Diabetologia 17, 121–126 (1979)

Diabetologia

Interactions of α-Ketoisocaproate, Glucose and Arginine in the Secretion of Glucagon and Insulin from the Perfused Rat Pancreas

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Summary. The effects of α-ketoisocaproate (KIC, 10 mmol/l) on glucagon and insulin release were studied in the in vitro perfused rat pancreas. The experiments were performed at low glucose concentration (3.3 mmol/l) in the absence or presence of arginine (10 mmol/l). In all the experiments KIC induced a marked and not rapidly reversible inhibition of glucagon release. This inhibition was more pronounced in the absence (76 percent) than presence of arginine (61 percent). These inhibitory patterns closely duplicated those which were seen in parallel experiments which included a rise in the concentration of glucose (from 3.3 to 11.1 mmol/l). KIC was also a potent stimulator of insulin release. The results are compatible with the view that the intracellular metabolism of KIC and glucose plays an essential role in the regulation of glucagon release by exogenous substrates.

Key words: α-ketoisocaproate, KIC, arginine, glucose, glucagon release, insulin release, perfusion, rat pancreas.

Glucose normally inhibits the secretion of glucagon, both in vivo and in vitro [7, 46, 47]. The intimate mechanisms by which glucose exerts its inhibitory effect are not fully elucidated but several hypotheses have been proposed. According to the glucoreceptor hypothesis, glucose interacts with specific receptors located at the A_2-cell plasma membrane [29, 31, 32]. Another hypothesis postulates that the A_2-cell is devoid of intrinsic glucose-sensing capacity and that the inhibitory effect of glucose is indirect and mediated by insulin [41, 47]. Finally, in the substrate-site hypothesis, it is believed that the metabolism of glucose in the A_2-cell plays an essential role in the regulation of glucagon release. In favour of the latter concept are the observations that the A_2-cells, although having a low glucose-phosphorylation capacity [18], possess a high enzymatic capacity for degradation of triose phosphates [19]. Moreover, metabolic studies conducted on A_2-cell-rich islets of streptozotocin-treated guinea pigs have shown that oxygen consumption, glucose oxidation, glucose utilization and ATP concentration were increased in the presence of elevated glucose levels [2, 3, 28, 38]. Other investigators, however, failed to detect major changes in metabolism, in particular in ATP concentration, in islets from rats treated with streptozotocin or alloxan [23, 24]. These negative findings may not invalidate the metabolic hypothesis since it is well established that the sensitivity of the A_2-cell to glucose is markedly impaired in these diabetic models, as in many other forms of chronic diabetes (e. g. 6–8, 23–25, 30, 47). Besides, ATP may not be the essential messenger mediating the effect of glucose upon glucagon release.

The present work was undertaken to gain additional information on the role of intermediary metabolism in the regulation of glucagon release from the normal rat pancreas. We have compared the effects of glucose and α-ketoisocaproate (KIC) upon glucagon release in the absence or presence of arginine. Both KIC, which is the first catabolic product of leucine, and glucose are potent insulinotropic secretagogues and are actively metabolized in rat islets [12, 13, 33, 34, 42, 43].

Materials and Methods

Fed female albino rats were used (mean body weight ± SEM: 251 ± 6 g; n = 19). The perfusion technique has been described previously [15]. The animals were anaesthetized with sodium pentobarbital (42 mg/kg intraperitoneally) and the pancreas was isolated,

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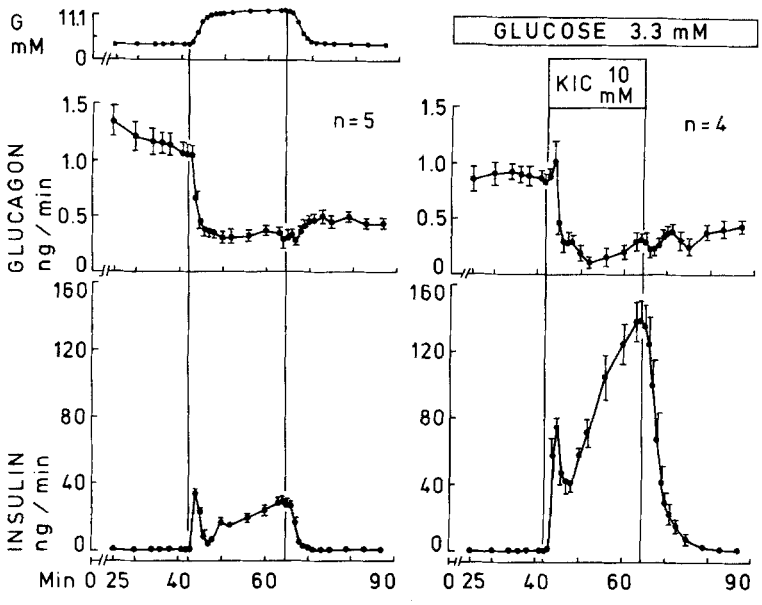

