In Vivo Induction of Insulin Secretion by Ornithine α-Ketoglutarate: Involvement of Nitric Oxide and Glutamine

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We previously demonstrated that ornithine α-ketoglutarate (OKG), known for its anabolic properties, induces insulin secretion in vitro. The present study was undertaken to further characterize this effect in vivo and investigate a possible interaction with glucose both in vivo and in vitro. Male Wistar rats received an intravenous bolus of OKG (25 mg/kg) and/or glucose (0.8 g/kg) or saline, and their plasma insulin and glucose levels were monitored for 30 minutes. OKG alone increased plasma insulin to a similar extent to glucose. In combination with glucose, OKG significantly increased glucose-induced insulin secretion in vivo and in vitro, and led to a significant increase in glucose utilization in vivo. The absence of significant variations in plasma arginine and glutamine suggests a direct effect of OKG on the pancreas. To assess the involvement of the synthesis of nitric oxide and glutamine in OKG-induced insulin secretion, the experiments were repeated in the presence of inhibitors of these 2 pathways, respectively L-nitroarginine-methylester (L-NAME) and methionine sulfoximine (MSO). Both inhibitors were able significantly to reduce OKG-induced insulin secretion without affecting either basal or glucose-induced insulin release. Thus OKG acts directly with glucose on islets to induce insulin secretion via mechanisms involving NO and glutamine synthesis. In addition, our results suggest that OKG and glucose act via separate pathways.

THE ANABOLIC/ANTICATABOLIC properties of ornithine α-ketoglutarate (OKG) came to light in the early 1960s in the improved nutritional status of patients with liver diseases given OKG.[1] This prompted various studies to evaluate the benefit of OKG supplementation of enteral and parenteral nutrition. OKG has been demonstrated to improve nitrogen balance in surgical,[2] burn,[3,4] septic, and polytrauma patients,[5] by either increasing protein synthesis or reducing protein breakdown.[5] Various mechanisms may be involved in these effects. OKG is a precursor of glutamine (Gln) and arginine (Arg), both of which play a key role in the control of protein metabolism and immune response.[6] Some evidence also suggests that endocrine processes may be involved, with possible interactions between OKG and insulin secretion and/or peripheral action. First, OKG could modulate the peripheral response to insulin action, as suggested by the OKG-induced improvement in glucose tolerance in burned patients in the absence of an increase in plasma insulin.[7] Second, an OKG-induced increase in plasma insulin has been demonstrated after intravenous and oral OKG administration in healthy subjects.[8,9] We have recently demonstrated, in isolated rat islets of Langerhans, that OKG stimulates insulin secretion in vitro dose-dependently[10] via the induction of 2 different pathways, namely, nitric oxide-synthase and Gln production.[11] In perifused rat islets of Langerhans we observed that neither α-ketoglutarate (α-KG) nor ornithine (Orn) alone, in concentrations equivalent to that of OKG, could account by themselves for the effect of OKG.[12] Interestingly, the action of OKG on plasma insulin in healthy volunteers is not reproduced by either α-KG or Orn alone.[9] Taken as a whole, these data suggest that interactions between the 2 components of OKG are required to induce this effect. Such an interaction, related to the fact that Orn and α-KG share a common metabolic pathway, has been found necessary for some of the anabolic/anticatabolic effects of OKG and the synthesis of Gln.[5,13] Additional mechanisms may be involved in the insulinotropic effect of OKG in vivo. First, as stated above, OKG is a precursor of Gln and Arg, 2 amino acids involved in insulin secretion (either directly or indirectly), and OKG administration has been demonstrated to increase their availability. Arg is known as a very potent non-glucose insulin secretagogue in vivo.[14] Although Gln given alone fails to induce insulin secretion in incubated islets[15] or decreases it in perifused islets,[16] it enhances insulin secretion induced by branched-chain amino acids in vitro[15,17] through a glutamate dehydrogenase-dependent process[18] and recent studies in vitro showed a direct involvement of glutamate, the metabolite of Gln, in insulin release.[19] This raises the question of a possible indirect effect of OKG on insulin secretion through the systemic generation of these amino acids.

Second, there is evidence that insulin secretory response to nonglucose stimuli may be affected by glucose (Glc).[14,20,21] Interestingly, it has been shown in healthy subjects that insulin secretory response to OKG is more than 2.5 times higher in the fed[22] than in the fasted state.[9] Thus OKG may act on insulin secretion either via an increase in systemic Arg and Gln availability or via a direct effect on the pancreas and an interaction with Glc secretory effect at the islet level.

