The Stimulation of Insulin Secretion in Non–Insulin-Dependent Diabetic Patients by Amino Acids and Gliclazide in the Basal and Hyperglycemic State

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Sulfonylureas stimulate insulin secretion as their predominant contribution toward decreasing blood glucose in diabetic patients. We studied eight gliclazide-treated, non–insulin-dependent diabetic patients on two occasions with a protocol of basal observation for 30 minutes, a 60-minute infusion of randomized leucine or arginine, and a further 90-minute hyperglycemic clamp. Basal glucose was the same on both occasions (mean, 7.82 mmol/L for leucine *v* 7.79 for arginine, P = NS), and glucose levels declined to 7.50 and 7.25 mmol/L, respectively, by 30 minutes. After leucine infusion, the decline of glucose continued, but stabilized or reversed with arginine such that by the end of the infusions, glucose levels were 6.63 ± 0.69 mmol/L for leucine and 7.62 ± 0.67 for arginine ($P < .02$). Arginine caused a sharp increase in insulin secretion (from 17.8 mU/L to 43.8 mU/L in 6 minutes) at the onset of the infusion, and thereafter insulin secretion was not significantly different throughout either the amino acid or hyperglycemic clamp periods (mean, 42.1 *v* 44.7 mU/L, respectively, P = NS). By contrast, the leucine infusion caused little acute change in secretion, but augmented it with time from the basal period (17.2 mU/L) to the end of the infusion (29.4 mU/L). During the hyperglycemic clamp period, there was significant further augmentation of insulin secretion, increasing to 81.6 ± 16 mU/L at the end of the study. Leucine significantly augmented insulin secretion compared with arginine (81.6 ± 16 *v* 54.0 ± 8.4 mU/L, respectively, $P < .002$). These data suggest that leucine is a better priming agent for sulfonylurea than arginine. Additive effects on insulin secretion may allow the use of combinations of branched chain amino acids (BCAAs) and sulfonylureas to augment insulin secretion in the presence of hyperglycemia.

SULFONYLUREAS stimulate insulin secretion as their predominant contribution toward decreasing blood glucose in diabetic patients.[1] They bind to a K^+ adenosine triphosphate channel on at least one sulfonylurea receptor[2] and block potassium efflux from the cell.[3] Insulin secretion from β cells is a function of blood glucose, exposure to sulfonylureas, exposure to other substrates, and the modulating effects of the activity of the neuroendocrine axis. These functions are not additive in a simple way. Indeed, in diabetic patients, insulin secretion as a response to the prevailing glucose may be blunted, while secretion stimulated by other secretagogues is intact.[4] Similarly, the response of β cells to amino acids need not necessarily be affected by sulfonylureas in the same way as the response to glucose. Different β-cell mechanisms are involved, and amino acids provide an independent stimulus to β cells.[5] The charged ionic amino acid arginine affects β cells apparently by depolarization mechanisms on the β cell, in contrast to the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine (Fig 1), which may affect β-cell function through metabolic pathways. Glucose-induced insulin secretion may be defective in early non–insulin-dependent diabetes mellitus while arginine responsiveness is retained.[5,6]

BCAAs seem to activate β cells through a pathway different from that of glucose stimuli, so synergism can be demonstrated between their actions.[7] Experimental evidence from perfusion of the rat pancreas in vitro suggests that both the slope of the response curve and its maximal stimulation can be increased by using amino acids with glucose.[8] In addition, the time-dependent inhibition of the response of the pancreas to arginine in normal subjects can be abolished by hyperglycemia,[9] suggesting that the synergistic mechanisms are robust and prevent downregulation of sensitivity.

Amino acid metabolism is in turn affected by insulin concentrations. In the fasting state, there is inhibition of hepatic uptake of alanine and other gluconeogenic amino acids, and peripheral uptake of BCAAs by skeletal muscle is enhanced in the postprandial state.[10-12] Gliclazide treatment reduces the amino acid concentrations during a test meal, and the mechanism of this is postulated to be via increased insulin availability.[13]

