Insulin-Mediated Increase in Sympathetic Nerve Activity Is Attenuated by C-Peptide in Diabetic Rats

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Connecting peptide (C-peptide) is secreted along with insulin in equimolar amounts into portal circulation in response to beta cell stimulation. The biological function of C-peptide had been mostly limited to establishing the secondary and tertiary structure of proinsulin. Recent studies have suggested that C-peptide can impact several functions, such as autonomic and sensory nerve function, insulin secretion, and microvascular blood flow. In this study we examined the effects of C-peptide in the presence or absence of insulin on cardiovascular and sympathetic nerve activity in both normal and streptozotocin (STZ)-induced diabetic Wistar rats. Animals were made diabetic by a single intravenous injection of STZ (50 mg/kg) and maintained for 6 weeks. The diabetic animals had higher plasma glucose, lower plasma insulin, and C-peptide, compared with the normal animals. To characterize cardiovascular and autonomic nervous responses, the animals were anesthetized with urethane/α-chloralose and instrumented for the recording of mean arterial pressure (MAP), heart rate (HR), and lumbar sympathetic nerve activity (LSNA). A bolus administration of C-peptide alone did not alter MAP, HR, or LSNA in normal or diabetic animals. The bolus administration of insulin alone increased HR and LSNA in normal and diabetic animals. However, the administration of insulin plus C-peptide attenuated the increase in HR in normals and the increase in LSNA in diabetic rats. We concluded that the C-peptides play a role in modulating the insulin-stimulated sympathetic nerve response. Exp Biol Med 229:80–84, 2004

Key words: diabetes; autonomic nervous system insulin; C-peptide

Connecting peptide (C-peptide) is a product of proinsulin processing to insulin and is secreted by the pancreatic beta cells in equimolar amounts along with insulin (1, 2). It was thought that C-peptide's main physiological role was in facilitating the folding of the proinsulin molecule in a manner that allowed for the appropriate formation of the disulfide bonds between the cysteine residues of the A- and B-chains of insulin (1, 2). Early studies with C-peptide focused on its effects on plasma glucose, insulin, and glucagon levels. These studies failed to report significant effects of C-peptide (3, 4). Recently, however, it has been demonstrated that C-peptide treatment of type 1 diabetic patients resulted in improved renal function, increased blood flow, augmented glucose utilization, and improved autonomic and somatic nerve function (1, 4–8). These beneficial effects correlated with stimulation of both Na^+/K^+-ATPase and endothelial nitric oxide synthase activities (1, 9, 10). Although C-peptide did not affect diabetic hyperglycemia, it was demonstrated to have other insulin-like effects (11).

In studies in which patients with peripheral neuropathy were treated with C-peptide, it was demonstrated that it significantly improved the heart rate variability associated with breathing. On the other hand, insulin treatment alone did not change heart rate variability; insulin may have caused minor deterioration (5). C-peptide administration has also been associated with improvement in sensory nerve function as assessed by temperature threshold discrimination measurements (12). Physiological concentrations of C-peptide (0.3–3 n*M*) has been demonstrated to activate insulin receptor tyrosine kinase, insulin receptor substrate-1 tyrosine phosphorylation, phosphatidylinositol 3-kinase, mitogen-activated protein kinase phosphorylation, p90Rsk, and GSK3 phosphorylation in rat L6 cells (11). Because of the above observation that C-peptide may exert selected effects primarily via the insulin-signaling pathway, we evaluated the effect of C-peptides on the insulin-mediated cardiovascular and autonomic nervous responses in normal and diabetic rats.

Materials and Methods

Male Wistar rats (Harlan, Indianapolis, IN) weighing 250–350 g were housed two to a cage in a temperature-controlled environment (23°C) with a cycle of light and darkness. The rats were given rodent chow and water ad libitum.

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Table 1. Body Weight and Basal Levels of Blood Insulin, C-Peptide, and Glucose in Nonfasted Normal and Diabetic Rats[a]

	Body weight (g)	Insulin (pmol/ml)	C-Peptide (pmol/ml)	Glucose (mg/dl)
Normal animal ($N = 13$)	355 ± 10	0.107 ± 0.132	1.66 ± .01	174 ± 4.99
Diabetic animal ($N = 13$)	273 ± 10*	0.031 ± 0.056*	0.302 ± 0.039*	503 ± 13.2*

[a] The values represent the mean ± SEM.
* $P < 0.05$ versus normal, Student's t test.