The aim of this study was first to confirm the effects of OKG on insulin secretion in vivo and then to evaluate both the influence of Glc on its secretory properties and the possible contribution of its conversion into Arg and Gln. We used an intravenous Glc tolerance test in the presence or absence of OKG. In these experiments, glucagon secretion was also evaluated, since amino acids such as Orn have been shown to induce in vivo glucagon secretion.[23] The interaction between

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OKG and Glc was also studied in vitro in a rat islet incubation model in order to gain insight at the cellular level. In addtion. intravenous Glc tolerance tests were repeated in the presence of L-nitroarginine-methyl-ester (L-NAME) or methionine sulfoximine (MSO), inhibitors of NO-synthase and Gln synthetase, respectively, 2 pathways potentially involved in OKG secretory effects.

Data presented here together with those published elsewhere[10-12] form a line of evidence that OKG directly stimulates insulin secretion, and that this effect is amplified by Glc and is mediated by Gln and NO-generating pathway.

MATERIALS AND METHODS

Animals

Seven- to eight-week-old nonfasted male Wistar rats (Iffa Credo, Lyon, France) weighing 250 to 300 g were used in all the experiments. They were housed under standard conditions and allowed free access to a standard regimen (UAR A04, UAR, Epinay-sur-Orge, France) and tap water until experimentation. Experiments complied with the ethical recommendations for the handling and care of laboratory animals of the French Ministry of Agriculture and Forestry.

Chemicals

L-NAME and MSO were obtained from Sigma Chemicals (St Louis, MO) and OKG was a generous gift from Chiesi S.A. (Courbevoie, France).

In Vitro Studies

Islet preparation. After anesthesia of the rat with sodium pentobarbital (40 mg/kg intraperitoneally) (Sanofi, Libourne, France) the pancreas was dissected according to a modified method of Lacy and Kostianovsky.[24] Briefly, after clamping of the common bile duct at a point close to the duodenum outlet, a retrograde injection of collagenase (Liberase, Boehringer Mannheim, Mannheim, Germany) in Hanks solution (Sigma Chemicals) was made into the bile duct. The pancreas was then dissected and incubated at 37°C. After filtering the crude preparation, the islets were washed twice with Hanks solution and hand-picked under a dissecting microscope.

Insulin secretion in vitro. All experiments were performed in 96-well filtration plates (Millipore, Molsheim, France) using 5 islets per well. Islets were transferred immediately after isolation into the plates and washed with basal Krebs Ringer Buffer (KRB, Glc 2.8 mmol/L) directly in the filtration plates. The islets were then incubated immediately with 200 μL of OKG and/or Glc in KRB for 90 minutes at 37°C under an atmosphere of 95% O_2 and 5% CO_2. At the end of the incubation period supernatants were collected and stored at −20°C until insulin determination. To investigate the effect of OKG on in vitro insulin secretion, islets were incubated in the presence or absence of OKG (0.5 and 1 mmol/L) and various Glc concentrations (2.8, 10, and 16.5 mmol/L).

In Vivo Studies

Experimental procedure. To determine OKG effects on insulin secretion in vivo and the influence of Glc, 3 experimental groups (6 rats per group) were studied: a Glc-treated group (0.8 g/kg), an OKG-treated group (25 mg/kg), and a group given both Glc (0.8 g/kg) and OKG (25 mg/kg). For these experiments, the rats were anesthetized by halothane inhalation (Zeneca Pharma, Cergy, France). Glc (Sigma Chemicals) and/or OKG were administered intravenously as a single bolus. A control group was studied in parallel, the rats receiving an equivalent volume of physiological saline (Sigma Chemicals). Heparinized venous blood samples were taken at the retro-orbital sinus just before and repeatedly after injection at times 1, 3, 5, 7, 10, 15, and 30 minutes. For the measurement of glucagon, blood samples were drawn on Iniprol (Trasylol, Boehringer Mannheim). After centrifugation, plasma samples were used either immediately for the measurement of Glc, or frozen at −20°C for insulin and glucagon determinations.

