Biochemical Pharmacology, Vol. 39, No. 3, pp. 537–547, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

STIMULUS-SECRETION COUPLING OF ARGININE-INDUCED INSULIN RELEASE

RESISTANCE OF ARGININE- AND ORNITHINE-STIMULATED GLUCAGON AND INSULIN RELEASE TO D,L-α-DIFLUOROMETHYLORNITHINE

V. LECLERCQ-MEYER, J. MARCHAND and W. J. MALAISSE*
Laboratory of Experimental Medicine, Brussels Free University, B-1000 Brussels, Belgium

(*Received* 12 *January* 1989; *accepted* 3 *September* 1989)

Abstract—In the isolated perfused rat pancreas, D,L-difluoromethylornithine, tested at a concentration of 3 mmol/L, failed to affect the release of glucagon and insulin caused, over 15 min stimulation, by either L-arginine or L-ornithine (2.0, 5.0 or 10.0 mmol/L) in the presence of either 3.3 or 5.6 mmol/L D-glucose. The inhibition of ornithine decarboxylase also failed to affect the release of glucagon provoked by either L-leucine (2 or 3 mmol/L) or L-glutamine (2 mmol/L) and the secretion of insulin stimulated by a rise in glucose concentration from 5.6 to 10.6 mmol/L. These data are interpreted to suggest that the rapid generation of polyamines from either L-arginine or L-ornithine does not play any significant role in the immediate glucagonotropic and insulinotropic action of these cationic amino acids.

In a recent study, it was reported that L-arginine and L-ornithine are effectively metabolized in pancreatic islet cells and lead to the *de novo* generation of the polyamines putrescine, spermidine and spermine [1]. The question was raised, therefore, whether such metabolic events play a role in the insulinotropic action of these cationic amino acids. When islet cells are removed from rats treated with 2-amino-isobutyric acid, only a minor increase in the secretory reponse to L-arginine or L-ornithine was observed, despite a dramatic increase in the activity of islet ornithine decarboxylase [2]. Likewise, when D,L-α-difluoromethylornithine (α-DFMO) was used as an inhibitor of the latter enzyme, only a partial and poorly reproducible inhibition of insulin release evoked by either L-arginine or L-ornithine was found in experiments performed with isolated rat islets over 90 min incubation [3]. The present experiments, conducted in the isolated perfused rat pancreas, were undertaken to further explore the effect of α-DFMO upon the secretory behaviour of the endocrine pancreas. The technique used offers two advantages. First, it allows examination of the effect of α-DFMO upon the early secretory response to selected secretagogues. Second, it allows exploration of the release of both glucagon and insulin simultaneously.

MATERIALS AND METHODS

Fed female Wistar rats of a mean body weight of 229 ± 3 g (N = 47) were used in the present study. A blood sample was obtained from the tail prior to anesthesia for glucose and insulin determinations in the plasma. The animals were anesthetized with sodium barbital (42 mg/kg, i.p.) and the pancreas was isolated from all adjacent organs (also duodenum) through appropriately placed ligatures. The isolated pancreas was perfused *in situ* through both the coeliac and superior mesenteric arteries via a cannula inserted into the aorta, as previously described [4].

The perfusion medium contained the following salts (in mmol/L): NaCl, 118.5; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 1; $NaHCO_3$, 25. It was supplemented with dextran (40 g/L, clinical grade, Sigma Chemical Co., St Louis, MO) and bovine serum albumin (5 g/L, fraction V, RIA grade, Sigma) and equilibrated against a mixture of O_2 and CO_2 (95:5) with a resulting pH of 7.4. A finite volume of water was left out from the basal perfusate in order to realize the final adjustment in volume during the perfusions (1.6 mL/min) through the side-arm infusion of two times 0.075 mL/min of either water alone (control periods or control perfusions) or water containing the dissolved stimuli. The latter included L-ornithine, L-arginine (both from Aldrich Chemie, Steinheim, F.R.G.), L-leucine, L-glutamine (both from Merck, Darmstadt, F.R.G.), D-glucose and α-DFMO (donated by Merrell Dow Institute, Strasbourg, France). All side-arm solutions were brought to a pH of 7.4 prior to their administration and infused using glass syringes mounted on an infusion pump (Braun, Melsungen, F.R.G.). In the experiments in which the effects of α-DFMO were examined, the infusion of the compound (3 mmol/L final concentration) was started at min 5 and then continued throughout the perfusions. Thus, at the time the first effluent sample was taken (min 25 on the graphs), the pancreas had already been exposed for 20 min to α-DFMO.

The perfusate reached the pancreas with a temperature of 37° and the pancreatic effluent was collected from the portal vein via a cannula and tubing connected to a fraction collector (Retriever II, Isco,

* To whom correspondence should be addressed at Laboratoire de Médecine Expérimentale, Boulevard de Waterloo 115, B-1000 Brussels, Belgium.

Table 1. Some functional parameters related to the animals used and the perfusions performed in the absence or presence of 3 mmol/L α-DFMO

	No α-DFMO (N = 24)	+ α-DFMO (N = 23)
Flow rate (mL/min)	1.54 ± 0.01	1.56 ± 0.01
Pressure (mm Hg) at min 25	27.5 ± 0.7	28.6 ± 1.0
at min 100	26.5 ± 0.6	27.2 ± 0.6
Rat body weight (g)	234 ± 4	223 ± 4
Plasma glucose (mg/100 mL)	141 ± 3	139 ± 2
Plasma insulin (μ units/mL)	42 ± 3	41 ± 4
Pancreas wet weight (g)	0.85 ± 0.03	0.85 ± 0.02
Pancreas glucagon content (μg)	5.5 ± 0.2	5.7 ± 0.4
(μg/g)	6.5 ± 0.3	6.8 ± 0.4
Pancreas insulin content (μg)	138.1 ± 6.2	131.7 ± 6.2
(μg/g)	164.5 ± 7.8	156.6 ± 7.9

No P value is indicated as there was no statistical difference at the P = 0.05 level between the two series of experiments.

U.S.A.). Effluent samples were obtained at the times shown on the Figures into glass tubes containing 2000 KIU aprotinin (Kallikrein Inhibitor Units, Trasylol, Bayer, Brussels, Belgium). The actual flow rate was monitored throughout the perfusions by weighing selected collecting tubes at min 25, 30, 45, 60, 75, 90 and 100. All collecting tubes were rapidly transferred to an iced water bath and frozen at −25° upon termination of the experiments. The pancreas was then dissected free from all surrounding fat, weighed and extracted by hand with a glass homogenizer using acidified ethanol [5]. After centrifugation the supernatant was kept at −25°.

Glucagon and insulin were estimated in the effluent samples using 0.05, 0.1 or 0.2 mL aliquots in dependence of the hormonal concentrations expected. Similar aliquots were used in the case of the pancreatic extract which, on the day of the assay, was neutralized and further diluted with the assay diluent (×4000 for glucagon, ×8000 for insulin). Both the glucagon and insulin radioimmunoassays were performed using a charcoal-dextran separation of the bound from the free hormone. The glucagon assay used a glycine buffer [6], aprotinin (1000 KIU/ml of buffer), [^{125}I]glucagon (obtained through the courtesy of Drs D. G. Pipeleers and C. F. H. Van Schravendijk, Vrije Universiteit Brussel, Brussels, Belgium), a C-terminal anti-glucagon serum (2601, our laboratory) and purified porcine glucagon as the standard (Novo, Bagsvaerd, Denmark). The insulin assay used a veronal buffer [7], aprotinin (500 KIU/mL of buffer), [^{125}I]insulin (porcine, same source as [^{125}I]glucagon), a guinea-pig anti-insulin serum (P3, our laboratory) and rat insulin as the standard (R 170, Novo). The diluent was supplemented with RIA grade bovine albumin (Sigma) in both assays. The sensitivity, the intra- and interassay coefficients of variation of these assays were reported previously [8]. Glucose was determined in the plasma using a hexokinase method (Sigma Diagnostics).

