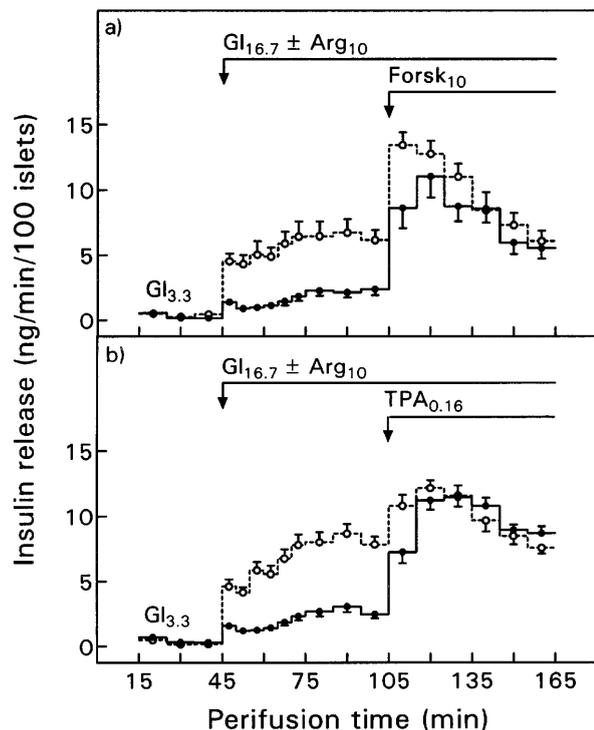


Figure 2 Effects of L-arginine on glucose-induced insulin secretion. After 45 min at 3.3 mmol/l glucose ($Gl_{3.3}$), islets were perfused at 16.7 mmol/l glucose ($Gl_{16.7}$) with (○) or without (●) 10 mmol/l L-arginine (Arg_{10}). Subsequently both media received 10 μ mol/l forskolin ($Forsk_{10}$) (a) or 0.16 μ mol/l TPA ($TPA_{0.16}$) (b) as indicated. Results are means \pm S.E.M. ($n = 7-14$).

insulin secretion was mimicked by the membrane depolarizing agent TEA (20 mmol/l) (Fig. 1b), which at 20 mmol/l closes both ATP-regulated, Ca^{2+} -activated and delayed rectifying K^+ channels in mouse pancreatic β -cells (23–25). Furthermore, TEA (20 mmol/l) failed to affect insulin release in the combined presence of glucose (16.7 mmol/l)+L-arginine (10 mmol/l) (Fig. 1a) and L-arginine (10 mmol/l) failed to affect insulin release in the combined presence of glucose (16.7 mmol/l)+TEA (20 mmol/l) (Fig. 1b). Thus L-arginine may potentiate glucose-induced insulin secretion by stimulation of membrane depolarization.

In the presence of the adenylate cyclase activator forskolin (10 μ mol/l) or the diacylglycerol analogue TPA (0.16 μ mol/l), L-arginine (10 mmol/l) was no longer able to potentiate glucose (16.7 mmol/l)-induced insulin secretion (Fig. 2). Thus maximum sensitization to glucose-induced Ca^{2+} influx by activation of protein kinase A or C (26) may obliterate the effect of L-arginine-induced Ca^{2+} influx on insulin secretion.

Effects of L-arginine at 3.3 mmol/l glucose

At 3.3 mmol/l glucose, L-arginine (10 mmol/l) failed to stimulate insulin secretion (Fig. 3). In accordance with

membrane depolarization by the electrogenic transport of this cationic amino acid, however, L-arginine (10 mmol/l) stimulation of insulin secretion was sizeable in the presence of the K^+ channel inhibitor TEA (20 mmol/l), which may potentiate membrane depolarization by L-arginine (Fig. 3a). In addition, L-arginine stimulation was observed in the presence of forskolin (10 μ mol/l) (Fig. 3b) and TPA (0.16 μ mol/l) (Fig. 3c), which lower the Ca^{2+} requirement in insulin secretion. Thus glucose may sensitize L-arginine stimulation of insulin secretion through potentiation of membrane depolarization and by activation of protein kinase A or C.