A second set of experiments was conducted to investigate plasma amino acid variations after Glc and/or OKG administration; the design of these experiments was the same as that described above. For amino acid measurement, plasma samples were deproteinized with sulfosalicylic acid (30 mg/mL) (Sigma Chemicals) and frozen at −80°C.

To assess the contribution of NO and Gln synthesis in OKG-induced insulin secretion, the experiments were repeated in the presence of inhibitors of these 2 pathways, namely, L-NAME (25 mg/kg)[25,26] and MSO (25 mg/kg),[27] administered intaperitoneally 15 minutes before glucose and/or OKG or saline. The concentrations of the inhibitors used were adapted from classical concentrations used in the literature after checking for the absence of direct toxicity and insulinotropic effects (data not shown).

Analytical Methods

Plasma Glc concentration was determined enzymatically by the Glc oxidase method using a Glc analyzer (Beckman, Palo Alto, CA). The measurements of in vitro insulin release and plasma insulin were performed by radioimmunoassay using a commercially available kit (Insik-5, Diasorin, Saluggia, Italy) with purified rat insulin as standard (Linco, St Charles, MO). For glucagon determination, a commercially available radioimmunoassay was used as well as the corresponding standard (Linco).

Individual free amino acids in plasma were assayed by cation-exchange chromatography on a Jeol Amino-Tac analyzer (Jeol-Europe, Croissy-sur-Seine, France) with ninhydrin postcolumn derivatization and spectrophotometric detection.

Calculations and Statistics

For statistical comparison, global insulin and glucagon secretions were evaluated by the area under the curve (calculated by the trapezoidal method) of plasma insulin and glucagon as a function of time.

For comparison of peripheral Glc utilization, the coefficient of peripheral assimilation of Glc (K) was calculated as the slope of the curve of plasma Glc over time (between 1 and 15 minutes after Glc load) plotted on a semi-logarithmic scale.[28]

To compare variations in plasma amino acid, data are presented as differences (Δ) from basal values.

All data are presented as the mean ± SEM of 6 experiments in each group. Statistical analysis was performed by analysis of variance (ANOVA) followed by a Fisher test (StatView, SAS Institutes Inc, Cary, NC) and $P < .05$ was considered as statistically significant.

RESULTS

Secretory Effects of OKG on Glc-Induced Insulin Secretion In Vitro

In incubation experiments, Glc 10 and 16.5 mmol/L induced its well-known stimulating effect on insulin release with a dose-effect relationship (Fig 1).

In basal KRB, ie, containing Glc 2.8 mmol/L, OKG 0.5 and 1 mmol/L induced a significant insulin secretory response. When associated with higher Glc concentrations (10 or 16.5 mmol/L), OKG-induced insulin secretion was significantly higher than that induced by Glc alone at an OKG concentration of 1 mmol/L (Fig 1).

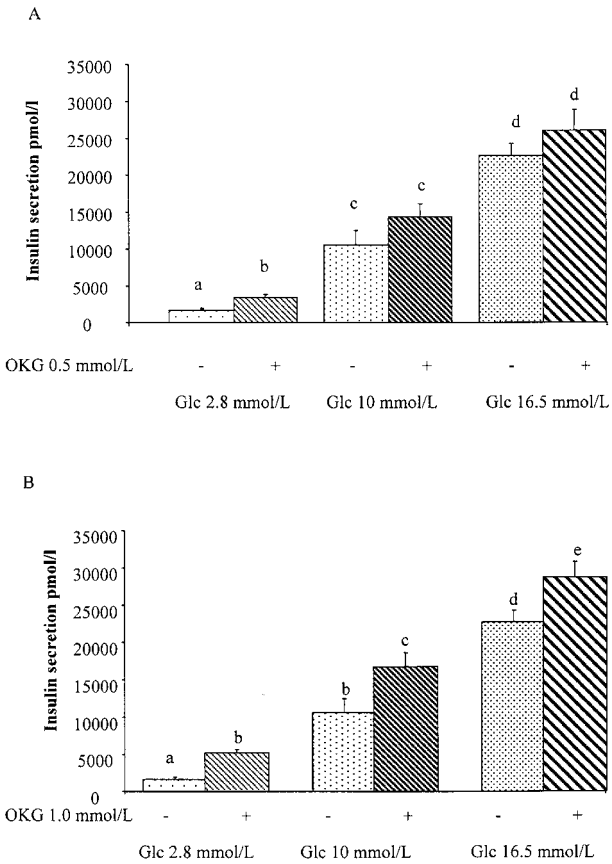

Fig 1. Effects of 2 different OKG concentrations on in vitro Glc-induced insulin secretion. Islets (5 islets per well, 8 wells per experimental condition) were incubated for 90 minutes at 37°C in KRB alone or in the presence of different Glc concentrations (2.8, 10, or 16.5 mmol/L) and/or OKG (A: 0.5, or B: 1 mmol/L). Results (mean ± SEM, 6 experiments per group) represent insulin concentration in the media at the end of the incubation period. Bars with different superscripts are significantly different at *P* < .05.

