The Stimulus-Secretion Coupling of Amino Acid-Induced Insulin Release

Biosynthetic and Secretory Responses of Rat Pancreatic Islet to L-Leucine and L-Glutamine

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Summary. L-glutamine dramatically augments insulin release evoked by L-leucine. The dynamics, specificity and environmental modifications of the insulin secretory response to the combination of L-glutamine and L-leucine were investigated. L-glutamine alone failed to stimulate proinsulin biosynthesis or insulin release in rat pancreatic islets, but augmented synthesis and secretion evoked by L-leucine. In relation to the secretory response, L-glutamine could be replaced by L-asparagine but not L-glutamate; L-leucine could be replaced by L-norvaline or L-isoleucine but not by L-valine, L-norleucine, glycine, L-serine, L-lysine, L-phenylalanine or L-arginine. The secretory response to L-leucine was rapid and biphasic, whereas the enhancing action of L-glutamine upon insulin release was progressive. The release of insulin evoked by the combination of L-leucine and L-glutamine was inhibited by antimycin A, menadione, ammonium ions, verapamil, adrenaline, and by the absence of extracellular Ca^{2+} . It was increased at high carbon dioxide tension, and by glucose, theophylline and cytochalasin B. It is concluded that the enhancing action of L-glutamine upon insulin release evoked by certain amino acids represents a phenomenon dependent on the integrity of oxidative metabolism and involving essentially the same sequence of metabolic, ionic and motile events as that characterizing the process of glucose-induced insulin release.

Key words: Insulin release, proinsulin biosynthesis, pancreatic islets, amino acids, L-leucine, L-isoleucine, L-norvaline, L-valine, L-norleucine, glycine, L-serine, L-lysine, L-phenylalanine, L-arginine, L-glutamine, L-asparagine, L-glutamate.

Amino acids stimulate insulin release [1]. The clinical relevance of such a phenomenon is best illustrated by the syndrome of leucine-induced hypoglycaemia, a condition which led to the discovery of the stimulant action of L-leucine and other amino acids upon insulin release [1].

It was recently reported that L-glutamine, which represents a major fuel for pancreatic islet cells but which fails to stimulate insulin release in the presence or absence of glucose [2], dramatically enhanced insulin secretion evoked by either L-leucine or its non-metabolised analogue b(-)-2-aminobicyclo-[2,2,1]heptane-2-carboxylic acid [3]. This finding is of considerable significance in elucidating the biochemical determinant(s) of amino acid-induced insulin release.

The aim of the present study was to characterize the dynamics, specificity and environmental modification of the secretory response to the combination of L-glutamine and L-leucine, and to investigate the effect of such a combination upon proinsulin biosynthesis in isolated rat pancreatic islets. In order to facilitate the interpretation of results, most experiments were performed in the absence of D-glucose, using concentrations of amino acids far above their physiological values.

Methods

The methods used to measure insulin release [4] and proinsulin biosynthesis [5] in isolated islets and insulin release in isolated perfused pancreases [6] removed from fed albino rats are described in detail elsewhere. Briefly, groups of eight islets each were incubated for 90 min in 1.0 ml of a medium containing Na^+ 139, K^+ 5, Ca^{2+} 1, Mg^{2+} 1, Cl^- 124 and HCO_3^- 24 mmol/l and bovine albumin (5 mg/ml). Except for the presence of dextran [7] in the perfusate, the media used for incubating islets or perfusing pancreases were of identical composition. In order to increase the CO_2 tension whilst maintaining extracellular pH unchanged (7.4),

Fig. 1. Effect of L-leucine and L-glutamine upon insulin release by the isolated perfused rat pancreas. Upper panel: effect of L-leucine (10 mmol/l: ●—●; $n = 5$; 15 mmol/l: ○—○; $n = 6$) administered from min 25 onwards upon insulin release in the presence of L-glutamine (10 mmol/l) administered throughout the perfusion period; control data obtained in the sole presence of L-glutamine (★—★; $n = 3$). Middle panel: effect of L-glutamine alone (10 mmol/l: ★—★; $n = 3$), L-Leucine alone (10 mmol/l: ●—●; $n = 3$; 15 mmol/l: ●—●; $n = 4$) and the combination of L-glutamine (10 mmol/l) and L-leucine (15 mmol/l: ○—○; $n = 4$), the amino acids being always administered from min 25 onwards. Lower panel: effect of L-glutamine (10 mmol/l: ●—●; $n = 6$) administered from min 25 onwards in the presence of L-leucine (10 mmol/l) administered throughout the perfusion period; control data obtained in the sole presence of L-leucine (10 mmol/l) are shown (●—●; $n = 3$)

the usual gas phase (O_2/CO_2 : 95/5%, v/v) was replaced by a mixture of 70% O_2 and 30% CO_2 , and the HCO_3^- concentration of the medium increased from 24 to 102 mmol/l at the expense of the Cl^- concentration [8].

