

Effects of ornithine α -ketoglutarate on insulin secretion in rat pancreatic islets: implication of nitric oxide synthase and glutamine synthetase pathways*

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Ornithine α -ketoglutarate (OKG) administration in human subjects elicits insulin secretion. We investigated whether this action was related to an effect of OKG on islets of Langerhans, and addressed the underlying mechanisms of action. For this purpose the influence of OKG on insulin secretion was measured in isolated rat islets of Langerhans under two different conditions. In incubated islets, OKG (0.25 to 2.5 mmol/l) significantly and dose-relatedly increased insulin secretion (1.7- to 4.2-fold; $P < 0.05$ v. basal). To study the kinetics of OKG-stimulated insulin secretion, perfusion experiments were performed, which showed that OKG affected insulin secretion in both initial and later phases. Experiments using α -ketoglutarate (α -KG) (1 mmol/l) or ornithine (Orn) (2 mmol/l) alone, in concentrations equal to that of OKG, showed that the OKG-induced insulin secretion could not be obtained by either component alone, suggesting that an α -KG–Orn interaction is mandatory for the insulin-secreting effect to occur. Since data obtained *in vivo* suggest that effects of OKG may depend on the synthesis of NO, glutamine and/or polyamines, three metabolic pathways potentially involved in insulin secretion, we then evaluated their contribution by means of their respective inhibitors: L-N^G-nitroarginine methyl ester (L-NAME), methionine sulfoximine (MSO) and difluoromethylornithine (DFMO). Both L-NAME and MSO were able significantly to reduce OKG-induced insulin secretion (30 and 40% respectively; $P < 0.05$), while DFMO was ineffective. Thus OKG is an effective stimulator of insulin secretion, requiring the joint presence of both Orn and α -KG, and acting mainly via the synthesis of NO and glutamine. A better understanding of OKG insulino-secretory properties and its mechanisms of action are a prerequisite for its use in insulin-compromised situations.

Nitric oxide: Polyamines: Glutamine

Ornithine α -ketoglutarate (OKG) is a salt composed of an α -ketoglutarate (α -KG) moiety and two ornithine (Orn) moieties, which dissociates completely in solution (Cynober, 1995). Its anabolic and anti-catabolic properties were highlighted in the early 1960s with the observation of an improvement in the nutritional status of patients with liver failure receiving OKG (see Gay *et al.* 1979),

prompting various studies to evaluate the benefit of OKG supplementation of enteral and parenteral nutrition. OKG has been demonstrated to improve N balance in surgical (Leander *et al.* 1985), burns (Cynober *et al.* 1984a, 1986), septic and polytrauma patients (Cynober, 1995), by either increasing protein synthesis or reducing protein breakdown (Cynober, 1995).

Abbreviations: Arg, arginine; DFMO, difluoromethylornithine; Glc, glucose; Gln, glutamine; α -KG, α -ketoglutarate; KRB, Krebs–Ringer buffer; L-NAME, L-nitroarginine methyl ester; MSO, methionine sulfoximine; OKG, ornithine α -ketoglutarate; Orn, ornithine.

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The effects of OKG may depend not only on the production of glutamine (Gln) and arginine (Arg), two amino acids that play important roles in protein anabolism and immune defence (Cynober, 1995; Le Boucher *et al.* 1999), but also on the production of other Orn metabolites such as polyamines. In addition, it has been argued that endocrine processes may be involved, with possible interactions between OKG and insulin secretion and/or peripheral action (Cynober, 1995). OKG may modulate the peripheral response to insulin action, as suggested by OKG-induced improvement in glucose tolerance in hyperinsulinaemic burn patients in the absence of any increase in insulinaemia (Vaubourdolle *et al.* 1987). However, an OKG-induced increase in insulinaemia has been demonstrated after both intravenous and oral OKG administration in healthy subjects (Krassowski *et al.* 1981; Cynober *et al.* 1990). An intriguing feature is that the action of OKG on insulinaemia (Cynober *et al.* 1990) could not be reproduced by administering either α -KG or Orn alone, suggesting that interactions between the two components of OKG are required to induce this effect. Such an interaction, related to the common metabolic pathway shared by Orn and α -KG, has been demonstrated to be essential for some of the anabolic and anti-catabolic effects of OKG and the synthesis of Gln (Cynober, 1995; Le Boucher *et al.* 1997).

In addition, it has been demonstrated that both Orn and α -KG may play a role in insulin secretion (Lenzen *et al.* 1986; Malaisse *et al.* 1989b). Malaisse *et al.* (1989a) have demonstrated in the pancreatic β -cell line RINm5F that Orn, like Arg, can stimulate insulin secretion by mechanisms independent of its conversion into Arg. Moreover, in both intact islets and islet homogenates, Orn is extensively metabolised in rat pancreatic islets (Malaisse *et al.* 1989b), suggesting that two metabolic pathways may be involved in the induction of insulin secretion, namely the generation of putrescine and other polyamines, and the conversion of Orn into L-glutamate (Malaisse *et al.* 1989a). On the other hand, studying mitochondrial homogenates of mice islets, Lenzen *et al.* (1986) have postulated a parallel between the generation of α -KG and the stimulation of insulin secretion by amino acids such as leucine.