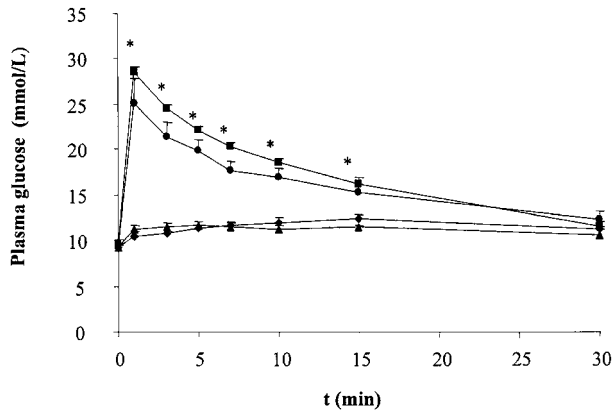

Fig 2. Effects of OKG on plasma Glc levels. Rats received a single bolus of either NaCl 0.9% (◆), Glc 0.8 g/kg (●), OKG 25 mg/kg (▲), or OKG 25 mg/kg + Glc 0.8 g/kg (■). Blood samples were taken at times 0, 1, 3, 5, 7, 10, 15, and 30 minutes for the measurement of plasma Glc. Results (mean ± SEM, 6 experiments) are expressed as mmol/L. *P < .05 Glc 0.8 g/kg and OKG 25 mg/kg + Glc 0.8 g/kg *v* NaCl 0.9 %.

Plasma Glucose

OKG treatment did not influence plasma Glc compared with the NaCl-treated group (Fig 2). In both groups plasma Glc remained unchanged compared with basal levels throughout the experimental period.

After bolus Glc administration with or without OKG, plasma Glc showed a maximum at t = 1 minute and decreased thereafter to reach basal values at t = 30 minutes (Fig 2). While plasma Glc was not different between the 2 groups at each time point, Glc disappearance rate (K) calculated over the first 15 minutes after Glc and Glc/OKG administration showed that peripheral Glc utilization was significantly improved by OKG administration compared with Glc alone (Fig 3).

Insulin Secretory Response to OKG Administration

OKG and/or Glc intravenous load caused a significant increase in plasma insulin compared with NaCl-treated rats. This response was characterized by an initial peak at t = 1 minute and a persistent increase in plasma insulin throughout the study period (Fig 4).

Both OKG and Glc initiated a first peak of insulin secretion of similar magnitude. In terms of total insulin secretion, represented by the area under the curve of plasma insulin over 30 minutes, Glc and OKG induced a similar significant increase in insulin release (Fig 5).

When associated with Glc, OKG caused a significantly higher insulin secretion than OKG or Glc alone, and this affected both the initial peak (Fig 4) and total insulin secretion (Fig 5).

Glucagon Secretory Response to OKG Administration

In parallel, the effect of OKG on glucagon secretion was determined; AUC of plasma glucagon over the 30-minute ex-

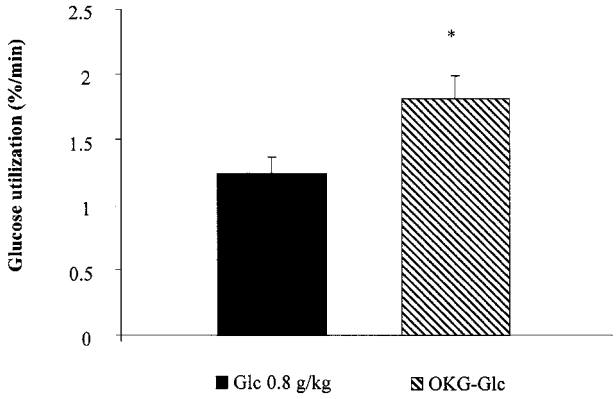

Fig 3. Effect of OKG on Glc disappearance rate (K) in Wistar rats. Rats received a single bolus administration of either NaCl 0.9%, Glc 0.8 g/kg, OKG 25 mg/kg, or OKG 25 mg/kg + Glc 0.8 g/kg. K was calculated between 1 and 15 minutes. Results (mean ± SEM of 6 experiments per group) are expressed as %/min. *P < .05 *v* Glc.

