Amelioration of Sensory Nerve Dysfunction by C-Peptide in Patients With Type 1 Diabetes

Karin Ekberg, Tom Brismar, Bo-Lennart Johansson, Björn Jonsson, Per Lindström, and John Wahren

Studies have demonstrated that proinsulin C-peptide stimulates the activities of Na^+,K^+-ATPase and endothelial nitric oxide synthase, both of which are enzyme systems of importance for nerve function and known to be deficient in type 1 diabetes. The aim of this randomized double-blind placebo-controlled study was to investigate whether C-peptide replacement improves nerve function in patients with type 1 diabetes. Forty-nine patients without symptoms of peripheral neuropathy were randomized to either 3 months of treatment with C-peptide (600 nmol/24 h, four doses s.c.) or placebo. Forty-six patients (15 women and 31 men, aged 29 years, diabetes duration 10 years, and HbA1c 7.0%) completed the study. Neurological and neurophysiological measurements were performed before and after 6 and 12 weeks of treatment. At baseline the patients showed reduced nerve conduction velocities in the sural nerve (sensory nerve conduction velocity [SCV]: 50.9 ± 0.70 vs. 54.2 ± 1.2 m/s, P < 0.05) and peroneal nerve (motor nerve conduction velocity: 45.7 ± 0.55 vs. 53.5 ± 1.1 m/s, P < 0.001) compared with age-, height-, and sex-matched control subjects. In the C-peptide treated group there was a significant improvement in SCV amounting to 2.7 ± 0.85 m/s (P < 0.05 compared with placebo) after 3 months of treatment, representing 80% correction of the initial reduction in SCV. The change in SCV was accompanied by an improvement in vibration perception in the patients receiving C-peptide (P < 0.05 compared with placebo), whereas no significant change was detectable in cold or heat perception. In conclusion, C-peptide administered for 3 months as replacement therapy to patients with early signs of diabetic neuropathy ameliorates nerve dysfunction. Diabetes 52: 536–541, 2003

Diabetic neuropathy is defined by the presence of detectable sensory, motor, and autonomic deficits on clinical examination, with or without the presence of dysesthetic or paresthetic symptoms (1). Factors of importance for the pathogenesis of diabetic neuropathy are formation of advanced glycation end products and polyol pathway activation following hyperglycemia, reduced nerve Na^+,K^+-ATPase activity, and microvascular abnormalities, e.g., reduced endoneurial perfusion (2). Although several studies demonstrate that it is possible to retard the progression of diabetic complications by intensified insulin treatment, development of neuropathy cannot be prevented (3–5). Recently, interest has focused on the possibility that C-peptide, previously considered to lack biological activity, may play a beneficial role in the treatment and prevention of diabetic complications (6).

During the last decade, C-peptide has been found to exert a number of physiological effects in patients with type 1 diabetes (6). These effects are probably mediated via a G-protein–coupled membrane receptor (7,8). Upon stimulation, a Ca^2+-dependent signaling pathway is activated, resulting in a stimulation of Na^+,K^+-ATPase and endothelial nitric oxide synthase (eNOS) activities (8,9). Both of these enzyme systems are known to be deficient in diabetes (10,11). C-peptide has been shown to exert beneficial effects on both diabetic nephropathy and neuropathy in animal models of type 1 diabetes and in patients with type 1 diabetes. These effects are partly related to the ability of C-peptide to stimulate blood flow and promote capillary recruitment in peripheral tissues, but possibly also to direct stimulation of Na^+,K^+-ATPase (8,12). In the early stages of diabetes C-peptide reduces glomerular hyperfiltration (13,14), and in patients with incipient diabetic nephropathy C-peptide replacement results in diminished urinary albumin excretion rate (15). Similar effects have been demonstrated during short- and long-term administration of C-peptide in streptozotocin (STZ)-induced diabetic rats (STZ rats), where it has been demonstrated that the beneficial effects of C-peptide are accompanied by corrections of morphologic abnormalities secondary to the diabetic state (16,17). C-peptide replacement in BB/Wor rats partially reverses acute and chronic metabolic, functional, and structural changes in peripheral nerves following the onset of diabetes (12). The diabetes-induced reduction in motor nerve conduction velocity was arrested by C-peptide, and the Na^+,K^+-ATPase activity of the
nerves was partly restored in rats treated with replacement doses of C-peptide during 3–8 months. Furthermore, C-peptide treatment resulted in decreased paranoval swelling and demyelination, decreased axonal degeneration, and augmented regenerative activity (12). In addition to the stimulation of Na⁺,K⁺-ATPase activity, the C-peptide effects on nerve function and structure may be mediated by an improved nerve microcirculation secondary to stimulation of vasa nervorum eNOS activity. In STZ rats treated with C-peptide for 2 weeks, the improvement in motor and sensory nerve conduction velocity was accompanied by a near normalization of nerve blood flow (18).