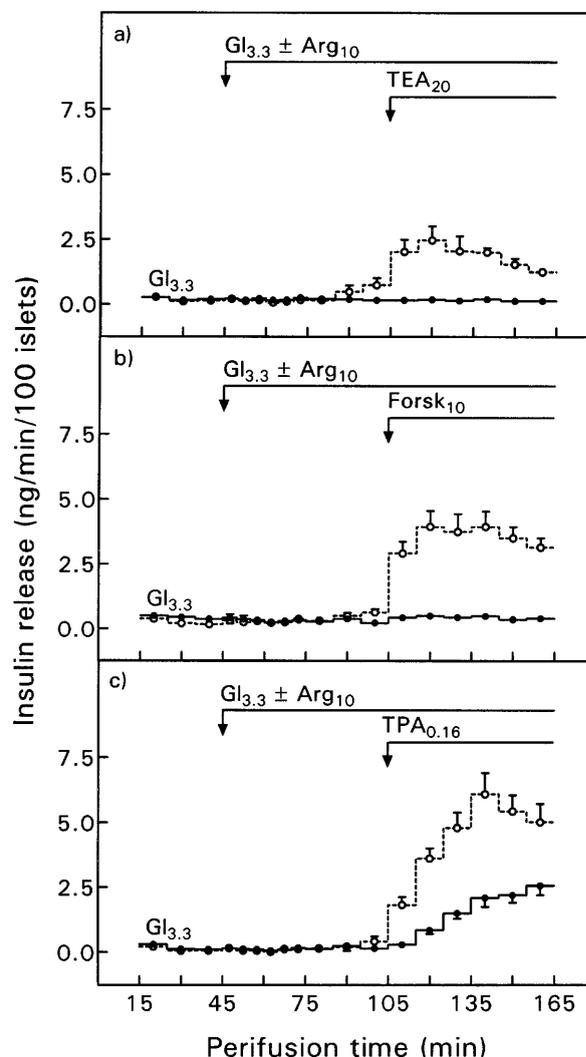


Figure 3 Effects of TEA, forskolin and TPA on L-arginine-induced insulin secretion. Islets were perfused at 3.3 mmol/l glucose ($Gl_{3.3}$) with (○) or without (●) 10 mmol/l L-arginine (Arg_{10}). Then both media were supplemented with 20 mmol/l TEA (TEA_{20}) (a), 10 μ mol/l forskolin ($Forsk_{10}$) (b) or 0.16 μ mol/l TPA ($TPA_{0.16}$) (c) as indicated. Results are means \pm S.E.M. ($n = 3-6$).

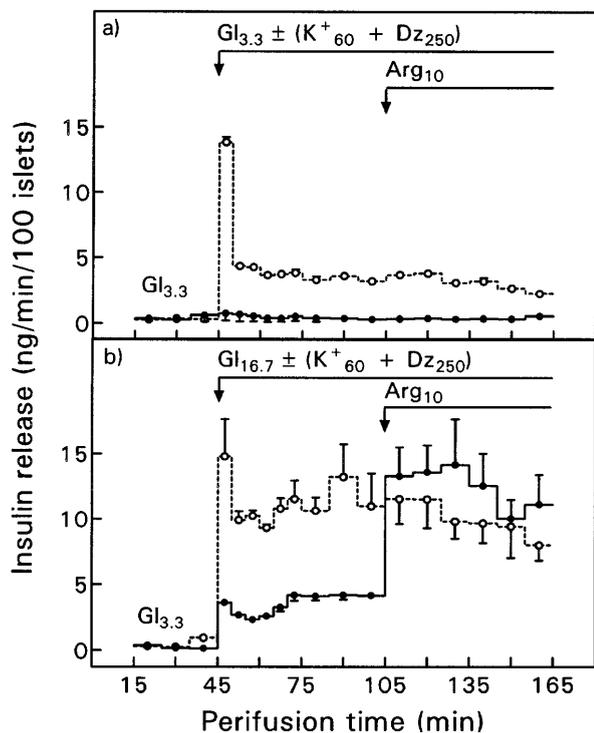


Figure 4 Effects of L-arginine and glucose on insulin secretion in the presence of K^+ +diazoxide. Islets were perfused at 3.3 mmol/l glucose ($G_{3.3}$) or 16.7 mmol/l glucose ($G_{16.7}$) and with (\circ) or without (\bullet) 60 mmol/l K^+ +250 μ mol/l diazoxide ($K^+_{60}+Dz_{250}$) as indicated. Subsequently 10 mmol/l L-arginine (Arg_{10}) was added to all media. Results are means \pm S.E.M. ($n = 3$).