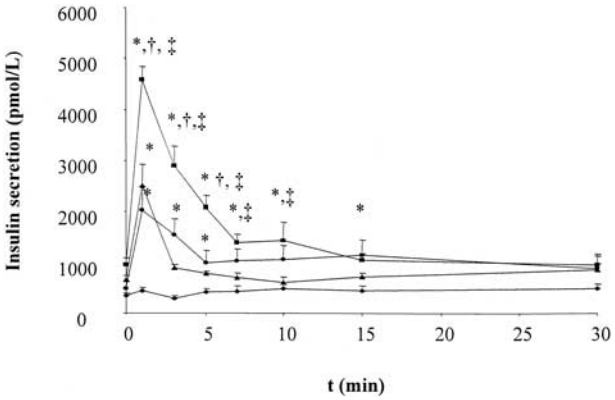

Fig 4. OKG effects on kinetics of insulin secretion. Rats received a single bolus administration of either NaCl 0.9% (◆), Glc 0.8 g/kg (●), OKG 25 mg/kg (▲), or OKG 25 mg/kg + Glc 0.8 g/kg (■). Blood samples were taken at times 0, 1, 3, 5, 7, 10, 15, and 30 minutes for the measurement of plasma insulin. Results (mean ± SEM of 6 experiments per group) are expressed as pmol/L. *$P < .05$ *v* basal secretion, †$P < .05$ *v* Glc 0.8 g/kg; ‡$P < .05$ *v* OKG 25 mg/kg.

perimental period showed a significant decrease in glucagon secretion only in the OKG group (Table 1).

Changes in Plasma Amino Acids

In OKG-treated animals a significant increase in plasma Orn was noted at t = 1 minute (ΔOrn: NaCl: −2 ± 3; Glc: −7 ± 1; OKG: +667 ± 101; OKG-Glc: +896 ± 104 μmol/L; n = 6 per group, $P < .05$ OKG and OKG-Glc *v* NaCl and Glc) and a progressive return to normal values at the end of the study period. Concerning Arg, in the different groups there was a gradual decrease in their plasma concentrations (−20 % in 30 minutes, $P < .05$), except for a slight transitory increase of Arg in the OKG group at t = 3 minutes (ΔArg: +6.2 ± 8.1 μmol/L,

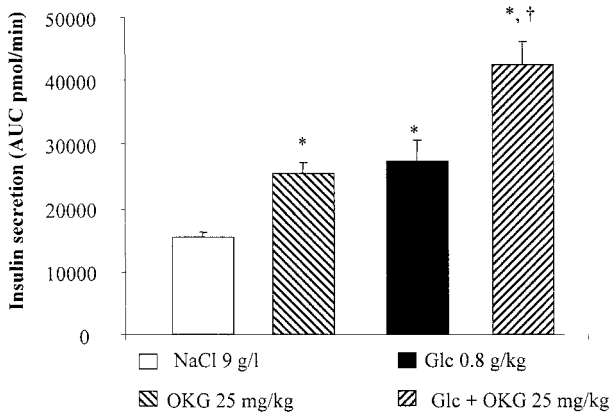

Fig 5. Effects of OKG on global insulin secretion. Rats received a single bolus administration of either NaCl 0.9%, Glc 0.8 g/kg, OKG 25 mg/kg, or OKG 25 mg/kg + Glc 0.8 g/kg. Blood samples were taken at times 0, 1, 3, 5, 7, 10, 15, and 30 minutes for the measurement of plasma insulin. Results (mean ± SEM, 6 experiments per group) represent AUC of plasma insulin during the 30-minute experiment. *$P < .05$ *v* NaCl 0.9%; †$P < .05$ *v* Glc 0.8 g/kg, and OKG 25 mg/kg.