Diabetes was induced in normal rats by a single intravenous tail vein injection of streptozotocin (STZ) (50 mg/kg dissolved in sodium citrate, 0.1 n*M*, pH 4.5). One week later a blood sample was collected from infraorbital bleeding and diabetes was confirmed by a blood glucose level >300 mg/dl. Glucose was measured using an analyzer (Yellow Springs Instrument Co, Yellow Springs, OH). Diabetic animals were maintained 6 weeks without insulin supplements. At the end of 6 weeks, plasma was collected and insulin (ICN Pharmaceuticals, Orangeburg, NY) and C-peptide (Linco Research, St. Charles, MO) concentrations were determined.

On the day of the study, normal or diabetic rats that were fasted for 18–24 hrs were anesthetized with urethane (500 mg/kg) and α-chloralose (80 mg/kg) and placed on a heating pad. A rectal thermometer (61161-280, VWR, Chicago, IL) was inserted, and body temperature was maintained at 37°C ± 1.0°C. A tracheotomy was performed to minimize respiratory difficulties. Catheters filled with heparinized saline (2000 U/ml) were inserted into the left femoral artery for monitoring the cardiovascular parameters and into the left and right femoral vein for hormone administration and the continuous infusion of anesthetic (α-chloralose [33 mg/ml per hour] and urethane [5.3 mg/ml per hour]), respectively.

Recording of sympathetic nerve activity was made from the lumbar sympathetic nerve as described in previous studies with minor modification (13). Following isolation and connecting the nerve for recording, the abdomen was closed to prevent evaporation. Lumbar sympathetic nerve activity (LSNA) was amplified (5,000–10,000 times) and filtered (low 30 Hz, high 1000 Hz) using a Grass RPS 107 amplifier and a Grass IR Z probe. The amplified and filtered signal was channeled to an oscilloscope HM205. An audio amplifier-loudspeaker (Grass model AM8 audio monitor) was used for auditory evaluation. Rectifying and integrating the action potentials in 1-sec intervals using data acquisition system obtained whole nerve activity. At the end of each experiment, the animal was sacrificed, and any residual output from the nerve was subtracted as noise when nerve activity was calculated. Because the absolute value of the nerve activity is dependent on the recording conditions (i.e., size of nerve bundle, amount of tissue fluid around the nerve), and these nonphysiological factors vary in different preparations, nerve activity data were normalized as a percentage of the baseline nerve activity for the graphs. Basal nerve activity calculated by using nerve activity minus death activity divided by amplification. Data for graphs were obtained by taking 3-min averages starting 21 mins before injection and going to 60 mins post injection.

Table 2. Basal Values for Heart Rate and Mean Arterial Pressure in Normal and Diabetic Rats[a]

	Heart rate (bpm/min)	Mean arterial pressure (mm Hg)
Normal animal ($N = 20$)	367 ± 13	92 ± 2
Diabetic animal ($N = 20$)	339 ± 9	89 ± 3

[a] The values represent the mean ± SEM.
* $P < 0.05$ versus normal, Student's t test.

Following establishing a control period for mean arterial pressure (MAP), heart rate (HR), and LSNA, a bolus of C-peptide was administered to achieve a plasma concentration of approximately 1.10 nmol/ml of plasma. In control studies, a scrambled version of the 31-amino acid C-peptide (scrambled C-peptide) was used. The total volume of each injection was 0.2 ml/animal. The MAP, HR, and LSNA were monitored continuously for 60 mins.

In other studies the animals were also given a bolus administration of insulin alone (16 nmol/ml) or bolus administration of insulin plus C-peptide. The MAP, HR, and LSNA were recorded as described above.

Data are reported as the mean ± SEM. Repeated-measures analysis of variance was used to compare different groups. A Student's t test was used to compare pairs of means at selected time points between two groups.

Results

Body weights and basal levels of plasma, glucose, insulin, and C-peptide in nonfasted normal and diabetic animals can be seen in Table 1. The diabetic animals were smaller and had significantly higher glucose, with lower insulin and C-peptide levels when compared with normal controls.

The basal MAP, HR, and average LSNA were not different in normal and diabetic animals (Table 2 and Fig.

