CONTINUOUS INTRAPERITONEAL INSULIN INFUSION PARTICLY RESTORES THE GLUCAGON RESPONSE TO HYPOGLYCAEMIA IN TYPE 1 DIABETIC PATIENTS

P.R. OSKARSSON (1), P.E. LINS (1), L. BACKMAN (2), U.C. ADAMSON (1)

SUMMARY - The glycaemic and hormonal responses to a hypoglycaemic event induced by an i.v. bolus of insulin was studied in seven type 1 diabetic patients treated first with continuous subcutaneous insulin infusion (CSII) and subsequently with continuous intraperitoneal insulin infusion (CIPII). Arterialised blood glucose and venous hormonal responses were analyzed. HbA1c was improved by CIPII. Although a regimen of a higher basal insulin infusion rate was applied during CIPII the basal peripheral venous insulin levels were lower. The i.v. bolus of insulin resulted in hypoglycaemia in both tests but was more pronounced during the CSII test expressed as a smaller area under the curve (AUC) for the first hour (13.0 ± 2.3 vs. 13.7 ± 1.2 mmol l⁻¹ h⁻¹, p=0.016, CSII vs. CIPII). The hypoglycaemia resulted in a significant and similar increase in the plasma levels of adrenaline, cortisol and growth hormone in both experiments. A significant increase in the glucagon level was only observed during CIPII. The incremental glucagon response was also significantly more pronounced in the CIPII test expressed as maximal responses (7.5 ± 3.0 vs. 17.0 ± 3.1 pg ml⁻¹, p = 0.048, CSII vs. CIPII) as well as incremental AUC (5.1 ± 3.2 vs. 13.4 ± 13.3 pg ml⁻¹ h⁻¹, p = 0.027, CSII vs. CIPII).

It seems that CIPII in type 1 diabetic patients could improve the glucagon release to hypoglycaemia. This observation may contribute in explaining why CIPII is associated with a lower incidence of hypoglycaemia in spite of an improvement in metabolic control.

Key-words: type 1 diabetes, insulin infusion, pump treatment, subcutaneous, intraperitoneal, glucagon.

RÉSUMÉ - L'administration continue d'insuline par voie intrapéritonéale restaure partiellement la réponse du glucagon à l'hypoglycémie chez les diabétiques de type 1.

Les réponses glycémiques et hormonales à une hypoglycémie induite par un bolus i.v. d'insuline ont été explorées chez sept diabétiques de type 1 traités par pompe à insuline continue, d'abord par voie sous-cutanée (CSII) puis par voie intrapéritonéale (CIPII). L'analyse a porté sur la glycémie du sang artérialisé et sur les réponses hormonales en sang veineux.

L'HbA1c était améliorée par le traitement CIPII. Bien qu'un plus fort débit basal d'insuline ait été appliqué dans le groupe CIPII, les taux de base d'insuline en sang veineux périphérique étaient plus faibles dans ce groupe. Le bolus i.v. d'insuline a induit une hypoglycémie dans chaque groupe, mais cette dernière était plus prononcée dans le groupe CSII, attestée par une aire sous la courbe glycémiante plus faible lors de la première heure (13.0 ± 2.3 vs. 13.7 ± 1.2 mmol l⁻¹ h⁻¹, p=0.016, CSII vs. CIPII). L'hypoglycémie a induit une augmentation significative et similaire dans chaque groupe des taux plasmatiques d'adrénaline, de cortisol et d'hormone de croissance. Une augmentation significative des niveaux de glucagon a été notée dans le seul groupe CIPII. La réponse incrémentale du glucagon était également significativement plus forte dans le groupe CIPII, qu'elle soit exprimée en réponse maximale (7.5 ± 3.0 vs. 17.0 ± 3.1 pg ml⁻¹, p = 0.048, CSII vs. CIPII) ou en aire sous la courbe incrémentale (5.1 ± 3.2 vs. 13.4 ± 13.3 pg ml⁻¹ h⁻¹, p = 0.027, CSII vs. CIPII).