Table 1. Effect of OKG on Glucagon Release

Group	Glucagon (pmol/L)
NaCl 0.9%	4,578 ± 544
Glc 0.8 g/kg	4,044 ± 486
OKG 25 mg/kg	3,314 ± 311*
OKG 25 mg/kg + Glc 0.8 g/kg	3,867 ± 151

NOTE. Rats received a single bolus administration of either NaCl 9 g/L, Glc 0.8 g/kg, OKG 25 mg/kg, or OKG 25 mg/kg + Glc 0.8 g/kg. Blood samples were taken at times 0, 1, 3, 5, 7, 10, 15, and 30 minutes for the measurement of plasma glucagon. Results (mean ± SEM of 6 experiments per group) represent global glucagon release calculated as the AUC of plasma glucagon during the 30-minute experiment.

*$p < .05$ NaCl 0.9% *v* basal secretion.

$P < .05$). Gln concentrations stayed unchanged in all groups throughout the experimental period.

Effect of L-NAME on OKG-Induced Insulin Secretion

Effects on insulin secretion of the administration of L-NAME, a NO-synthase inhibitor, before Glc and/or OKG load, are expressed as the AUC of plasma insulin during the 30-minute experimental period, and presented in Fig 6. L-NAME by itself had no effect on basal or on Glc-induced insulin release. In contrast, OKG-induced insulin release was completely suppressed by L-NAME. This inhibitory effect was also observed in the Glc-OKG-treated group.

Effect of MSO on OKG-Induced Insulin Secretion

The influence of the inhibition by MSO of the Gln synthetase pathway on OKG-induced insulin secretion is presented in Fig 7 as AUC of plasma insulin for the whole experimental period. MSO did not affect basal or Glc-induced insulin secretion over

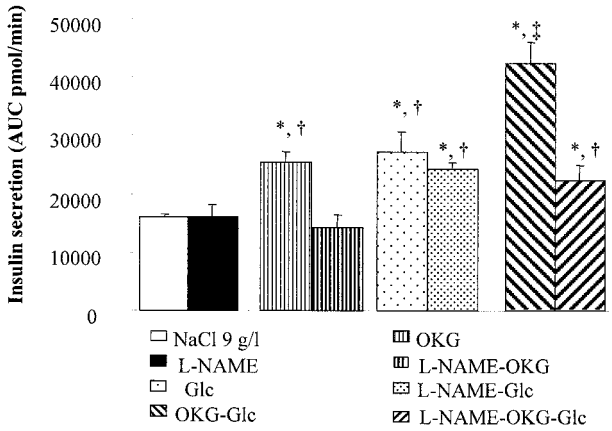

Fig 6. Inhibition of OKG-induced insulin secretion by L-NAME. Rats received a single bolus administration of either NaCl 0.9%, Glc 0.8 g/kg, OKG 25 mg/kg, or OKG 25 mg/kg + Glc 0.8 g/kg. In L-NAME-treated animals, L-NAME (25 mg/kg) was administered 15 minutes before effector administration. Blood samples were taken at times 0, 1, 3, 5, 7, 10, 15, and 30 minutes for the measurement of plasma insulin. Results (mean ± SEM of 6 experiments per group) represent global insulin secretion calculated as the AUC of plasma insulin during the 30-minute experiment. *$P < .05$ *v* NaCl 0.9%; †$P < .05$ *v* OKG 25 mg/kg-NAME; ‡$P < .05$ *v* OKG-Glc-NAME.

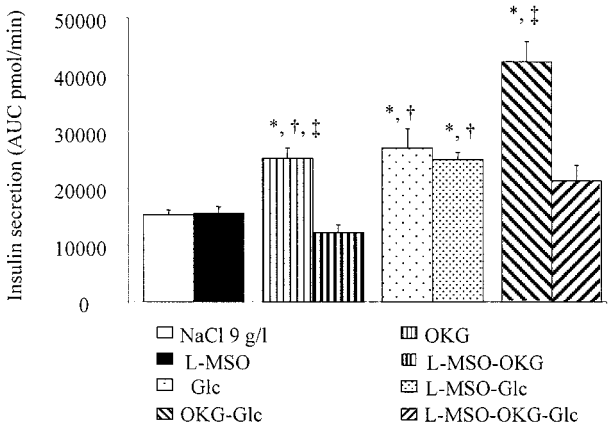

Fig 7. Inhibition of OKG-induced insulin secretion by MSO. Rats received a single bolus administration of either NaCl 0.9%, Glc 0.8 g/kg, OKG 25 mg/kg, or 25 OKG mg/kg + Glc 0.8 g/kg. In MSO-treated animals, MSO (25 mg/kg) was administered 15 minutes before effector administration. Blood samples were taken at times 0, 1, 3, 5, 7, 10, 15, and 30 minutes for the measurement of plasma insulin. Results (mean ± SEM of six experiments per group) represent global insulin secretion calculated as the AUC of plasma insulin during the 30-minute experiment. *$P < .05$ *v* NaCl 0.9%; †$P < .05$ *v* OKG 25 mg/kg MSO; ‡$P < .05$ *v* OKG-Glc-MSO.