Fig. 1. The effects of α-ketoisocaproate (KIC, 10 mmol/l) upon glucagon and insulin release from the rat pancreas perfused in the presence of a low concentration of glucose (3.3 mmol/l, panel to the right). On the panel to the left are illustrated the effects of a rise in the concentration of glucose (from 3.3 to 11.1 mmol/l)

Table 1. Glucagon secretory rates (ng/min) from the perfused rat pancreas. The results represent the mean (± SEM) of (n) experiments and refer to the upper panels of Fig. 1 (parts A and B) and Fig. 2 (parts C and D)

Part	(n)	Substrates present in basic perfusate	Substrates added in infusion period	Glucagon secretory rates (ng/min) Equilibration period min 25 to 42	Infusion period min 47 to 66	Post infusion period min 66 to 87
A	(5)	Glucose 3.3 mmol/l	Glucose 7.8 mmol/l (→ 11.1 mmol/l)	1.17±0.11	0.33±0.04	0.44±0.04
B	(4)	Glucose 3.3 mmol/l	KIC 10.0 mmol/l	0.89±0.08	0.21±0.06	0.34±0.04
C	(6)	Glucose 3.3 mmol/l + ARG 10.0 mmol/l	Glucose 7.8 mmol/l (→ 11.1 mmol/l)	4.12±0.55	0.86±0.11	1.74±0.24
D	(4)	Glucose 3.3 mmol/l + ARG 10.0 mmol/l	KIC 10.0 mmol/l	2.83±0.56	1.11±0.14	1.43±0.13
Non paired *t* test			B *vs* A	NS	NS	NS
			D *vs* C	NS	NS	NS
			C *vs* A	$P < 0.001$	$P < 0.01$	$P < 0.01$
			D *vs* B	$P < 0.02$	$P < 0.01$	$P < 0.001$

all adjacent organs, including the duodenum, being excluded in order to avoid interference in the assay of enteric glucagon-like immunoreactivity. The rats were given intravenous heparin sodium (125 U) at the end of the isolation procedure. The whole pancreas was perfused in situ without recycling, through the coeliac and mesenteric arteries via a cannula inserted into the aorta.

The perfusion medium comprised the following in mmol/l: NaCl, 118.5; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2, $NaHCO_3$, 25; $CaCl_2$, 2. It was supplemented with glucose (3.3 mmol/l), dextran (40 g/l, T70, Pharmacia, Uppsala, Sweden) and bovine albumin (5 g/l, fraction V, Sigma, St. Louis, U. S. A.). The medium was equilibrated against a mixture of oxygen and carbon dioxide (95:5, v/v), with a resulting pH of 7.4, and entered the pancreas with a temperature of 37.5 °C. The flow rate was set around 2 ml/min, with a resulting (mean ± SEM) perfusion pressure of 4.47 ± 0.20 kPa (33.5 ± 1.5 mmHg). When needed the substrates L(+)-arginine (Merck, Darmstadt, West Germany), glucose (Merck) and KIC (Sigma, St. Louis, U. S. A.) were dissolved in twice distilled water and administered through a side-arm syringe at a flow rate of 0.075 ml/min (Braun infusion pump, Melsungen, West Germany). In those experiments which were performed in the presence of arginine (final concentration in the perfusate : 10 mmol/l), the infusion of the amino acid was maintained throughout the perfusions. In the experiments which included an exposure to glucose (perfusate concentration : 11.1 mmol/l) or the Na salt of KIC (perfusate concentration : 10 mmol/l), the infusion of these substrates was started after a 40 min equilibration period and thereafter continued for 25 min. In all cases the increase in osmolarity induced by the secretagogues was corrected by a 10 mmol/l decrease in the amount of NaCl in the perfusate.