However, data from the literature concerning OKG action on insulin secretion are only descriptive, with no information on the underlying mechanisms. The aim of the present study was thus to evaluate the effects of OKG at concentrations similar to those observed during *in vivo* OKG administration, and the contribution of Orn and α -KG, its two components, on insulin secretion, and to investigate the metabolic pathways potentially involved in this action. For this purpose, rat islets of Langerhans were used under two experimental conditions: the static model of incubated islets; the dynamic model of perfused islets. Since the synthesis of Gln, NO and polyamines has been demonstrated to be involved in OKG effects (Vaubourdolle *et al.* 1990; Moineard *et al.* 2002) and may also be implicated in OKG-induced insulin secretion, specific inhibitors of the suggested pathways, namely L-N^G-nitroarginine methyl ester (L-NAME), an effective inhibitor of NO synthase, methionine sulfoximine (MSO), a glutamine synthetase inhibitor, and difluoromethylornithine (DFMO),

an inhibitor of polyamine synthesis, were used in order to evaluate their contribution.

Materials and methods

Animals

Seven- to eight-week-old non-fasted male Wistar rats (Charles River, St-Aubin-les-Elbeuf, France) weighing 250–300 g were used in all the experiments. They were housed under standard conditions and allowed free access to food (A04; UAR, Epinay-sur-Orge, France) and tap water until the time of islet isolation.

The study complied with general recommendations for animal care and handling. Three of the investigators (S.D., L.C. and G.R.) are officially authorised by the French Ministry of Agriculture and Forestry to perform experimental research on rats.

Reagents

Arg, Orn, α -KG, L-NAME and MSO were obtained from Sigma-Aldrich (St Louis, MO), DFMO was purchased from Calbiochem (San Diego, CA) and OKG was a generous gift from Chiesi S.A. (Courbevoie, France). Analytical grade reagents for the preparation of buffer solutions were obtained from Sigma Aldrich.

The basal Krebs–Ringer buffer (KRB) (pH 7.4) contained 118.5 mmol NaCl/l, 2.54 mmol CaCl₂/l, 1.19 mmol KH₂PO₄/l, 4.74 mmol KCl/l, 25 mmol NaHCO₃/l, 1.19 mmol MgSO₄/l, bovine serum albumin (5 mg/ml), D-glucose (Glc, 2.8 mmol/l) and 10 mmol 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid/l (Marie *et al.* 2000).

Method of islet isolation

After anaesthesia of the rat with sodium pentobarbital (40 mg/kg intraperitoneally) (Sanofi, Libourne, France) the pancreas was dissected according to a modified method of Lacy & Kostianovsky (1967). Briefly, after clamping the common bile duct at a point close to the duodenum outlet, a retrograde injection of 10 ml Hanks' solution (Sigma Aldrich) containing 2.5 mg collagenase (Liberase[®]; Boehringer Mannheim, Mannheim, Germany) was made into the duct. The pancreas was then dissected and incubated for 20 min at 37°C.

After filtration of the crude preparation, the islets were washed twice with Hanks' solution and hand-picked under a dissecting microscope. About 400 islets were isolated from each animal. Only freshly isolated islets were used for perfusion or incubation experiments.

Incubation system

All the experiments were carried out in ninety-six-well filtration plates (Millipore, Molsheim, France) using five islets per well and eight wells per experimental condition. The islets were transferred to the plates immediately after isolation and washed three times with basal KRB directly in the filtration plates. The islets were incubated

immediately with 200 μ l of the effectors in KRB for 90 min at 37°C under an atmosphere of 95% O₂ and 5% CO₂ (v/v). For each experiment, two control groups were always studied in parallel: one in basal KRB, to determine basal insulin secretion; one with 16.5 mmol Glc/l as a functional control eliciting maximal insulin secretion. In all the experiments, islets were incubated in parallel with 10 mmol Arg/l, since the effect of this amino acid on insulin secretion is well known (Malaisse *et al.* 1989a,b) and can be considered as a functional reference for amino acid-induced insulin secretion. At the end of the incubation period, supernatant fractions were collected and stored at -80°C until insulin determination.

For experiments with metabolic inhibitors, a 30 min pre-incubation period was used, islets being incubated in KRB with or without L-NAME (5 mmol/l), an inhibitor of NO synthase (Schmidt *et al.* 1992), MSO (5 mmol/l), an inhibitor of Gln synthase (Minet *et al.* 1997), or DFMO (5 mmol/l), an inhibitor of ornithine decarboxylase (Blachier *et al.* 1989a). After this pre-incubation period, the medium was changed and the islets were further incubated in the same medium with each of the effectors, 16.5 mmol Glc/l, 10 mmol Arg/l and 1 mmol OKG/l, alone or in the presence of inhibitors.