We have previously examined the insulinogenic effects of gliclazide in normal and diabetic patients with an infusion of mixed amino acids (Synthamin; Travenol Laboratories, Thetford, Norfolk, UK),[14] and have shown that gliclazide has marked effects on insulin secretion when amino acid concentrations increase. Insulin secretion increases toward normal with gliclazide therapy, and amino acids potentiate this response. Insulin secretion can be quadrupled at high glucose concentrations when amino acids are infused. On the other hand, amino acids have little effect on insulin secretion when glucose is near normal. This implies that amino acids may have a special role in selectively augmenting insulin secretion in the presence of hyperglycemia and gliclazide, but this effect "degrades" as blood glucose returns toward normoglycemia. Thus, a combination of amino acids and gliclazide may act more efficiently than gliclazide alone. Synthamin is a mixture of amino acids used in parenteral nutrition. It consists of both BCAAs and polar amino acids, and it was not possible with this protocol to decide whether either type or both types of amino acids caused the effect.

We therefore undertook a comparison of infusions of leucine and arginine to examine the extent of their synergistic effects on the insulinogenic action of gliclazide in subjects with non–insulin-dependent diabetes.

PATIENTS AND METHODS

Eight non–insulin-dependent diabetic patients already treated with gliclazide were recruited from our diabetic clinic. After provision of informed consent, they were treated with gliclazide (median dose, 80

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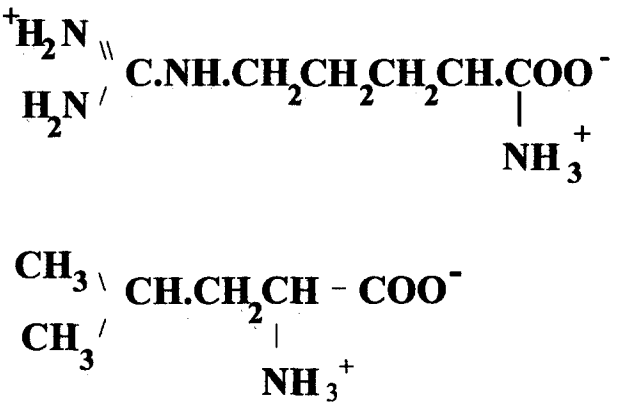

Fig 1. Secondary structure of arginine (top) and leucine (below). The charged groups on arginine render it ionic at pH 7. Leucine is one of 3 BCAAs involved in human metabolism.

mg daily) in an open manner, and all patients continued to take their regular daily dose of gliclazide during the whole of the study. Each patient was studied on two occasions at an interval of 1 to 2 weeks. Subject characteristics are listed in Table 1. All participants completed both arms of the study. On the day of the study, patients were admitted at 8 AM in the fasting state and then took their usual dose of gliclazide.

Two intravenous cannulae were inserted under local anesthetic. One distal cannula was attached to an automatic sampling device previously described,[15] and the other was used for administration of amino acids and glucose. Either leucine or arginine was given in random order on the first occasion, and the other amino acid on the second. The investigation was a double-blind crossover study.

Basal sampling was undertaken for 30 minutes, followed by an amino acid infusion for 60 minutes. Amino acid solutions were given in equal molar and volume doses over a period of 60 minutes. The doses were as follows: L-arginine 0.26555 g/kg ideal body weight (IBW) or L-leucine 0.200 g/kg IBW. This was approximately a 20-g load of L-arginine and a 15-g load of leucine for an average man. Following the infusion (at time 90 minutes), a hyperglycemic clamp (11 mol/L) was then performed over 90 minutes using an iterative computer program.[16] The overall study length was 3 hours; samples were taken every 2 minutes for glucose and every 6 minutes for insulin, C-peptide, and amino acids.

Food and Drugs–grade (conforms to UK Medicine Act, 1996) amino acids were supplied by Sigma (Poole, Dorset, UK). The amino acids were made up as sterile, pyrogen-free solutions of equal molarity (0.152 mol/L) in 0.45% saline (Mandeville Medicines, Aylesbury, Buckinghamshire, UK). These solutions were L-arginine (free weight, 174.2) 26.555 g/L and L-leucine (free weight, 131.2) 20 g/L. The study fulfilled the criteria of the Declaration of Helsinki for studies in man. Full ethical approval was obtained through the Central Oxford Research Ethical Committee. All participants provided written informed consent before inclusion in the study.