Samples of the effluent were collected at 1 min intervals in

Table 2. Insulin secretory rates (ng/min) from the perfused rat pancreas. The results represent the mean (± SEM) of (n) experiments, and refer to the lower panels of Fig. 1 (parts A and B) and Fig. 2 (parts C and D)

Part	(n)	Substrates present in basic perfusate	Substrates added in infusion period	Insulin secretory rates (ng/min)		
				Equilibration period min 25 to 42	Infusion period Early phase min 42 to 47	Infusion period Late phase min 47 to 66
A	(5)	Glucose 3.3 mmol/l	Glucose 7.8 mmol/l (→ 11.1 mmol/l)	0.3±0.1	12.9± 0.8	19.6± 1.9
B	(4)	Glucose 3.3 mmol/l	KIC 10.0 mmol/l	0.2±0.1	40.8± 4.4	99.3±11.2
C	(6)	Glucose 3.3 mmol/l + ARG 10.0 mmol/l	Glucose 7.8 mmol/l (→ 11.1 mmol/l)	22.9±4.1	123.6±14.4	109.0±13.9
D	(4)	Glucose 3.3 mmol/l + ARG 10.0 mmol/l	KIC 10.0 mmol/l	24.2±2.5	142.2±19.9	114.1±15.2
Non paired *t* test			B *vs* A	NS	P < 0.001	P < 0.001
			D *vs* C	NS	NS	NS
			C *vs* A	P < 0.001	P < 0.001	P < 0.001
			D *vs* B	P < 0.001	P < 0.01	NS

chilled tubes containing 2,000 KIU aprotinin (Trasylol; donated by Drs. G. Wald and A. Pirard, Bayer, Brussels, Belgium) and frozen at −25 °C until assay. At the times shown on the figures, glucagon (0.2 ml aliquots) and insulin (0.01 to 0.2 ml aliquots) were measured with individual radioimmunoassays for these hormones [17]. The tracers were ^{125}I-glucagon (480 to 490 mCi/mg, CNTS, France) and ^{125}I-insulin (124 to 132 mCi/mg, SORIN, Italy). The standards were beef-pork glucagon (lot 258-234-13-161-1, a gift from Dr. M. A. Root, Eli Lilly and Co., Indianapolis, U.S.A.) and rat insulin (lot R 170, a gift from Dr. J. Schlichtkrull, Novo, Bagsvaerd, Denmark). Glucose was assayed in the pancreatic effluent with a glucose-oxidase method (Boehringer-Mannheim) using an AAI Technicon analyzer.

The rates of glucagon and insulin secretion were expressed as ng/min. Total glucagon or insulin release was calculated from the areas under the curves. All results in the text and the figures are given as the mean ± SEM of n experiments. Statistical analyses were conducted using two-tailed paired or non-paired *t* tests [44]. Except if otherwise stated, results given in the text refer to the non-paired *t* test.

Results

Effects of KIC on Glucagon and Insulin Release in the Presence of 3.3 mmol/l Glucose

The results of these experiments are shown in Figure 1 and Tables 1 and 2. The infusion of KIC first produced a slight increase in glucagon release (1.02 ± 0.18 ng/min at min 44) which, however, was not significantly different from the mean output during the equilibration period (paired *t* test : $P > 0.2$). Thereafter, the infusion of KIC provoked a marked inhibition of glucagon release (paired *t* test *vs* equilibration period : $P < 0.001$). Upon the arrest of KIC infusion, the glucagon secretory rates showed some oscillations but remained extensively depressed (paired *t* test *vs* equilibration period : $P < 0.001$). These results with KIC closely duplicated those with 11.1 mmol/l glucose, except that the inhibition of glucagon release occurred 1 min earlier in the case of glucose (Fig. 1; Table 1, part A). At no time was there any significant difference between the glucagon secretory rates recorded in the KIC and glucose experiments.

Concomitantly with the effects on glucagon, KIC induced a biphasic stimulation of insulin release which was much greater than that induced by glucose (Fig. 1, Table 2). Thus, in the presence of KIC the insulin secretory rate was 3 (early phase) to 5 times (late phase) as high as that seen in the presence of glucose. The stimulation of insulin release ceased abruptly upon terminating the infusion of KIC or glucose.

Effects of KIC on Glucagon and Insulin Release in the Presence of 3.3 mmol/l Glucose and 10 mmol/l Arginine

The results of the experiments performed with KIC in the presence of arginine are shown in Figure 2 and Tables 1 and 2.