Perifusion system

Four perifusion chambers (Swinnex 13; Millipore Corp., Bedford, MA) were run simultaneously, the islets (eighty islets per chamber) being placed on Whatman filters (mesh 0.45 μ m; Whatman Ltd., Maidstone, Kent, UK). Immediately after isolation, the islets were transferred into the perifusion chambers and perifused with basal KRB, equilibrated with 95% O₂ and 5% CO₂ (v/v) by continuous bubbling; the flow rate was set at 1 ml/min. After a 60 min wash-out period during which the perfusate was continuously discarded, the perifusion was carried out with basal KRB for 30 min (basal period) followed by a perifusion with a stimulating concentration of 16.5 mmol Glc/l, OKG (0.25, 0.5 and 1 mmol/l), Orn (2 mmol/l) or α -KG (1 mmol/l) for a 30 min stimulated period. The perfusate was then switched back to basal KRB and perifusion was continued for 60 min. Perfusate was collected in fractions of 5 ml for the two basal KRB periods and 2 ml for the stimulated period, and immediately stored at -80°C until insulin determination.

Analytical methods

Insulin was determined by radioimmunoassay using the INSIK-5 Kit (Diasorin, Saluggia, Italy) and rat insulin (Linco, St Charles, MO) as standard.

Calculations and statistics

All data are presented as the mean values and standard errors of the mean of four to eleven experiments. Unless stated otherwise, insulin secretion during incubation is presented in μ U/islet per 90 min and during perifusion in μ U/islet per min. For the perifusion experiments, basal and stimulated insulin secretions were evaluated by the area under the

curve of insulin secretion as a function of time calculated by the trapezoidal method during the basal period (i.e. between time 0 and time 30 min after the start of the perifusion) and during the stimulated period (i.e. between time 32 min and 62 min). To compare the early and late phase insulin secretion in the perifusion experiments, the stimulated period was divided into two 15 min periods, and insulin secretion (area under the curve) was calculated separately. These data are presented as the ratio to the corresponding basal value of insulin secretion.

Statistical analysis was performed by ANOVA followed by a Fisher test (StatView; SAS Institutes Inc., Cary, NC, USA); $P < 0.05$ was considered statistically significant.

Results

Secretory response to ornithine α -ketoglutarate: dose-response study

To investigate the effects of OKG on insulin secretion, OKG concentrations ranging from 0.25 to 2.5 mmol/l were first screened using the incubation method. In this set of experiments (n 11), islets of Langerhans were incubated for 90 min in the presence of various OKG concentrations or Glc (16.5 mmol/l) or Arg (10 mmol/l). Results of insulin secretion were compared with basal conditions. These data are presented in Fig. 1.

At 16.5 mmol/l, Glc exhibits its well-known stimulating effect on insulin secretion, which was increased nine-fold compared with basal concentration. Also, OKG significantly stimulated insulin release. Whereas the increase in insulin secretion was significant for all OKG concentrations *v.* basal secretion, insulin secretion induced by higher OKG concentrations (1.0 and 2.5 mmol/l) were significantly increased *v.* lower OKG doses. A dose-effect relationship was demonstrated by a close linear relationship between OKG concentration and insulin secretion (r 0.98; $P = 0.003$). Arg (10 mmol/l) induced an insulin secretion comparable to that elicited by OKG (2.5 mmol/l).

Kinetics of ornithine α -ketoglutarate action

The effect of OKG was further characterised by perifusion experiments using OKG at concentrations that can be achieved *in vivo* during OKG administration (Cynober *et al.* 1984b, 1990). Islets of Langerhans were thus perifused (six experiments) with 16.5 mmol Glc/l or increasing concentrations of OKG (0.25, 0.5 and 1.0 mmol/l). Results of insulin secretion during the stimulated period were compared with those observed during the basal period (Fig. 2 (a) and (b)). In the presence of OKG, insulin secretion increased with OKG concentrations and showed a significant first initial peak at 32–34 min ($P < 0.05$ *v.* basal insulin secretion). The peak value tended to increase as a function of OKG concentration (0.95 (SEM 0.34), 1.19 (SEM 0.35) and 1.45 (SEM 0.50) μ U/islet per min respectively, NS). The OKG-induced insulin secretion remained significantly elevated ($P < 0.05$ *v.* basal) throughout the stimulated period (Fig. 2 (a)).

Perifusion with 16.5 mmol Glc/l resulted in a biphasic stimulation of insulin secretion with a first significant