Table 1. Description of the Study Group

Characteristic	Value
No. of subjects	8
Age (yr)	
Mean	62
Range	53-67
Sex ratio (male:female)	6:2
Duration of diabetes (yr)	
Mean	4
Range	1-9
Gliclazide dose (mg/d)	
Median	80
Range	40-320
Fasting blood glucose (mmol/L)*	8.2 ± 0.9

*Mean ± SD.

Insulin was assayed using a Pharmacia radioimmunoassay with Sepharose separation. The coefficient of variation (CV) was 3.3% at 40.5 mU/L.[17] C-peptide radioimmunoassays used a Novo Nordisk (Copenhagen, Denmark) K6 antibody using Sepharose separation. The CV was 4.2% at 1.32 nmol/L.[17] Glucose levels were measured by an automated MIRA hexokinase method using Boehringer (Mannheim, Germany) gluco-quant with a CV of 1.8% at 10.5 mmol/L.[17]

Data were verified by sequential graphical techniques and analyzed statistically using paired *t* tests for paired data from the same subjects. The power of the study is that using eight subjects and an insulin assay CV of 10%, a 1-mU/L change about a mean of 10 mU/L would be significant at *P* less than .05.

RESULTS

There were no significant differences between the basal periods on the two study occasions for glucose, insulin, or C-peptide. Glucose concentrations for the infusion and clamp periods are shown in Fig 2. Basal glucose was the same on both occasions (mean, 7.82 mmol/L for leucine *v* 7.79 mmol/L for arginine, P = NS), and glucose declined to 7.50 and 7.25 mmol/L, respectively, by 30 minutes. After leucine infusion, the decline of glucose continued, but it stabilized or reversed with arginine such that by the end of the infusions glucose levels were 6.63 ± 0.69 mmol/L for leucine and 7.62 ± 0.67 mmol/L for arginine ($P < .02$). The hyperglycemic clamp period from 90 minutes onward was successfully maintained in the two arms of the study (P = NS).

The insulin data are shown in Fig 3. Arginine caused a sharp increase in insulin secretion (from 17.8 mU/L to 43.8 mU/L in 6 minutes) at the onset of the infusion, and thereafter insulin secretion was not significantly different throughout either the amino acid or hyperglycemic clamp periods (mean, 42.1 *v* 44.7 mU/L respectively, P = NS). By contrast, leucine infusion caused little acute change in secretion, but augmented it with time from the basal of 17.2 mU/L to the end of the infusion (29.4 mU/L). During the hyperglycemic clamp period, there was significant further augmentation of insulin secretion, increasing to 81.6 ± 16 mU/L at the end of the study. Leucine significantly augmented insulin secretion as compared with arginine (81.6 ± 16 *v* 54.0 ± 8.4 mU/L, respectively, $P < .002$).

C-peptide data are shown in Fig 4. They demonstrate concordance with the insulin data, indicating that the observed changes in insulin were secretory rather than clearance phenomena. Glucose infusion data (Fig 5) were similar for both studies, with no significant differences in glucose requirements to maintain hyperglycemia.

Amino acid concentrations were not measured directly, but with infusions of similar concentrations of a mixture of amino acids, plasma concentrations approximately double from a median of 3.5 mmol/L (normal physiological level) to 7 mmol/L.

DISCUSSION

In man, amino acids have divergent properties on the pancreatic β-cell that are related to their secondary structure. Valine, leucine, and isoleucine as a group are termed BCAAs

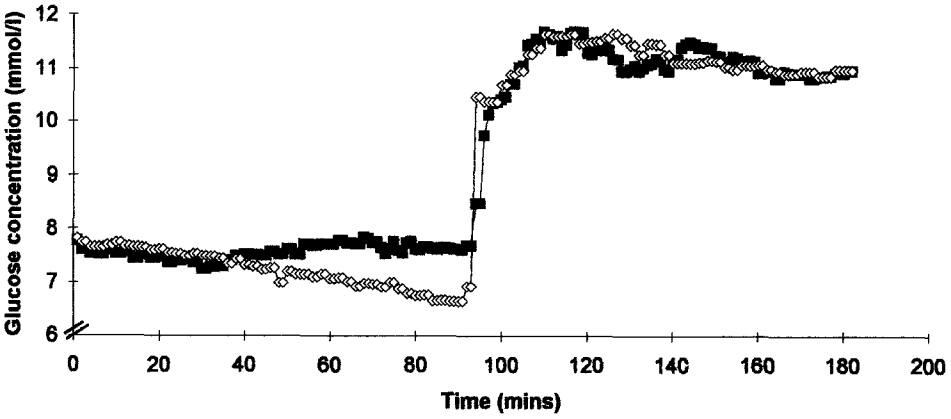

Fig 2. Mean glucose concentrations during the study: ◇, leucine study; □, arginine study. Amino acids were infused from time 30 to 90, and a hyperglycemic clamp was established at 90 minutes and continued to 180 minutes.