L-leucine, L-norvaline, L-isoleucine, L-valine, L-norleucine, glycine, L-serine, L-phenylalanine, L-arginine and L-glutamate

were obtained from Merck, Darmstadt, FRG; L-glutamine, antimycin A and menadione from Sigma Chemicals, St. Louis, Missouri; theophylline from Schwartz/Mann, Organeburg, New York; verapamil from Knoll A. G., Ludwigshafen, FRG; adrenaline from Federa, Brussels, Belgium; the trifluoromethylphenothiazine derivative of domperidone from Janssen Pharmaceutica, Beerse, Belgium; cytochalasin B and L-asparagine from Aldrich, Milwaukee, Wisconsin, L-Lysine from Koch-Light Laboratories, Colnbrook, Bucks, UK; and L-[2,4,6- 3H]phenylalanine from The Radiochemical Centre, Amersham, UK.

All results are expressed as mean $\pm$ SEM together with the number of individual determinations (n). Except for the perfusion experiments, the comparison of control and experimental values is restricted to data obtained within the same experiment(s), using Student's t -test for group comparison. In each experiment, all islets were derived from the same preparation and, usually, an equal number of individual determinations were performed under control and experimental conditions.

Results

Dynamics of the Secretory Response to L-Leucine and L-Glutamine

The dynamics of the secretory response to L-leucine and L-glutamine were investigated in the isolated perfused pancreas (Fig. 1). When L-glutamine (10 mmol/l) was administered alone either throughout the 60 min or over the last 35 min of perfusion, the rate of insulin release remained close to basal value. Likewise, when L-leucine (10 mmol/l) was administered alone throughout the 60 min or over the last 35 min of perfusion, the rate of secretion was only slightly and occasionally higher than basal value. The concentration of L-leucine had to be raised to 15 mmol/l to evoke an easily discernible biphasic secretory response.

The secretory response to L-leucine was considerably augmented in the presence of L-glutamine. The enhancing action of L-glutamine was most evident when the latter amino acid was circulated for 25 min before stimulation with L-leucine (Fig. 1, upper panel). Under these conditions, both the initial and late secretory responses to L-leucine were considerably enhanced. The rate of secretion was 7–20 times higher than in the absence of L-glutamine (Table 1).

The enhancing action of L-glutamine was much less marked when both amino acids were introduced together at min 25 of the perfusion. Indeed, in this case, the secretory response to L-leucine was increased mostly during the late phase of stimulation, and the integrated output of insulin was only double that in the absence of L-glutamine (Table 1).

When the pancreases were first exposed to L-leucine before introduction of L-glutamine, an enhancing action of the latter amino acid was again

Table 1. Insulin output evoked by L-glutamine and L-leucine in the isolated perfused rat pancreas

L-glutamine (mmol/l)	L-leucine (mmol/l)	Insulin output ^a
10 (25–60) ^b	—	0.18±0.03 (3)
—	10 (25–60)	0.24±0.13 (3)
—	10 (0–60)	0.32±0.09 (3)
10 (25–60)	10 (0–60)	1.68±0.43 (6)
10 (0–60)	10 (25–60)	5.07±0.50 (5)
—	15 (25–60)	1.32±0.07 (4)
10 (25–60)	15 (25–60)	2.54±0.32 (4)
10 (0–60)	15 (25–60)	9.63±0.68 (6)

^a Results are expressed as mU/pancreas and represent the integrated output of insulin between min 25 and min 60 of perfusion. For purpose of comparison, the integrated output evoked by D-glucose 16.7 mmol/l (administered from min 25–60 averaged 13.60±2.97 mU/pancreas ($n = 5$). Mean values ± SEM are shown together with the number of individual determinations

^b The period (min) during which each amino acid was administered is shown in parentheses

evident, but the secretory rate increased in a progressive manner, in contrast to the biphasic pattern usually evoked by nutrient secretagogues.

Specificity of the Secretory Response to L-Leucine and L-Glutamine

Further experiments were performed with isolated rat pancreatic islets. We have previously shown that the secretory response to leucine and glutamine is stereospecific for the L-form of these amino acids [3].

L-glutamine could not be replaced by L-glutamate in its capacity to potentiate L-leucine-induced insulin release (Table 2, experiment 4). L-glutamate did not prevent L-glutamine from augmenting L-leucine-stimulated secretion. L-asparagine, like L-glutamine, was able to cause a dose-related potentiation of L-leucine-induced insulin release (Table 2, experiment 3). In this respect and on a molar basis, L-glutamine was more potent than L-asparagine. L-asparagine, like L-glutamine [2], did not affect insulin release in the absence of another nutrient (Table 2, experiment 1) and failed to enhance significantly glucose-induced insulin release (Table 2, experiment 2). L-asparagine did not prevent L-glutamine from augmenting L-leucine-induced insulin release (Table 2, experiment 3). Several amino acids other than L-glutamine and L-asparagine were able to augment L-leucine-induced insulin release (see below). In contrast to L-glutamine and L-asparagine, all these other amino acids also significantly enhanced D-glucose-stimulated insulin secretion. Moreover, none of these other amino acids enhanced L-leucine-induced insu-

lin release to the same extent as L-glutamine or even L-asparagine.