Only little information is available with regard to the possible influence of C-peptide on nerve function in humans. However, C-peptide augments the capacity of the heart to adjust to fluctuations in venous inflow during deep breathing in patients with early signs of autonomic neuropathy, as indicated by improved heart rate variability during respiratory excursions (19). After only a few years of type 1 diabetes, asymptomatic patients present with reduced nerve conduction velocities (20). The aim of the present study was to examine whether C-peptide may exert a positive influence on early peripheral nerve function abnormalities in patients with type 1 diabetes. Specifically, the effect of 3 months of C-peptide treatment on peripheral nerve conduction velocity and other early signs of diabetic neuropathy were investigated.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Approximately 200 patients with type 1 diabetes, who previously reported for participation in scientific studies, were screened for the following inclusion criteria: aged 20–40 years, diabetes duration 5–15 years, BMI <30 kg/m², HbA₁c <10%, serum creatinine <120 μmol/l, and plasma C-peptide <0.2 nmol/l. The patients did not have any symptoms of diabetic peripheral polyneuropathy and were not on treatment that might influence nerve function, e.g., cytostatic, tricyclic antidepressive, or antiepileptic agents, nor were they on treatment with sympatheticomimetic agents, ACE inhibitors, β-blockers, or Ca²⁺-channel blockers. A total of 54 type 1 diabetes patients were enrolled for the study, and 49 patients were randomized to either active therapy or placebo. None of the patients had signs of diabetic nephropathy, but eight (six in the placebo group) had diabetic simplex retinopathy and one had diabetic autonomic neuropathy.

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**Protocol.** The study was carried out in a double-blind placebo-controlled randomized fashion with two study arms: treatment with either C-peptide or placebo for 3 months. After giving informed consent, patients underwent a physical examination, including a neurological evaluation, electrocardiogram at rest and during deep breathing, blood pressure measurement, and clinical chemistry laboratory testing. In addition, samples for C-peptide levels in plasma and urine were collected. Furthermore, a neurophysiological examination was carried out including measurements of nerve conduction velocities (NCV), and sensory function was assessed by quantitative sensory testing (QST). The patients found to fit the inclusion criteria were randomized to either of the treatment groups; 60% were assigned to treatment with C-peptide and 40% to placebo. The patients were instructed to take the trial medication at least 1 h after food. Intravenous injections of 120 nmol C-peptide in connection with their regular insulin administration in the morning, at lunch, and at dinner and 240 nmol C-peptide at bedtime or an equivalent volume of C-peptide diluent (placebo). C-peptide was recombinantly produced and kindly provided by Schwarz Pharma, Monheim, Germany. This C-peptide dose regimen, 600 nmol/24 h, is equimolar to a dose of 100 IU insulin and is expected to result in C-peptide plasma levels within the physiological range during 18–20 h per day. After 6 weeks of treatment, renewed assessments of NCV were performed, and after 12 weeks, NCV and QST were measured again. Thereafter, the study was ended and the trial medication discontinued. Patient compliance with the treatment was checked by visual control of the returned medication vials and on the basis of analyses of C-peptide concentration in urine and plasma samples during and at the end of the study (evaluation performed by an independent analyst). Randomization and blinding of the trial medication was performed by the Karolinska Hospital Pharmacy, and source data verification was monitored by an external monitor (Vindslås Konsult, Sollentuna, Sweden).