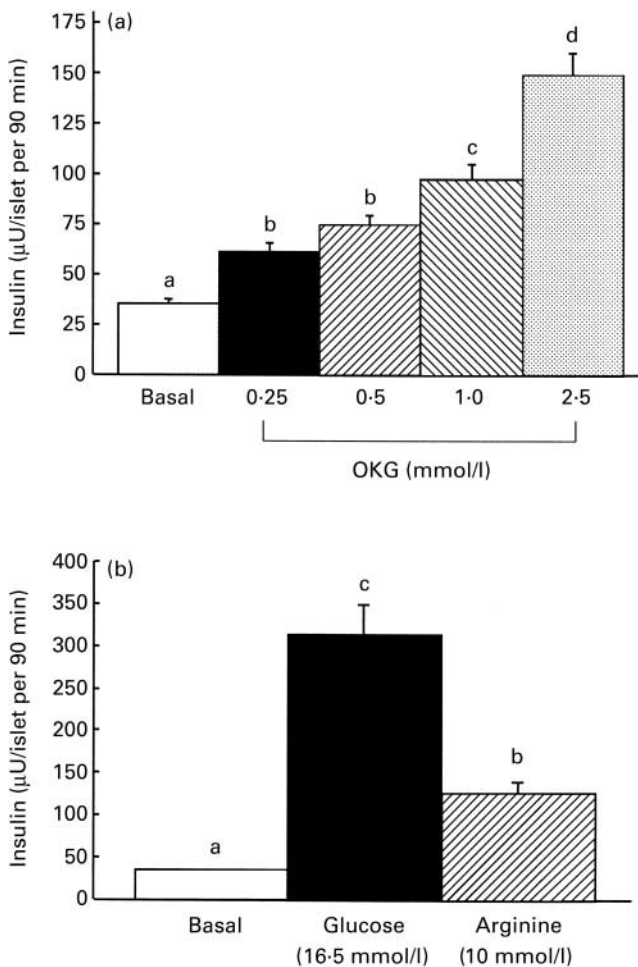


Fig. 1. Stimulating effects of ornithine α -ketoglutarate (OKG) on insulin secretion by isolated rat Langerhans islets. Islets (five islets per well, eight wells per experimental condition) were incubated for 90 min at 37°C in basal Krebs–Ringer buffer: (a) alone or in the presence of OKG concentrations ranging from 0 to 2.5 mmol/l; (b) alone or in the presence of 16.5 mmol glucose/l or 10 mmol arginine/l. Mean values of insulin concentration in the media at the end of the incubation period are shown with the standard errors of the mean represented by vertical bars for eleven experiments per group. ^{a,b,c,d} Groups with unlike superscript letters were significantly different ($P < 0.05$).

initial peak at 32 min and a significantly elevated late phase reaching a maximum at 52 min (Fig. 2 (b)).

To compare the early and late phases of insulin secretion, the stimulated period was divided into two periods of 15 min. Perfusion with different OKG concentrations showed a significant stimulation in the early phase for the three OKG concentrations used (ratio to basal insulin secretion: 0.25 mmol OKG/l, 1.79 (SEM 0.31), $P < 0.05$; 0.5 mmol OKG/l, 2.14 (SEM 0.15), $P < 0.05$; 1 mmol OKG/l, 3.41 (SEM 0.68), $P < 0.05$). On the other hand, only at the 0.5 and 1.0 mmol/l concentrations did OKG maintain a significant stimulation in the late phase until the end of stimulation (ratio to basal insulin secretion: 0.25 mmol OKG/l, 1.41 (SEM 0.17), NS; 0.5 mmol OKG/l, 1.79 (SEM 0.13), $P < 0.05$; 1 mmol OKG/l, 2.59 (SEM 0.34), $P < 0.05$).

Contribution of α -ketoglutarate and ornithine to ornithine α -ketoglutarate-induced insulin secretion

To evaluate the respective contribution of Orn and α -KG, the two components of OKG, islets were perfused (six experiments) in the presence of 1 mmol OKG/l, 1 mmol α -KG/l or 2 mmol Orn/l, allowing for the presence of 2 mol Orn and 1 mol α -KG in 1 mol OKG (six experiments).

As in previous experiments, OKG significantly stimulated insulin secretion. Both α -KG and Orn stimulated the early and late phase of insulin secretion significantly ($P < 0.05$) compared with basal secretion (Fig. 3 (a)), but the effects of both α -KG and Orn were significantly lower than that of OKG (Fig. 3 (b)).

Effect of L-nitroarginine methyl ester on ornithine α -ketoglutarate-induced insulin secretion

To investigate the possible role of NO in the secretory response to OKG, incubation experiments ($n = 6$) were repeated with or without L-NAME, an NO synthase inhibitor. Results are presented in Fig. 4 (a) and (b).

While L-NAME alone at a concentration of 5 mmol/l tended to increase basal insulin secretion, it produced a significant reduction in both OKG (1 mmol/l) and Arg (10 mmol/l) effects on insulin secretion. On the other hand, in the presence of 16.5 mmol Glc/l, L-NAME did not modify Glc-induced insulin secretion.

Effects of methionine sulfoximine on ornithine α -ketoglutarate-induced insulin secretion

To examine the possible involvement of the Gln synthetase pathway in OKG effects, islets of Langerhans were incubated in the presence of 5 mmol MSO/l (seven experiments per group). MSO did not affect either basal or Arg-stimulated insulin secretion. The results are presented in Fig. 5 (a) and (b). In the presence of MSO, OKG-induced insulin secretion was significantly reduced (40%; $P < 0.05$).

Effects of difluoromethylornithine on ornithine α -ketoglutarate-induced insulin secretion

To investigate the possible involvement of the polyamine pathway, incubation experiments were carried out in the presence of 5 mmol DFMO/l (four experiments per group).