The specificity towards L-leucine was explored by testing the capacity of L-glutamine to augment insulin release in the presence of each of ten different amino acids (Table 3). L-glutamine significantly enhanced insulin release in the presence of L-leucine, L-norvaline and L-isoleucine. The enhancing action of L-glutamine was most marked with L-leucine and least marked with L-isoleucine. L-glutamine failed to enhance significantly insulin release in the presence of L-valine, L-norleucine, glycine, L-serine, L-lysine, L-phenylalanine or L-arginine.

The magnitude of the glutamine-induced increment in insulin output was not closely related to the capacity of a given amino acid to stimulate secretion in the absence of any other exogenous nutrient. For instance, in the presence of 5 mmol/l L-leucine, 20 mmol/l L-norvaline and 20 mmol/l L-lysine, comparable values for insulin output were found in the absence of any other nutrient (i. e. 34.5 ± 2.6 , 39.5 ± 2.8 and 44.7 ± 5.0 μ U/islet per 90 min, respectively), whereas the response to L-glutamine (10 mmol/l) was strikingly variable: the glutamine-induced increase in insulin output was more than double in the presence of 5 mmol/l L-leucine (data not shown) than 20 mmol/l L-norvaline, and no effect of L-glutamine could be detected in the presence of 20 mmol/l L-lysine.

With the exception of L-valine and L-arginine, all amino acids listed in Table 3 augmented significantly either L-leucine- or D-glucose-stimulated insulin release. L-valine failed to affect secretion whether in the presence of L-leucine or D-glucose. L-arginine dramatically enhanced secretion in the presence of D-glucose, but failed to do so and even occasionally inhibited release in the presence of L-leucine. There was no obvious link between the capacity of L-glutamine to augment secretion evoked by a given amino acid and the aptitude of that same amino acid to augment release in the presence of either L-leucine or D-glucose (Table 3).

Environmental modification of the secretory response to L-Leucine and L-Glutamine

The release of insulin evoked by the combination of L-leucine and L-glutamine (10 mmol/l each) was significantly lower than that induced by D-glucose 16.7 mmol/l ($p < 0.001$; $df = 82$). D-glucose caused a dose-related increase in the secretory response to the combination of L-leucine and L-glutamine (Fig. 2). Even at a 16.7 mmol/l concentration of glucose, the secretory rate was significantly higher in the presence of the two amino acids than in their absence

Table 2. Effects of D-glucose and various amino acids upon insulin release

Experiment no.	D-glucose (mmol/l)	L-leucine (mmol/l)	L-glutamate (mmol/l)	L-glutamine (mmol/l)	L-asparagine (mmol/l)	Insulin output ^a (μU/islet per 90 min)
1	—	—	—	—	—	11.3± 1.2 (62)
	—	—	—	—	10.0	15.2± 3.7 (9)
	—	—	—	10.0	—	11.3± 0.9 (136)
	—	—	—	10.0	10.0	12.7± 4.9 (10)
2	8.3	—	—	—	—	81.0± 7.1 (27)
	8.3	—	—	—	1.0	76.5± 7.5 (9)
	8.3	—	—	—	3.0	79.1± 7.8 (9)
	8.3	—	—	—	10.0	109.3±10.9 (28)
3	—	10.0	—	—	—	49.5± 3.6 (36)
	—	10.0	—	—	1.0	54.4± 5.4 (9)
	—	10.0	—	—	3.0	76.8± 7.3 (18)
	—	10.0	—	—	10.0	111.8± 8.2 (44)
	—	10.0	—	3.0	—	193.7± 6.2 (9)
	—	10.0	—	3.0	3.0	194.8± 3.2 (9)
	—	10.0	—	10.0	—	247.1± 8.8 (17)
4	—	20.0	—	—	—	76.2± 5.1 (17)
	—	20.0	—	10.0	—	184.1± 5.8 (17)
	—	20.0	10.0	—	—	84.8± 9.8 (10)
	—	20.0	5.0	5.0	—	173.9± 5.1 (10)

^a Mean values ± SEM are shown with the number of individual determinations in parentheses

Table 3. Effect of amino acids upon insulin release in the absence of any other nutrient and in the presence of either L-glutamine, L-leucine or D-glucose