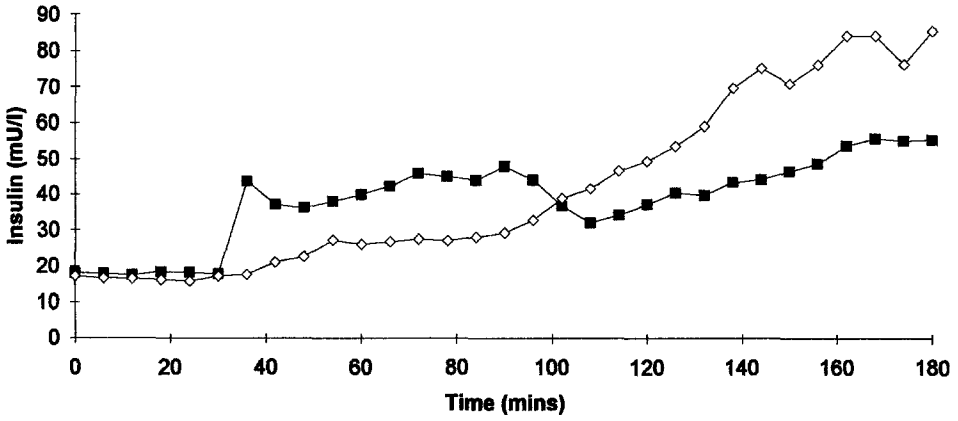

Fig 3. Mean insulin data: ◇, leucine study; ■, arginine study. Amino acids were infused from time 30 to 90 minutes, and a hyperglycemic clamp was established at 90 minutes and continued to 180 minutes.

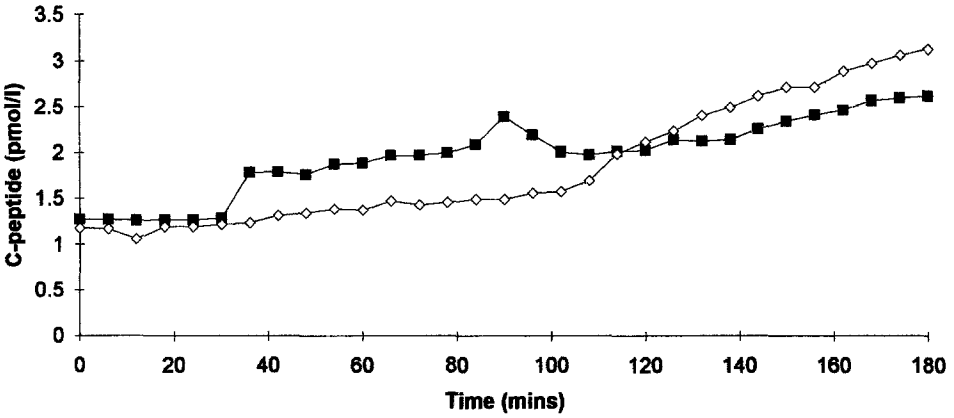

Fig 4. Mean C-peptide data: ◇, leucine study; ■, arginine study. Amino acids were infused from time 30 to 90 minutes, and a hyperglycemic clamp was established at 90 minutes and continued to 180 minutes.