The results in the text, tables and figures are presented as the mean ±SE, together with the number of individual determinations or experiments performed (N). Integrated hormonal secretory rates were computed from the areas under the curves. In the case of glucagon release, the secretory data were, on occasions, expressed in terms of Δ output in subtracting the basal from the stimulated output. These Δ values are indicated only in the text. Such calculations were not done for insulin release in which case subtraction of the low basal output observed in the experimental conditions used (glucose 3.3 and 5.6 mmol/L) had little influence on the stimulated output values. The effects of α-DFMO upon glucagon and insulin release were evaluated both in terms of observed secretory data (graphs, text and tables) and percentage (text and tables) relative to the mean secretory data (to which was ascribed a 100% value) obtained in the control perfusions performed in the absence of α-DFMO. The latter percentage mode of comparison, however, was unsuitable when the hormonal output was at the limit of detection of the assay, for instance, in the case of insulin release at low glucose (3.3 mmol/L) and low amino acid (2 mmol/L) concentrations (see Results). Statistical comparisons were conducted using the two-tailed non-paired *t*-test.

As shown in Table 1, there was no difference in the experimental parameters related to the animals (body weight, plasma glucose, plasma insulin) between the control (No α-DFMO, N = 24) and the α-DFMO experiments (N = 23). α-DFMO was also without influence on flow rate, pressure, pancreas wet weight, and glucagon or insulin content at the end of the perfusions.

RESULTS

Influence of α-DFMO on basal glucagon and insulin release

As expected, the basal output of glucagon during the early period of perfusion (min 30–41) in the control experiments performed in the absence of α-DFMO (Figs 1–5, upper panels, closed circles; Table 2) was lower at 5.6 (0.14 ± 0.02 ng/min, N = 14, Figs 1, 2 and 5) than at 3.3 mmol/L glucose (0.28 ng ± 0.03 ng/min, N = 10, Figs 3 and 4, P < 0.001). In the presence of α-DFMO (upper panels and open circles on Figs 1–5; Table 2), the glucagon secretory rates were slightly depressed to 0.13 ± 0.02 ng/min (Figs 1, 2, and 5, N = 13) and 0.21 ± 0.03 ng/min (Figs 3 and 4, N = 10) at 5.6 and

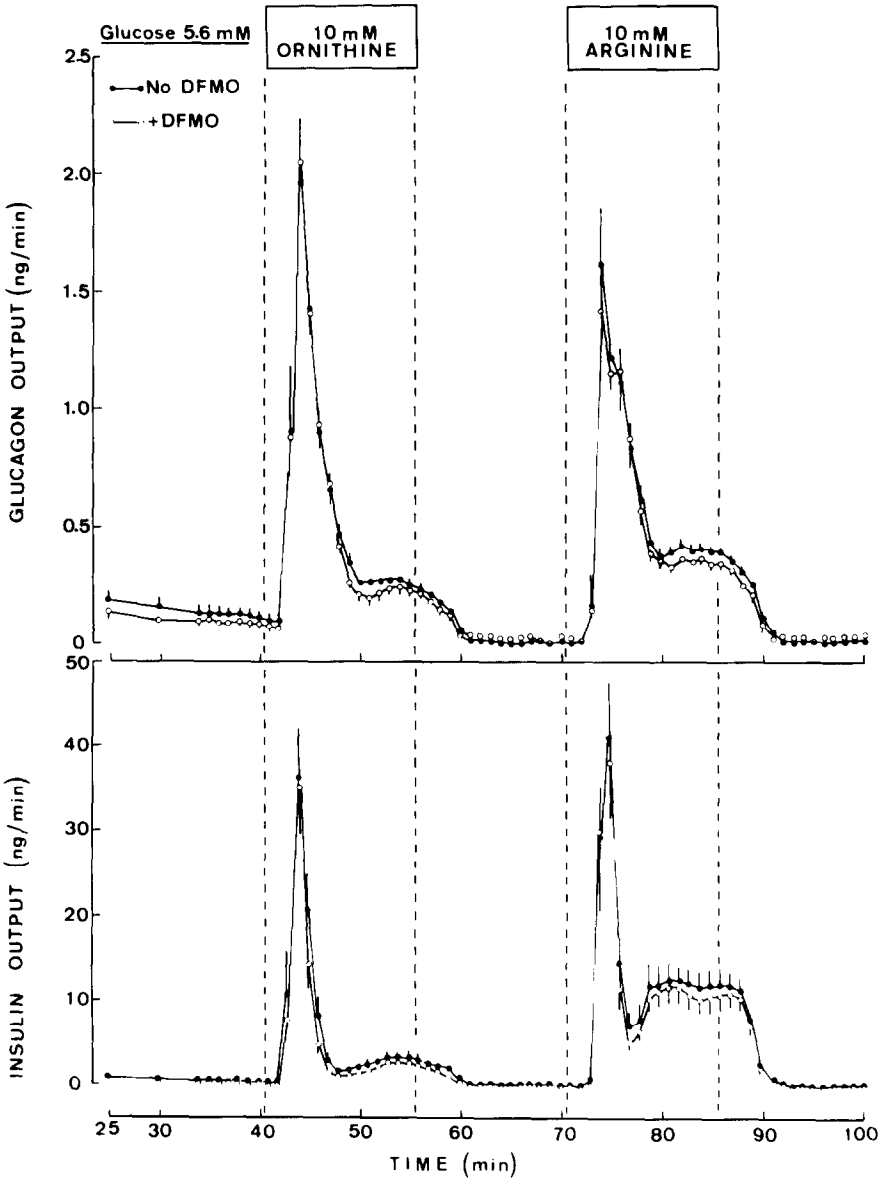

Fig. 1. The effects of 10 mmol/L ornithine and 10 mmol/L arginine upon glucagon (upper panel) and insulin release (bottom panel) from the rat pancreas perfused at 5.6 mmol/L glucose and absence (closed circles, N = 4) or presence of 3 mmol/L α-DFMO (open circles, N = 4). The vertical dotted lines indicate the times at which the infusion was switched from a control to a stimulus-containing syringe or *vice versa*.

3.3 mmol/L glucose, respectively. As a whole, the basal output of glucagon in the presence of α-DFMO represented 83.4 ± 7.8% (N = 24) of that observed in the control perfusions (100.0 ± 7.7%, N = 23; Table 2). Neither the secretory rates nor the percentage values were significantly different.

α-DFMO also slightly depressed the basal output of insulin during the early period (min 30–41) at 5.6 mmol/L glucose, although this was little evidenced on the graphs due to the scale employed (Figs 1, 2 and 5, bottom panels). Thus, the insulin secretory rates amounted to 0.27 ± 0.05 ng/min and represented 82.0 ± 14.6% (N = 13) in the presence of α-DFMO relative to the values seen in the control perfusions (0.33 ± 0.04 ng/min and 100.0 ± 12.6%, N = 14; Table 2). Such limited depression was not significant. At the low 3.3 mmol/L glucose level, α-DFMO appeared to slightly elevate the basal output in some of the experiments (Figs 3 and 4, bottom panels; Table 2). These differences again were not significant, the results being at the limit of detection of the insulin assay (see end of Materials and Methods section).