Nutrient (mmol/l)	Nil	L-glutamine (10 mmol/l)	L-leucine (10 mmol/l)	D-glucose (8.3 mmol/l)
Nil	15.4±1.8 (56)	13.6± 2.2 (40)	54.0±2.5 (74)	97.2± 4.1 (132)
L-leucine (10)	54.0±2.5 (74) ^b	196.1±13.1 (28) ^b	84.7±4.1 (20) ^{a b}	199.7±13.3 (27) ^b
L-norvaline (20)	39.5±2.8 (39) ^b	93.1± 8.1 (19) ^b	93.7±6.0 (10) ^b	162.3± 4.4 (20) ^b
L-isoleucine (20)	15.7±1.4 (18)	50.2± 5.5 (28) ^b	86.7±4.9 (20) ^b	142.6± 6.0 (20) ^b
L-valine (20)	14.8±2.1 (20)	21.8± 3.9 (20)	52.6±5.7 (10)	101.0± 5.9 (20)
L-norleucine (20)	12.7±1.6 (9)	14.6± 2.2 (17)	88.1±6.9 (10) ^b	124.5±11.1 (10) ^d
Glycine (10)	18.0±2.7 (19)	25.1± 2.2 (9)	99.4±1.8 (9) ^b	125.5± 6.0 (18) ^b
L-serine (10)	20.2±2.5 (10)	19.7± 2.1 (10)	99.7±4.7 (9) ^b	118.8± 4.6 (19) ^c
L-lysine (20)	40.8±4.0 (40) ^b	40.3± 7.9 (10)	77.6±6.0 (9) ^c	151.0± 9.9 (18) ^b
L-phenylalanine (10)	20.1±3.4 (10)	23.0± 2.4 (10)	86.3±5.8 (9) ^b	140.9± 7.0 (9) ^b
L-arginine (10)	21.6±2.4 (19)	21.9± 3.0 (10)	42.9±3.0 (29)	214.5± 5.5 (9) ^b

^a The result shown at the intersection of L-leucine 10 mmol/l (third column) and L-leucine 10 mmol/l (second line) corresponds to the effect of L-leucine 20 mmol/l

Levels of statistical significance (^b: $p < 0.001$; ^c: $p < 0.005$; ^d: $p < 0.05$) refer either to the capacity of the amino acid to augment insulin output relative to the control value shown on the first line (column 1, 3 and 4) or to the capacity of L-glutamine 10 mmol/l to augment amino acid-induced insulin release (column 2). Insulin release is expressed as μU/islet per 90 min. Mean values ± SEM are shown with the number of individual determinations in parentheses

($p < 0.001$; $df = 64$). We have characterized previously the effect of L-glutamine [2] and L-leucine [9] upon insulin release at increasing concentrations of D-glucose. When tested within the same experiments, the release of insulin evoked by L-leucine 10 mmol/l was virtually identical in the presence of D-glucose 8.3 mmol/l or L-glutamine 10 mmol/l, the

latter secretory rate averaging $98.2 \pm 1.1\%$ ($n = 27$) of the former ($100.0 \pm 1.1\%$, $n = 27$). This contrasts with the vastly different secretory capacity of D-glucose and L-glutamine when tested in the absence of L-leucine (Table 3).

As shown in Table 4, the release of insulin evoked by L-leucine and L-glutamine was abolished

by antimycin A (10 $\mu\text{mol/l}$), the absence of extracellular Ca^{2+} , and the presence of either verapamil (10 $\mu\text{mol/l}$) or adrenaline (5 $\mu\text{mol/l}$). It was inhibited in a dose-related fashion by increasing concentrations of menadione (Table 4) or decreasing concentrations of extracellular Ca^{2+} (Fig. 3), with an apparent "Ki" for menadione close to 6 $\mu\text{mol/l}$ and an apparent "Km" for Ca^{2+} close to 0.2 mmol/l. The secretory response to the combination of L-leucine and L-glutamine was decreased in the presence of a potential calmodulin-antagonist, the trifluoromethylphenothiazine derivative of domperidone (1 $\mu\text{mol/l}$), slightly but significantly diminished in the presence of NH_4^+ (0.5 mmol/l), slightly but significantly enhanced by an increase in pCO_2 , and dramatically potentiated by either theophylline (1.4 mmol/l) or cytochalasin B (20 $\mu\text{mol/l}$). None of the agents listed in Table 4 affect basal insulin release.

Although the combination of L-leucine and L-glutamine (both 10 mmol/l) displayed an insulintropic capacity comparable to that of the combination of L-leucine (10 mmol/l) and D-glucose (8.3 mmol/l), the influence of verapamil or of Ca^{2+} removal upon insulin release was not identical in the presence of these two combinations. The rate of insulin release was indeed significantly lower in the presence of verapamil or absence of extracellular Ca^{2+} when L-leucine was used in combination with L-glutamine as distinct from D-glucose (Table 5).