DFMO did not affect either basal insulin release or that induced by 16.5 mmol Glc/l or Arg under these experimental conditions (data not shown). OKG-induced insulin secretion was not significantly modified by DFMO (basal secretion: 29 (SEM 7) μ U/islet per 90 min; OKG: 78 (SEM 22) μ U/islet per 90 min; OKG + DFMO: 72 (SEM 23) μ U/islet per 90 min; $P < 0.05$ OKG and OKG + DFMO *v.* basal secretion).

Discussion

Our aim was to investigate the insulinotropic effect of OKG and to study its possible metabolic pathways in isolated islets of Langerhans. OKG stimulates insulin secretion dose-dependently, inducing both the first and

the second phase of insulin release. However, neither Orn nor α -KG alone, at concentrations equivalent to that of OKG, reproduces the effects of OKG.

In both incubation and perfusion experiments, we observed a dose-dependent induction of insulin release by OKG, already significant at a concentration of 0.25 mmol/l. This is in line with the OKG-induced increase in insulinaemia observed *in vivo* in healthy subjects (Cynober *et al.* 1984b, 1990). This effect was expected because numerous studies have pointed to the involvement of both α -KG and Orn, the two components of OKG, in insulin secretion (Lenzen *et al.* 1986; Malaisse *et al.* 1989a). However, most of these findings were based on effector concentrations higher than those used in the present experiments. Experimental conditions such as basal Glc concentration may partly explain some of the differences between our

results and those reported in the literature. For example, Blachier *et al.* (1989b) showed a marked increase in insulin release in incubated and perfused rat islets in the presence of 10 mmol Orn/l and 5.6 mmol Glc/l, while other experiments failed to demonstrate any Orn effect below 3 to 5 mmol/l. Our findings indicate that Orn alone or as its α -KG salt stimulates insulin secretion significantly in a range of concentrations (i.e. 0.5 to 1.0 mmol/l) achieved during *in vivo* administration (Cynober, 1995), but neither α -KG nor Orn by itself reproduces OKG-induced insulin secretion. Similar results were obtained in islets from pigs (data not shown) suggesting that the observed effects are not species-related. Orn and α -KG induced the first initial peak and the second phase of insulin secretion, insulin release during each of these two periods being approximately half of total release during the stimulated period.

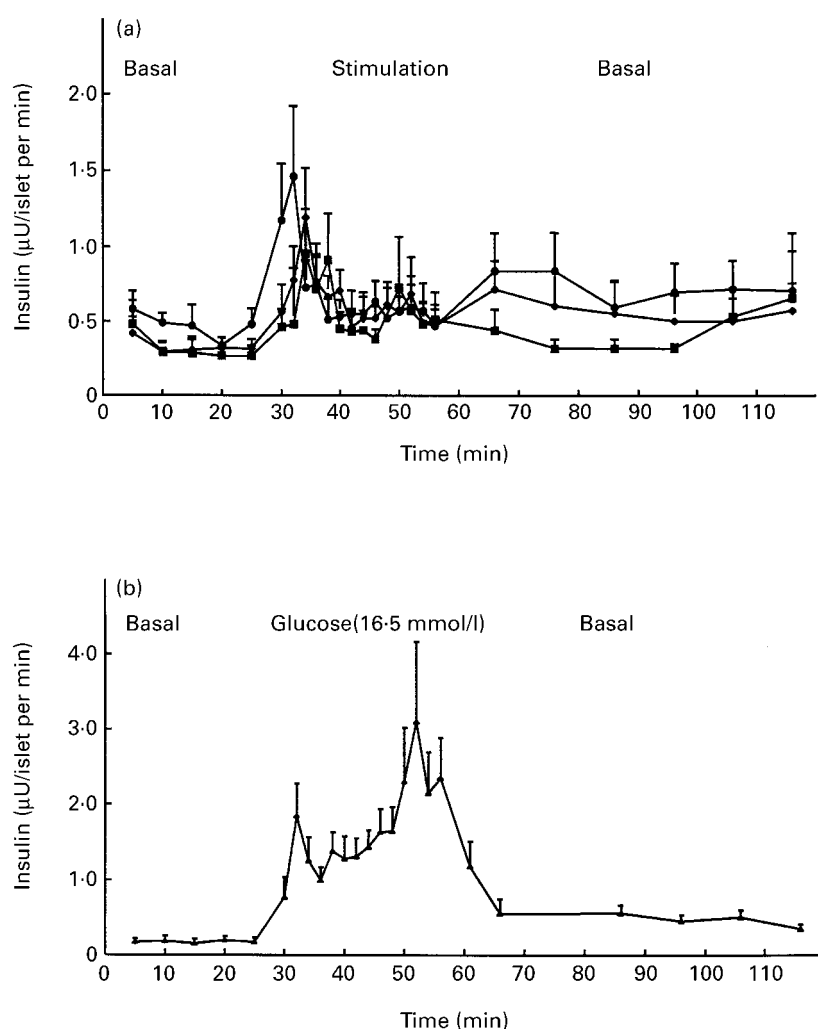


Fig. 2. Kinetics of ornithine α -ketoglutarate (OKG) action on insulin secretion in perfused Langerhans islets. After a basal period in Krebs–Ringer buffer (30 min), islets (eighty islets per chamber and per experimental condition) were perfused for 30 min in the presence of either: (a) OKG at one of three different concentrations ((\blacksquare), 0.25; (\blacklozenge), 0.5; (\bullet), 1.0 mmol/l); (b) 16.5 mmol glucose/l (\blacktriangle). Mean values of insulin secretion (six experiments per group) in the perfusion media during basal and stimulated perfusion periods are shown, with the standard errors of the mean represented by vertical bars. There were no statistical differences between groups during the basal and post stimulation periods.