**Sensory function and neurophysiological assessments.** Motor nerve conduction velocity (MCV) and compound muscle action potential amplitude (CMAP) in the peroneal nerve and sensory nerve conduction velocity (SCV) and sensory nerve action potential amplitude (SNAP) in the sural nerve were measured bilaterally as previously described (20) using surface electrodes and digital equipment for stimulation and recording (Neuropak 2; Nihon-Kohden, Kyoto, Japan). The assessments were performed under strictly standardized conditions in a warm room, with the legs warmed with heat pads for at least 10 min before the nerve conduction measurements in order to obtain skin temperatures at ±3°C. The coefficient of variation (CV) for MCV and SCV, respectively, was 2.7 and 4.9%, based on determinations made in the same individual on separate occasions. The QST included measurements of perception thresholds for vibration, heat and cold. A vibrating probe (Vibratometer; Somedic, Stockholm, Sweden) was applied over the first metatarsal to allow evaluation of the vibration perception. Temperature threshold determinations were done with the Marstock technique using a temperature-regulated probe (Thermostat, Somedic, Stockholm, Sweden) (21). The probe was applied over the dorsum of the feet, and the patient reported temperature sensations by pressing a button on perception of heat, cold, etc. All respective measurements of perception thresholds were done bilaterally and in triplicate, and the mean of all six measurements was used in the statistical analysis.

**Analyses.** Clinical chemistry variables, including HbA₁c, were determined according to the standard procedures at the Department of Clinical Chemistry at the Karolinska Hospital. C-peptide concentrations in plasma and urine were analyzed by radioimmunoassay using a commercial kit (Eurodiagnostica, Malmö, Sweden).

**Statistical methods.** Power analysis was performed on the basis of the results published by Hylliemark et al. (20), who reported that the difference in nerve conduction velocity (NCV) in type 2 diabetes patients treated with C-peptide compared to placebo was 4 m/s, with a 0.05 two-sided significance level, would have 80% power to detect the difference at a 5% change of NCV in the C-peptide–treated group and no change in the placebo group, assuming that the common standard deviation (SD) was 4 m/s.

All data are presented as means ± SE. Statistical tests were performed using nonparametrical methods. The Mann-Whitney two-sample test was applied in comparisons between groups. When comparing changes within groups, Wilcoxon’s signed-rank test was used. The data were analyzed on a per protocol basis, i.e., including only those subjects of the full analysis set who did not show major protocol violations. The decision whether a protocol violation should be considered minor or major was made by a panel including the trial manager and the investigators before the randomization code was opened. The safety analysis data set included all subjects who received at least one dose of C-peptide.

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TABLE 1
Characteristics of the patient population before the start of the study

<table>
<thead>
<tr>
<th></th>
<th>C-peptide</th>
<th>Placebo</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30 ± 1.4</td>
<td>28 ± 1.4</td>
</tr>
<tr>
<td>Proportion of women</td>
<td>7/26</td>
<td>8/20</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>76 ± 1.7</td>
<td>75 ± 2.9</td>
</tr>
<tr>
<td>Body height (m)</td>
<td>1.77 ± 0.02</td>
<td>1.76 ± 0.02</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Daily insulin requirement (units/24 h)</td>
<td>9.7 ± 0.7</td>
<td>10 ± 0.7</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121</td>
<td>123 ± 2.9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73.0</td>
<td>75 ± 1.6</td>
</tr>
</tbody>
</table>

Data are means ± SE. None of the variables differed significantly between the two patient groups.

RESULTS
The baseline characteristics of the patients are presented in Table 1. There were no statistically significant differences between the two treatment groups. Twenty-two of the patients in the C-peptide group (85%) and 12 in the placebo group (60%) had plasma C-peptide levels at baseline below the detection limit of the assay (<0.10 nmol/l), and in the remaining patients C-peptide ranged between 0.13 and 0.20 nmol/l. During the C-peptide treatment, plasma C-peptide levels were restored to physiological levels (1.3 ± 0.15 nmol/l; measured ~3 h after subcutaneous injection of C-peptide). The level of metabolic control, as reflected by HbA1c, was similar in the two groups (Table 1), and there was no change in this variable during the study; after 12 weeks of treatment HbA1c was 7.0 ± 0.27 vs. 6.6 ± 0.14% (NS) in the C-peptide and placebo groups, respectively. No adverse drug reactions, adverse events that could be related to the trial medication, or significant changes in safety variables (blood chemistry and vital signs) were observed during the study period.