Effects of L-arginine at 60 mmol/l K^+ +250 μ mol/l diazoxide

When the β -cell membrane is depolarized with 60 mmol/l K^+ and the ATP-sensitive K^+ channels are opened with 250 μ mol/l diazoxide, glucose stimulation is confined to a potentiation of Ca^{2+} -induced insulin secretion (27–29). Under these conditions, glucose (16.7 mmol/l) stimulated, but L-arginine (10 mmol/l) failed to affect insulin secretion (Fig. 4), suggesting that maximum membrane depolarization by K^+ may obliterate the stimulatory effect of L-arginine on insulin secretion. Thus at 3.3 mmol/l glucose, L-arginine (10 mmol/l) failed to potentiate K^+ (60 mmol/l)+diazoxide (250 μ mol/l)-induced insulin secretion (Fig. 4a). Furthermore at 16.7 mmol/l glucose, K^+ (60 mmol/l)+diazoxide (250 μ mol/l) eliminated the stimulatory effect of L-arginine (10 mmol/l) on glucose-induced insulin secretion (Fig. 4b). L-Arginine may, therefore, only stimulate insulin secretion through stimulation of membrane depolarization.

Effects of L-NAME at 16.7 mmol/l glucose

L-NAME (5, 10 mmol/l) and L-arginine (5, 10 mmol/l) equally potentiated both phases 1 and 2 of glucose-induced insulin secretion, in a way which was

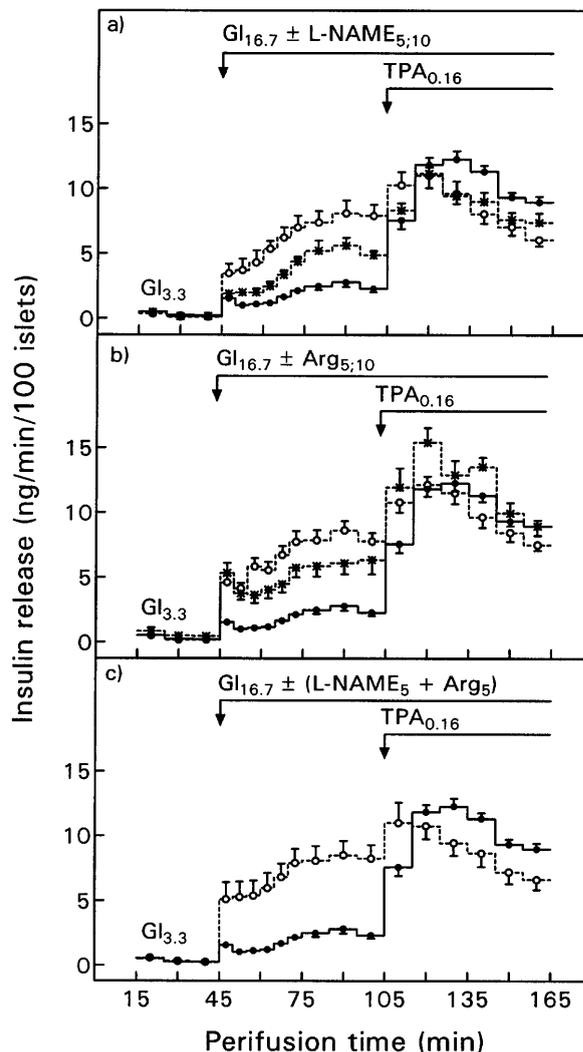


Figure 5 Effects of L-NAME and L-arginine on glucose-induced insulin secretion. After 45 min at 3.3 mmol/l glucose ($G_{3.3}$), islets were perfused at 16.7 mmol/l glucose ($G_{16.7}$) in the absence (\bullet) or presence of L-NAME and/or L-arginine as outlined. (a) L-NAME at 5 mmol/l ($*$) or 10 mmol/l (\circ) ($L-NAME_{5,10}$); (b) L-arginine at 5 mmol/l ($*$) or 10 mmol/l (\circ) ($Arg_{5,10}$); (c) 5 mmol/l L-NAME+5 mmol/l L-arginine (\circ) ($L-NAME_5+Arg_5$). At the indicated time point 0.16 μ mol/l TPA ($TPA_{0.16}$) was added to all media. Results are means \pm S.E.M. ($n = 6-20$).

obliterated in the presence of TPA (0.16 μ mol/l) (Fig. 5). Thus L-NAME, which like L-arginine stimulates membrane depolarization but unlike L-arginine inhibits cNOS, mimicked the pattern of L-arginine stimulation of insulin secretion. Furthermore, the combined addition of L-NAME (5 mmol/l) and L-arginine (5 mmol/l) did not further augment insulin secretion as compared with L-arginine (10 mmol/l) alone, suggesting that L-arginine and L-NAME have additive effects in stimulation of insulin secretion (Fig. 5).