These results suggest that both compounds are involved in the OKG effect, and that the simultaneous action of α -KG and Orn is required for the insulinotropic effect of OKG. Since OKG completely dissociated in aqueous solution, the effect of simultaneous addition of α -KG (as its sodium salt) and Orn (as its hydrochloride salt) was not tested. However, Fig. 3 shows that α -KG and Orn by themselves account respectively for nearly 30 and 60% of OKG effects, suggesting that in these conditions the effects of α -KG and Orn are merely additive. Nevertheless, further experiments are needed to clarify this point. Moreover, as the insulin secretory properties of non-Glc secretagogue is largely influenced by Glc concentration, it would be interesting to evaluate the effects of Orn and α -KG in the presence of different Glc concentrations.

An α -KG–Orn interaction has been suggested to induce several anabolic effects of OKG by promoting Arg and Gln synthesis (Cynober *et al.* 1990; Le Boucher *et al.* 1997),

based on a metabolic pathway common to Orn and α -KG. When these two compounds are administered simultaneously, saturation of this common pathway favours the utilisation of α -KG and Orn in other pathways, such as Arg, Gln or polyamine synthesis (Cynober, 1999), both potentially involved in insulin secretion (Malaisse *et al.* 1982; Welsh & Sjöholm, 1988; Schmidt *et al.* 1992).

Our data, which confirm *in vivo* observations of OKG-induced insulin secretion in healthy subjects, led us to investigate the underlying mechanisms. Three different pathways of OKG metabolism may be involved: (i) the production of NO through the synthesis of Arg; (ii) the production of glutamate via transamination of both Orn and α -KG; (iii) the decarboxylation of Orn into polyamines.

The first of these pathways we studied was the induction of NO production by exogenous Orn, since some data suggest that OKG effects may be related to the generation of NO (Moinard *et al.* 2002). This effect was evaluated

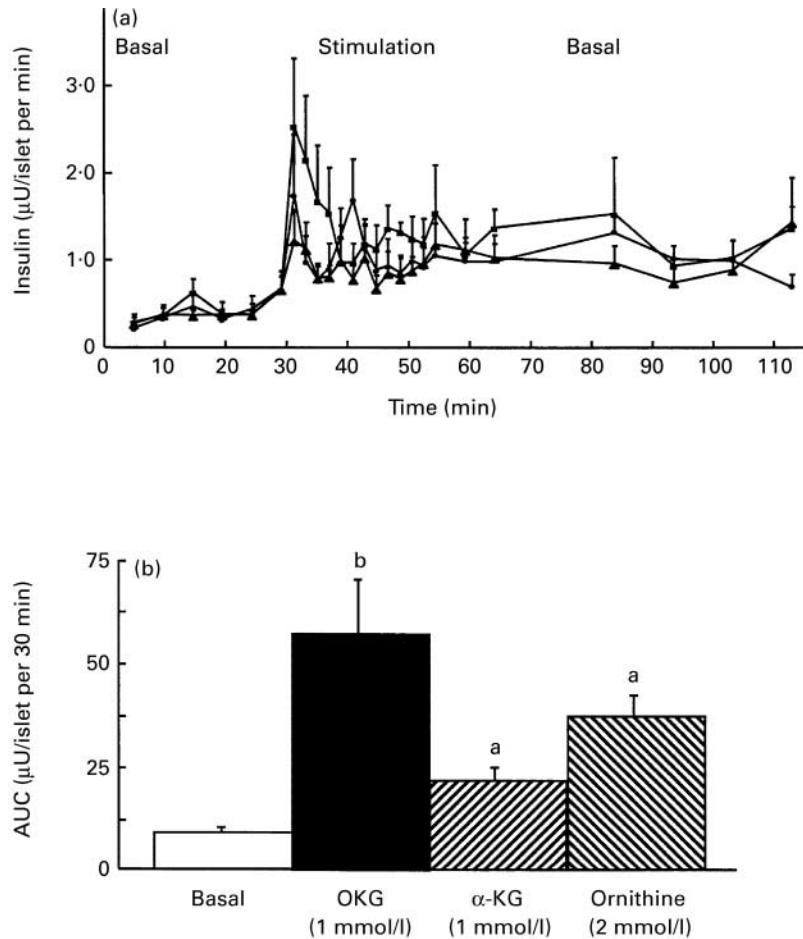


Fig. 3. Effects of ornithine α -ketoglutarate (OKG) and equivalent concentration of α -ketoglutarate (α -KG) and ornithine on insulin secretion by perfused rat Langerhans islets. After a basal period in Krebs–Ringer buffer (30 min), islets (eighty islets per chamber and per experimental condition) were perfused for 30 min with 1 mmol OKG/l (—■—), 2 mmol ornithine/l (—●—) or 1 mmol α -KG/l (—▲—). (a), Insulin secretion as a function of time; (b), area under the curve (AUC) of insulin production as a function of time during perfusion. Mean values of insulin secretion (six experiments per group) in the perfusion media during basal and stimulated perfusion periods are shown, with the standard errors of the mean represented by vertical bars. ^{a,b}Groups with unlike superscript letters were significantly different ($P < 0.05$).