Influence of α-DFMO on ornithine- and arginine-induced glucagon release

In the control perfusions, the rat pancreas

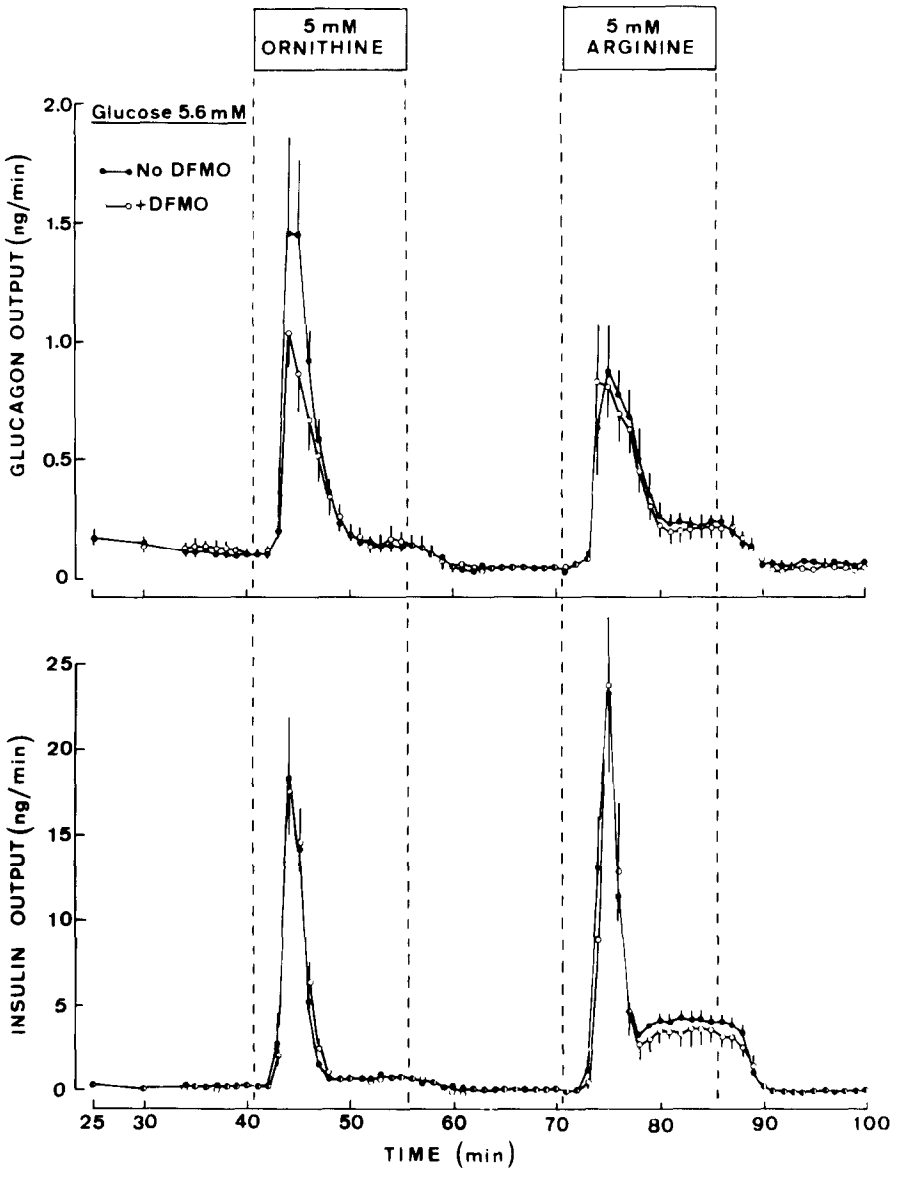

Fig. 2. The effects of 5 mmol/L ornithine and 5 mmol/L arginine upon glucagon (upper panel) and insulin release (bottom panel) from the rat pancreas perfused at 5.6 mmol/L glucose and absence (closed circles, N = 4) or presence of 3 mmol/L α-DFMO (open circles, N = 4). Vertical dotted lines as in Fig. 1.

Table 2. Basal glucagon and insulin output from the rat pancreas perfused in the absence or presence of 3 mmol/L α-DFMO

	ng/min		Per cent (relative to no α-DFMO)	
	No α-DFMO	+ α-DFMO	No α-DFMO	+ α-DFMO
Basal glucagon output				
Glucose 5.6 mmol/L	0.14 ± 0.02 (14)	0.13 ± 0.02 (13)	100.0 ± 11.6 (14)	90.8 ± 11.6 (13)
Glucose 3.3 mmol/L	0.28 ± 0.03 (10)	0.21 ± 0.03 (10)	100.0 ± 9.7 (10)	73.7 ± 9.7 (10)
Pooled data			100.0 ± 7.7 (24)	83.4 ± 7.8 (23)
Basal insulin output				
Glucose 5.6 mmol/L	0.33 ± 0.04 (14)	0.27 ± 0.05 (13)	100.0 ± 12.6 (14)	82.0 ± 14.6 (13)
Glucose 3.3 mmol/L	0.18 ± 0.02 (10)	0.30 ± 0.12 (10)	—	—

The results were computed from the areas under the curves during min 30–41. No P value is indicated, as in Table 1.

