Effects of dipeptide administration on hypoglycaemic counterregulation in type 1 diabetes

J M’Bemba, L Cynober, P de Bandt, M Taverna, A Chevalier, C Bardin, G Slama, JL Selam

SUMMARY

Objectives: To investigate if a dipeptide made of glutamine and alanine is able to contribute to the recovery from insulin-induced hypoglycaemia in type 1 diabetes. Research design and methods: Fifteen adult type 1 patients were randomly assigned to study group (n = 7): intravenous infusion of 20 g Dipeptiven\(\text{T}\) in normal saline (i.e., 8 g alanine and 13 g glutamine), or control group (n = 8): same infusion, normal saline only. A 150 min gradual hypoglycaemic hyperinsulinemic clamp was administered after 2h of infusion. Counterregulatory hormones, symptoms, and cognitive function (4 choice reaction test) were regularly measured during the study.

Results: Blood glucose and glucose infusion rates were similar in the 2 groups. Circulating levels of alanine and glutamine peaked at 90 min and remained elevated throughout the test. This was associated with significant differences in: glucagonemia 107 ± 20 vs 58 ± 8 pg/ml, and neuroglycopenic symptoms scores: 7 ± 3 vs 18 ± 13, at t 150 min, in study and control group, \(p < 0.05\). Dysautonomic symptoms, cognitive tests as well as epinephrine, norepinephrine, cortisol and growth hormone were similar between groups.

Conclusion: Intravenous infusion of a dipeptide made of alanine and glutamine is capable to reactivate glucagon secretion during insulin-induced hypoglycaemia and to reduce hypoglycaemic symptoms.

Key-words: Hypoglycaemia · Aminoacids · Dipeptides · Type 1 Diabetes.

REUME

Effets de l’administration de dipeptide sur la contre-régulation de l’hypoglycémie chez le diabétique de type 1

Objectifs : Evaluer si un dipeptide composé de glutamine et alanine peut contribuer à lutter contre l’hypoglycémie insulinique chez le diabétique de type 1.

Matériel et méthodes : Quinze diabétiques de type 1, adultes, ont été repartis de façon randomisée dans le groupe d’étude (n = 7) avec perfusion intraveineuse de 20g de Dipeptiven\(\text{T}\) dilué dans du sérum salé isotonique (soit 8 g d’alanine et 13 g de glutamine), ou dans le groupe contrôle: perfusion de sérum salé isotonique. Après 2 heures de perfusion, un clamp hypoglycémique graduel de 150 minutes est pratiqué. Les hormones de contre-régulation, les symptômes hypoglycémiques et les fonctions cognitives évaluées par le test de réaction à la stimulation lumineuse, sont mesurées de façon répétée pendant l’étude.

Résultats : Les glycémies et les débits de glucose sont similaires dans les 2 groupes. Les taux plasmatiques d’alanine et glutamine montrent un pic à 90 min et restent ensuite élevés pendant le test. À l’inverse, des différences significatives sont notées pour la glucagonémie: 107 ± 20 vs 58 ± 8 pg/ml, et pour les symptômes neuroglycopeniques: 7 ± 3 vs 18 ± 13 à 150 minutes, pour le groupe d’étude et le groupe contrôle, respectivement, \(p < 0.05\). Les symptômes dysautonomiques, les scores des tests cognitifs, ainsi que les taux d’adréna- line, noradrénaïline, cortisol et hormone de croissance sont similaires dans les deux groupes.

Conclusion : La perfusion intraveineuse d’un dipeptide composé d’alanine et glutamine est capable de réactiver la sécrétion de glucagon au cours de l’hypoglycémie insulinique, et de réduire les symptômes hypoglycémiques.

Mots-clés : Hypoglycémie · Acides aminés · Dipeptides · Diabète de type 1.
Risk of severe hypoglycaemia is increased with current methods of intensive insulin therapy [1]. Besides conventional methods (education, diet, etc), new prevention techniques have been investigated, e.g. meticulous avoidance of minor hypoglycaemias, [2], brain [3-5] or autonomous system [6, 7] stimulants. The latter option includes alanine administration that has been intensively investigated by Cryer et al. [8-10]. They showed that alanine stimulates glucagon secretion, even in long duration diabetes with absence of glucagon response to hypoglycaemia [8]. They also showed in diabetic patients that 40 g alanine given orally may prevent nocturnal hypoglycaemia [10] or restore normoglycaemia more rapidly [9]. However, bad taste and high cost of oral alanine makes this solution poorly practicable. In addition alanine has a high osmolarity and large intake may be responsible of diarrhea. Alanine is also a gluconeogenic substrate per se. This latter mechanism may possibly contribute, as much as the induction of glucagon secretion, to the anti hypoglycaemic properties of alanine. However, while alanine is one of the main gluconeogenic acid, it is not the most potent glucagon secretagogue [11, 12].