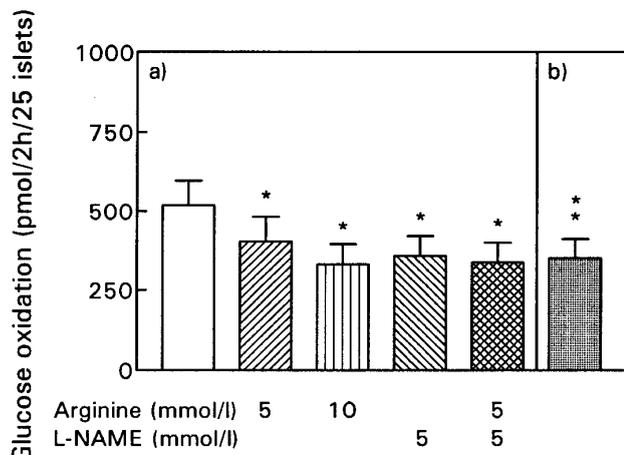


Figure 6 Effects of L-arginine and L-NAME on glucose oxidation. Glucose oxidation at 16.7 mmol/l glucose was determined with or without L-arginine and/or L-NAME (a) and without extracellular K⁺ (b) as indicated. Results are means \pm s.e.m. ($n = 3-9$). * $P < 0.001$ versus control; ** $P < 0.05$ versus control.

Effects of L-arginine and L-NAME on glucose oxidation

Both L-arginine (5, 10 mmol/l) and L-NAME (5 mmol/l) inhibited glucose oxidation at 16.7 mmol/l glucose (Fig. 6a). Since L-NAME inhibits NO production, these data do not suggest that L-arginine may lower glucose oxidation through production of NO. Thus, L-arginine (5 mmol/l) and L-NAME (5 mmol/l) in combination did not alleviate inhibition, but rather appeared to have additive effects in inhibition of glucose oxidation. The mechanism of this L-arginine inhibition of glucose oxidation is not known, but it may be related to L-arginine stimulation of K⁺ efflux, since K⁺ omission likewise lowered glucose oxidation (Fig. 6b).

Discussion

In accordance with previous data from several laboratories (1-6), this study suggests that the electrogenic transport of the cationic amino acid L-arginine potentiates glucose-induced insulin secretion by membrane depolarization. Thus L-arginine stimulation of both phase 1 and phase 2 of glucose-induced insulin secretion was mimicked by the membrane depolarizing agent TEA which, at variance from the direct effect of L-arginine on membrane potential may depolarize the plasma membrane by inhibition of ATP-regulated, Ca²⁺-activated and delayed rectifying K⁺ channels in mouse pancreatic β -cells (23-25). Furthermore, maximum sensitization to glucose-induced Ca²⁺ influx by forskolin or TPA obliterated the effect of L-arginine on insulin secretion.

Previous data have questioned membrane depolarization as the sole mechanism in L-arginine potentiation of insulin secretion, since L-arginine stimulation of

glucose-induced insulin secretion was found to persist in the presence of a high K⁺ concentration, which was suspected to cause maximum membrane depolarization and stimulation of Ca²⁺ influx through voltage-sensitive Ca²⁺ channels independent of L-arginine (3). However, this finding was not confirmed by the present study in which depolarization by K⁺ was found to prevent L-arginine from further stimulating insulin secretion.

Glucose stimulates insulin secretion by means of a synergistic interaction between at least two signalling pathways. One, the ATP-sensitive K⁺ channel-dependent pathway, stimulates Ca²⁺ influx through voltage-gated channels by closure of the ATP-sensitive K⁺ channels and depolarization of the β -cell membrane. The resulting increase in intracellular Ca²⁺ induces insulin secretion. The other, the ATP-sensitive K⁺ channel-independent, Ca²⁺-dependent pathway then augments the Ca²⁺-stimulated release (27-29). When the β -cell membrane is depolarized with K⁺ and the ATP-sensitive K⁺ channels are opened with diazoxide, only the ATP-sensitive K⁺ channel-independent, Ca²⁺-dependent effect of glucose on insulin secretion persists (27-29). The finding that L-arginine under these conditions, with maximum depolarization with K⁺, failed to affect insulin secretion suggests that L-arginine only potentiates glucose-induced insulin secretion through stimulation of membrane depolarization.