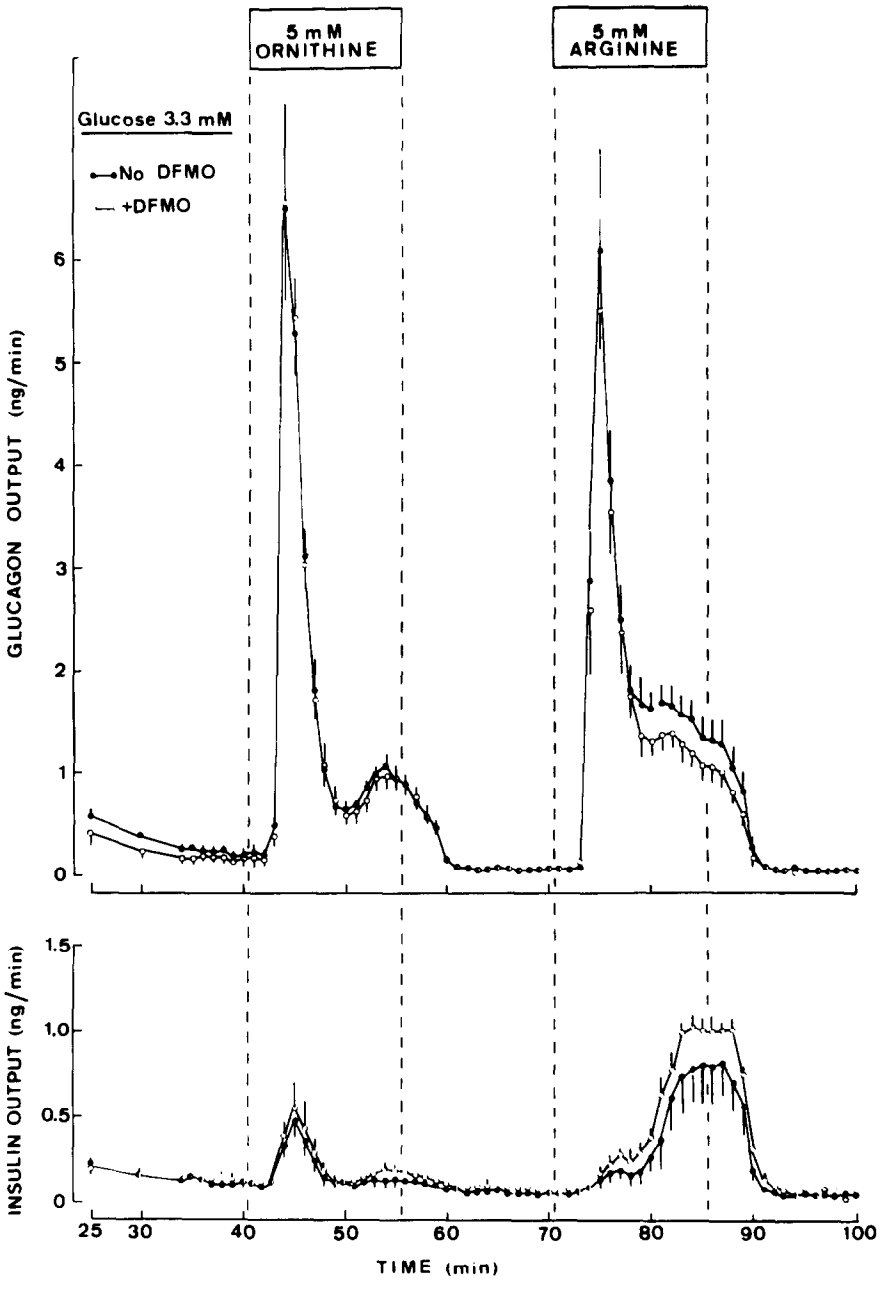

Fig. 3. The effects of 5 mmol/L ornithine and 5 mmol/L arginine upon glucagon (upper panel) and insulin release (bottom panel) from the rat pancreas perfused at 3.3 mmol/L glucose and absence (closed circles, N = 4) or presence of 3 mmol/L α-DFMO (open circles, N = 4). Vertical dotted lines as in Fig. 1.

responded in a dose-dependent and negatively glucose-modulated manner to both ornithine and arginine (Figs 1–3, upper panels, closed circles; Table 3).Thus, at 5.6 mmol/L glucose, the stimulated glucagon output (area under the curve) decreased from a value of 9.2 ± 0.4 to 6.7 ± 1.2 and 4.0 ± 0.3 ng in response to 10, 5 and 2 mmol/L ornithine, and from a value of 10.0 ± 0.8 to 6.2 ± 1.0 and 3.2 ± 0.7 ng in response to the same concentrations of arginine. At 3.3 mmol/L glucose, the output of glucagon, which represented 2–5 times that seen at 5.6 mmol/L glucose, decreased from a value of 26.9 ± 1.4 to 12.8 ± 1.6 ng in presence of 5 and 2 mmol/L ornithine, and from a value of 32.9 ± 3.8 to 9.1 ± 1.0 ng in the presence of the same concentrations of arginine. Of interest was the observation that, at the two glucose levels used, comparable concentrations of ornithine and arginine enhanced the total output of glucagon to nearly the same extent. Indeed, the overall responses to arginine represented

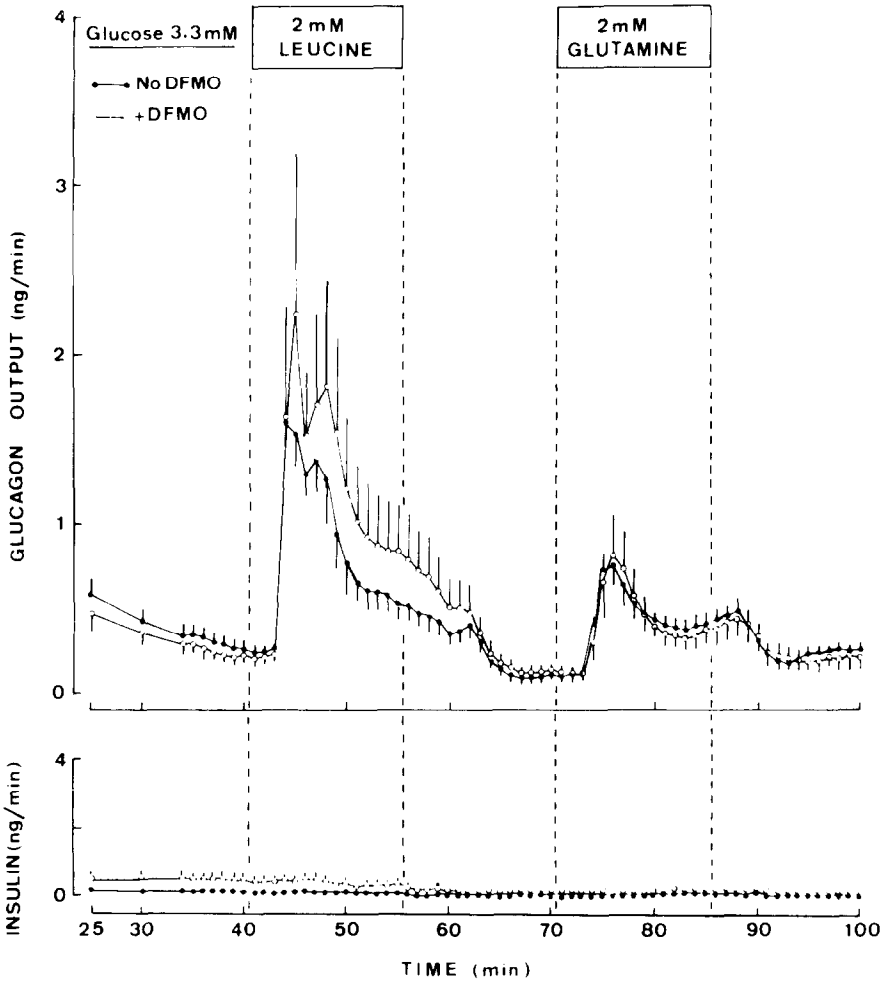

Fig. 4. The effects of 2 mmol/L leucine and 2 mmol/L glutamine upon glucagon (upper panel) and insulin release (bottom panel) from the rat pancreas perfused at 3.3 mmol/L glucose and absence (closed circles, N = 4) or presence of 3 mmol/L α-DFMO (open circles, N = 4). Vertical dotted lines as in Fig. 1.

99.9 ± 7.0% of those to ornithine (100.0 ± 4.5%, N = 16 in each case). During the late phase, however, the output of glucagon was notably higher in presence of arginine than in that of ornithine. For instance, using a 5 mmol/L concentration of amino acids, the late output of glucagon in response to arginine (min 80–86) amounted to 0.24 ± 0.04 ng/min at 5.6 mmol/L glucose (Fig. 2) and 1.54 ± 0.19 ng/min at 3.3 mmol/L glucose (Fig. 3), whereas in response to ornithine (min 50–56), it only reached values of 0.15 ± 0.02 (Fig. 2) and 0.87 ± 0.08 ng/min (Fig. 3) at these glucose levels, respectively (N = 4 in each case). As a whole, the late phase of arginine-induced glucagon release represented 144.6 ± 12.0% relative to that induced by ornithine (100.0 ± 4.2%, N = 16 in each case, $P < 0.01$).