Therefore, while alanine is one of the main gluconeogenic substrate e.g. alanine. This latter mechanism may possibly contribute, as much as the induction of glucagon secretion, to the anti hypoglycaemic properties of alanine. However, while alanine is one of the main gluconeogenic acid, it is not the most potent glucagon secretagogue [11, 12]. Finally, only Nair et al. have studied the effects of a mixture of amino acids (including glutamine but excluding alanine) infused intravenously, on counterregulatory mechanisms during a hypoglycaemic clamp [13], though in normal subjects. They showed that aminoacids pre clamp do not elevate blood glucose but stimulate glucagon secretion during hypoglycaemic clamp.

Dipeptides compared to aminoacids have similar metabolic effects [14] and several potential advantages: lower osmolarity thus better intestinal tolerance, less bad taste, more efficient intestinal transport, more prolonged effects on plasma amino acids levels [15]. Finally, one can select a dipeptide combining a strong glucagon secretagogue amino acid e.g arginine or glutamine [16], and a strong neoglucogenetic substrate e.g. alanine.

In this study, we have analysed the effects of a dipeptide made of glutamine and alanine given intravenously to diabetic subjects on glycaemic counterregulation during a clamp-induced hypoglycaemia.

Materials and methods

Patients

Fifteen type 1 diabetic patient gave informed written consent and were randomly assigned to study group (n = 7, dipeptide) and control group (n = 8, placebo). Clinical characteristics (Tab I) showed that subgroups were comparable in terms of age, duration, insulin doses and HbA1c levels. Perception of hypoglycaemia, assessed during hypoglycaemic clamp (see below), began at the same glycaemic levels in the 2 groups (Tab I).

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 ± 9</td>
<td>38 ± 10</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>16 ± 9</td>
<td>15 ± 10</td>
</tr>
<tr>
<td>Insulin dose (u/24h)</td>
<td>50 ± 19</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.5 ± 1.1</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>Hypoglycaemia perception</td>
<td>3.0 ± 0.3</td>
<td>3.1 ± 0.3</td>
</tr>
</tbody>
</table>

Hypoglycaemic clamp

The gradual clamp technique has been described elsewhere in details [17-19]. Briefly, normoglycaemia was maintained overnight using subcutaneous insulin supplements, frequent capillary glucose measurements, and hypoglycaemias were avoided.

In the morning, usual insulin dose and breakfast were omitted, and the subjects were trained at least four times at performing 4RT in order to achieve stable recordings. Then, two intravenous catheters were inserted, one in an antebrachial vein for infusion of glucose and insulin, and the other in a dorsal hand vein for blood sampling. This hand was placed in a heated (60-65°C) box to arterialize venous blood. Rapid-acting human insulin (Actrapid, Novo Nordisk) in a 0.9% sodium chloride was infused during a 2h basal period to achieve euglycaemic levels, then set at a fixed rate of 1 mU/Kg/min. Using a lap-top computer program (John Robert, Necker Hospital, Paris, unpublished), loaded regularly with the plasma glucose measurements, the infusion rate of a 30% glucose solution was automatically adjusted every 3-5 min to gradually reach a plasma glucose level of 2.2 mM in 0.5-0.6 mM steps (4.4,3.9,3.4,2.8 and 2.2 mM) after 120 min and to be maintained at this latter level for 30 min. On completion of the clamp, IV insulin was withdrawn, plasma glucose was restored to ≈ 8 mM, and the patients took a rest and ate their lunch before returning to their usual insulin treatment.

From t-2h, dipeptide or placebo were infused intravenously for 5 hours, i.e. 2 hours before hypoglycaemia (euglycaemic clamp) and during the 3 hours of the hypoglycaemic clamp. Dipeptide infusion consisted of Dipeptiven® 100 ml (Fresenius AG, Germany) diluted in 500 ml of normal saline. It contained 20 g of dipeptide made of L-alanine (8g) and L-glutamine (12 g). Placebo was made of normal saline only (600 ml infused over 5 hours).