Previous data have suggested that the permissive effect of glucose on L-arginine stimulation of insulin secretion is mediated by potentiation of L-arginine-induced membrane depolarization and stimulation of Ca²⁺ influx (4). In agreement with these data L-arginine stimulation of insulin secretion at low glucose concentrations was also allowed by TEA which, by closure of K⁺ channels may potentiate the direct effect of the cationic amino acid on membrane potential. This may, however, not be the sole mechanism by which glucose permits L-arginine stimulation of insulin secretion.

It is well established that glucose, in addition to inhibition of ATP-sensitive K⁺ channels and stimulation of Ca²⁺ influx, may also activate adenylate cyclase, stimulate cAMP accumulation and therefore activate protein kinase A in mouse and rat islets (30). In addition, recent data suggest that glucose may activate protein kinase C through stimulation of Ca²⁺ influx (31-33). Thus glucose stimulation of adenylate cyclase may occur in coordination with activation of protein kinase C, which activates adenylate cyclase in islets (30).

An implication of both protein kinase A and C in the permissive effect of glucose on L-arginine stimulation is substantiated by the ability of both the adenylate cyclase activator forskolin and the diacylglycerol analogue TPA to sensitize L-arginine stimulation of insulin secretion. Neither of these agents affect the membrane potential at 3.3 mmol/l glucose (34, 35), and most likely, these effects of forskolin and TPA are mediated by lowering of the Ca²⁺ requirement for insulin secretion through

sensitization of the exocytotic machinery to even small changes in Ca^{2+} influx in response to L-arginine (26). Thus glucose may sensitize L-arginine stimulation of insulin secretion by three mechanisms, i.e. stimulation of membrane depolarization, activation of protein kinase A and activation of protein kinase C.

Altogether the present data do not point to a significant stimulatory or inhibitory role of L-arginine-derived NO in L-arginine-induced insulin secretion.

Recent data have demonstrated that pancreatic islets express the constitutive, Ca^{2+} /calmodulin-sensitive NOS, and that glucose-induced Ca^{2+} influx therefore may stimulate NO production in the presence of L-arginine (16, 20). Several studies have not, however, been able to demonstrate that the small changes in NO accumulation, which may follow L-arginine stimulation, affect glucose-induced insulin secretion (6, 14). A few studies have suggested that L-arginine-derived NO may inhibit glucose-induced insulin secretion, since L-NAME at 5 mmol/l in these studies failed to stimulate insulin secretion, but potentiated a rather weak effect of 5 mmol/l L-arginine on glucose-induced insulin secretion (18–20). In this regard, it should be emphasized that L-arginine *per se* has been demonstrated to require a certain threshold concentration for stimulation of insulin secretion (3), suggesting that combined effects of threshold concentrations of L-arginine and L-NAME in these studies may represent additive effects in stimulation of insulin secretion (18–20). Thus, potentiation of L-arginine-induced insulin secretion by the cNOS inhibitor L-NAME in the present study appeared to rely solely on additive effects of these cationic agents on membrane depolarization since L-NAME potentiation of both phase 1 and phase 2 of glucose-induced insulin secretion were additive with L-arginine and were obliterated in the presence of TPA.

In agreement with several previous studies (36, 37), L-arginine appeared to have a small inhibitory effect on glucose oxidation in the present study. NO has been demonstrated to inhibit glucose-induced insulin secretion through inhibition of phosphofructokinase and aconitase (38, 39). The inhibitory effect of L-arginine on glucose oxidation may, however, not represent NO inhibition of phosphofructokinase and aconitase, since L-NAME showed a similar tendency and did not relieve L-arginine-induced inhibition of glucose oxidation. Since L-arginine has previously been shown not to be metabolised in islets (3) and since L-NAME is most likely not metabolised in islets, the inhibitory effect of these agents on glucose metabolism may also be associated with membrane depolarization. Thus membrane depolarization by L-arginine is accompanied by a large stimulation of K^+ efflux through activation of delayed rectifying K^+ channels (4, 34), which may lower cellular K^+ and inhibit pyruvate kinase (40). K^+ omission showed a similar ability to inhibit glucose oxidation, suggesting that L-arginine may inhibit glucose metabolism through lowering of cellular K^+ .

The possible significance of this small albeit significant inhibition of glucose metabolism in the interplay between glucose and L-arginine in the stimulation of insulin secretion remains to be fully established.

In conclusion this study has clearly demonstrated that L-arginine, independent of NO, potentiates glucose-induced insulin secretion through stimulation of membrane depolarization, and that glucose may sensitize L-arginine stimulation by three mechanisms i.e. by potentiation of membrane depolarization, by activation of protein kinase A and by activation of protein kinase C.

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