EFFECTS OF L-LEUCINE AND α-KETOISOCAPROIC ACID UPON INSULIN SECRETION AND METABOLISM OF ISOLATED PANCREATIC ISLETS

U. PANTEN, E.v. KRIEGSTEIN, W. POSER, J. SCHÖNBORN and A. HASSELBLATT

Institut für Pharmakologie und Toxikologie der Universität Göttingen, Germany

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1. Introduction

L-Leucine has been shown to stimulate insulin release *in vivo* [1,2] and *in vitro* [3,4]. It is unknown whether L-leucine must be metabolized to elicit this effect. From *in vivo* experiments Knopf et al. [2] suggested that α-ketoisocaproic acid (α-KIC) has no hypoglycemic action of its own but must be transaminated to leucine in order to stimulate insulin secretion. Recently the nonmetabolized, but transported, leucine analogue 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid was found to induce insulin release *in vivo* [5] and *in vitro* [6]. From these experiments it was concluded that the receptor sites for the stimulation of insulin release by L-leucine may be transport sites.

Since the above-mentioned experiments did not rule out that L-leucine or α-KIC trigger insulin release by metabolic events in the β-cell the present studies were performed. α-KIC induced a marked stimulation of insulin release by isolated pancreatic islets. The accompanying increase of fluorescence of reduced pyridine nucleotides demonstrated prompt changes of islet cell metabolism. In pancreatic islets α-KIC enhanced the tissue level and the production of leucine. All other tested leucine metabolites did not stimulate insulin release significantly.

2. Experimental

2.1. *Perifusion of pancreatic islets*

6–8 mon old obese-hyperglycemic mice of both sexes were starved for 20-28 hr and killed by decapitation. Pancreatic islets were dissected within 35 min at 2° in Krebs-Ringer phosphate buffer containing 3.3 mM glucose [7]. The islets were transferred into a small perifusion chamber in which the medium gently floated them against thin filaments [8]. Fluorimetric measurements were done through a glass window above the filaments. The media consisted of Krebs-Ringer bicarbonate buffer containing the tested substances. The medium flowed through the chamber at 85 ul/min, controlled by means of a valve without dead space (Labotron, Gelting). The complete system was maintained at 36° and gassed continuously with $O_2 + CO_2$ (95:5, v/v). When insulin secretion was measured, the media were supplemented with 2 mg/ml bovine serum albumin and collected in 4 min fractions.

2.2. *Freeze stop*

Groups of 4–10 islets were perifused for 45 min in a polythene chamber without the window holding part. Then the chamber was pushed quickly into Freon 12 kept at –150°. Thus the islets were chilled to –20° in less than 2 sec as measured with a thermocouple. At –25° the end of the chamber was cut off (3 mm length) and placed in a freeze drying unit at –40° and 0.001 Torr for 24 hr. The freeze-dried islets were freed from the surrounding salt crystals and weighed on a micro balance (Type 4125 Sartorius, Göttingen). About 50 islets were transferred to a small conical glass tube and extracted twice with 50 μl 5% trichloroacetic acid using a modification of Eichner's [9] homogenization technique. The combined supernatants were freeze dried, redissolved in 50 μl distilled water and chromatographed.