Measurements and calculations

All blood samples were centrifuged immediately at 4°C and the plasma was kept frozen at -20°C until analysis, except for epinephrine and norepinephrine that was processed...
Immediately. Blood glucose levels were measured by a glucose oxidase method (autoanalyser II, Beckman Instruments, Brea, CA, USA) at 3 to 5 min intervals. Additional blood samples were taken at t-2h t-30 min then every 30 min during gradual hypoglycaemia and every 15 min during the 2.2 mM hypoglycaemic plateau for free insulin, glucose and counterregulatory hormone measurements. Plasma free insulin levels were measured by radioimmunoaassay (RIA — Pasteur, Paris, France) after immediate antibody extraction with polyethylene glycol [20]. Glucagon samples were collected into aprotinin-EDTA and measured by radioimmunoaassay [21]. Cortisol was measured by radioimmunoaassay on serum and growth hormone by immunoradiometric assay (K.G.M.M Alberti, Newcastle, England, unpublished). Catecholamines samples were collected on reduced gluthation and EGTA and measured by a radioenzymatic method [22]. Alanine and glutamine levels were measured at -2h, t-30, t30 and t180 min by automated ion-exchange chromatography [23] using a Jeol 500 (Jeol Europe, Croissy, France) analyzer, with ninhydrin detection.

Hypoglycaemic feelings were recorded every 30 min, and every 15 min during the 2.2 mM hypoglycaemic plateau. To evaluate hypoglycaemic symptoms, a 9-item questionnaire derived from Hepburn [24] was administered every 30 min, and every 15 min during the 2.2 mM hypoglycaemic plateau. The questionnaire included assessment of four autonomic and four neuroglycopenic symptoms, each one being scored according to intensity, from 0 (absent) to 7 (very severe). Autonomic symptoms scores were calculated from the scores of sweating, trembling, feeling hungry, and heart pounding, and neuroglycopenic symptoms scores from the scores of inability to concentrate, dizziness, motor incoordination and difficulty in speaking. Symptoms present at baseline were not counted in final scoring.

After the completion of each questionnaire, a 4 RT test was performed using a Reaction Timer device (AMPD-PM- 2217, Central Sheffield University Hospital, UK). Briefly, the device records the accuracy (number of errors), and time (milliseconds, ms) for the subject to clear a visual symbol appearing randomly in one of four quadrants of a computer screen, by pressing the corresponding key on a keypad [25, 26]. 500 measurements were made on each occasion and averaged.

The glycaemic thresholds for the different hormones were defined as the glucose levels at which an hormone achieved a defined increment over the baseline, in at least two successive samples. The increments were: > 410 pmol/l for epinephrine, > 0.33 nmol/l for norepinephrine, > 192 nmol/l for cortisol, and > 18 µU/l for growth hormone. For glucagon, the threshold was defined as the glucose level at which glucagon exceeded the basal values by 2 SD, in at least 2 consecutive measurements. The above criteria have been used by other authors because they correspond to physiologically significant hormone responses to hypoglycaemia [27, 28].

A rise in symptom scores of two points or more over the score at normoglycaemia, on at least two successive measurements was considered significant. Threshold of awareness was set when the answer about feeling hypoglycaemia was affirmative, in at least two consecutive occasions.

Plasma glucose thresholds for cognitive dysfunction were determined in two ways: 1) in statistical terms, as a rise of at least 2 SD above mean basal values, on at least two successive recordings, and 2) as a change in performance test of known physiological significance. This change was defined as an increase over baseline of > 2% for speed, 02 of > 5% for accuracy, in at least two consecutive measurements, as recommended by Maran et al. [29].

If a significant change did not occur during hypoglycaemia, the glucose nadir was entered as the threshold for the statistical procedures.

Statistical analysis

Except for population data, which are given as mean ± SD, all results are expressed as means ± SEM. Significance of comparisons between study and control patients were assessed using unpaired Student’s t test.

Results

Glucose and insulin profiles

The hypoglycaemic clamp procedures reduced plasma glucose levels in a nearly similar fashion in each of the two populations (Fig 1A). The insulin infusion produced a sustained increase in plasma insulin levels that were not statistically different between the groups (maximum levels 624 ± 54 and 606 ± 96 pM in study and control group, respectively).

