C-peptide increases forearm blood flow in patients with type 1 diabetes via a nitric oxide-dependent mechanism

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Johansson, Bo-Lennart, John Wahren, and John Pernow. C-peptide increases forearm blood flow in patients with type 1 diabetes via a nitric oxide-dependent mechanism. Am J Physiol Endocrinol Metab 285: E864–E870, 2003. First published June 10, 2003; 10.1152/ajpendo.00001.2003.—Proinsulin C-peptide has been shown to increase muscle blood flow in type 1 diabetic patients. The underlying mechanism is not fully understood. The aim of this study was to evaluate if the vasodilator effect of C-peptide is mediated by nitric oxide (NO). Eleven type 1 diabetic patients were studied two times and randomized to administration of intravenous and intra-arterial infusion of C-peptide or saline. Forearm blood flow (FBF) was measured by venous occlusion plethysmography during infusion of C-peptide or saline before, during, and after NO synthase (NOS) blockade. Endothelium-dependent and -independent vasodilatation was evaluated by administration of acetylcholine and sodium nitroprusside, respectively. FBF increased by 35% during intravenous C-peptide (P < 0.01) but not during saline infusion (−2%, not significant). NOS blockade resulted in a more pronounced reduction in FBF during intravenous C-peptide than during saline infusion (−41% vs. −26%, P < 0.05). Intra-arterial C-peptide failed to increase FBF during NOS blockade. However, when C-peptide was given after the recovery from NOS blockade, FBF rose by 26% (P < 0.001). The vasodilator effects of acetylcholine and nitroprusside were not influenced by C-peptide. It is concluded that the stimulatory effect of C-peptide on FBF in type 1 diabetic patients is mediated via the NO system and that C-peptide increases basal endothelial NO levels.

acetylcholine; insulin; Nω-monomethyl-L-arginine; sodium nitroprusside; venous occlusion plethysmography

A series of recent studies demonstrates that proinsulin C-peptide has the ability to exert several physiological effects in patients with type 1 diabetes. Thus short (1- to 3-h)- and long (3-mo)-term administration of C-peptide in physiological concentrations improves renal and nerve function in type 1 diabetic patients (4, 16–18, 21) and animals (12, 33, 36). Moreover, C-peptide increases blood flow, improves capillary diffusion capacity, and stimulates oxygen uptake in forearm skeletal muscle at rest (5, 20) and during exercise in type 1 diabetic patients but not in healthy controls (19). It also stimulates myocardial function and blood flow (10, 22).

The mechanism behind the effects seen by C-peptide has not been fully determined. C-peptide shows specific binding to human cell membranes (32), and its binding curve demonstrates full saturation at a plasma concentration of 0.9 nmol/l (32). C-peptide dose-dependently stimulates Na+/K+-ATPase activity in renal tubule segments (28), erythrocytes (26), and peripheral nerves (36, 15). The activity of this enzyme is reduced in several tissues in diabetes with signs of diabetic late complications (7, 30). In addition, C-peptide activates endothelial nitric oxide synthase (eNOS) under both in vitro (25) and in vivo conditions (3, 34). There are several reports suggesting that endothelial function is impaired in diabetes. The impairment may be because of reduced availability of nitric oxide (NO) secondary to either decreased eNOS activity or enhanced inactivation of the NO produced (1, 23, 24, 29, 39, 40). Whether C-peptide has the ability to enhance the levels of NO for mediating vasodilatation in vivo in humans has not been examined before. On the basis of the above considerations, it can be speculated that the vasodilator effect elicited by C-peptide is mediated by NO. Consequently, the aim of the present study was to evaluate this hypothesis and examine whether C-peptide improves endothelial-dependent vasodilation in type 1 diabetic patients.