the 30-minute study period. However, MSO led to a significant decrease in OKG-induced insulin secretion, in both the OKG-treated and the Glc-OKG-treated groups.

DISCUSSION

The main purpose of the present work was to show in vivo the consequence of OKG administration in the presence of Glc on insulin secretion and to characterize the pathways that might be implicated. These data indicate that OKG is a potent inducer of insulin secretion in vivo. Our results also suggest that OKG potentiates Glc-induced insulin secretion and improves Glc utilization. The underlying mechanisms seem to be independent of a systemic increase in Gln and Arg bioavailability, and instead to involve the intra-insular generation of NO and Gln.

As is evident from Glc tolerance test, OKG induced a rapid increase in plasma insulin with a maximum already reached at t = 1 minute. Comparison of the area under the curve after different treatments indicates that the quantity of insulin secreted in response to 25 mg/kg OKG is comparable to that of Glc (0.8 g/kg). These data can be compared to those obtained by Krassowski et al[8] after parenteral administration of OKG. These authors showed that an infusion of 20 g/m^2 OKG over a 30-minute period markedly increased plasma insulin levels for about 90 minutes. Kinetics of insulin response differed somewhat from those obtained in the present study, but the experimental conditions can explain these differences: in Krassowski's study OKG was infused over a 30-minute period compared with a bolus administration in the present study. Also, very large amounts of OKG were administered, corresponding to the total daily dosage given in clinical practice,[29] whereas in our experimental conditions, administration of 25 mg/kg OKG led to an increase in plasma Orn that was similar to that reached during therapeutic OKG administration.[29]

Another major difference concerns OKG effects on glucagon secretion. Krassowski et al[8] observed a significant increase in plasma glucagon, whereas in the present experiments, OKG administration decreased glucagon secretion. Different hypotheses can be advanced. First, a very large increase in plasma insulin in fasted subjects could trigger a decrease in plasma Glc and reduce glucagon secretion.[9] Second, Orn by itself, or more probably via the formation of Arg, which is favored by the very large dose of OKG administered,[8,9] could act on glucagon secretion. Third, in Krassowski's study, human volunteers were studied in the postabsorptive state, whereas in the present study rats were used in the fed state. This would also explain why Glc administration did not further decrease plasma glucagon, glucagon secretion being already repressed in the basal fed state.

As stated above, OKG may have exerted its effects through the systemic generation of Arg and Gln. Although in our experiments insulin secretion was effectively stimulated, this occurred in the absence of significant variations in plasma Gln. A slight transient increase in plasma Arg was observed only in the OKG-treated group. Given the large dose of Arg (>3 mmol/L) needed to initiate an insulin secretion,[30,31] the magnitude of the increase in plasma Arg (+6 μmol/L) in our experiments seems unable to account for the effect of OKG.[32] Hence these data suggests an endocrine effect of OKG via a direct effect on islets of Langerhans.