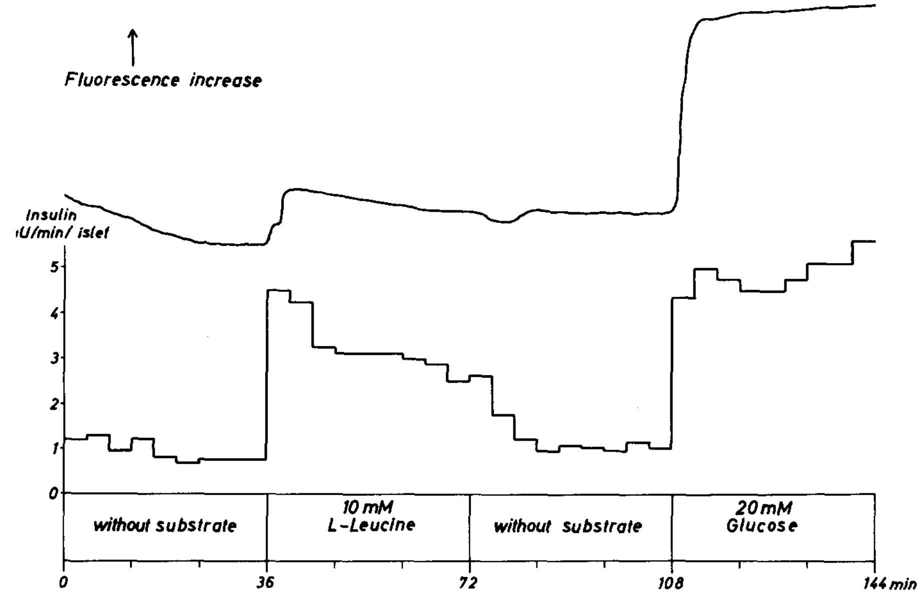

Fig. 1. Effect of L-leucine on PNH-fluorescence trace (upper curve) of a single perifused islet. In a parallel experiment the amount of insulin released was immunoassayed.

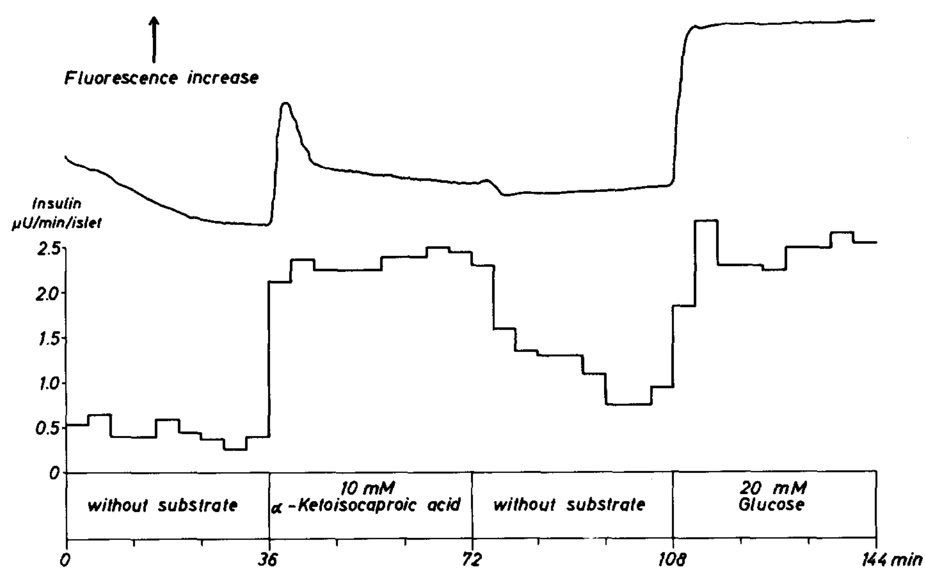

Fig. 2. Effect of α-KIC on PNH-fluorescence trace (upper curve) of a single perifused islet. In a parallel experiment the amount of insulin released was immunoassayed.

Table 1
Effect of α-ketoisocaproic acid on levels of amino acids in pancreatic islets.

Additions to medium	n	Taurine	Glycine	Alanine	Leucine	γ-Amino-butyrate	Arginine
				mmoles/kg dry weight			
None	3	49.7 ± 4.8	3.01 ± 0.77	1.35 ± 0.18	0.70 ± 0.12	5.51 ± 0.97	1.39 ± 0.11
α-Ketoisocaproic acid (10 mM)	3	51.3 ± 3.0	3.63 ± 0.11	1.25 ± 0.21	1.70 ± 0.16*	4.28 ± 0.25	0.86 ± 0.06

Islets were perifused for 30 min without substrate, followed by 15 min with 10 mM α-KIC. Controls were perifused for 45 min without substrate.
* Value differs significantly from the corresponding control value ($p < 0.01$).

2.3. *Incubation of tissue*

3–10 islets or pieces of exocrine pancreas of similar size were incubated in 100 μl Krebs-Ringer bicarbonate buffer for 3 hr at 36° as described in [10]. Metabolism was stopped by addition of 20 μl 3 M perchloric acid. The tissue was rapidly squashed with a small glass rod and immediately centrifuged at 15,000 *g* for 2 min. The supernatant was neutralized with 2 M K_2HPO_4 or 1 M KOH.

2.4. *Analytical*

Acetoacetate [11], DNA [12] and immunologically reactive insulin [13] were measured as described. Salmon sperm DNA and ox insulin were used as standards. Fluorescene of reduced pyridine nucleotides (PNH) was recorded with the method of Chance et al. [14] as described [8], using a ST 41 mercury arc (Original Hanau). Amino acids were measured by micro column chromatography [15], using a commercially available analyzer (Type Chromatocord, Labotron, Gelting).