Biosynthetic Response to L-Leucine and L-Glutamine

L-glutamine (10 mmol/l) failed to stimulate proinsulin biosynthesis as judged from the incorporation of L-[2,4,6- ^3H]phenylalanine in immunoreactive insulin-like peptides (Table 6). L-leucine (10 mmol/l) stimulated the biosynthesis of both proinsulin and other islet peptides, with a preferential effect on proinsulin synthesis ($p < 0.005$). L-glutamine significantly augmented L-leucine-stimulated total protein and proinsulin biosynthesis, with again a preferential effect on the synthesis of the hormonal peptides ($p < 0.01$).

Discussion

The present findings confirm that L-glutamine markedly potentiates L-leucine-induced insulin release [3]. The concentrations of L-leucine and L-glutamine used in most of the present experiments largely exceed physiological values. However, at a physiological glucose concentration, the stimulant effect of L-leucine upon insulin release is virtually propor-

Fig. 2. Effect of increasing concentrations of D-glucose upon insulin release in the absence (○—○) and presence (●—●) of the combination of L-glutamine and L-leucine (10 mmol/l each). Mean $\pm$ SEM refer to 10–43 individual determinations

Table 4. Effect of several agents upon insulin release evoked by the combination of L-glutamine and L-leucine (both 10 mmol/l)

Agent	Insulin output ^a	p^c
Nil	100.0 $\pm$ 1.2 (71)	
Antimycin A (10 $\mu\text{mol/l}$)	10.7 $\pm$ 3.7 (10)	<0.001
O ₂ 70%–CO ₂ 30%	118.0 $\pm$ 4.6 (15)	<0.005
Menadione (1 $\mu\text{mol/l}$)	97.3 $\pm$ 2.5 (9)	NS
Menadione (3 $\mu\text{mol/l}$)	68.8 $\pm$ 5.1 (9)	<0.001
Menadione (10 $\mu\text{mol/l}$)	35.8 $\pm$ 4.0 (19)	<0.001
NH ₄ ⁺ (0.5 mmol/l)	85.9 $\pm$ 4.8 (18)	<0.02
No Ca ²⁺ + EGTA (0.5 mmol/l)	12.5 $\pm$ 1.9 (10)	<0.001
Verapamil (10 $\mu\text{mol/l}$)	8.1 $\pm$ 1.4 (9)	<0.001
Theophylline (1.4 mmol/l)	241.7 $\pm$ 3.5 (10)	<0.001
Adrenaline (5 $\mu\text{mol/l}$)	9.0 $\pm$ 2.2 (10)	<0.001
DP ^b (1 $\mu\text{mol/l}$)	60.5 $\pm$ 7.1 (10)	<0.001
Cytochalasin B (20 $\mu\text{mol/l}$)	200.6 $\pm$ 10.1 (10)	<0.001

^a Mean values $\pm$ SEM are expressed in percentage of the mean control value (first line) found within the same experiment(s). Such a control value averaged 224.5 $\pm$ 4.6 $\mu\text{U/islet}$ per 90 min ($n = 71$). Number of individual determinations in parentheses

^b DP: Trifluoromethylphenothiazine derivative of domperidone

^c Statistical significance of differences between control and experimental values

tional to the concentration of this amino acid, without any obvious threshold phenomenon (data not shown). Likewise, the facilitation of L-leucine-stimulated insulin output by L-glutamine is dose-related in the physiological range of L-glutamine concentrations [10]. The enhancing action of L-glutamine upon insulin release differs from that of other nutrients in its specificity for a few amino acids. Indeed, L-glutamine fails to stimulate insulin release in the presence of D-glucose, whatever the concentration of

Fig. 3. Effect of increasing concentrations of extracellular Ca^{2+} upon insulin release evoked by the combination of L-leucine and L-glutamine (10 mmol/l each). Mean $\pm$ SEM refer to 9–36 individual measurements and are expressed in percentage of the mean control value found within the same experiment(s) at a 1.0 mmol/l concentration of Ca^{2+} . Such a reference value averaged 238.6 ± 12.5 $\mu\text{U}/\text{islet}$ per 90 min ($n = 45$)

the sugar used to stimulate secretion [2]. L-glutamine also fails to affect insulin release in the presence of glycine, L-lysine, L-valine, L-norleucine, L-serine, L-phenylalanine and L-arginine (Table 3). The failure of L-glutamine to affect insulin release in the presence of these amino acids is independent of either their own capacity to stimulate secretion or their ability to augment L-leucine- and D-glucose-stimulated insulin output (Table 3). Among the amino acids so far investigated, only L-leucine, its non-metabolizable analogue b(-)-2-aminobicyclo-[2,2,1]heptane-2-carboxylic acid and, to a lesser extent, L-norvaline and L-isoleucine allowed L-glutamine to cause an increment in insulin release [3]. These are also the only amino acids so far found to act as allosteric activators of glutamate dehydrogenase in islet homogenates, suggesting that this activation plays an essential role in the secretory response to L-glutamine [3, 10].