Moreover, there is evidence that insulin secretory response to non-glucose stimuli such as Arg is dependent on the Glc concentration to which islets are exposed in vitro[20] and in healthy subjects.[21,33] Our data indicate that OKG potentiates Glc-induced insulin secretion. This observation is strengthened by our results of in vitro incubation experiments in which we observed a significant stimulation by 1 mmol/L OKG of Glc-induced insulin secretion at 10 and 16.5 mmol/L Glc concentrations. Notably, this concentration of OKG is similar to that found in the plasma of subjects receiving 10 g OKG.[29] In addition, these findings are in line with the observation in previous in vitro studies that in basal KRB, OKG (0.25 to 2.5 mmol/L) induced a dose-dependent insulin secretion (C. Schneid et al; submitted for publication). Taken as a whole, these data support the idea that OKG acts directly on the pancreatic β cell and that this effect does not depend on its systemic metabolism. It is also noteworthy that Blachier et al[34] have shown a Glc dependency of in vitro Orn and Arg-stimulated insulin secretion. However, in this latter in vitro study, a combination of a pharmacological concentration of Orn (10 mmol/L) and of 16.7 mmol/L Glc was used, which produced only the Glc-induced insulin secretion,[34] and not the significant potentiation between Glc and Orn shown in our study. This further emphasizes the importance of α-KG/Orn interaction in this effect. As the amount of Glc supplied by the intravenous bolus is such as to induce maximal insulin secretory response, our results suggest that Glc and OKG may act on insulin secretion via different mechanisms. Notably, one consequence of this potentiating effect of OKG on Glc-induced insulin secretion is a significant increase in Glc disappearance rate when Glc load is associated with OKG. This suggests that OKG also promotes Glc utilization by peripheral tissues, as already suggested by different studies.[7,35] It seems that the increase in plasma insulin can account for this observation.

Three mechanisms can be proposed to explain the potentiating effect of OKG on Glc-induced insulin secretion. First, a direct effect of Orn may play a role, since Orn is known to be a potent insulin secretagogue.[23] However, experiments in healthy human volunteers have shown that at the plasma level obtained in our study, Orn fails to affect insulin secretion.[9] Second, OKG may induce insulin secretion through its oxidation and adenosine triphosphate (ATP) synthesis (ie, fuel effect).[36] Taking into account the respective amounts of OKG and Glc supplied as individual boluses (0.6 μmol/kg OKG and 4.6 mmol/kg Glc) the size of the insulin secretory effect enables us to rule out an effect of OKG via its utilization for ATP generation. A third possibility, namely, a specific role for the interaction between Orn and α-KG, has therefore to be considered, and the determination of the underlying mechanism deserves further study.

The involvement of NO synthesis in insulin secretion is a subject of debate[30,31,37] and indeed we did not observe any effect of L-NAME on Glc-induced insulin secretion. However, some data support an involvement of NO synthesis in Orn-induced insulin release[31] while several studies point to a role of NO in some metabolic effects of OKG.[29,38] The first possible mechanism involved in OKG-induced insulin secretion could thus be an increase in Arg availability (related to the α-KG/Orn interaction) promoting the synthesis of NO. This was investigated through the use of L-NAME. The significant inhibition of OKG-induced insulin secretion by L-NAME, while both basal- and Glc-induced secretion remained unchanged, supports the involvement of NO in the insulinotropic effects of OKG. These observations are consistent with the suggested role of NO generation within islets of Langerhans in Arg-induced insulin secretion.[30] As Malaisse et al[39] failed to demonstrate any significant synthesis of Arg from Orn in a tumoral cell line (RINm5F) and in isolated islets, it can be suggested that OKG may have a sparing effect on Arg metabolism, leading to increased Arg availability for NO production.

Finally, the metabolism of both α-KG and Orn could lead to glutamate and thus to Gln, which is also involved in some of the anabolic properties of OKG.[38] In experiments testing this hypothesis not only were basal and Glc-induced insulin secretion unaffected by MSO, but also OKG-induced insulin release was significantly decreased. On the other hand, it has been shown that glutamate alone did not induce any insulin secretion in incubated islets of Langerhans,[32] which points to a possible channeling of intra-insular α-KG/Glu/Gln metabolism,[40] as suggested in other cells.[41] Thus it can be proposed that the inhibition of Gln-synthase by MSO affects an intracellular glutamate pool involved in the regulation of insulin secretion.[42]

In conclusion, this study reveals that OKG-induced insulin secretion is comparable to that induced by the administration of Glc at 0.8 g/kg. Beside the potent induction of insulin secretion by OKG, this study also shows that OKG potentiates Glc-induced insulin secretion both in vivo and in vitro, this potentiating effect leading to an improvement in Glc utilization. This induction seems related to a direct effect of OKG on islets of Langerhans via 2 metabolic pathways, NO-synthase and Gln synthetase. Moreover, the specific effects of the inhibitors L-NAME and MSO suggest that the mechanisms of OKG-induced insulin secretion are independent of Glc-induced release. Lastly, this action is also independent of changes in either plasma amino acids or glucagon secretion.

It would be of clinical interest to evaluate whether these effects are maintained in insulin-compromised situations, eg, in type 2 diabetes.

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