PATIENTS AND METHODS

Patients

Eleven male type 1 diabetic patients were studied on two different occasions. Their mean age and diabetes duration were 37 ± 4 and 23 ± 3 yr, respectively. Their body mass index was 24.9 ± 0.7 kg/m². Plasma C-peptide levels in the fasting state were undetectable except in two patients showing values just above the detection limit (0.20 and 0.22 nmol/l). All patients received subcutaneous injections of insulin 4 times/day (short-acting insulin before meals and intermediate-acting insulin at bedtime with a mean insulin dosage of 0.7 ± 0.05 U·kg⁻¹·24 h⁻¹). The mean glycosylated hemoglobin (Hb A₁c) was 7.2 ± 0.7% (reference value 3.5–5.5%). Five patients exhibited signs of retinopathy, graded as simplex retinopathy in three patients and as preproliferative changes in two. The latter two had been treated with laser photocoagulation and were on therapy with angiotensin-
converting enzyme (ACE) inhibitors because of hypertension and microalbuminuria. Autonomic nerve dysfunction, as evidenced by decreased heart rate variability during deep breathing, was also present in four of the five patients with retinopathy, but not in the others. Apart from insulin (and ACE inhibitors in 2 patients), no other medication was used. The protocol was reviewed and approved by the institutional ethics committee. All patients were informed of the nature, purpose, and possible risks of the study before giving their voluntary consent to participate.

**Experimental Procedures**

The patients arrived at 7:30 AM to the laboratory after an overnight fast without having taken their morning insulin dose. Teflon catheters were introduced percutaneously under local anesthesia in the brachial artery (4–6 cm in the retrograde direction) and in a deep antecubital vein (4–6 cm in the distal direction) in the nondominant arm. A catheter was also placed in a superficial vein near the antecubital fossa of the contralateral arm for infusion of insulin and C-peptide or saline. After the catheterization, an intravenous low-dose insulin infusion (0.2 mU·kg⁻¹·min⁻¹) was started and so regulated that euglycemia (4–6 mmol/l) was achieved and maintained during the study. Forearm blood flow (FBF) was measured by venous occlusion plethysmography with an air-filled cuff applied to the widest part of the left forearm. A venous occlusion pressure of 50 mmHg was applied on the upper arm, and the circulation of the hand was occluded by inflation of a wrist cuff to 240 mmHg during the recording of inflow curves (6). Intra-arterial blood pressure was recorded continuously, except during the intra-arterial infusion periods, using a pressure sensor system (Senso Nor 840; Senso Nor, Horten, Norway). Heart rate was followed continuously by electrocardiogram recordings.

**Series A.** Five patients were studied two times according to the study protocol presented in Fig. 1A. The patients were randomized to receive C-peptide (6 pmol·kg⁻¹·min⁻¹) intravenously on one occasion and saline on the other with a time interval of 1–2 mo. Blood flow measurements started 30 min after insertion of the catheters and continued at timed intervals during the study. Saline, the endothelium-dependent vasodilator acetylcholine (ACh; 3, 10 and 30 μg/min), and the endothelium-independent vasodilator sodium nitroprusside (SNP; 10 μg/min) were administered in the brachial artery for 2 min each, whereas the NOS inhibitor N⁵G-monomethyl-L-arginine (L-NMMA) was given for 20 min. L-NMMA was administered in the brachial artery at a dose of 2 mg/min for 8 min followed by a dose of 1 mg/min for 12 min. To avoid an influence on FBF by the infusion itself, all intra-arterial infusions were given at a constant rate of 2.5 ml/min. Thus, when ACh (10 μg/min) and SNP (10 μg/min) were coinfused with L-NMMA (1 mg/min), each substance was given at 1.25 ml/min, resulting in an infusion rate of 2.5 ml/min. During each of the intra-arterial infusion periods, blood flow was recorded for 5 min. Blood flow was calculated as the mean value from the plethysmographic inflow curves recorded every 15 s (inflow 10 s and deflation 5 s) during the 2nd and 3rd min (8 curves) of the ACh and SNP infusion periods and

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**Fig. 1.** A: study design for series A. Five patients were studied on two occasions and randomized to receive iv C-peptide infusion on one occasion and saline infusion on the other occasion. B: study design for series B. Six patients were studied two times and randomized as in series A. L-NMMA, N⁵G-monomethyl-L-arginine; SNP, sodium nitroprusside; I.a., intra-arterial.

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during the 4th to 5th min (8 curves) of the saline and L-NMMA infusion periods. 