L-glutamine at a close-to-physiological concentration represents a major fuel to cover the basal energy expenditure of islet cells [2]. Its influence

Table 5. Influence of Ca^{2+} removal and verapamil upon insulin release evoked by L-leucine in the presence of L-glutamine or D-glucose

Experimental variation (mmol/l)	L-leucine (10 mmol/l) and L-glutamine (10 mmol/l)	L-leucine (10 mmol/l) and D-glucose (8.3 mmol/l)	<i>p</i>
Nil	207.2 $\pm$ 3.2 (18) ^a	214.6 $\pm$ 3.3 (18)	>0.10
Ca^{2+} absent	10.3 $\pm$ 3.8 (9)	23.6 $\pm$ 4.8 (9)	<0.05
Verapamil (0.01 mmol/l)	17.4 $\pm$ 3.0 (9)	34.9 $\pm$ 3.3 (9)	<0.005

^a Mean values $\pm$ SEM are expressed as $\mu\text{U}/\text{islet}$ per 90 min with the number of individual determinations in parentheses

Table 6. Effects of L-glutamine and L-leucine upon biosynthetic activity

Nutrient(s) (mmol/l)	[³ H] IRI (fmol/islet)	[³ H] TCA-precipitable (fmol/islet)	Ratio ^c (%)
Nil	1.70 $\pm$ 0.15 ^a	15.32 $\pm$ 0.38	11.1 $\pm$ 0.9
L-glutamine (10)	1.66 $\pm$ 0.15	18.29 $\pm$ 0.90	9.3 $\pm$ 0.7
L-leucine (10)	3.31 $\pm$ 0.20	21.38 $\pm$ 1.27	15.6 $\pm$ 0.8
L-glutamine (10) + L-leucine (10)	5.39 $\pm$ 0.29	26.82 $\pm$ 1.08	20.1 $\pm$ 0.8

^a Mean values $\pm$ SEM refer to eight individual determinations

^b The incorporation of L-[2,4,6-³H]phenylalanine (132 mmol/l) in immunoreactive insulin (IRI) and TCA-precipitable peptides is expressed as fmol/islet per 90 min (TCA: trichloroacetic acid)

^c Ratio of [³H] IRI/[³H]TCA-precipitable peptides

upon the fuel-sensor B cell may thus represent an essential feature of the physiological regulation of insulin release by circulating nutrients.

These considerations amply justify a detailed study of the biochemical, biophysical and functional events evoked by L-glutamine in islets exposed to L-leucine. The present study deals solely with the functional events, i.e. biosynthetic and secretory phenomena, evoked by the combination of L-glutamine and L-leucine in the endocrine pancreas. There is very little published information concerning the effect of L-glutamine upon insulin release. However, the capacity of distinct amino acids to affect insulin release in the presence of D-glucose or L-leucine, as reported in this study, is in agreement with data previously collected from studies on rat [11] and rabbit [12] pancreatic tissue, except for the failure of L-arginine to augment L-leucine-induced insulin secretion in the present system.

Our data suggest that the secretory response evoked by the combination of L-leucine and L-glutamine somehow depends on the metabolism of

the latter amino acid. L-glutamate, which does not easily penetrate islet cells [13], failed to reproduce the effect of L-glutamine (Table 2). However, L-asparagine, which like L-glutamine is readily deamidated in several tissues, shared with L-glutamine the property of enhancing L-leucine-induced insulin release whilst failing to significantly affect D-glucose-stimulated secretion [2].

It is also conceivable that the catabolism of L-leucine participates in the secretory phenomenon. However, this is rather unlikely because, in the presence of L-glutamine, the non-metabolised analogue of L-leucine, *b*(-)-2-aminobicyclo[2,2,1]heptane-2-carboxylic acid, is as potent as L-leucine itself in stimulating insulin release [3, 10]. Other indications that the metabolic situation of islets exposed to both L-glutamine and L-leucine differs from that of islets incubated with L-leucine alone [9] arise from their vastly different sensitivity to exogenous NH_4^+ or changes in pCO_2 . At a 0.5 mmol/l concentration of NH_4^+ , L-leucine-induced insulin release is virtually abolished [14], whereas the release of insulin evoked by the combination of L-leucine and L-glutamine was reduced by no more than $14.1 \pm 4.8\%$. Likewise, the intracellular acidification resulting from the exposure of islets to a high pCO_2 [8] doubled L-leucine-stimulated insulin release [14], but only caused an $18.0 \pm 4.6\%$ increase in insulin output in the presence of both L-leucine and L-glutamine (Table 4).

These findings are compatible with the fuel hypothesis for insulin release [15], in which the secretory response to nutrients is thought to be associated with an increase in catabolic fluxes. In the present study, the release of insulin provoked by the combination of L-leucine and L-glutamine appeared to be dependent on the integrity of oxidative processes, because it was suppressed by antimycin A (Table 4). Insulin release was also decreased by menadione, which inhibits nutrient-induced insulin release by lowering the islet content of reduced pyridine nucleotides [16, 17]. As is the case with other nutrient secretagogues, the functional response to the combination of L-leucine and L-glutamine included stimulation of biosynthetic activity, with a preferential effect on proinsulin synthesis (Table 6).

