Effect of Interleukin-6 (IL-6) on the Vascular Smooth Muscle Contraction in Abdominal Aorta of Rats with Streptozotocin-Induced Diabetes

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Abstract

Patients with type 1 diabetes are at a risk of hypertension. However, the mechanisms behind the findings are not completely known. The aim of the present study was to investigate involvement of interleukin-6 (IL-6) on the contraction of abdominal aorta in rats with type 1 diabetes. IL-6 levels in the plasma of rats with streptozotocin (STZ)-induced diabetes were determined by ELISA. The abdominal aorta was dissected free of fat and connective tissues and then cut into spiral rings. The endothelium-denuded strip was vertically suspended in tissue chambers containing 5 ml Krebs solution at 37°C and bubbled continuously with 95% O2-5% CO2. The effects of phenylephrine (Phe) on the contractile responses of abdominal aorta were recorded. The effects of IL-6 and anti-rat IL-6 antibody on the Phe-induced response were also examined. Plasma levels of IL-6 increased time-dependently in rats with STZ-induced diabetes. Phe caused concentration-dependent contraction in aortic rings. Phe-induced contractions were higher in vascular strips of STZ-induced diabetic rats than that of control rats. Pretreatment of vascular strips with IL-6 for 1 h did not cause contraction but enhanced the contraction in response to Phe. Treatment of the vascular strips with an anti-IL-6 antibody for 1 h decreased the Phe-induced contractions. These results suggest that IL-6 causes vascular smooth muscle contraction in abdominal aorta of rats with type 1 diabetes.

Key Words: abdominal aorta, smooth muscle contraction, interleukin-6, type 1 diabetes

Introduction

Vascular complications are the major causes of increased mortality and morbidity in diabetic patients (2). Abnormalities in blood vessel constriction or dilatation (vasomotion), detected in early diabetes, can result in blood flow dysregulation and increased peripheral resistance thereby contributing to diabetic retinopathy, nephropathy, neuropathy and a higher rate of hypertension. Patients with type 1 diabetes are also at a substantially increased risk of hypertension. However, the cellular and molecular mechanisms of these effects are not completely known.

Type 1 diabetes is associated with increased...
cytokine-mediated inflammation. Monocyte interleukin (IL)-6 levels are significantly elevated in type 1 diabetic subjects (4), and plasma concentrations of IL-6 are high in type 1 diabetic patients (1, 10, 16). IL-6 is increased during hyperglycemia in young and children patients with type 1 diabetes (7, 15). Lower heart rate variability is associated with higher plasma concentrations of IL-6 in type 1 diabetes (6). Hypertensive patients have high circulating levels of IL-6. IL-6 overexpression induces pulmonary hypertension (17). A role for IL-6 in hypertension is suggested by its contractile effects on smooth muscle, including vascular smooth muscle.

IL-6 enhances α(1)-adrenergic receptor-mediated contraction in the corpus cavernosum of the rat (12). IL-6 directly impairs endothelium-dependent relaxation and enhances vascular contraction in systemic vessels of pregnant rats (13). IL-6 might play a role in the vascular contraction of type 1 diabetes. In this study, we have investigated the involvement of IL-6 on the contraction of abdominal aorta in rats with type 1 diabetes.

**Materials and Methods**

**Animals**

Experiments were performed on male Wistar rats. Animals were 4-6 weeks old and weighed 180 ± 10 grams at the beginning of the experiment. Rats were housed in a temperature (22°C)-controlled environment. The use and treatment of animals followed the guidelines of the International Animal Care and Use Committee of Shandong University. All animals were cared for in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals published by the National Natural Science Foundation of PRC.

Diabetes was induced with an intraperitoneal injection of streptozotocin (STZ, 60 mg/kg) in sodium citrate buffer (0.1 M, pH 4.2). Control animals were injected with the sodium citrate buffer alone. Body weight and blood glucose levels were measured at the beginning of the experiment. Glucose levels were measured with glucose test strips (Bayer, Elkhart, IN, USA). Blood glucose levels were measured again, after 1 week, to confirm the development of hyperglycemia.

Four weeks after the induction of diabetes, body weight and blood glucose levels were measured, the animals were sacrificed, and tissue samples were collected for in vitro studies. A second group of animals was killed after 16 weeks to measure IL-6 levels in blood. A third group of untreated animals was killed at 10-12 weeks of age. Aortic samples from these animals were used to study the effect of IL-6 on contractile responses.