Series B. Six patients were studied on two different occasions with an interval of 1–2 mo according to the protocol shown in Fig. 1B. Blood flow measurements started 30 min after the introduction of the catheters. Saline and ACh (10 μg/min) were infused in the brachial artery for 2 min each. L-NMMA was given for 24 min at a dose of 2 mg/min during the first 5 min followed by 1 mg/min for the remaining 19 min. Depending on the randomization, C-peptide (7 μg/min) or saline was coadministered with L-NMMA in the brachial artery for 5 min starting at 5 min of L-NMMA administration. At the end of the 5-min intra-arterial coinfusion period with L-NMMA and C-peptide, an intravenous infusion of C-peptide (6 pmol·kg⁻¹·min⁻¹) was started and continued during the rest of the study day. On the other study day, when the 5-min intra-arterial coinfusion period with L-NMMA and saline was ended, an intravenous infusion of saline was given. At 15 min of the L-NMMA infusion period, ACh (10 μg/min) was coinfused. After (30 min) cessation of the L-NMMA infusion, ACh (10 μg/min) was administered again for 2 min followed by intra-arterial infusion of SNP (10 μg/min) for 2 min. Finally, during the last 5 min of the study protocol, C-peptide (7 μg/min) was given in the brachial artery for 5 min (Fig. 1B). As for series A, all intra-arterial infusions were given at a constant rate of 2.5 ml/min to avoid an influence on FBF. Blood flow was measured during each intra-arterial infusion period and calculated as in series A.

In both series A and series B, blood samples were drawn from the deep forearm vein at the beginning and at the end of the study for analyses of plasma glucose, insulin, and C-peptide concentrations.

Blood Sample Analyses

Plasma glucose was measured by a glucose oxidase method using a glucose analyzer (Beckman Glucostat). Hb A₁c was determined with a liquid-chromatographic assay, and normal reference values were 3.5–5.5% (15). Plasma immunoreactive free insulin was analyzed after immediate polyethylene glycol precipitation (2). Plasma C-peptide was assessed by a radioimmunological technique using a commercial kit (C-peptide RD 315; MILAB, Malmo, Sweden). 

Drugs

Human biosynthetic C-peptide was obtained from Eli Lilly (Indianapolis, IN). L-NMMA was from Alexis (Laufelfingen, Switzerland). L-NMMA was dissolved in 0.9% sterile NaCl, passed through a Millipore (Millex-GS) filter, and tested for bacterial toxins before infusion. ACh (Mioclor; OMJ Pharmaceuticals, San German, Puerto Rico) and SNP (Nitopress; Abbott Laboratories, N. Chicago, IL) were diluted in saline before infusion.

Calculations and Statistical Analyses

Changes in FBF are expressed both in percent and in absolute values (37). The data are given as means ± SE. Statistical differences were calculated using Wilcoxon’s matched pairs test.

RESULTS

Venous Plasma Glucose, Insulin, and C-peptide Concentrations

Venous plasma glucose and insulin concentrations were stable during the study both in series A and series B (Table 1). During intravenous infusion of C-peptide, postprandial levels (2.4–2.9 nmol/l) were obtained.

Blood Flow

Series A. On the intravenous C-peptide infusion day, C-peptide resulted in a 35 ± 10% (P < 0.05) increase in FBF (from 2.6 ± 0.3 to 3.6 ± 0.6 ml·min⁻¹·100 ml⁻¹ at 100 min of C-peptide infusion), whereas it remained unaltered during the intravenous saline infusion day.
MECHANISM OF C-PEPTIDE-INDUCED INCREASE IN FOREARM BLOOD FLOW

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Fig. 3. Series A. Change in forearm blood flow evoked by ia infusion of L-NMMA during simultaneously ongoing iv infusion of either C-peptide or saline. Data (means ± SE; n = 5) are expressed as percent change in blood flow from the previous basal (NaCl) period. A statistical difference (P < 0.05) was found between the C-peptide and saline study day.