Le traitement des diabétiques de type 1 par CIPII pourrait améliorer la réponse du glucagon à l'hypoglycémie. Cette observation peut contribuer à expliquer pourquoi le traitement par CIPII est associé à une plus faible incidence d'hypoglycémie malgré l'amélioration du contrôle métabolique.

Mots-clés : diabète de type 1, infusion d'insuline, traitement par pompe, sous-cutanée, intrapéritonéale, glucagon.
Hypoglycaemia is a clinical problem in the treatment of type 1 diabetic patients, especially if the metabolic target is near-normoglycaemia [1]. Intraperitoneal insulin treatment with implantable pumps (CIPII) is reported to result in fewer hypoglycaemic events compared with other types of intensive insulin therapy such as continuous subcutaneous insulin infusion (CSII) [2, 3]. The mechanism behind this favourable effect of CIPII remains unclear but may involve factors such as more predictable [4] and/or more reproducible insulin profiles [5]. The portal route of infused insulin may also be of importance, since it directs insulin predominantly to the liver, yielding a more physiologic hepatic insulinisation at a lower peripheral insulinemia due to first-passage hepatic insulin extraction [6]. Hitherto only one study is available comparing the intraperitoneal (i.p.) route with the intravenous (i.v.) route during hypoglycaemia [7]. In that study when hypoglycaemia was induced by an infusion of insulin either i.p. or i.v. for 150 min using the same delivery rates, it induced a less pronounced inhibition of the hepatic glucose production in spite of the predicted more prominent hepatic insulinisation. The mechanism behind this favourable counterregulatory effect was not explained. In order to further elucidate the reason behind the reduced frequency of hypoglycaemia during CIPII the present experimental study was performed. The intention was to expose the patients to an identical hyperinsulinemic challenge with special emphasis on the glucagon response in the same patients during continuous treatment with CSII and CIPII.

**Patients and Methods**

Seven type 1 diabetic patients (two females) on CSII for at least one year with unsatisfactory metabolic control for at least 6–12 months (Table I) participated in the study. All patients had meal-stimulated C-peptide < 0.2 nM. Four of the patients had non-proliferative retinopathy and one patient had microalbuminuria, otherwise none had any sign of late diabetic complications. Prior to the study the patients had been treated with CSII (Minimed, model 506, Sylmar, USA). The patients were offered intraperitoneal insulin treatment with an implantable insulin pump (Minimed, model 2001, Sylmar, USA) since their metabolic control was unsatisfactory (HbA1c > 7.2%, i.e. 2.0% above non-diabetic range) during the last 12 months and blood glucose monitoring displaying instability. The patient’s blood glucose stability (SDBG) was determined from a calculation of the standard deviation of 70 values of home monitoring of blood glucose, according to a method evaluated in our laboratory [8]. In brief the method is based on the measurements obtained at specific time points (in the morning, before lunch, before dinner, 2 h after dinner and before bed) every other day during one month. The SDBG is normally distributed in type 1 diabetic patients with a mean of 3.9 mmol l⁻¹. The incidence of biochemical hypoglycaemia (blood glucose < 3.0 mmol l⁻¹ according to home blood glucose monitoring) was also calculated from the same time period as used for the calculation of SDBG. Due to ethical reasons it was not possible to do the experiment in random order since it would require a cessation of the infusion rate of the implantable pump during the CSII period which results in an increasing risk of an occlusion of the infusion catheter. The study protocol was approved by the local ethical committee and all patients gave their informed consent to participate in the study.