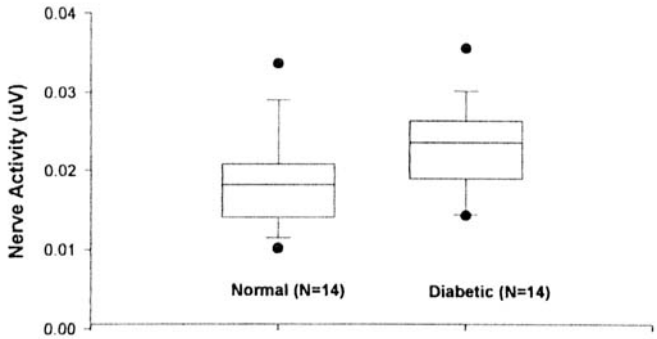

Figure 1. Basal value of LSNA (microvolts) normal and diabetic animal. Nerve activity, expressed as microvolts, was calculated using the basal nerve activity minus the death time activity divided by the amplification. $N = 14$ for controls and 14 for diabetics, respectively.

1). The administration of C-peptide alone or scrambled C-peptide (control) did not affect MAP, HR, and LSNA in normal or diabetic rats (Figs. 2 and 3, respectively).

The bolus administration of insulin with or without C-peptide significantly increased LSNA in normal rats 18 mins post injection and remained increased for the duration of the experiment ($P < 0.05$; Fig. 4A). Also, the bolus administration of insulin with or without C-peptide significantly increased MAP in normal rats for only the first 5–7 mins post injection ($P < 0.05$). MAP returned to baseline and did not change for the duration of the experiment (Fig. 4B). The

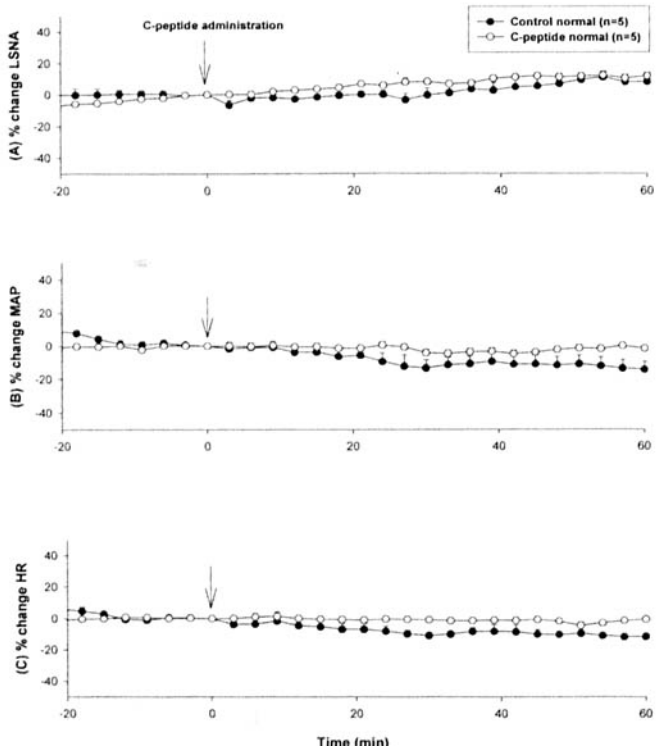

Figure 2. Effect of C-peptide administration or scrambled C-peptide ($C\text{-peptide}_{scr}$) on LSNA (A), MAP (B), and HR (C) in normal male Wistar rats. Arrow denotes time of administration of C-peptide or scrambled C-peptide. Values are expressed as percent change from baseline at time 0. $N = 5$ for both groups.