(3.7 ± 0.3 vs. 3.6 ± 0.4 ml·min⁻¹·100 ml⁻¹). The difference in blood flow response on the two study days was 38 ± 12%, P < 0.05. Intra-arterial infusion of ACh (3, 10, and 30 μg/min) evoked a dose-dependent increase in FBF (from 3.4 ± 0.2 to 7.0 ± 1.9, 8.8 ± 1.7, and 19.6 ± 5.8 ml·min⁻¹·100 ml⁻¹ at the three different dosages, respectively, on the C-peptide study day and from 3.5 ± 0.3 before to 8.7 ± 2.1, 11.1 ± 2.7, and 17.8 ± 3.6 ml·min⁻¹·100 ml⁻¹ on the saline study day). There was no difference in the response to ACh between the two study days, nor was the response to SNP influenced by C-peptide (Fig. 2). Intra-arterial infusion of L-NMMA significantly reduced FBF during the intravenous infusion of C-peptide (−41 ± 2%, from 3.6 ± 0.6 to 2.1 ± 0.4 ml·min⁻¹·100 ml⁻¹, P < 0.05) and saline (−26 ± 7%, from 3.6 ± 0.4 to 2.8 ± 0.5 ml·min⁻¹·100 ml⁻¹). The reduction was significantly more pronounced during the C-peptide infusion period (Fig. 3). As expected, administration of L-NMMA blunted the increase in FBF to 10 μg ACh (increased from 3.6 ± 0.6 to 6.0 ± 1.9 and from 3.6 ± 0.4 to 5.1 ± 2.1 ml·min⁻¹·100 ml⁻¹ on the C-peptide and saline study days, respectively) but not the response to SNP (from 3.6 ± 0.6 to 19.6 ± 1.0 and from 3.6 ± 0.4 to 18.5 ± 2.5 ml·min⁻¹·100 ml⁻¹ on the C-peptide and saline study days, respectively; Fig. 2).

Series B. Table 2 shows the mean FBF recorded at different time points before, during, and after L-NMMA administration. Infusion of L-NMMA in the brachial artery reduced FBF by 38 ± 2% (from 3.3 ± 0.3 to 2.3 ± 0.3 and from 3.1 ± 0.3 to 2.5 ± 0.3 ml·min⁻¹·100 ml⁻¹ on the C-peptide and saline study days, respectively, P < 0.03). On the C-peptide study day, a 5-min intra-arterial infusion of C-peptide during coadministration of L-NMMA failed to increase FBF (Table 2 and Fig. 4). In contrast, when a 5-min intra-arterial C-peptide infusion was given after washout of L-NMMA at the end of the study on the saline day, a marked rise in FBF was observed (Table 2 and Fig. 4). In contrast, on the day C-peptide was infused intravenously, there was a significant increase in FBF at 45 min after the end of L-NMMA infusion (P < 0.03), whereas no further increase was obtained by the intra-arterial C-peptide infusion given at the end of the study (Table 2 and Fig. 5).

Intra-arterial administration of ACh (10 μg/min) induced a fourfold increase in FBF from 3.3 ± 0.3 to 13.8 ± 3.8 ml·min⁻¹·100 ml⁻¹ during the C-peptide study day and from 3.1 ± 0.3 to 11.2 ± 3.9 ml·min⁻¹·100 ml⁻¹ during the saline study day (Table 3). As expected, the increase in FBF by ACh was inhibited by 90% during the subsequent L-NMMA infusion on both study occasions (increase in FBF from 3.3 ± 0.3 to 3.9 ± 0.6 ml·min⁻¹·100 ml⁻¹ during the C-peptide study day and from 3.1 ± 0.3 to 3.7 ± 1.0 ml·min⁻¹·100 ml⁻¹ during the saline study day; Table

Table 2. Forearm blood flow recorded in series B during ia infusion of saline, L-NMMA, L-NMMA plus C-peptide, and C-peptide alone during the study days with iv infusions of C-peptide or saline

<table>
<thead>
<tr>
<th>C-Peptide or Saline iv</th>
<th>Saline Basal</th>
<th>L-NMMA 4-min Infusion</th>
<th>C-Peptide (35 μg) or Saline ia</th>
<th>L-NMMA 24-min infusion + saline</th>
<th>Saline 45 min after L-NMMA</th>
<th>C-Peptide (35 μg ia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-peptide day</td>
<td>3.3 ± 0.3</td>
<td>2.3 ± 0.3*</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>3.7 ± 0.6*</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Saline day</td>
<td>3.1 ± 0.3</td>
<td>2.3 ± 0.4*</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>3.9 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are ml·min⁻¹·100 ml⁻¹. L-NMMA, N⁴-monomethyl-L-arginine. A 5-min ia infusion of C-peptide (on the C-peptide day) or saline (on the saline day) started during the administration of L-NMMA, immediately followed by an iv infusion of the same substance given during the rest of the study. On both study days, a 5-min ia infusion of C-peptide was given at the end of the study. *Statistically significant difference (P < 0.03) in forearm blood flow from the previous infusion period.