3. Results and discussion

Insulin secretion of the perifused pancreatic islet was stimulated by 10 mM L-leucine with an initial overshoot (fig. 1). Increase of PNH-fluorescence began 10–15 sec after arrival of L-leucine at the islet, stopped 1 min later for 20–30 sec and reached its maximum 4 min after medium change (fig. 1). Withdrawal of L-leucine caused a small temporary decrease of PNH-fluorescence. 10 mM L-valine had no effect on insulin release and PNH-fluorescence. The islet responded to 10 mM α-KIC with a marked stimulation of insulin secretion (fig. 2).

A concomitant overshooting increase of PNH-fluorescence took place, which started 10–15 sec after medium change and had its peak 3 min later (fig. 2). 10 mM α-ketoisovalerate did not elicit insulin release and induced only a small rise of the PNH-fluorescence. Each experiment was repeated 5 times using different islets (0.3–0.6 mm, longest diameter). The described typical effects on insulin secretion and PNH-fluorescence were always seen.

If one accepts the assumption that the biochemically related substances L-leucine and α-KIC stimulate insulin release by the same mechanism, there are two ways to explain their action: first both substances could fit to the same receptor site for insulin secretion. The fading of insulin release induced by L-leucine may indicate that, in contrast to α-KIC (10 mM), L-leucine (10 mM) is not a substrate yielding sufficient metabolic energy. Secondly, L-leucine or α-KIC or both may trigger insulin secretion after being metabolized.

Rapid metabolic events in the pancreatic islet brought about by L-leucine and α-KIC are shown by the changes of the PNH-fluorescence. It is likely that the latter primarily reflect mitochondrial NADH production by action of α-KIC dehydrogenase. This view was supported by the lack of changes of PNH-fluorescence after exposing the islets to 20 mM acetate, 20 mM D,L β-hydroxybutyrate, 10 mM acetoacetate and 10 mM glutamate. Moreover, islets perifused with a medium containing 10 mM acetoacetate responded to addition of 10 mM α-KIC with the typical PNH-fluorescence kinetics. The idea that

Table 2
Acetoacetate and leucine production by pancreas tissue.

Additions to medium	Endocrine islets		Exocrine tissue	
	Acetoacetate	Leucine	Acetoacetate	Leucine
	nmoles/hr/g DNA			
None	< 0.10 (5)	0.24 ± 0.02 (5)	0.58 ± 0.06 (5)	0.12 ± 0.03 (5)
Isovalerate (10 mM)	< 0.10 (5)		0.56 ± 0.05 (5)	
α-Ketoisocaproic acid (10 mM)	0.35 ± 0.05 (8) *	1.21 ± 0.06 (5)*	2.43 ± 0.37 (5) *	1.14 ± 0.22 (5) *
L-Leucine (10 mM)	0.42 ± 0.04 (8) *		4.58 ± 0.49 (5) *	

The islets and the exocrine pancreas contained 22.3 ± 2.2 or 18.8 ± 1.9 g DNA/kg dry weight, respectively.
* Values differ significantly from the corresponding control values ($p < 0.01$).

degradation of α-KIC elicited insulin release became less likely by the following results: 10 mM isovalerate, 10 mM pyruvate and 10 mM D,L mevalonate had no effect, and 3 mM acetoacetate had only a slight stimulatory effect on insulin release of islets previously perifused with medium supplemented with 5 mM glucose.

The possibility that L-leucine is the true trigger of insulin secretion induced by α-KIC was tested by measuring content and production of amino acids from isolated pancreatic islets (tables 1 and 2). The tissue levels of γ-aminobutyrate, which so far has been found in high amounts only in nervous tissue, and of arginine were lowered slightly by 10 mM α-KIC, whereas the leucine content rose more than 2-fold. 10 mM α-KIC enhanced by 5-fold leucine production by islets. Transamination of α-KIC or inhibited oxidation of leucine produced by proteolysis could be the causes. Proteolysis probably remained unchanged as the basal arginine production was not altered significantly by 10 mM α-KIC (0.11 ± 0.02 or 0.13 ± 0.01 mmoles/hr/g DNA, respectively). These rates were at least 4 times higher than the arginine oxidation rate of islets [16]. The results are in accordance with the view that L-leucine and α-KIC stimulate insulin release by mechanisms depending on the transmembrane leucine gradient. Thus the different kinetics of insulin secretion induced by L-leucine and α-KIC may reflect changes of this gradient.

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