A number of other analogues exist between insulin release evoked by L-glutamine and L-leucine and that evoked by other nutrient secretagogues. For instance, both represent calcium-dependent phenomena. In this respect, however, the insulin release evoked by L-glutamine and L-leucine together differed from that evoked by either D-glucose alone [18] or the association of D-glucose and L-leucine (Table 5). The insulin release evoked by

L-glutamine and L-leucine showed greater sensitivity to the absence of extracellular Ca^{2+} or presence of the organic calcium-antagonist verapamil, and a lower apparent "Km" for extracellular Ca^{2+} (i. e. 0.2 mmol/l in the presence of both L-leucine and L-glutamine, (Fig. 3), as distinct from 0.5 mmol/l in the sole presence of D-glucose [18]). These findings suggest that different nutrients may exert separate effects upon calcium handling by islet cells. Furthermore, L-glutamine (even in the presence of L-leucine) fails to reproduce the inhibitory effect of D-glucose or L-leucine upon ^{45}Ca efflux from perfused islets (unpublished observations).

The process of nutrient-induced insulin release is thought to involve activation of both adenylate cyclase and protein phosphorylation by endogenous calmodulin [19–22]. A comparable phenomenon may be involved in the secretory response to L-glutamine and L-leucine. Indeed, such a response can be potentiated by theophylline, and inhibited by either adrenaline or the trifluoromethylphenothiazine derivative of domperidone, a presumed calmodulin-antagonist (P. Laduron, personal communication). The enhancing action of cytochalasin B upon insulin release caused by the combination of L-leucine and L-glutamine points to the participation of microfilaments in the secretory process, as previously documented in the case of D-glucose- or L-leucine-induced insulin release [6, 23].

Although these data indicate that insulin release provoked by the combination of L-glutamine and L-leucine shares several features in common with that provoked by D-glucose or other nutrient secretagogues, a striking difference between these two processes should be underlined. Whereas the stimulation of insulin release by most nutrients (e. g. D-glucose, D-glyceraldehyde, D-erythrose, pyruvate, lactate, L-leucine, α -ketoisocaproate, α -ketocaproate; [24–28] is characterized by a biphasic pattern, the enhancement by L-glutamine of L-leucine-stimulated insulin release was a steadily increasing phenomenon. In the pancreas already exposed to L-leucine, no initial secretory peak occurred in response to L-glutamine administration (Fig. 1). During prolonged exposure to both amino acids, the rate of secretion increased progressively and much more markedly than during prolonged exposure to D-glucose or other nutrient secretagogues. In our opinion, this unusual secretory pattern suggests that much may be learned about the intimate mechanism of insulin release by further investigations of the biochemical and biophysical effects of L-glutamine and L-leucine on pancreatic islets.

In conclusion, the present work indicates that L-glutamine markedly augments L-leucine-stimulated

insulin release. This phenomenon appears to reflect both the capacity of L-glutamine to act as a fuel and the capacity of L-leucine to activate glutamate dehydrogenase in islet cells. The interaction between these two amino acids is thus compatible with the concept that the nutritional regulation of insulin release is tightly dependent on metabolic events evoked by nutrients in the pancreatic B cell [15].