The baseline NCV in the sural nerve (SCV) in the diabetic patients was 50.9 ± 0.70 m/s, which was significantly lower than in the healthy control subjects (54.2 ± 1.24 m/s, P < 0.05) (Table 2). Conduction velocity in the peroneal nerve (MCV) was also significantly reduced in the diabetic patients (45.7 ± 0.55 vs. 53.5 ± 1.13 m/s, P < 0.001). There was no significant difference in either SCV or MCV between the two treatment groups at baseline. Neither CMAP nor SNAP showed statistically significant differences between groups or between diabetic patients and control subjects. During the study there was a significant increase in SCV in the C-peptide group after only 6 weeks of treatment (Table 3), and after 12 weeks this improvement amounted 2.7 ± 0.85 m/s (P < 0.01), corresponding to an increase of 5% (Fig. 1). The improvement in SCV was greater in patients with low SCV at baseline compared with those with a less marked SCV reduction initially. No statistically significant change in SCV was seen in the placebo-treated patients, and the response in the C-peptide–treated group was significantly different from that in the placebo-treated group (P < 0.05). Regarding MCV, after 6 weeks of C-peptide treatment, there was a small significant change in the MCV (P < 0.05), but this improvement was not apparent at 12 weeks, nor was the change statistically different from that of the placebo group.

C-PEPTIDE AND SENSORY NERVE FUNCTION

The baseline NCV in the sural nerve (SCV) in the diabetic patients was 50.9 ± 0.70 m/s, which was significantly lower than in the healthy control subjects (54.2 ± 1.24 m/s, P < 0.05), whereas similar values for cold and vibration perception were found in the diabetic patients and healthy control subjects (Table 2). After C-peptide or placebo treatment there was no significant change in temperature perception. However, after 12 weeks of C-peptide treatment the vibration threshold had decreased from 0.63 ± 0.07 to 0.48 ± 0.05 arbitrary units (P < 0.05, Table 3), which was a statistically significant improvement compared with placebo (P < 0.05).

DISCUSSION
The type 1 diabetic patients included in the study did not present with symptoms of diabetic neuropathy, and their average diabetes duration was only 10 years. Yet, in comparison with the group of healthy control subjects, the diabetic patients showed significantly reduced SCV and MCV and diminished sensory perception. These changes are likely to reflect early circulatory and metabolic disturbances of the peripheral nerves rather than structural changes, and the sensory defects indicate the presence of subclinical neuropathy. The present study shows for the first time in patients that C-peptide in replacement doses has the capacity to improve the early neurological changes that accompany type 1 diabetes, as previously demonstrated in rat models of type 1 diabetes (12,18,22). The NCV in the sural nerve had increased significantly after only 6 weeks of C-peptide treatment. This effect was further accentuated at 12 weeks of treatment, and the response was statistically different from that in the control group (P < 0.05). The C-peptide–induced improvement amounted to 2.7 m/s, which corresponds to an 80% restoration of the initial SCV deficit in these patients (50.9 ± 0.70 m/s before treatment vs. 54.2 ± 1.24 m/s in the healthy subjects). SNAP was in the normal range in the diabetic patient group, and C-peptide treatment was not accompanied by an increase in this variable. In the placebo group SNAP increased during the study, but the change during the treatment period was not statistically different in the two study groups. Blood glucose control was good in all patients and unchanged throughout the study. Conse-

TABLE 2
Neurophysiological and sensory variables in patients and healthy control subjects at baseline

<table>
<thead>
<tr>
<th></th>
<th>Healthy control subjects</th>
<th>C-peptide</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>MCV (m/s)</td>
<td>53.5 ± 1.13*</td>
<td>44.9 ± 0.79</td>
<td>46.7 ± 0.71</td>
</tr>
<tr>
<td>CMAP (mV)</td>
<td>8.38 ± 0.55</td>
<td>6.96 ± 0.50</td>
<td>7.60 ± 0.41</td>
</tr>
<tr>
<td>SCV (m/s)</td>
<td>54.2 ± 1.24†</td>
<td>50.5 ± 0.89</td>
<td>51.4 ± 1.12</td>
</tr>
<tr>
<td>SNAP (µV)</td>
<td>19.2 ± 2.37</td>
<td>16.3 ± 1.81</td>
<td>15.8 ± 1.92</td>
</tr>
<tr>
<td>Cold threshold</td>
<td>2.03 ± 0.17</td>
<td>3.4 ± 0.55</td>
<td>2.1 ± 0.17</td>
</tr>
<tr>
<td>Heat threshold</td>
<td>6.46 ± 0.51†</td>
<td>8.0 ± 0.43</td>
<td>7.0 ± 0.47</td>
</tr>
<tr>
<td>Vibration threshold</td>
<td>0.52 ± 0.10</td>
<td>0.63 ± 0.07</td>
<td>0.48 ± 0.07</td>
</tr>
</tbody>
</table>