The presence of 3 mmol/L α-DFMO during the perfusions did not alter the dose-dependency and negative glucose-modulation of the glucagon responses to ornithine and arginine (Figs 1–3, upper panels, open circles; Table 3). Thus, at 5.6 mmol/L glucose, the stimulated glucagon output decreased from a value of 8.8 ± 0.4 to 5.4 ± 1.1 and 4.6 ± 0.4 ng in response to 10, 5 and 2 mmol/L ornithine, and from a value of 9.3 ± 0.5 to 6.0 ± 1.1 and 4.0 ± 0.9 ng in response to the same concentrations of arginine. At the low 3.3 mmol/L glucose level, the glucagon responses, as in the absence of α-DFMO, were 2–5 times higher than at 5.6 mmol/L glucose and decreased from a value of 26.6 ± 2.3 to 9.6 ± 1.6 ng in response to 5 and 2 mmol/L ornithine, and from 28.7 ± 2.4 to 8.5 ± 1.5 ng in response to the same concentrations of arginine. α-DFMO also had no influence on the secretory patterns of the glucagon responses to ornithine and arginine. Thus, as in the control experiments performed at 5.6 or 3.3 mmol/L glucose, comparable concentrations of ornithine and arginine enhanced the total glucagon output to the same extent. Thus, the overall responses to arginine represented 102.2 ± 6.1% of those to ornithine (100.5 ± 5.2%,

Table 3. Glucagon output from the rat pancreas perfused in the absence or presence of 3 mmol/L α-DFMO

Glucagon output	ng		Per cent (relative to no α-DFMO)	
	No α-DFMO	+ α-DFMO	No α-DFMO	+ α-DFMO
A. Glucose 5.6 mmol/L				
Ornithine 10 mmol/L	9.2 ± 0.4 (4)	8.8 ± 0.4 (4)	100.0 ± 4.1 (4)	95.5 ± 4.6 (4)
5 mmol/L	6.7 ± 1.2 (4)	5.4 ± 1.1 (4)	100.0 ± 18.1 (4)	81.3 ± 16.1 (4)
2 mmol/L	4.0 ± 0.3 (2)	4.6 ± 0.4 (2)	100.0 ± 7.4 (2)	114.3 ± 9.4 (2)
Arginine 10 mmol/L	10.0 ± 0.8 (4)	9.3 ± 0.5 (4)	100.0 ± 7.6 (4)	92.8 ± 5.1 (4)
5 mmol/L	6.2 ± 1.0 (4)	6.0 ± 1.1 (4)	100.0 ± 16.9 (4)	96.5 ± 17.0 (4)
2 mmol/L	3.2 ± 0.7 (2)	4.0 ± 0.9 (2)	100.0 ± 23.0 (2)	123.4 ± 26.7 (2)
Leucine 5 mmol/L	5.6 ± 1.4 (4)	6.0 ± 1.1 (3)	100.0 ± 25.4 (4)	108.1 ± 19.2 (3)
Glucose +5 mmol/L	0.5 ± 0.1 (4)	0.3 ± 0.2 (3)	100.0 ± 22.7 (4)	129.6 ± 36.6 (3)
B. Glucose 3.3 mmol/L				
Ornithine 5 mmol/L	26.9 ± 1.4 (4)	26.6 ± 2.3 (4)	100.0 ± 5.1 (4)	98.9 ± 8.6 (4)
2 mmol/L	12.8 ± 1.6 (2)	9.6 ± 1.6 (2)	100.0 ± 12.7 (2)	75.5 ± 12.4 (2)
Arginine 5 mmol/L	32.9 ± 3.8 (4)	28.7 ± 2.4 (4)	100.0 ± 11.6 (4)	87.3 ± 7.3 (4)
2 mmol/L	9.1 ± 1.0 (2)	8.5 ± 1.5 (2)	100.0 ± 10.9 (2)	92.6 ± 16.3 (2)
Leucine 2 mmol/L	14.4 ± 2.0 (4)	20.0 ± 7.0 (4)	100.0 ± 13.7 (4)	139.4 ± 48.6 (4)
Glutamine 2 mmol/L	8.2 ± 1.3 (4)	7.9 ± 2.1 (4)	100.0 ± 16.0 (4)	96.8 ± 25.9 (4)
Pooled data				
at 5.6 mmol/L glucose	ornithine and arginine stimuli		100.0 ± 5.0 (20)	97.0 ± 5.6 (20)
	non ornithine and arginine stimuli		100.0 ± 15.8 (8)	118.9 ± 19.1 (6)
at 3.3 mmol/L glucose	ornithine and arginine stimuli		100.0 ± 4.3 (12)	90.1 ± 4.9 (12)
	non ornithine and arginine stimuli		100.0 ± 9.7 (8)	118.1 ± 26.7 (8)
at both glucose levels	ornithine and arginine stimuli		100.0 ± 3.5 (32)	94.4 ± 3.9 (32)
	non ornithine and arginine stimuli		100.0 ± 8.9 (16)	118.4 ± 26.7 (14)

The results were computed from the areas under the curves, whether during the entire (amino acids) or the later periods (glucose) of administration of stimulus (see the text). No P value is indicated, as in Table 1.

N = 16 in each case). The late phase of arginine-induced glucagon release also was higher than that seen in response to ornithine. As an example, using the 5 mmol/L concentration of amino acids, the late output of glucagon in response to arginine (min 80–86) amounted to 0.22 ± 0.05 ng/min at 5.6 mmol/L glucose (Fig. 2) and 1.26 ± 0.15 ng/min at 3.3 mmol/L glucose (Fig. 3), whereas in response to ornithine (min 50–56) it amounted to 0.16 ± 0.04 (Fig. 2) and 0.82 ± 0.12 ng/min (Fig. 3) at these glucose levels, respectively. As a whole, in the presence of α-DFMO, the late phase of arginine-induced glucagon release represented 144.3 ± 12.7% of that induced by ornithine (100.0 ± 8.2%, N = 16 in each case, $P < 0.01$). Such a value was comparable to that obtained in the control experiments.

The pooling of the glucagon secretory data obtained with ornithine and arginine (bottom of Table 3) indicated that the stimulatory effects of these two amino acids in the presence of α-DFMO represented 94.4 ± 3.9% relative to those observed in the control experiments (100.0 ± 3.5%, N = 32 in each case, NS). There also was no statistical difference when the results were expressed in terms of Δ output (107.5 ± 7.2 and 100.0 ± 4.8% in the presence and absence of α-DFMO, respectively).

Influence of α-DFMO on ornithine- and arginine-induced insulin release

In the control perfusions, the rat pancreas responded in a dose-dependent and positively glucose modulated manner to both ornithine and arginine (Figs 1–3, bottom panels, closed circles; Table 4). Thus, at 5.6 mmol/L glucose, the stimulated insulin output (area under the curve), decreased from a value of 112.2 ± 18.6 to 51.7 ± 7.9 and 21.7 ± 3.2 ng in response to 10, 5 and 2 mmol/L ornithine, and from a value of 233.1 ± 33.7 to 99.7 ± 9.6 and 30.9 ± 3.7 ng in response to the same concentrations of arginine. At 3.3 mmol/L glucose, the insulin response to a 5 mmol/L concentration of these amino acids was of a limited amplitude and represented 3.1 ± 0.7 and 7.8 ± 2.2 ng in the case of ornithine and arginine, respectively (note the expanded scale at the bottom of Fig. 3). The 2 mmol/L concentrations of ornithine and arginine barely influenced the secretion of insulin at this low 3.3 mmol/L glucose level. The latter results therefore were not considered in the calculations and thus, not reported in Table 4). In contrast to the observation made in the case of glucagon release, the total amount of insulin released in response to arginine was, at the two glucose levels used and when observed, about twice that released in response to the same concentration of ornithine. Thus, the overall insulin responses to arginine represented 208.5 ± 23.0% of those to ornithine (100.0 ± 8.2%, $P < 0.001$, N = 14 in each case). A late phase of insulin release was not observed using the lower 2 mmol/L concentration of the two amino acids. When observed, however, such late phase was markedly higher in response to arginine than to ornithine. For instance, in taking the same example as in the case of glucagon release (i.e. the 5 mmol/L concentration of amino acids), the late output of insulin in response to arginine (min 80–86) amounted to 4.21 ± 0.48 ng/min at 5.6 mmol/L