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3). The vasodilator response to ACh had returned at 30 min after cessation of the L-NMMA infusion on both study occasions (Table 3). After the L-NMMA infusion period, intra-arterial infusion of SNP (10 μg/min) resulted in a nearly sevenfold increase in FBF both during the C-peptide (from 3.3 ± 0.3 to 18.5 ± 3.3 ml·min⁻¹·100 ml⁻¹) and saline (from 3.1 ± 0.3 to 17.3 ± 2.7 ml·min⁻¹·100 ml⁻¹) study days (Table 3).

**DISCUSSION**

The main findings of this study are that the increase in FBF induced by C-peptide in type 1 diabetic patients is inhibited by blockade of NO production (series B) and that the reduction in FBF induced by NOS blockade during C-peptide administration is augmented (series A). These observations suggest that C-peptide increases NO levels, which results in enhanced resting blood flow. It has previously been demonstrated that young type 1 diabetic patients without signs of late diabetic complications have a reduced FBF compared with healthy controls both at rest (5, 20) and during exercise (19). Intravenous infusion of C-peptide in physiological amounts evokes forearm vasodilatation in the patients but not in healthy controls (19). Furthermore, the reduced myocardial perfusion and function, also found in young otherwise healthy type 1 diabetic patients, is improved by C-peptide replacement (10, 22). The mechanisms underlying the stimulatory effects of C-peptide on blood flow in type 1 diabetic patients is not fully understood. However, on the basis of the findings by Rigler et al. (32), demonstrating specific binding for C-peptide and a binding curve with full saturation at low physiological concentrations (0.9 nmol/l), it may be postulated that C-peptide does not give rise to blood flow increments in healthy subjects because all binding sites are already occupied before C-peptide is given. This is not the case in type 1 diabetic patients who lack endogenous C-peptide. Observations in isolated arterioles from the rat (33) and bovine aortic endothelial cells (25) indicate that C-peptide causes release of NO. The hypothesis behind the present study was therefore that the C-peptide-induced increase in FBF in type 1 diabetic patients involves stimulation of the NO system. We observed that intra-arterial administration of C-peptide in series B failed to increase FBF in the presence of the NO inhibitor L-NMMA. On the other hand, at 60 min after administration of L-NMMA when the blockade had disappeared, intra-arterial C-peptide increased FBF by 38%, which is similar to the response obtained by intravenous C-peptide obtained in the present (series A) and previous (5, 19, 20) studies. The increase in flow induced by ACh was blocked by 90% in the presence of L-NMMA, which demonstrates a high degree of NOS blockade by L-NMMA. Furthermore, the finding that the response to ACh had recovered at the time of the second intra-arterial administration of C-peptide demonstrates that the NOS blockade by L-NMMA, when given as an intra-arterial infusion, was of short duration and had disappeared at this time. These observations suggest that the increase in FBF by C-peptide in type 1 diabetic patients is mediated by NO in agreement with the results from series A demonstrating that the reduction in blood flow evoked by L-NMMA was greater during intravenous infusion of C-peptide than during saline infusion and previous in vitro observations (14). Because the response to L-NMMA reflects basal levels of endogenous NO, the enhanced response to L-NMMA in the presence or C-peptide indicates that more endogenous NO is available for maintenance of an elevated level of blood flow. Taken together, these data provide evidence that C-peptide enhances the basal level of NO, which results in increased FBF and a more marked fall in blood flow in response to NOS blockade.

Table 3. Series B: percent change in forearm blood flow from basal in response to ia infusion of ACh before, during, and after L-NMMA infusion and to SNP after infusions of L-NMMA during the two different study days with iv C-peptide or saline infusion

<table>
<thead>
<tr>
<th></th>
<th>ACh Before L-NMMA</th>
<th>ACh During L-NMMA</th>
<th>ACh After L-NMMA</th>
<th>SNP After L-NMMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-peptide day</td>
<td>323 ± 119*</td>
<td>30 ± 25</td>
<td>214 ± 97*</td>
<td>589 ± 88*</td>
</tr>
<tr>
<td>Saline day</td>
<td>281 ± 130*</td>
<td>27 ± 33</td>
<td>215 ± 83*</td>
<td>616 ± 86*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are % change. Dose of ACh was 10 μg/min. SNP, sodium nitroprusside. *Statistically significant changes in blood flow response induced by ACh and SNP (P < 0.05). No statistical differences were found in the ACh or SNP responses between the two study days.
Several previous studies have established that patients with type 1 diabetes have impaired endothelial function, which usually is assessed by determination of endothelium-dependent vasodilatation stimulated with various agonists. The present study allows evaluation of whether administration of C-peptide improves endothelial function; this was done by administration of the endothelium-dependent vasodilator ACh in series A. However, the vasodilator effect of ACh was not increased by C-peptide, which contrasts with the observation that the vasoconstrictor response to NOS blockade was enhanced by C-peptide. The findings may be taken to indicate that basal but not stimulated synthesis of NO is enhanced by C-peptide. In addition, the response to the endothelium-independent vasodilator SNP was unaffected by C-peptide, suggesting that the sensitivity of the vascular smooth muscle to NO was not altered by C-peptide.