Data are means ± SE for both legs. *P < 0.001; †P < 0.05 for difference between diabetic patients and healthy control subjects. None of the variables differed significantly between the two patient groups.
quently, metabolic control is unlikely to have influenced NCV in this study. In accordance with previous observations in type 1 diabetic patients (20), the NCV deficit in the peroneal nerve before treatment was more pronounced than in the sural nerve. After 6 weeks of treatment, there was a modest effect of C-peptide on MCV (P < 0.05), but this effect was no longer apparent after 12 weeks. The difference in motor and sensory nerve response may be related to different mechanisms underlying the conduction defect in diabetes, including reductions in NCV either induced by hyperglycemia or related to the characteristic distal axonal degeneration of dying back type in this disorder (23). It is conceivable that hyperglycemia or related factors are responsible for the decrease in MCV, which appears early in diabetes in absence of clinical symptoms, and which shows a relationship to the level of metabolic control (20). In contrast, the pathogenesis of the distal axonopathy of dying back type involving the sensory nerves in diabetes may be different and include factors such as hypoxia, reduced levels of Na⁺,K⁺-ATPase, and possibly altered operation of nerve growth factors (rev. in 23). In the present study, the significant improvement of SCV after 6 and 12 weeks of treatment showed no correlations to HbA₁c, and it is suggested that C-peptide is capable of influencing the latter pathogenic factors.

Analogous with the findings for NCV, there was a proportionate response in the QST results. C-peptide treatment for 3 months resulted in an improved vibration perception, which is in good agreement with the previous observations (24), the C-peptide treatment resulted in increased nerve Na⁺,K⁺-ATPase activity (12). Recently, it was demonstrated that improvements in MCV and SCV following C-peptide replacement in rats with STZ-induced diabetes was accompanied by a marked improvement of nerve blood flow (18). This improvement was completely abolished in the presence of a NOS blocker, and the effect is thus most likely mediated via C-peptide’s ability to stimulate endothelial nitric oxide production and subsequently blood flow, as previously demonstrated for other tissues (9,25–28).

It is primarily in C-peptide–deficient patients that a series of different physiological effects of C-peptide have been demonstrated. Healthy subjects, with normal C-peptide plasma concentrations, show no detectable response to C-peptide administration. A possible explanation to this phenomenon derives from in vitro experiments, in which half-saturation of C-peptide binding was already demonstrated at 0.3 nmol/l and full saturation at 0.9 nmol/l (7). Because full saturation of C-peptide binding sites already occurs at the ambient C-peptide concentration in healthy

**Table 3**

Change from baseline in neurophysiological and sensory variables after 6 and 12 weeks of C-peptide and placebo

<table>
<thead>
<tr>
<th></th>
<th>C-peptide (n = 26)</th>
<th>Placebo (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>MCV (m/s)</td>
<td>0.8 ± 0.32*</td>
<td>0.1 ± 0.29</td>
</tr>
<tr>
<td>CMAP (mV)</td>
<td>0.4 ± 0.36</td>
<td>−0.1 ± 0.29</td>
</tr>
<tr>
<td>SCV (m/s)</td>
<td>1.6 ± 0.76*</td>
<td>2.7 ± 0.85†‡</td>
</tr>
<tr>
<td>SNAP (µV)</td>
<td>0.6 ± 1.10</td>
<td>0.7 ± 1.19</td>
</tr>
<tr>
<td>Cold threshold (AU)</td>
<td>−</td>
<td>0.3 ± 0.46</td>
</tr>
<tr>
<td>Heat threshold (AU)</td>
<td>−</td>
<td>0.3 ± 0.31</td>
</tr>
<tr>
<td>Vibration threshold (AU)</td>
<td>−0.2 ± 0.07†‡</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SE. Improvements in nerve function are indicated by positive differences for MCV, CMAP, SCV, and SNAP and negative differences for cold, heat, and vibration perception thresholds. AU, arbitrary units. *P < 0.05 and †P < 0.01 for change from baseline. ‡P < 0.05 for change greater than in the placebo group.

The progression of diabetic neuropathy as evaluated by sciatic tibial MCV measurements, and significant improvement in MCV was seen after 8 months of treatment. In addition, examination of sensory nerve (sural) morphology after C-peptide treatment demonstrated significantly less marked structural alterations (reduced paranodal swelling and demyelination, increased number of intercalated nodes, and regenerating fibers) compared with untreated diabetic rats. Furthermore, and in agreement with previous observations (24), the C-peptide treatment resulted in increased nerve Na⁺,K⁺-ATPase activity (12).
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