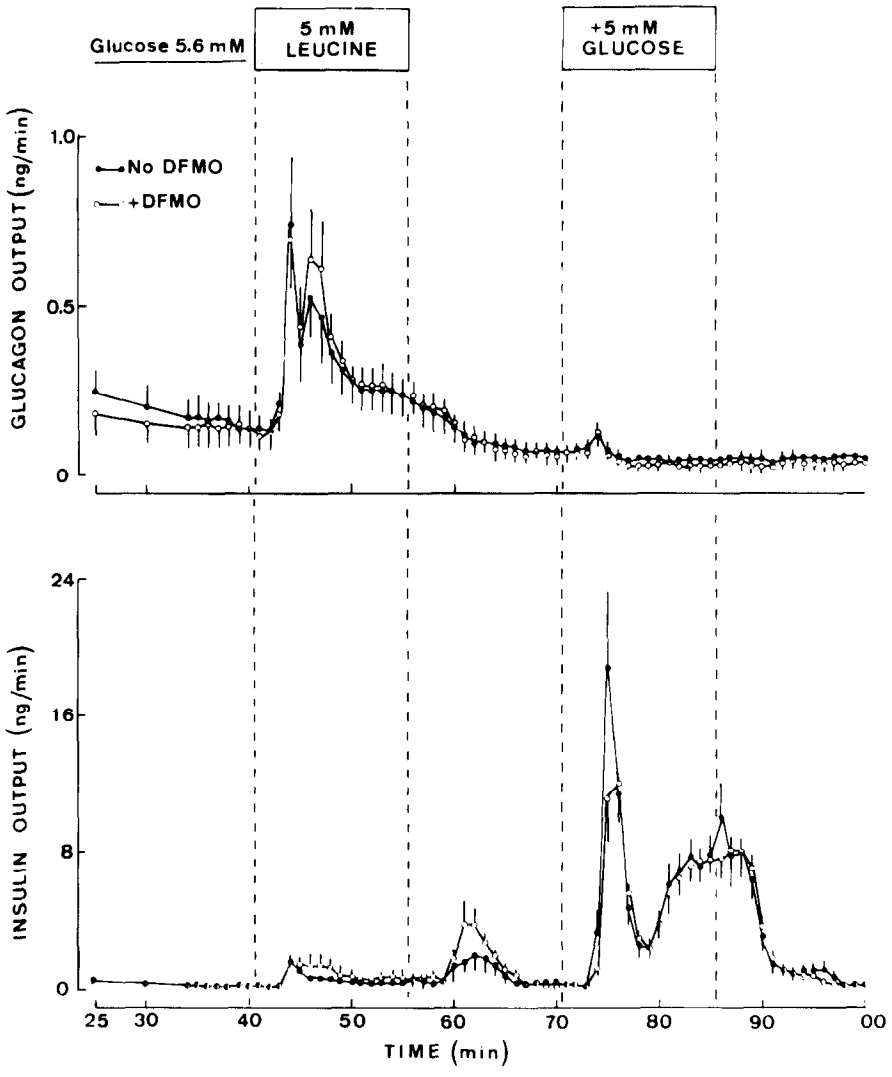

Fig. 5. The effects of 5 mmol/L leucine at 5.6 mmol/L glucose and of an elevation in the concentration of glucose from 5.6 to 10.6 mmol/L upon glucagon (upper panel) and insulin release (bottom panel) from the rat pancreas perfused in the absence (closed circles, N = 4) or presence of 3 mmol/L α-DFMO (open circles, N = 3). Vertical dotted lines as in Fig. 1.

glucose (Fig. 2) and 0.66 ± 0.20 ng/min at 3.3 mmol/L glucose (Fig. 3), whereas in response to ornithine (min 50–56), it reached values of only 0.85 ± 0.22 (Fig. 2) and 0.12 ± 0.03 ng/min (Fig. 3) at these glucose levels, respectively. As a whole, the late phase of arginine-induced insulin release represented 478.0 ± 57.9% relative to that induced by ornithine (100.0 ± 12.5%, N = 12 in each case, $P < 0.001$). It also was markedly more pronounced than that which was observed in response to arginine relative to ornithine in the case of glucagon release (144.6 ± 12.0%, N = 16, $P < 0.001$).

The presence of 3 mmol/L α-DFMO during the perfusions did not modify the dose-dependency and positive glucose modulation of the insulin responses to ornithine and arginine (Figs 1–3, bottom panels, open circles; Table 4). Thus, at 5.6 mmol/L glucose, the stimulated insulin output decreased from a value of 86.5 ± 14.6 to 52.6 ± 5.9 and 36.3 ± 3.0 ng in response to 10, 5 and 2 mmol/L ornithine, and from a value of 210.0 ± 32.2 to 103.8 ± 25.0 and 35.2 ± 12.0 ng in response to the same concentrations of arginine. At 3.3 mmol/L glucose, as in the absence of α-DFMO, there was no detectable insulin secretory response to the low 2 mmol/L concentration of amino acids, and that to 5 mmol/L was of a limited amplitude, with values of 3.9 ± 0.7 and 10.5 ± 0.7 ng in the case of ornithine and arginine, respectively (bottom of Fig. 3). α-DFMO also had no influence on the secretory patterns of the insulin responses to ornithine and arginine. Thus, as in the control experiments, the total amount of insulin released in response to arginine represented, at the two glucose levels used and when observed, about twice that released in response to the same concentration of ornithine (217.2 ± 22.6 relative to 100.0 ± 7.1%, N = 14 in each case, $P < 0.001$). As in the absence of α-DFMO, no late phase in insulin

Table 4. Insulin output from the rat pancreas perfused in the absence or presence of 3 mmol/L α-DFMO