In agreement with the well known inverse relationship between the effects of glucose and arginine in the alpha cell, the output of glucagon before KIC infusion was higher in the presence of arginine than in the absence of the amino acid (Table 1, equilibration period, D *vs* B). The infusion of KIC markedly reduced these elevated secretory rates (paired *t* test *vs* equilibration period : $P < 0.05$). This inhibition of

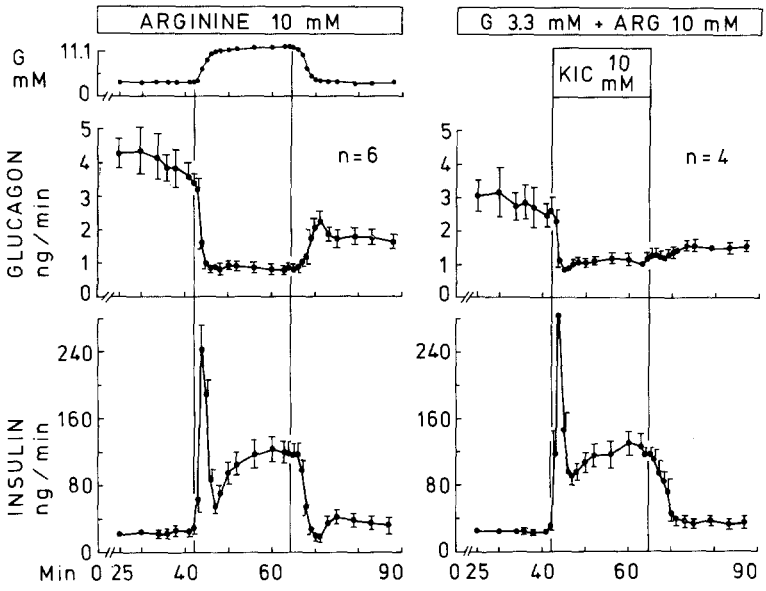

Fig. 2. The effects of α-ketoisocaproate (KIC, 10 mmol/l) upon glucagon and insulin from the rat pancreas perfused in the presence of a low concentration of glucose (3.3 mmol/l) and arginine (10 mmol/l) (panel to the right). On the panel to the left are illustrated the effects of a rise in the concentration of glucose (from 3.3 to 11.1 mmol/l) in the presence of arginine (10 mmol/l)

glucagon release was only partially reversed upon the cessation of KIC infusion. Thus, despite a slight increase, the output of glucagon remained significantly lower during the postinfusion period than during the equilibration period (paired *t* test : $P < 0.05$). This overall pattern of glucagon release mimicked the one obtained when 11.1 mmol/l glucose was applied to the pancreas in the presence of arginine (Fig. 2; Table 1, part C). In the glucose experiments, the glucagon secretory rate during the equilibration period was slightly higher than in the KIC experiments. The difference was not significant, however (Table 1, equilibration period, D *vs* C). Glucose abruptly inhibited this arginine-induced glucagon release to a rate which was comparable to that seen upon the KIC infusion (Table 1, infusion period, D *vs* C). Following the arrest of the glucose infusion, a limited biphasic glucagon response was noticed. Despite this slight increase the late glucagon output recorded in the glucose experiments was not significantly higher than the late glucagon output in the KIC experiments (Table 1, postinfusion period, D *vs* C). It is of interest to note that both glucose and KIC inhibited the secretion of glucagon to a much messer extent in the presence of arginine (Table 1, infusion period, C *vs* A, and D *vs* B).

As for insulin release, in the presence of arginine KIC exerted stimulatory effects which were superimposable on those of 11.1 mmol/l glucose (Fig. 2). Thus, at no time throughout the perfusions was there any difference between the insulin secretory rates in the two types of experiments (Table 2, D *vs* C). It should be emphasised that arginine markedly augmented insulin release both at low (3.3 mmol/l) and high (11.1 mmol/l) glucose concentrations (Table 2, equilibration and infusion periods, C *vs* A; D *vs* B). Arginine also facilitated the early phase of KIC-induced insulin release (Table 2, early phase of infusion period, D *vs* B) but did not change the late phase (Table 2, late phase of infusion period; D *vs* B).