**Study protocol**

The patients were studied on two occasions in the Department of Medicine, Danderyd Hospital, Stockholm. The first test was performed after the decision was made to change to CIPII while still being treated on CSII. The second test was intended to be performed about 6–9 months later. However at this time, the CIPII therapy was affected with problems worldwide due to increased insulin aggregation in the system leading to decreased delivery rates. Unfortunately none of the pumps were found to function accurately at this time. The problems could temporarily be solved by special rinsing procedures and in some instances by changing the catheters. Adequate delivery, (refill difference i.e. the amount programmed related to the delivered amount) could be tested at the refill procedure which was performed every 4-5 weeks. At the scheduled time all patients had a refill difference above 10% (range 12.6–69.1). For that reason the second test was done later than planned with a mean of 16 (11–20) months and at that time the refill difference was less than 10% (range 1.0–6.0).

No severe hypoglycaemic episode was reported the week preceding each test and no symptomatic hypoglycaemia was allowed the last 24-h preceding each test. In the morning after an overnight fast the patient came to laboratory at 07.30 a.m. and was placed in a comfortable, semi-recumbent position. A short teflon catheter was inserted into an antecubital vein on both sides, one used for blood sampling for the analyses of hormones and the other for injection of a bolus of insulin. A short teflon cannula was placed in a dorsal vein on the right hand which was placed in a warm-air box (Department of Medical Physics, Queens Medical Centre, Nottingham, UK), heated to 55-60°C to arterialize the venous blood samples for analyses of blood glucose. If a patient had a high glucose at admittance an i.v. infusion of short acting insulin to reach a level close to 6 mmol/l was given.

After a rest for approximately 30 min and at a capillary blood glucose level close to 6 mmol l⁻¹, an intravenous bolus of insulin 0.05 U kg⁻¹ was given in
order to induce a blood glucose level below 3 mmol l⁻¹. No change in the ongoing insulin substitution therapy was otherwise undertaken so that the first test was performed when insulin was administered by CSII and the second by CIPII at each patients individually determined basal rate.

Arterialised blood samples for the analysis of blood glucose were obtained every 15 min and venous blood samples for the analyses of plasma concentrations of adrenaline, C-peptide, cortisol, glucagon, growth hormone, noradrenaline and free insulin were obtained every 30 min during 180 min. Blood pressure and pulse rate were recorded at 15 min intervals during the same period. Hypoglycaemic symptoms were registered at 15 min intervals on a visual analogic scale. Symptoms such as palpitation, trembling, perspiration and hunger were considered as neurogenic while inability to concentrate, fatigue and tingling around the mouth were held to be neuroglucopenic. During the procedure the patients were allowed to drink water but otherwise no oral consumption was allowed.

Analyses

Blood glucose was determined by a glucose analyzer (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Plasma free insulin was determined according to Nakagawa et al., using commercial radioimmunoassay (RIA) kits (Pharmacia Diagnostics AB, Uppsala, Sweden) after precipitation of the antibody bound insulin with 25% polyethylene glycol immediately following the blood collection [9]. Plasma adrenaline and noradrenaline were analyzed by high performance liquid chromatography (HPLC) with electrochemical detection [10]. Plasma C-peptide [11], cortisol [12] and growth hormone [13] were analyzed by radioimmunoassays. Glucagon levels were measured with double-antibody radioimmunoassay in duplicate using guinea pig anti-human glucagon antibodies specific for pancreatic glucagon, ¹²⁵I-glucagon as tracer, and glucagon standard (Linco, St Charles, Mo., USA) [14]. Blood samples for the measurement of hormones were collected in plastic tubes containing EDTA or heparin and for plasma glucagon 250 KIU of aprotinin/mL (Trasylol, Bayer, Leverkusen, Germany) was added. After gently mixing, the tubes were immediately placed on ice and afterwards centrifuged at 4°C. Aliquots of plasma were stored at -70°C until assay. All samples from each individual were analyzed in the same determination. The intra- and inter-assay coefficients of variation of determination were 8% and 14% for catecholamines, 6% and 7% for free insulin, and 3% and 7% for glucagon.