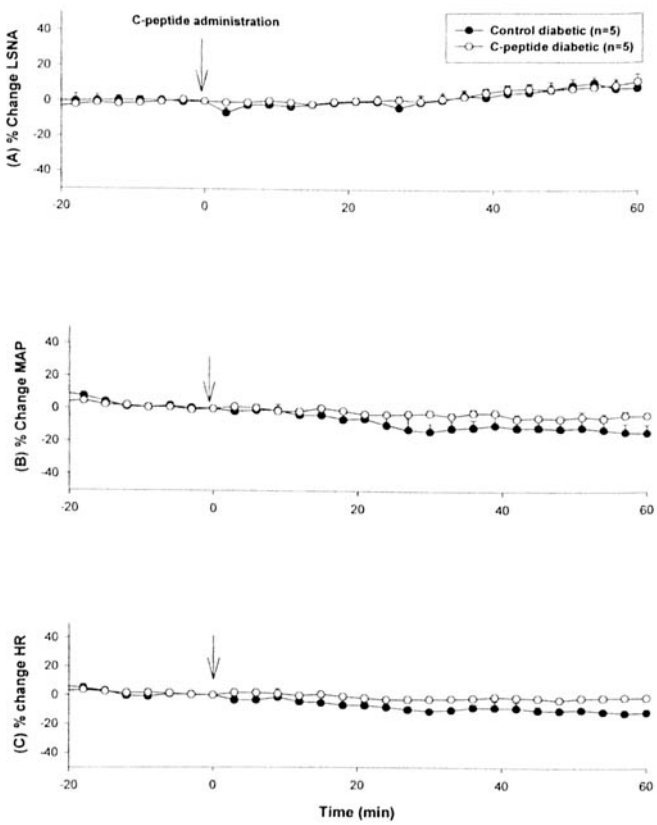

Figure 3. Effect of C-peptide administration or scrambled C-peptide ($C\text{-peptide}_{scr}$) on LSNA (A), MAP (B), and HR (C) in diabetic male Wistar rats. Arrow denotes time of administration of C-peptide or scrambled C-peptide. Values are expressed as percent change from baseline at time 0. $N = 5$ for both groups.

bolus administration of insulin significantly increased heart rate in normal rats after 20 mins post injection and remained increased for the duration of the experiment ($P < 0.05$; Fig. 4C). However, the bolus administration of insulin plus C-peptide significantly attenuated the insulin induced increase in heart rate in normal rats (Fig. 4C). The administration of insulin in diabetic animals again increased the LSNA over basal levels, but the addition of C-peptide significantly attenuated the insulin-induced increase (Fig. 5A). The MAP and HR of the diabetic rat were not affected by insulin alone or insulin plus C-peptide (Fig. 5B and 5C).

Discussion

We observed that C-peptide alone did not affect MAP, HR, or LSNA. However, the major effect of C-peptide was on insulin-induced cardiovascular and nervous responses. When C-peptide was administered in conjunction with insulin, we observed an attenuation of the insulin-induced increase in HR in normal and sympathetic activity in the diabetic animal. This study is consistent with other studies that have demonstrated the permissive role insulin plays with respect to C-peptide's physiological actions (1, 6, 14).

It has previously been demonstrated in our laboratory that insulin can act to increase both LSNA and HR in normal

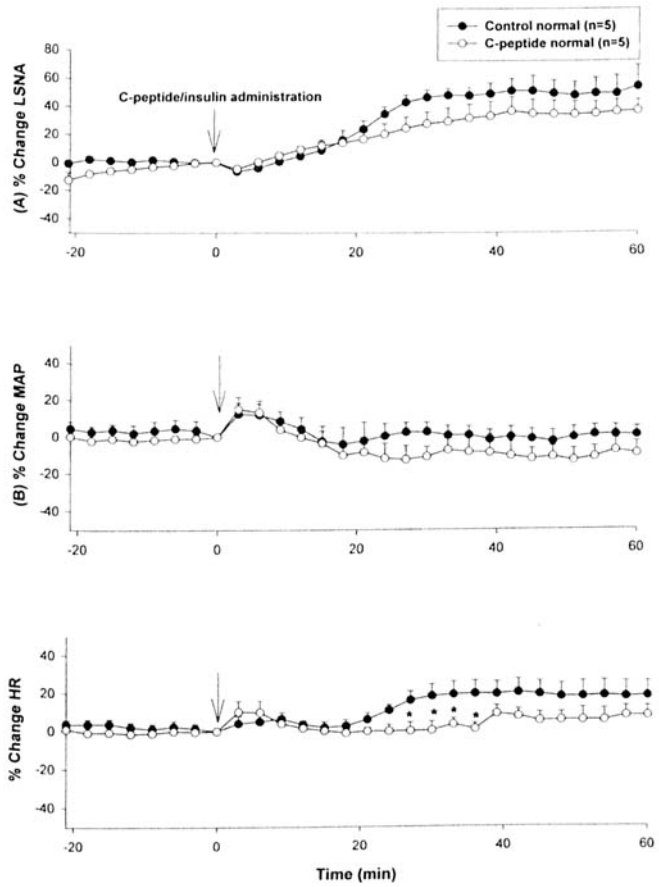

Figure 4. Effect of C-peptide with or without insulin (2 U/kg) LSNA (A), MAP (B), and HR (C) on in normal male Wistar rats. Arrow denotes time of insulin or insulin plus C-peptide administration. Values are expressed as percent change from baseline at time 0. $N=5$ for both groups. *, $P < 0.05$ versus insulin alone.