using L-NAME, an NO-synthase inhibitor. In incubation experiments, L-NAME (5 mmol/l) significantly decreased OKG effects on insulin secretion, indicating an involvement of the NO synthase pathway and NO production in OKG-induced insulin secretion. These data suggest that conversion of Orn into Arg may be involved. However, Malaisse *et al.* (1989b) failed to demonstrate any significant synthesis of Arg from Orn in a tumoral cell line (RINm5F) and in isolated islets. In addition, Sener *et al.* (1988) reported that rat islets are devoid of detectable ornithine transcarbamylase activity. On the other hand, recent studies suggest an implication of NO in Orn-induced insulin secretion, whereby sufficient amounts of Orn could be converted into Arg to enable NO production (Gross *et al.* 1997). Thus two hypotheses may be advanced. First, the NO synthase pathway may be quantitatively minor but metabolically significant. Second, Orn may have a sparing effect on Arg metabolism, leading to increased Arg avail-

ability for NO production. Further work is required to clarify this issue.

The second mechanism we studied was the consequence of the interaction between α -KG and Orn on glutamine metabolism (De Bandt & Cynober, 1998). While the contribution of glutamate to the regulation of insulin secretion has been recently challenged (MacDonald & Fahien, 2000), several studies emphasise the involvement of this amino acid in the regulation of insulin secretion (Malaisse, 1991; Maechler & Wollheim, 1999; Rorsman & Renström, 1999). On the other hand, as mentioned earlier, OKG exerts some of its metabolic effects via the synthesis of Gln, and could be a better precursor for Gln than Glc owing to transporter specificity and intracellular channeling (Cynober, 1999). We therefore studied insulin secretion in the presence of MSO, an inhibitor of Gln synthase. Whereas MSO was without effect on basal or Arg-stimulated insulin secretion, it produced a significant

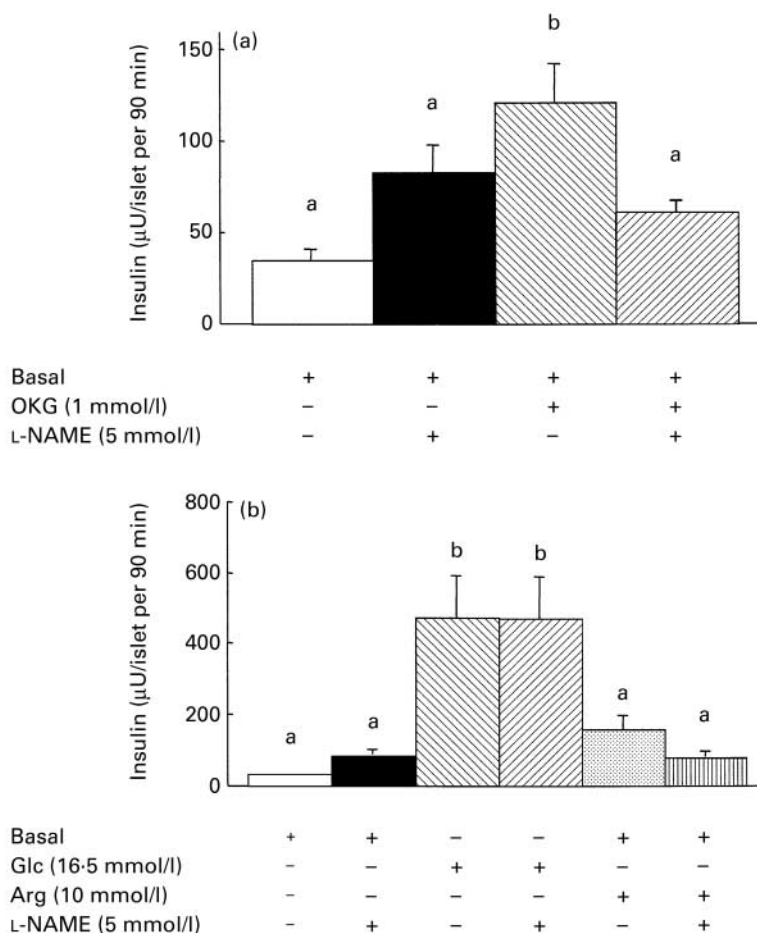


Fig. 4. Inhibition of ornithine α -ketoglutarate (OKG)-induced insulin secretion by L-nitroarginine methyl ester (L-NAME) in incubated islets of Langerhans. Islets (five islets per well, eight wells per experimental condition) were incubated for 90 min at 37°C with or without 5 mmol L-NAME/l in basal Krebs–Ringer buffer: (a) alone or in the presence of 1 mmol OKG/l; (b) alone or in the presence of glucose (Glc, 16.5 mmol/l) or arginine (Arg, 10 mmol/l). Mean values of insulin concentration (six experiments per group) in the media at the end of the incubation period are shown, with the standard errors of the mean represented by vertical bars. ^{a,b}Groups with unlike superscript letters were significantly different ($P < 0.05$).