Statistical analyses

All results are expressed as mean ± SEM unless otherwise stated. After validation for normal distribution using the Shapiro-Wilk’s test, analysis of variance (ANOVA) repeated measures design and Student’s two-tailed t-test for paired observations were used for statistical evaluation, when applicable. Wilcoxon signed rank test was used for the analysis of the symptoms. The area under the curve was calculated by

<table>
<thead>
<tr>
<th>Table I. Clinical data of the seven type 1 diabetic patients. Data are expressed as mean ± SEM. Data within parenthesis indicate range.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Duration (years)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
</tr>
<tr>
<td>Retinopathy</td>
</tr>
<tr>
<td>Nephropathy</td>
</tr>
<tr>
<td>HbA1c(% ref.&lt;5.2 %)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Basal rate (U/h)</td>
</tr>
<tr>
<td>Bolus doses (U/24h)</td>
</tr>
<tr>
<td>Insulin dose (U/24h)</td>
</tr>
<tr>
<td>SD BG (mmol/l)</td>
</tr>
<tr>
<td>Blood glucose values &lt; 3.0 mmol/l during 4 weeks</td>
</tr>
</tbody>
</table>
the trapezoidal rule. P-values less than 0.05 were considered significant.

RESULTS

The shift from CSII to CIPII reduced the HbA1c level and improved the blood-glucose stability (Table I). No severe hypoglycaemia was reported for the year prior to and after the change in therapy. The incidence of biochemical hypoglycaemia, which was calculated from the same time period as used for the calculation of SD\textsubscript{BG}, showed a tendency to decrease during CIPII (Table I). The total 24-h insulin dose did not change between the periods (Table I). The basal insulin infusion rate was increased during CIPII (Table I), while on these infusion rates the basal peripheral venous insulin levels were lower during CIPII (35.8 ± 2.9 vs. 53.4 ± 3.8 pmol l\textsuperscript{-1}, p<0.01, CIPII vs. CSII). The basal blood glucose levels were similar in the two tests (6.3 ± 0.3 vs. 6.2 ± 0.4 mmol l\textsuperscript{-1}, p=0.66, CIPII vs. CSII).

The identical i.v. bolus of insulin resulted in the expected reduction in blood glucose in all patients during CSII and in 6/7 patients during CIPII. One patient had a nadir blood glucose of only 3.5 mmol l\textsuperscript{-1}, and for that reason he was excluded from further analysis.

In the remaining 6 patients the nadir blood glucose was not different (2.4 ± 0.3 vs. 2.7 ± 0.3 mmol l\textsuperscript{-1}, p=0.17, CSII vs. CIPII, Fig. 1). However when the area under the curve (AUC) for the first hour was calculated, patients on CSII displayed significantly lower blood glucose values (13.0 ± 2.3 vs. 13.7 ± 1.2 mmol l\textsuperscript{-1}h\textsuperscript{-1}, p=0.016, CSII vs. CIPII). The AUC for the full 180 min was not different (42.0 ± 2.3 vs. 40.9 ± 3.1 mmol l\textsuperscript{-1}h\textsuperscript{-1}, p=0.40, CSII vs. CIPII).

A significant increase in the adrenaline, cortisol and growth hormone levels occurred in both experiments with peak values at 60 min (Table II). There was no difference in the responses of growth hormone, cortisol and adrenaline during the CSII and CIPII experiment.

In the CIPII experiment a significant increase in the glucagon level was observed while no such increase was seen during the test on CSII (Table II and Fig. 1). The incremental glucagon response was also significantly more pronounced in the CIPII experiment, expressed as maximal responses (7.5 ± 3.0 vs. 17.0 ± 3.1 pg ml\textsuperscript{-1}, p=0.048, CSII vs. CIPII, Table II) as well as incremental AUC (5.1 ± 12.0 vs. 44.4 ± 13.2 pg ml\textsuperscript{-1}h\textsuperscript{-1}, p=0.027, CSII vs. CIPII). Individual data of ∆ peak glucagon revealed higher values in all patients but one during CIPII, while no such increase in individual data of ∆ peak adrenaline was seen (Fig. 2).