**Measurement of Plasma IL-6**

IL-6 levels in blood were measured with an ELISA system (Cytoscreen, Biosource International, Camarillo, CA, USA). The assay is a solid-phase sandwich-type system that utilizes a specific anti-rat IL-6 antibody coated onto the wells of microtiter plates. Serum samples (50 µl) and standards were pipetted in triplicate into appropriate microtiter wells, and the assay was performed according to manufacturer’s instructions. The sensitivity of this IL-6 ELISA system is 0.7 pM, and the upper limit of detection is 150 pM.

**Tissue Culture**

Strips of the abdominal aorta were placed in Krebs solution containing 119 mM NaCl, 4.75 mM KCl, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 1.5 mM MgSO₄, 2.5 mM CaCl₂ and 11 mM glucose. They were dissected free of fat and connective tissues and then cut into 2 × 8 mm spiral strips. The endothelium was removed by rubbing the vessel interior with a wet cotton swab. Removal of the endothelium was verified by the absence of ACh relaxation in vascular strips precontracted with phenylephrine (Phe).

Vascular strips were studied in an apparatus in which one end of the loop was attached to a glass hook and the other end was connected to an external isometric force transducer (JH-2B, Beijing, PRC). They were stretched to an initial tension of 1 g and allowed to equilibrate for 30 min in a tissue bath filled with 5 ml Krebs solution continuously bubbled with 95% O₂-5% CO₂ at 37°C. The changes in isometric force were recorded using an MFlab system with SMUP-PC amplifier (Fudan University, Shanghai, PRC).

**Dose-Response Effects of Phe on the Contraction of Abdominal Aorta in STZ-Diabetic Rats**

Aortic rings from 10- to 12-week-old STZ-diabetic and age-matched control rats were mounted on the apparatus, left to equilibrate for 30 min in a tissue bath filled with 5 ml Krebs solution continuously bubbled with 95% O₂-5% CO₂ at 37°C. The changes in isometric force were recorded using an MFlab system with SMUP-PC amplifier (Fudan University, Shanghai, PRC).

**Effects of IL-6 on Phe-Induced Abdominal Aorta Contraction in Normal Rats**

In another set of experiments, intact 10- to 12-week-old male Wistar rats were killed and aortic rings were prepared. The strips were either nontreated,
or treated with IL-6 (0.1 µg/ml) for 1 h. Then, increasing concentrations of Phe (0.001-3 µM) were applied, and the effects on Phe-induced contraction were measured.

Effects of Anti-Rat IL-6 Antibody on Phe-Induced Abdominal Aorta Contraction in STZ-Diabetic Rats

The effect of anti-IL-6 antbody was tested on aortic rings from 10- to 12-week-old STZ-diabetic and age-matched controls. Aortic rings were either nontreated or treated with an anti-rat IL-6 antibody (10 ng/ml) for 1 h, following which the dose-response curves were recorded following Phe administration.

Chemicals

STZ and Phe were obtained from Sigma Co. Ltd. (St. Louis, MO, USA). Recombinant rat IL-6 and anti-rat IL-6 antibody were purchased from Peprotech Inc. (Rocky Hill, NJ, USA). STZ was dissolved in sodium citrate buffer (0.1 M, pH 4.2). Phe, IL-6 and anti-rat IL-6 antibody were prepared in distilled water.

Data Analysis

Data are presented as means ± SEM, with n indicating the number of rats. A paired t-test was used to test for a significant difference between two treatments in one group. ANOVA repeat measures were used to evaluate differences among more than two groups. A probability level of P < 0.05 was considered to be significant for statistical analysis.

Results

Blood Glucose and Body Weight Levels in STZ-Diabetic and Control Rats

Four weeks after induction of hyperglycemia, plasma glucose concentrations in STZ-treated rats were significantly greater than in the control animals, while body weight of the diabetic animals was significantly less than the controls (Fig. 1, A and B).

Plasma Concentrations of IL-6 in STZ-Diabetic and Control Rats

In the 4-week STZ diabetic animals, the plasma concentration of IL-6 was significantly higher than that of the controls (the value in STZ rats was 44.58 ± 3.18 pg/ml; the value in control rats was 11.99 ± 1.9 pg/ml, P < 0.05). In the 16-week STZ animals, the plasma concentration of IL-6 in type 1 diabetic rats increased to 81.97 ± 5.00 pg/ml which was significantly higher than the 4-week group (Fig. 1C, P < 0.05).
Effects of Phe on the Contraction of Abdominal Aorta in STZ-Diabetic Rats

The contractile responses of one aortic strip to increasing doses of Phe (0.001-3 µM) are shown in Fig. 2A. Data from 6-12 rats are summarized in Fig. 2B. Low concentrations of Phe (0.001-0.01 µM) failed to elicit effects on contraction of abdominal aorta strips. Phe (0.01-3 µM) dose-dependently increased the contractions of abdominal aorta strips. Phe (0.01-3 µM)-induced contraction appeared to be higher in the vascular strips isolated from STZ-diabetic rats (*P < 0.05 compared with gender/age-matched control rat).