Discussion

The results clearly demonstrate that in the perfused rat pancreas, in both the absence and presence of arginine, KIC (10 mmol/l) induces an extensive and not rapidly reversible inhibition of glucagon release. These inhibitory effects closely resembled those seen upon the administration of an almost equimolar glucose stimulus (11.1 mmol/l). Concomitantly, KIC induced a striking stimulation of insulin release, in agreement with previous observations [12, 13, 33, 42, 43]. The fact that, at a nearly equimolar concentration and in the absence of arginine, KIC was more potent than glucose in enhancing insulin release (Fig. 1) also corroborates previous findings [12, 42].

The present data are compatible with the hypothesis that changes in intermediary metabolism of the A_2-cells play a role in the regulation of glucagon release by exogenous nutrients. Proof of such an assumption awaits metabolic investigations on A_2-cell-rich islets, since a dynamic study of the whole pancreas cannot bring any information as to the specific metabolic changes in the A_2-cells. Nevertheless, the data available in the literature, as already stated in the introduction, suggest that there is no major difference in the metabolic handling of glucose in A_2-cell-rich and whole pancreatic islets, respectively. It is conceivable that the metabolism of KIC is also similar in the A_2-cells as in intact islets, in which

KIC has been shown to increase the rates of oxygen consumption, to be converted to acetoacetate, CO_2 and H_2O, and to increase the concentration of reduced pyridine nucleotides [12, 13]. Moreover, other findings from the literature regarding glucagon release seem to support the metabolic hypothesis. Thus, α-D-glucose, which is preferentially metabolized in islet cells [20, 21], is more effective than β-D-glucose in inhibiting glucagon release [9, 22, 40]. An inhibition of glucagon release is also observed in the presence of metabolic intermediates such as glyceraldehyde and dihydroxyacetone [10, 11, 27]. In contrast, metabolic inhibitors, such as mannoheptulose, 2-deoxyglucose, iodoacetamide, iodoacetate, 2-4-dinitrophenol and malonate, were reported to enhance glucagon secretion or to prevent glucose from suppressing amino acid induced glucagon release [4, 10, 26, 29, 48].

Whether insulin participated in the KIC-induced inhibition of glucagon release cannot be decided by our experiments. Such a possibility exists, since marked elevations of insulin were observed during the KIC infusions. The least that can be said is that insulin is not the key or sole determinant of glucagon release. At the same steady-state level of insulin release (19.6 ± 1.9 and 23.4 ± 2.5 ng/min), a ten-fold increase in glucagon output from 0.33 ± 0.04 (n = 5) to 3.60 ± 0.40 ng/min (n = 10) occurs when glucose 11.1 mmol/l is substituted by glucose 3.3 mmol/l plus arginine 10 mmol/l. Conversely, glucagon release may occur at the same rate (1.04 ± 0.08 and 1.11 ± 0.14 ng/min) despite a more than four-hundred-fold increase in steadystate insulin output from 0.26 ± 0.05 (n = 9) to 114.1 ± 15.2 (n = 4) ng/min due to the addition of α-ketoisocaproate and arginine (both 10 mmol/l) to a medium of low glucose concentration (3.3 mmol/l).

A last question raised by our experiments is whether the inhibitory effect of α-ketoisocaproate upon glucagon release is relevant to the effect of leucine upon glucagon output. The effects of leucine on the secretion of glucagon have not been studied in our laboratory. The data in the literature are conflicting. Thus, the in vivo administration of L-leucine did not influence the plasma levels of glucagon [5, 35, 39], except when infused intrapancreatically [14]. In vitro, in the sole presence of glucose, leucine has been found to enhance glucagon release [1, 36, 37, 45]. In contrast, however, some authors reported inhibition of glucagon release upon the infusion of leucine when the perfusate was supplemented with a mixture of fumarate, glutamate and pyruvate [45]. Such an inhibition is thus comparable to that obtained with KIC in the presence of arginine, for we have shown previously that arginine and a mixture of fumarate, glutamate and pyruvate exhibited equivalent glucagonotropic properties [16].

In conclusion, several questions remain to be answered in relation to the intimate mechanisms involved in the inhibitory action of KIC in the A_2-cell. That the inhibitory effect of KIC parallels that of glucose, however, suggests an essential role of the intracellular metabolism of these substrates in the regulation of glucagon release.

Acknowledgements. This work was supported in part by grants 3.4527.75 and 3.4537.76 from the Belgian Foundation for Scientific Medical Research (F. R. S. M., Brussels, Belgium). We are grateful to Mrs C. Demesmaeker for secretarial help.

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Received: February 12, 1979,
and in revised form: May 10, 1979

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