Symptom scores were not different (data not shown).

Fig. 1. Values of arterialised blood glucose, venous plasma free insulin and incremental venous glucagon obtained at insulin induced hypoglycaemia in 6 type 1 diabetic patients during continuous subcutaneous insulin infusion (CSII) (■) and continuous intraperitoneal insulin infusion (CIPII) (□). Values are mean ± SEM. * = p < 0.05, ** = p < 0.01 between the CSII and CIPII experiment. The arrow (†) indicates the i.v. bolus of insulin. The line (—) indicates continuous basal rate insulin infusion.
DISCUSSION

The main finding of this study was that CIPII partly restored the glucagon response to insulin-induced hypoglycaemia in patients with type 1 diabetes. This is an interesting finding since it is well known that the alpha cell is ‘blind’ to hypoglycaemia in patients with type 1 diabetes already after the first 1-2 years of disease duration [15]. All the patients in this study with one exception had much longer disease duration. The exception was one male patient with two years disease duration. However this patient failed to display any glucagon response during the CSII test.

FIG. 2. Values of peak minus basal venous plasma glucagon (left) and peak minus basal venous plasma adrenaline (right) obtained at insulin induced hypoglycaemia in 6 type 1 diabetic patients during continuous subcutaneous insulin infusion (CSII) (■) and continuous intraperitoneal insulin infusion (CIPII) (□). The line (—) indicates the mean value.

TABLE II. Changes in plasma hormone levels from basal level at normoglycaemia to peak level.

<table>
<thead>
<tr>
<th></th>
<th>CSII</th>
<th>CIPII</th>
<th>Mean differences in change between clamps, CSII and CIPII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline, nmol l⁻¹</td>
<td>2.92 (-0.49 ± 6.33) p = 0.04</td>
<td>2.05 (+1.04 ± 3.07) p = 0.004</td>
<td>-0.87 (-3.94 ± 2.21) p = 0.50</td>
</tr>
<tr>
<td>Noradrenaline, nmol l⁻¹</td>
<td>0.74 (-0.24 ± 1.73) p = 0.11</td>
<td>0.91 (+0.46 ± 1.34) p = 0.003</td>
<td>0.17 (-0.76 ± 1.08) p = 0.68</td>
</tr>
<tr>
<td>Glucagon, pg ml⁻¹</td>
<td>7.5 (-0.3 ± 15.3) p = 0.06</td>
<td>17.0 (+9.1 ± 24.9) p = 0.003</td>
<td>9.5 (+0.1 ± 18.9) p = 0.048</td>
</tr>
<tr>
<td>Cortisol, nmol l⁻¹</td>
<td>277 (+199 ± 355) p = 0.0003</td>
<td>286 (+204 ± 369) p = 0.0003</td>
<td>9 (-66 ± 83) p = 0.77</td>
</tr>
<tr>
<td>Growth hormone, mg ml⁻¹</td>
<td>19.3 (+3.3 ± 35.2) p = 0.03</td>
<td>13.4 (+3.6 ± 23.2) p = 0.02</td>
<td>-5.9 (-20.3 ± 8.6) p = 0.34</td>
</tr>
<tr>
<td>Insulin, pmol l⁻¹</td>
<td>42.4 (+13.8 ± 70.9) p = 0.03</td>
<td>66.9 (+24.8 ± 119.0) p = 0.01</td>
<td>14 (-35.2 ± 84.3) p = 0.32</td>
</tr>
<tr>
<td>C-peptide, nmol l⁻¹</td>
<td>0.05 (-0.09 ± 0.20) p = 0.74</td>
<td>0.02 (-0.02 ± 0.05) p = 0.30</td>
<td>-0.03 (-0.17 ± 0.09) p = 0.44</td>
</tr>
</tbody>
</table>

Data are means of differences (n) with 95% confidence interval (range), and p-values denoting the statistical significance of difference (n).
A number of mechanisms can be discussed in relation to the observed improvement in the glucagon secretory response during CIPII. It was obvious that the patients improved their metabolic control during CIPII. However several studies have failed to show any association between the improvement in the metabolic control per se and the glucagon response to hypoglycaemia [16]. It is well known that there is an association between improved metabolic control and an increasing risk for hypoglycaemic events [1].