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References

- Fajans SS, Floyd JC Jr, Knopf RF, Pek S, Weissman P, Conn JW (1972) Amino acids and insulin release in vivo. *Israel J Med Sci* 8: 233–243
- Malaisse WJ, Sener A, Carpinelli AR, Anjaneyulu K, Lebrun P, Herchuelz A, Christophe J (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLVI. Physiological role of L-glutamine as a fuel for pancreatic islets. *Mol Cell Endocrinol* 20: 171–189
- Sener A, Malaisse WJ (1980) Activation allostérique de la déshydrogénase glutamique dans les îlots de Langerhans: son rôle dans la libération d'insuline induite par les acides aminés. *CR Acad Sci Paris* 290: 1135–1137
- Malaisse WJ, Brisson GR, Malaisse-Lagae F (1970) The stimulus-secretion coupling of glucose-induced insulin release. I. Interaction of epinephrine and alkaline earth cations. *J Lab Clin Med* 76: 895–902
- Malaisse WJ, Malaisse-Lagae F, Walker MO, Lacy PE (1971) The stimulus-secretion coupling of glucose-induced insulin release. V. The participation of a microtubular-microfilamentous system. *Diabetes* 20: 257–265
- Van Obberghen E, Somers G, Devis G, Vaughan GD, Malaisse-Lagae F, Orci L, Malaisse WJ (1973) Dynamics of insulin release and microtubular-microfilamentous system. I. Effect of cytochalasin B. *J Clin Invest* 52: 1041–1051
- Leclercq-Meyer F, Marchand J, Leclercq R, Malaisse WJ (1976) Glucagon and insulin release by the in vitro perfused rat pancreas. Influence of the colloid composition of the perfusate. *Diab Metab* 2: 57–65
- Carpinelli AR, Sener A, Herchuelz A, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLI. Effect of intracellular acidification upon calcium efflux from islet cells. *Metabolism* 29: 540–545
- Malaisse WJ, Hutton JC, Carpinelli AR, Herchuelz A, Sener A (1980) The stimulus-secretion coupling of amino acid-induced insulin release. I. Metabolism and cationic effects of leucine. *Diabetes* 29: 431–437
- Sener A, Malaisse WJ (1980) L-leucine and a non-metabolizable analogue activate pancreatic islet glutamate dehydrogenase. *Nature* 288: 187–189
- Malaisse WJ, Malaisse-Lagae F (1968) Stimulation of insulin secretion by noncarbohydrate metabolites. *J Lab Clin Med* 72: 438–448
- Milner RDG (1970) The stimulation of insulin release by essential amino acids from rabbit pancreas in vitro. *J Endocr* 47: 347–356
- Sehlin J (1972) Uptake and oxidation of glutamic acid in mammalian pancreatic islets. *Hormones* 3: 156–166
- Sener A, Malaisse WJ (1980) The stimulus-secretion coupling of amino acid-induced insulin release. II. Sensitivity to K^+ , NH_4^+ and H^+ of leucine-stimulated islets. *Diab Metab* 6: 97–101
- Malaisse WJ, Sener A, Herchuelz A, Hutton JC (1970) Insulin release: the fuel hypothesis. *Metabolism* 28: 373–386
- Malaisse WJ, Sener A, Boschero AC, Kawazu S, Devis G, Somers G (1978) The stimulus-secretion coupling of glucose-induced insulin release. XXX. Cationic and secretory effects of menadione in the endocrine pancreas. *Eur J Biochem* 87: 111–120
- Malaisse WJ, Hutton JC, Kawazu S, Sener A (1978) The stimulus-secretion coupling of glucose-induced insulin release. XXXI. Metabolic effect of menadione in isolated islets. *Eur J Biochem* 87: 121–130
- Malaisse WJ, Hutton JC, Sener A, Levy J, Herchuelz A, Devis G, Somers G (1978) Calcium antagonists and islet function. VII. Effect of calcium deprivation. *J Membrane Biol* 38: 193–208
- Valverde I, Vandermeers A, Anjaneyulu R, Malaisse WJ (1979) Calmodulin activation of adenylate cyclase in pancreatic islets. *Science* 206: 225–227
- Sharp GWG, Wiedenkiller DE, Kaelin D, Siegel EG, Wollheim CB (1980) Stimulation of adenylate cyclase by Ca^{2+} and calmodulin in rat islets of Langerhans. *Diabetes* 29: 74–77
- Gagliardino JJ, Harrison DE, Christie MR, Gagliardino EE, Ashcroft SJH (1980) Evidence of the participation of calmodulin in stimulus-secretion coupling in the pancreatic β -cell. *Biochem J* 192: 919–927
- Schubart UK, Erlichman J, Fleischer N (1980) The role of calmodulin in the regulation of protein phosphorylation and insulin release in hamster insulinoma cells. *J Biol Chem* 255: 4120–4124
- Levy J, Herchuelz A, Sener A, Malaisse-Lagae F, Malaisse WJ (1976) Cytochalasin B-induced impairment of glucose metabolism in islets of Langerhans. *Endocrinology* 98: 429–437
- Malaisse WJ, Herchuelz A, Levy J, Sener A, Pipeleers DG, Devis G, Somers G, Van Obberghen E (1976) The stimulus-secretion coupling of glucose-induced insulin release. XIX. The insulinotropic effect of glyceraldehyde. *Molec Cell Endocrinology* 4: 1–12
- Sener A, Devis G, Somers G, Malaisse WJ (1977) The insulinotropic action of D-erythrose. *Diabetologia* 13: 125–130
- Sener A, Kawazu S, Hutton JC, Boschero AC, Devis G, Somers G, Herchuelz A, Malaisse WJ (1978) The stimulus-secretion coupling of glucose-induced insulin release. XXXIII. Effect of exogenous pyruvate on islet function. *Biochem J* 176: 217–232
- Malaisse WJ, Kawazu S, Herchuelz A, Hutton JC, Somers G, Devis G, Sener A (1979) The stimulus-secretion coupling of glucose-induced insulin release. XXXIV. Effect of lactate upon islet function. *Arch Biochem Biophys* 194: 49–62
- Hutton JC, Sener A, Herchuelz A, Atwater I, Kawazu S, Boschero AC, Somers G, Devis G, Malaisse WJ (1980) Similarities in the stimulus-secretion coupling mechanisms of glucose- and 2-keto acid-induced insulin release. *Endocrinology* 106: 203–219

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