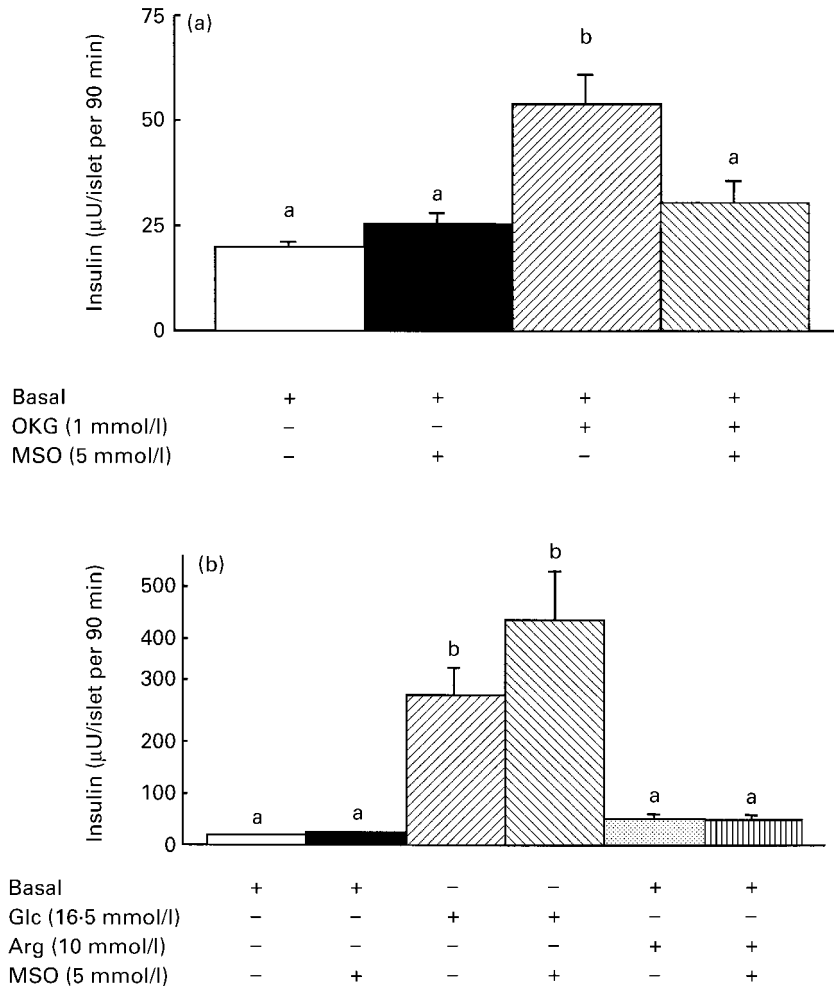


Fig. 5. Inhibition of ornithine α -ketoglutarate (OKG)-induced (1 mmol/l) insulin secretion by methionine sulfoximine (MSO) in incubated islets of Langerhans. Islets (five islets per well, eight wells per experimental condition) were incubated for 90 min at 37°C with or without 5 mmol MSO/l in basal Krebs–Ringer buffer: (a) alone or in the presence of 1 mmol OKG/l; (b) alone or in the presence of glucose (Glc, 16.5 mmol/l) or arginine (Arg, 10 mmol/l). Mean values of insulin concentration (six experiments per group) in the media at the end of the incubation period are shown, with the standard errors of the mean represented by vertical bars. ^{a,b}Groups with unlike superscript letters were significantly different ($P < 0.05$).

reduction (40%) in OKG-induced insulin release. This indicates that OKG acts partially on insulin-secretion via Gln synthesis. Whether this is related to intracellular channelling requiring a prior conversion of OKG into Gln or to some other mechanism related to glutamate availability remains to be determined.

Finally, the conversion of Orn into polyamines, a reaction catalysed by ornithine decarboxylase, was investigated. Polyamines seem to be involved in the regulation of Glc-sensitive insulin release, insulin biosynthesis and insulin mRNA content (Hoogarden *et al.* 1986; Welsh & Sjöholm, 1988). Also, polyamines have been identified as major mediators of OKG-induced proliferation of fibroblasts in culture (Vaubourdolle *et al.* 1990). However, no effect of ornithine decarboxylase inhibition by DFMO on OKG-induced insulin release was observed in the present work. This suggests that the formation of polyamines does not play any role in the secretory response to OKG.

In conclusion, for the first time a direct effect of OKG on insulin secretion in isolated islets of Langerhans has been demonstrated. This effect seems to be due to the induction of both NO and Gln synthesis. Although the mechanisms by which the production of Gln contributes to insulin secretion remain to be fully elucidated, these findings contribute to a better understanding of the mechanisms of OKG action, and suggest that OKG administration may be useful in situations where insulin secretion is compromised.

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