The ability of C-peptide to increase the basal level of NO may be of pathophysiological importance. Thus replacement of C-peptide for 2–8 mo to diabetic animals results in arrest of the development of neuropathy and improvement of both functional and structural nerve variables (36). Likewise, replacement of C-peptide in type 1 diabetic patients is accompanied by improved peripheral and autonomic nerve function (4, 16, 17). Although the mechanism behind the effects of C-peptide on peripheral and autonomic diabetic neuropathy is not fully established, C-peptide has been shown to significantly augment nerve nutritive blood flow in animals with experimental diabetes (3). Moreover, when C-peptide and the eNOS blocker are coadministered to diabetic animals, all the beneficial effects of C-peptide are abolished (3). These findings provide evidence that the C-peptide effects are mediated via the NO system. In addition, it is well documented that NO exerts several tissue protective effects. Besides being a potent vasodilator, NO is an anti-inflammatory mediator. It inhibits platelet aggregation, inactivates superoxide, and inhibits vascular smooth muscle growth. NO inhibits leukocyte-endothelium interaction, at least in part, by attenuating the expression of various cell adhesion molecules on the endothelium (34). In this context, it is interesting to note that C-peptide has been demonstrated to inhibit leukocyte-endothelium interaction via an NO-dependent mechanism in the rat mesenteric microcirculation (34). Moreover, C-peptide exerts cardioprotective effects during myocardial ischemia and reperfusion via an action related to NO and reduced infiltration of leukocytes (35). Another important effect of NO is to scavenge superoxide. Excess vascular superoxide production is suggested to impair endothelial function in diabetes (36). Thus, by enhancing the available level of NO, C-peptide may not only increase blood flow but also inhibit several events of pathophysiological importance such as inflammation, free radical accumulation, and vascular proliferation known to contribute to diabetic microvascular complications.

The mechanism by which C-peptide enhances NO production in diabetic patients remains to be established. C-peptide has been shown to activate Ca\(^{2+}\)-dependent intracellular signaling pathways in vitro systems (28, 35). The stimulatory effects by C-peptide on the NO release in vitro models is blocked by a calcium-binding agent (EDTA; see Refs. 3 and 25), suggesting that the C-peptide effect on the NO system is elicited via a Ca\(^{2+}\)-mediated signal. Furthermore, C-peptide is reported to stimulate Na\(^+-K^+\)-ATPase activity under both in vitro (28) and in vivo (36, 13) conditions. This activity of this enzyme is reduced in the diabetic state (7, 30). Interaction between the NO system and Na\(^+-K^+\)-ATPase activity has been reported; Na\(^+-K^+\)-ATPase activity has been found to modulate endothelium-dependent smooth muscle relaxation (31), possibly indicating that an increase in Na\(^+-K^+\)-ATPase activity may enhance NO bioactivity. Conversely, other studies demonstrate that NO may stimulate the activity of Na\(^+-K^+\)-ATPase (8) and that the level of NO is an important determinant of vascular smooth muscle Na\(^+-K^+\)-ATPase activity. It may therefore be hypothesized that the vasodilator effect of C-peptide is elicited via its stimulatory effect on Ca\(^{2+}\) influx, activating NO formation directly via stimulation of eNOS, and possibly also indirectly via activation of Na\(^+-K^+\)-ATPase.

In conclusion, the increase in FBF mediated by C-peptide in patients with type 1 diabetes is blocked by inhibition of NO production. In addition, the vasoconstrictor effect of NOS blockade is enhanced by C-peptide. These observations suggest that C-peptide enhances basal synthesis of NO, resulting in increased blood flow. Considering the beneficial effects of NO, these results may have implications for the therapeutic potential of C-peptide in type 1 diabetic patients.

**DISCLOSURES**

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