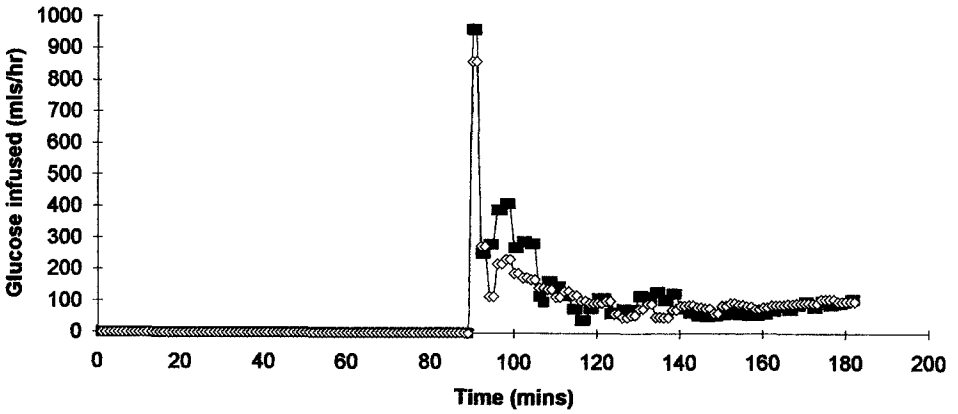

Fig 5. Mean glucose infused: ◇, leucine study; ■, arginine study. Amino acids were infused from time 30 to 90 minutes, and hyperglycemic clamp was established at 90 minutes and continued to 180 minutes.

(Fig 1). The two side chains in the amino acid sequence of leucine are nonpolar, and it remains un-ionized at pH 7. The α-amino and α-carboxyl groups are charged positively and negatively, respectively, but overall, the molecule has no net polarity. By contrast, arginine, one of the basic amino acids, has a positively charged group at pH 7 in addition to the α-amino group. The polarity of arginine confers significantly different properties on the molecule that affect its mechanism of action in insulin release from the β cell.

Leucine is probably metabolized in the β cell, allowing facultative insulin release by a pathway that converges with the glycolytic pathway at the level of acetyl coenzyme A. Arginine infusion acts rapidly to cause insulin release—an effect previously used in tests of dynamic β cell function in both non–insulin-dependent[5] and insulin-dependent[6,18] diabetes mellitus. Arginine has been thought to have these rapid effects on β-cell insulin production because of the nature of its polarity. The effect seems to be directly on the membrane and the depolarization phase of the β cell, facilitating the entry of Ca^{2+} and subsequent insulin release.[19] This has been challenged as being the only way that arginine produces effects,[20] but it remains likely that a substantial proportion of the insulinogenic effect is direct rather than indirect. Whether this effect is on the sulfonylurea gate mechanism or on other parts of the membrane is still uncertain.

In our studies, insulin secretion was augmented rapidly with arginine and then continued approximately constant from a few minutes after initiation of arginine infusion despite a subsequent hyperglycemic clamp. One conclusion that might be drawn from this is that arginine saturated the insulin secretory capacity of the β cell, so further secretory stimuli do not have additive or multiplicative effects. By contract, leucine caused a much slower incremental increase in insulin secretion which one can therefore conclude is not saturating the secretory capacity, since the hyperglycemic clamp following leucine administration produces a large augmentation of total insulin output reflected in the mean concentrations of both insulin and C-peptide. Glucose concentrations continued to decrease during leucine infusion in the presence of some increment of insulin secretion. By contrast, with initiation of the arginine infusion, the decline of glucose was reversed despite a doubling of the insulin concentration. This may well reflect the effect of activation of α cells producing glucagon, since the depolarizing effects of arginine in the islet are likely to be nonspecific. An increase in glucagon has been described with alanine infusion,[21] whereas in the rat, others have noted a suppression of arginine-stimulated glucagon secretion in the presence of gliclazide.[22]

We have previously demonstrated that glucose, amino acids, and gliclazide interreact to produce at least additive effects in diabetes.[14] The amino acids in these previous studies used a mixture (Synthamin 14) that contains both polar amino acids and BCAAs. The current study was designed to elucidate whether these multiplicative effects were more likely to be related to the monoamine monocarboxylic amino acids or the basic amino acids. The data presented suggest that the BCAA leucine has insulinogenic effects of its own that are augmented by glycemia. Thus, leucine is a candidate for priming β cells in the presence of sulfonylureas, in contrast to arginine, which has insulinogenic effects but cannot be regarded as a priming agent.

In conclusion, previous data have demonstrated the multiplicative effect of the sulfonylurea gliclazide, amino acids, and glucose on insulin secretion. These data suggest that leucine is a better priming agent than arginine. Additive effects on insulin secretion may allow the use of combinations of BCAAs and sulfonylureas to augment insulin secretion in the presence of hyperglycemia.

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