Glucose infusion rates were not significantly different between groups: 514 ±140 and 400 ± 26 umol/kg/min in study and control patients at t 180 min.

Aminoacids levels

Alanine and glutamine plasma levels were not different between groups at baseline (Fig 1B and 1C), i.e. before dipeptide infusion, and rose significantly during dipeptide infusion (maximum values at t-30 i.e. 90 min after the beginning of dipeptide infusion had begun: alanine 694 ± 70 vs 155 ±6, p<0.01; glutamine 1045 ± 90 µmol/l at t 325 ± 64, p<0.01).

Counter regulatory hormones

Basals hormones levels did not differ between groups. Glucagon levels were significantly higher during the 2 mM
hypoglycaemic plateau in study patients: 107 ± 20 vs 58 ± 8 pg/ml in control patients at t 150 min, p < 0.05 (Fig 1D). Other hormones showed no significant difference between groups: maximum levels epinephrine 329 ± 133 vs 647 ± 150 pg/ml at t 180 min, norepinephrine 655 ± 20 vs 830 ± 47 pg/ml at t 180 min, cortisol 675 ± 48 vs 809 ± 53 nM at t 180 min, growth hormone 70 ± 17 vs 107 ± 24 mU/l at t 120 min in study and control group, respectively. Glycaemic thresholds for the rise of all above hormones were not different between groups.

Symptoms and cognitive function
Symptoms showed significantly lower (p < 0.05) scores for neuroglycopenic symptoms and a non significant trend to lower scores for dysautonomic symptoms in the study group during the 2 mM hypoglycaemic plateau (Fig 1E and 1F). Glycaemic thresholds for a significant rise in symptoms score were not different between groups. Cognitive function measurements showed no difference between groups, though plasma glucose thresholds for speed change from baseline was significantly higher in the study group 3.3 ± 0.2 vs 2.8 ± 0.2 mM. (p < 0.05).

Discussion
We have found that intravenous infusion of a dipeptide made of 8 g alanine and 12 g glutamine was able to better stimulate glucagon secretion during a hyperinsulinemic
clamped hypoglycaemia in type 1 diabetic patients when compared to patients administered with the hypoglycaemic stimulus alone.

These findings agree with those of Wiethop and Cryer who demonstrated a clear glucagon secreting effect of oral alanine in type 1 diabetic patients subjects [8]. Our data also confirm those of Nair showing that intravenous infusion of aminoacids in the absence of hypoglycaemia (our pre-clamp phase) does not create glucagon hypersecretion which could potentially elevate blood glucose levels when not needed [13].

Finally, we also confirmed that aminoacid infusion does not accelerate glucose recovery, probably because of the dominant inhibitory effect of clamped hyperinsulinemia on glucose production [13]. Wiethop and Cryer found the opposite, i.e. faster glucose recovery with oral alanine addition, but hypoglycaemia was produced with subcutaneous injection of insulin inducing a non sustained hyperinsulinemia [9]. We also found that other counterregulatory parameters e.g. glucose infusion and hormones were not altered by dipeptide infusion. Noteworthy, our results were obtained with a significantly lower aminoacids quantity than other above cited authors, who used either 20-40 g alanine [8-10] or 60 g aminoacid mixture [13].

Importantly, symptoms scores (especially neuroglycopenic symptoms) were reduced during hypoglycaemia in the patients receiving dipeptide infusion. A hypothetical mechanism is a potential utilisation of aminoacids by the brain as alternate energetic substrates, though the clinical reality of such phenomena is controverted [30, 31]. It is also intriguing that we were unable to show any cognitive dysfunction differences between dipeptide infused and control patients, though changes in symptoms thresholds and cognitive impairment threshold may not be necessarily parallel [19].

Finally, we still need to confirm potential advantages of oral dipeptide over free aminoacids e.g. better taste and gastric tolerance. We also need to show that oral dipeptide is able to reproduce the effect of intravenous dipeptide before such product may be envisaged as a potential preventive or curative tool against insulin induced hypoglycaemia.

**Acknowledgements** – Dr N Neveux and Mrs C Genton for their expert amino-acid analysis, Laboratory of biological nutrition, University of Paris 5.

**References**