Insulin output	ng		Per cent (relative to no α-DFMO)	
	No α-DFMO	+ α-DFMO	No α-DFMO	+ α-DFMO
A. Glucose 5.6 mmol/L				
Ornithine 10 mmol/L	112.2 ± 18.6 (4)	86.5 ± 14.6 (4)	100.0 ± 16.7 (4)	77.8 ± 13.2 (4)
5 mmol/L	51.7 ± 7.9 (4)	52.6 ± 5.9 (4)	100.0 ± 15.2 (4)	101.7 ± 11.4 (4)
2 mmol/L	21.7 ± 3.2 (2)	36.3 ± 3.0 (2)	100.0 ± 14.8 (2)	167.1 ± 13.7 (2)
Arginine 10 mmol/L	233.1 ± 33.7 (4)	210.0 ± 32.2 (4)	100.0 ± 14.5 (4)	90.1 ± 13.8 (4)
5 mmol/L	99.7 ± 9.6 (4)	103.8 ± 25.0 (4)	100.0 ± 9.6 (4)	104.1 ± 25.1 (4)
2 mmol/L	30.9 ± 3.7 (2)	35.2 ± 12.0 (2)	100.0 ± 12.0 (2)	114.0 ± 21.8 (2)
Leucine 5 mmol/L	9.2 ± 3.3 (4)	13.3 ± 7.7 (3)	100.0 ± 35.6 (4)	144.4 ± 83.7 (3)
Glucose +5 mmol/L	119.8 ± 21.3 (4)	107.8 ± 18.3 (3)	100.0 ± 17.8 (4)	90.0 ± 15.7 (3)
B. Glucose 3.3 mmol/L				
Ornithine 5 mmol/L	3.1 ± 0.7 (4)	3.9 ± 0.7 (4)	100.0 ± 21.6 (4)	126.8 ± 23.3 (4)
Arginine 5 mmol/L	7.8 ± 2.2 (4)	10.5 ± 0.7 (4)	100.0 ± 28.1 (4)	134.4 ± 8.5 (4)
Pooled data				
at 5.6 mmol/L glucose	ornithine and arginine stimuli		100.0 ± 5.3 (20)	102.9 ± 8.4 (20)
	non ornithine and arginine stimuli		100.0 ± 18.4 (8)	117.2 ± 40.0 (6)
at 3.3 mmol/L glucose	ornithine and arginine stimuli		100.0 ± 16.4 (8)	130.6 ± 11.6 (8)
at both glucose levels	ornithine and arginine stimuli		100.0 ± 5.8 (28)	110.8 ± 7.2 (28)
	non ornithine and arginine stimuli		100.0 ± 18.4 (8)	117.2 ± 40.0 (6)

The results were computed from the areas under the curves during the entire periods of stimulation (see the text). No P value is indicated, as in Table 1.

release was observed using the lower 2 mmol/L concentration of amino acids. When observed, it was higher in response to arginine than to ornithine. For instance, in the presence of the 5 mmol/L concentration of amino acids, the late output of insulin in response to arginine (min 80–86) amounted to 3.52 ± 0.73 ng/min at 5.6 mmol/L glucose (Fig. 2) and 0.87 ± 0.07 ng/min at 3.3 mmol/L glucose (Fig. 3), whereas in response to ornithine (min 50–56), it only reached values of 0.83 ± 0.15 (Fig. 2) and 0.16 ± 0.02 ng/min (Fig. 3) at these glucose levels, respectively. As a whole, the late phase of arginine-induced insulin release represented 479.5 ± 38.2% relative to that induced by ornithine (100.0 ± 7.5%, N = 12 in each case, $P < 0.001$). This percentage value was comparable to that seen in the control experiments. It also was markedly more pronounced than that observed for arginine relative to ornithine in the case of glucagon release in presence of α-DFMO (144.3 ± 12.7%, N = 16, $P < 0.001$).

The pooling of the secretory data obtained with ornithine and arginine (bottom of Table 4) indicated that the stimulatory effects of these two amino acids in the presence of α-DMFO represented 110.8 ± 7.2% relative to those recorded in the control experiments (100.0 ± 5.8%, N = 28 in each case). The difference in these percentage values was not statistically significant.

Influence of α-DFMO on the release of glucagon in response to leucine, glutamine and glucose

In the control perfusions, the administration of leucine provoked a triphasic and inversely glucose-modulated enhancement of glucagon release (Figs 4 and 5, upper panels, closed circles; Table 3). Thus, the output of glucagon was significantly higher using the 2 mmol/L concentration of leucine at 3.3 mmol/L glucose (14.4 ± 2.0 ng) than with the 5 mmol/L concentration of amino acid at 5.6 mmol/L of the hexose (5.6 ± 1.4 ng, N = 4 in each case, $P < 0.02$). α-DFMO did not modify the dynamic patterns and the inverse glucose modulation of the glucagon responses to leucine (Figs 4 and 5, upper panels, open circles; Table 3). Thus, the output of glucagon in response to 2 and 5 mmol/L leucine amounted to 20.0 ± 7.0 (N = 4) and 6.0 ± 1.1 ng (N = 3) at 3.3 and 5.6 mmol/L glucose, respectively. Overall, the output of glucagon recorded in response to leucine and presence of α-DFMO appeared to be slightly elevated when compared to that seen in the control experiments (123.7 ± 24.4 relative to 100.0 ± 13.4%, N = 8 and N = 6, respectively). The difference in these percentage values, however, was not significant.

The glucagon response to 2 mmol/L glutamine in the control experiments was biphasic and of a lower amplitude (8.2 ± 1.3 ng) than that which had been obtained with 2 mmol/L leucine at 3.3 mmol/L glucose (Fig. 4, upper panel, closed circles; Table 3). The secretory pattern as well as the amount of glucagon released upon stimulation with glutamine (7.9 ± 2.1 ng) were strictly comparable in the α-DFMO experiments (Fig. 4, upper panel, open circles; Table 3).

As expected, an increase in the glucose concentration from 5.6 to 10.6 mmol/L normally inhibited the output of glucagon to the very low level of 0.5 ± 0.1 ng during the later time of the infusion of the hexose (min 77–86, Fig. 5, upper panel, closed circles; Table 3). In the presence of α-DFMO, glucose inhibited the release of glucagon to a similar extent at a comparable time (0.3 ± 0.2 ng, Fig. 5, upper panel, open circles; Table 3).

As a whole, in pooling all the results obtained for glucagon release, the effects of leucine, glutamine (stimulation) and glucose (inhibition) represented

118.4 ± 16.7% (N = 14) in the presence relative to the absence of α-DFMO (100.0 ± 8.9%, N = 16). These percentage values were not statistically different. They also were not significantly different when the results were expressed in terms of Δ output, although in that case, the effects of the three stimuli appeared slightly more pronounced in the presence as compared to the absence of α-DFMO (138.4 ± 25.9 relative to 100.0 ± 12.5%, N = 14 and N = 16, respectively).

Influence of α-DFMO on the release of insulin in response to leucine, glutamine and glucose

In the control experiments performed at 5.6 mmol/L glucose, the 5 mmol/L leucine concentration enhanced the secretion of insulin to a limited value of 9.2 ± 3.3 ng, with an off response being noted upon the arrest of the leucine infusion (Fig. 5, bottom panel, closed circles; Table 4). No insulin secretory response was observed in response to the lower 2 mmol/L leucine concentration or to 2 mmol/L glutamine at 3.3 mmol/L glucose (Fig. 4, lower panel, closed circles). In the presence of α-DFMO, the insulin response to 5 mmol/L leucine at 5.6 mmol/L glucose was comparable to that seen in the control experiments (13.3 ± 7.7 ng, Fig. 5, bottom panel open circles; Table 4). There also was no enhancement of insulin secretion upon the administration of the lower 2 mmol/L concentrations of leucine or glutamine at 3.3 mmol/L glucose (Fig. 4, bottom panel, open circles).

Finally, the elevation in the concentration of glucose from 5.6 to 10.6 mmol/L provoked a classical biphasic stimulation of insulin release in both the absence and presence of α-DFMO (Fig. 5, bottom panel, closed and open circles), respectively. The total insulin output values during stimulation also were comparable (119.8 ± 21.3 relative to 107.8 ± 18.3 ng in the presence or absence of α-DFMO, respectively; Table 4).

The pooling of all secretory data obtained for insulin release with leucine and the elevation of the glucose levels (bottom of Table 4) indicated that the positive effects of these stimuli observed in the presence of α-DFMO represented 117.2 ± 40.0% (N = 6) relative to those recorded in the control experiments (100.0 ± 5.9%, N = 8). The difference in these percentage values was not statistically significant.