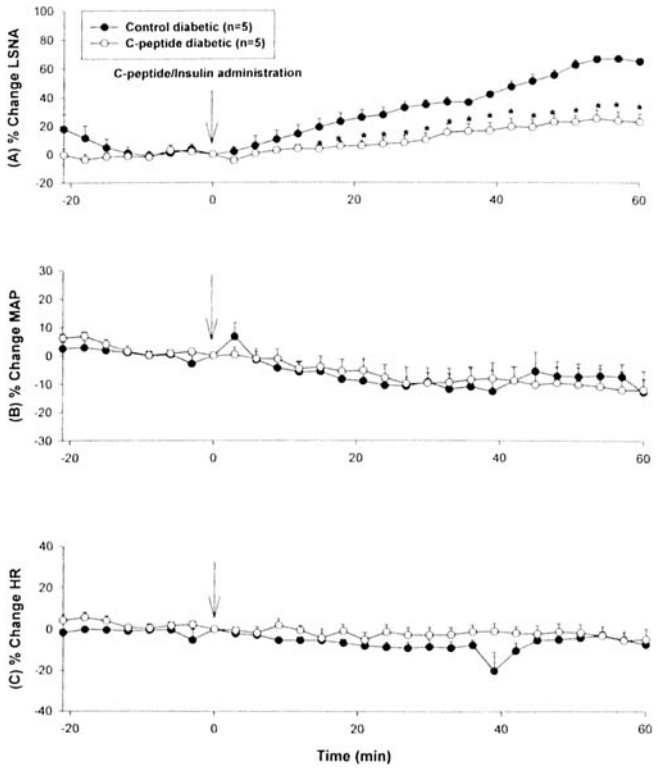

Figure 5. Effect of C-peptide with or without insulin (2 U/kg) on LSNA (A), MAP (B), and HR (C) in diabetic male Wistar rats. Arrow denotes time of insulin or insulin plus C-peptide administration. Values are expressed as percent change from baseline at time 0. $N=5$ for both groups. *, $P < 0.05$ for diabetic; +, C-peptide and insulin versus insulin alone.

animals but decrease MAP (15–17). There have been continuing reports suggesting that hyperinsulinism is associated with sympathetic stimulation, and this increased sympathetic activity is related to cardiovascular morbidity (18).

Studies have provided evidence that diabetic complications differ with respect to the two different types of diabetes (types 1 and 2). Studies suggest that the differences in the degree and type of complications can be accounted for by the presence or absence of insulin and C-peptide (19). The differences have been demonstrated in both nerve structural changes as well as and electrophysiological changes that are associated with diabetic neuropathies in the two types of diabetes (7, 20). Both insulin and C-peptide have been observed to exert neuroprotective effects, and deficiency in either or could contribute to the differences observed between diabetic polyneuropathy in the two types of diabetes (9). Comparison between diabetic polyneuropathy in rat models of the two types of diabetes show less severe axonal degeneration in type 2 models (8, 20). These findings suggest that in addition to the hyperglycemic state, the absence or presence of other factors, such as C-peptide, may play a role leading to enhanced complication.

Previous studies demonstrating C-peptide's effect on renal function, autonomic and sensory nerve function, insulin secretion, glucose uptake, and the modulation of microvascular blood flow in skin and skeletal muscle are consistent with a C-peptide–induced decrease in autonomic sympathetic tone (4–8, 21–23). A possible reason for the minimum influence of C-peptide in normal animals may be that these animals already have adequate normal levels of circulating C-peptide and maybe enough to saturate the putative C-peptide receptors. In the present study, we observed that the STZ diabetic animals with significantly lower levels of circulating C-peptide have an enhanced sensitivity to a C-peptide–mediated response. Thus, the replacement of C-peptide along with insulin might provide better metabolic control and especially the autonomic nervous-mediated responses. We suggest that C-peptide's ability to attenuate sympathetic responses in type I diabetes could decrease vascular tone and augment blood flow in affected tissues.

In summary, our results are the first to demonstrate that C-peptide plays a role in attenuating the insulin-induced activation of the sympathetic nervous system especially in a type I diabetic animal model. Thus, C-peptide may be important in modulating the function of the autonomic nervous responses.

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