Furthermore it has repeatedly been demonstrated that one hypoglycaemic episode may attenuate the counterregulatory response to following episodes. One interesting study demonstrated that the meticulous avoidance of hypoglycaemia for three months by increasing the HbA1c level by about 1% partly restored the glucagon response in patients with type 1 diabetes of 4–7 years disease duration [17]. It is obvious that a similar mechanism might operate also in the present study as the CIPII therapy is known to be associated with a reduction in the risk for hypoglycaemic episodes [2, 3]. In this study we cannot rule out such an effect, but in order to find such a difference one would probably require more or less continuous recordings of the blood glucose. In the absence of such measurements we observed a non-significant tendency towards less episodes of biochemical hypoglycaemia during home blood glucose monitoring for the estimation of the blood glucose stability index. It should be mentioned that our overall frequency of episodes of hypoglycaemia was low compared to other studies [18, 19] and that no severe episodes, defined as an episode for which the treatment required help of another person, was reported.

The autonomic nervous system is of importance for an intact glucagon response to hypoglycaemia [20] and autonomic neuropathy has been associated with defects in the counterregulatory response to hypoglycaemia [21]. It has been demonstrated that an improvement in metabolic control can delay the progression of autonomic neuropathy [22], but to the best of our knowledge there is no evidence of an improvement of the function of the autonomic nervous system by intensified insulin therapy. Adrenaline is another known factor that has been shown both in vitro [23] and in vivo [24] to stimulate glucagon secretion. This mechanism may be of importance in patients with type 1 diabetes [25]. However, in the present study the adrenaline response was of similar magnitude in both tests. Therefore, adrenaline could not be the explanation for the improved glucagon response during CIPII. Several previous studies have shown that a high concentration of circulating insulin may decrease the release of glucagon to hypoglycaemia. This was shown in healthy subjects as well as in type 1 diabetes [26, 27]. In the present study a significant glucagon rise was seen at 60 min and onwards, at a time when the insulin concentration returned towards the low basal range, as a result of the continuous administration of insulin via the s.c. and i.p. routes, respectively. The lower peripheral circulating insulin levels during CIPII [28], might therefore have contributed in explaining the partly restored glucagon release during hypoglycaemia. In a recent study it was speculated that the hepatic sensitivity to glucagon might be enhanced when the patients were exposed to a less pronounced chronic hyperinsulinemia [29]. Certainly if such an effect could be verified it would indicate a more important role for glucagon in the counterregulatory machinery. We believe that the partly restored glucagon response, and perhaps the enhanced hepatic sensitivity to glucagon, contributed to the less pronounced hypoglycaemic effect by i.v. insulin during the CIPII test. This effect occurred in spite of the higher basal rate of insulin infusion by the pump during the CIPII test.

The magnitude of the glucagon response in this study could seem small, but it has been previously shown that a minimal increase in glucagon levels can have major effects on hepatic glucose production [30]. Thus, in this previous study a similar rise in the plasma glucagon level as in the present study resulted in the hepatic glucose production transiently increasing by about 50%.

In conclusion it seems that CIPII in type 1 diabetic patients could improve the glucagon release and result in a less pronounced fall in blood glucose to an i.v. bolus of insulin. These findings may contribute in explaining why CIPII is associated with a lower incidence of hypoglycaemia in spite of an improvement in metabolic control.

Acknowledgement – This work was supported by grants from the Bert von Kantzow Foundation, the Karolinska Institute and the Swedish Medical Research council (Grant No. 19x-6389).

REFERENCES