DISCUSSION

In several cell types, the rapid generation of polyamines was proposed to play a key role in cell activation [9, 10]. The major aim of the present study was to investigate the possible role of the rapid *de novo* formation of polyamines in the insulin and glucagon secretory responses of the endocrine pancreas to L-arginine and L-ornithine. For this purpose, α-DFMO was used as an inhibitor of ornithine decarboxylase, the latter drug indeed suppressing the formation of polyamines from the cationic amino acids in isolated islet cells [1]. In all present experiments, α-DFMO was tested at a concentration of 3 mmol/L, in far excess of the K_i (5.6 μmol/L) for the inhibition of ornithine decarboxylase by this agent [2]. It should be kept in mind, however, that the effect of α-DFMO upon polyamine generation by islet cells in the isolated perfused rat pancreas was not assessed.

The other experimental conditions were selected in order either to optimize the secretory response of glucagon-producing cells (e.g. in the presence of 3.3 mmol/L D-glucose) and insulin-producing cells (e.g. in the presence of 5.6 mmol/L D-glucose) or to explore the effects of other secretagogues than the cationic amino acids (e.g. L-leucine or L-glutamine). The present study indicates that L-ornithine and L-arginine stimulate, in a rapid and sustained manner, the release of both glucagon and insulin, the magnitude of such a secretory response being negatively related to the ambient glucose concentration (3.3–5.6 mmol/L) in the case of glucagon secretion, but positively related to the hexose concentration in the case of insulin release. Our results also confirm that L-leucine (2.0–5.0 mmol/L) and L-glutamine (2.0 mmol/L) are able to stimulate glucagon release [8, 11, 12]. With the cationic amino acids, the concentration–response relationship was not identical in the case of glucagon release and insulin secretion, respectively. Thus, when tested at a low concentration of 2.0 mmol/L, both L-arginine and L-ornithine caused a sizeable stimulation of glucagon release but virtually failed to affect insulin release (data not shown), at least in the presence of 3.3 mmol/L D-glucose. In the light of a current hypothesis, which ascribes the secretory effects of cationic amino acids to the accumulation of these positively charged molecules in islet cells with subsequent depolarization of the plasma membrane [13–15], the above-mentioned difference in concentration–response relationship could be ascribed to the difference in functional status (stimulated vs resting) of glucagon- and insulin-producing cells, respectively, at the low concentrations of D-glucose used in our experiments. It is noteworthy, however, that the activity of ornithine decarboxylase appears higher in A-cells located at the periphery of rat islets than in the centrally located B-cells [16]. Incidentally, during the late phase of exposure to cationic amino acids, the secretory response of both glucagon- and insulin-producing cells was more marked in the presence of L-arginine than L-ornithine. A comparable situation prevailed when the order of administration of these two amino acids was reversed (data not shown).

No significant effect of α-DFMO upon hormonal release could be detected in the present study, whatever the experimental conditions used to stimulate the release of glucagon and insulin. These negative findings argue against the view that the *de novo* generation of polyamines in islet cells plays any major role in the immediate and short-term secretory effects of L-arginine and L-ornithine in the endocrine pancreas. This conclusion is not meant to deny that the chronic depletion of polyamine content of islet cells may exert untoward effects such as an impairment of their biosynthetic and mitotic activity [17].

Acknowledgements—This study was supported by grants from the Belgian Foundation for Scientific Medical

Research and Ministry of Scientific Policy. We thank C. Demesmaeker for secretarial help.

REFERENCES

1. Malaisse WJ, Blachier F, Mourtada A, Camara J, Albor A, Valverde I and Sener A, Stimulus-secretion coupling of arginine-induced insulin release. Metabolism of L-arginine and L-ornithine in pancreatic islets. *Biochim Biophys Acta* **1013**: 133–143, 1989.
2. Sener A, Owen A, Malaisse-Lagae F and Malaisse WJ, Stimulus-secretion coupling of arginine-induced insulin release. Effect of 2-aminoisobutyric acid upon ornithine decarboxylase activity and insulin secretion. *Res Commun Chem Pathol Pharmacol* **65**: 65–80, 1989.
3. Blachier F, Leclercq-Meyer V, Marchand J, Woussen-Colle M-C, Mathias PCF, Sener A and Malaisse WJ, Stimulus-secretion coupling of arginine-induced insulin release. Functional response of islets to L-arginine and L-ornithine. *Biochim Biophys Acta* **1013**: 144–151, 1989.
4. Leclercq-Meyer V, Marchand J, Leclercq R and Malaisse WJ, Glucagon and insulin release by the *in vitro* perfused rat pancreas. Influence of the colloid composition of the perfusate. *Diabète Metab* **2**: 57–65, 1976.
5. Malaisse WJ, Malaisse-Lagae F and Wright PH, Effect of fasting upon insulin secretion in the rat. *Am J Physiol* **213**: 843–848, 1967.
6. Harris V, Faloona GR and Unger RH, Glucagon. In: *Methods for Hormone Radioimmunoassay* (Eds. Jaffe BM and Behrman HR), 2nd Edn, pp. 643–656. Academic Press, New York, 1978.
7. Leclercq-Meyer V, Marchand J, Rebolledo O, Malaisse WJ and Leclercq R. A combined radioimmunoassay for glucagon and insulin. *Diabetologia* **11**: 419–425, 1975.
8. Leclercq-Meyer V, Marchand J, Woussen-Colle M-C, Giroix M-H and Malaisse WJ, Multiple effects of leucine on glucagon, insulin, and somatostatin secretion from the perfused rat pancreas. *Endocrinology* **116**: 1168–1174, 1985.
9. Pegg AE and McCann PP, Polyamine metabolism and function. *Am J Physiol* **243**: C212–C221, 1982.
10. Koenig H, Goldstone A and Lu CY, Polyamines regulate calcium fluxes in a rapid plasma membrane response. *Nature* **305**: 530–534, 1983.
11. Eisenstein AB and Strack I, Amino acid stimulation of glucagon secretion by perifused islets of high-protein-fed rats. *Diabetes* **27**: 370–376, 1978.
12. Assan R, Marre M and Gormley M, The amino acid-induced secretion of glucagon. In: *Handbook of Experimental Pharmacology, Glucagon, Vol. 66/II* (Ed. Lefèbbre PJ), pp. 19–41. Springer, Berlin, 1983.
13. Henquin JC and Meissner HP, Effects of amino acids on membrane potential and $^{86}Rb^+$ fluxes in pancreatic β-cells. *Am J Physiol* **240**: E245–E252, 1981.
14. Charles S, Tamagawa T and Henquin JC, A single mechanism for the stimulation of insulin release and $^{86}Rb^+$ efflux from rat islets by cationic amino acids. *Biochem J* **208**: 301–308, 1982.
15. Blachier F, Mourtada A, Sener A and Malaisse WJ, Stimulus-secretion coupling of arginine-induced insulin release. Uptake of metabolized and non-metabolized cationic amino acids by pancreatic islets. *Endocrinology* **124**: 134–141, 1989.
16. Dorn A and Bernstein H-G, Remarkable localization of ornithine decarboxylase in rat pancreas. *Eur J Cell Biol* **41**: 127–129, 1986.
17. Welsh N and Sjöholm Å, Polyamines and insulin production in isolated mouse pancreatic islets. *Biochem J* **252**: 701–707, 1988.