Effect of IL-6 on Phe-Induced Abdominal Aorta Contraction in Normal Rats

IL-6 (0.1 µg/ml) had no effect on the tension of abdominal aorta strips (P > 0.05, n = 6). However, pretreating rings with IL-6 for 1 h increased the response to Phe (Fig. 3, P < 0.05).

Effect of Anti-Rat IL-6 Antibody on Phe-Induced Contraction in STZ-Diabetic Rats

To test the effect of anti-rat IL-6 antibody on contractile responses of aortic strips, strips were treated with an anti-rat IL-6 antibody (10 ng/ml) for 1 h following which Phe (0.001-3 µM)-induced contractions of the vascular strips in type 1 diabetes rats were studied (Fig. 4A). Antibody treatment alone had no effect on the tension of the abdominal aorta strips (P > 0.05, n = 6). However, the IL-6 antibody treatment reduced the Phe contractile response in the STZ rats (Fig. 4B). At a dose of 0.01 µM, Phe-induced contraction of the STZ vascular strips was 1.16 ± 0.09 g, which was lower than the Phe response in controls (1.62 ± 0.14 g, P < 0.05). At 0.03 µM, the Phe-induced response of the anti-rat IL-6 group was almost the same with that of controls (1.17 ± 0.09 g, P > 0.05).
Concentrations of plasma IL-6 in type 1 diabetic rats are increased, [2] IL-6 enhances the contraction of abdominal aorta strips in rats, and [3] IL-6 is involved in the enhanced vascular contraction of rats with type 1 diabetes.

In diabetes, early inflammation causes the release of chemokines such as monocyte chemoattractant protein 1 (MCP-1) in vascular endothelium and in adipose tissues. These factors increase expression of interstitial and vascular cellular adhesion molecules and attract monocytes and immunocytes. They undergo chemokine-induced proliferation and proinflammatory gene activation producing cytokines like tumor necrosis factor α (TNFα), interleukin-1 (IL-1), IL-6, IL-18, interferon (IFN) and others. Many of these molecules enter the circulation where their levels correlate with the degree of inflammatory activity. Among these cytokines, IL-6 appears to be a very important cytokine associated with type 1 diabetic (5). Consistent with previous studies on diabetic patients (1, 10, 16), we found that blood level of IL-6 elevates in the rats with type 1 diabetes.

The risk of peripheral vascular disease is increased in diabetic patients in whom it occurs earlier and is often more severe and diffuse (2). Data in patients with type 1 diabetes mellitus show a significant increase in arterial pressure (11). Endothelial dysfunction, vascular smooth muscle cell dysfunction, inflammation and hypercoagulability are the key factors in diabetic arteriopathy (8). We compared the Phe-induced vascular contraction between type 1 diabetic and control rats. The results showed that the vascular contractions were higher in type 1 diabetic rats than in the control rats suggesting that diabetes-associated vascular smooth muscle shows hyper-contractility.

Derosa et al. (3) evaluated systolic blood pressure (SBP), diastolic blood pressure (DBP) and IL-6 in the diabetic hypertensive (DH) patients. They found that SBP, DBP and IL-6 were significantly higher in the DH group. After 6 months of candesartan therapy, IL-6 decreased. Furthermore, there was a significant decrease of SBP and DBP values in the DH group. The results suggested the involvement of IL-6 in the diabetic hypertensive patients. Iversen et al. (9) investigated the effect of IL-6 on vascular tone in isolated human arterial and venous segments from various organs. They found that IL-6 induced contraction of arterial but not of venous segments. Studies in pregnant rats showed that IL-6 increased both systolic blood pressure and isometric contraction of isolated aortic strips (14). IL-6 was also reported to enhance vasomotor reactivity of the corpus cavernosum in rats (12). In our studies, we first observed the effect of IL-6 on Phe-induced abdominal aorta contraction in normal rats, and that IL-6 increased the Phe-induced contraction. We further observed the effect of anti-rat IL-6 antibody treatment on Phe-induced contraction in type 1 diabetic rats, and that
anti-rat IL-6 antibody partly decreased Phe-induced contraction. These results suggest that IL-6 is involved in the vascular hyper-contractility of rats with type 1 diabetes. IL-6 might increase the rate of diabetic hypertension via vascular hyper-contractility.

In conclusion, IL-6 excites the vascular smooth muscle contraction in abdominal aorta of the rats with type 1 diabetes.

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References


