Chronic hyperglycemia impairs functional vasodilation via increasing thromboxane-receptor-mediated vasoconstriction

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Submitted 12 June 2006; accepted in final form 1 August 2006

Xiang L, Naik JS, Abram SR, Hester RL. Chronic hyperglycemia impairs functional vasodilation via increasing thromboxane-receptor-mediated vasoconstriction. Am J Physiol Heart Circ Physiol 292: H231–H236, 2007. First published August 11, 2006; doi:10.1152/ajpheart.00623.2006.—Individuals with hyperglycemia exhibit impaired exercise performance and functional vasodilatory response. Based on the importance of arachidonic acid (AA) metabolites in functional vasodilation and the increased thromboxane-to-prostacyclin ratio in diabetes, we hypothesized that chronic hyperglycemia in diabetes increases thromboxane-receptor (TP)-mediated vasoconstriction, resulting in an attenuated functional vasodilation. Three groups of lean Zucker rats (8 wk) were used to test the effects of chronic hyperglycemia on endothelial function: normal, streptozotocin (STZ; 70 mg/kg ip), and STZ + insulin (2 U/day). After 4 wk of treatment, spinotrapezius arcade arterioles were chosen for microcirculatory observation. Arteriolar diameter was measured following muscle stimulation and 10 μM AA application in the absence and presence of 1 μM SQ-29548 (TP antagonist). STZ rats exhibited significantly higher fasting glucose levels and attenuated functional and AA-induced dilation compared with normal animals. SQ-29548 improved the vasodilatory responses in STZ rats but had no effect in controls. Insulin treatment normalized both the glucose levels and the vasodilatory responses, and SQ-29548 treatment had no effect on functional or AA-mediated vasodilation in STZ + insulin animals. These results suggest that the impaired functional vasodilation in diabetic rats is due to hyperglycemia-mediated increases in TP-mediated vasoconstriction.

Arachidonic acid; prostacyclin; diabetes

HYPERGLYCEMIA IS A HALLMARK OF BOTH METABOLIC SYNDROME AND DIABETES MELLITUS. Endothelial dysfunction is a key feature of hyperglycemic individuals and is thought to be a major cause of the associated vascular complications. Both in vivo and in vitro studies have demonstrated that hyperglycemia directly induces endothelial dysfunction and attenuates endothelium-dependent relaxation (4, 18, 19). In a previous study, our group showed in hyperglycemic obese Zucker rats that functional dilation and acetylcholine-induced arteriolar dilation in the spinotrapezius muscle were impaired (36). In addition, exercise training normalized blood glucose levels and improved the vasodilatory responses in this model of diabetes and obesity (36). These results suggested a possible relationship between the impaired functional dilation and hyperglycemia-induced endothelial dysfunction. Many factors have been proposed to contribute to the hyperglycemia-induced endothelial dysfunction, such as enhanced reactive oxygen species (22, 32), reduced nitric oxide (NO) bioavailability (15), and upregulated protein kinase C (6, 32). However, the role of chronic hyperglycemia in the impairment of functional vasodilation is not known.

Our previous studies (11, 26, 27) have shown that, under normal conditions, arachidonic acid (AA) metabolite(s) plays an important role in functional hyperemia. Vasoactive AA metabolites include vasoconstrictors such as prostacyclin (PGI2) and/or vasoconstrictors such as thromboxane A2 (TXA2) and its precursor prostaglandin H2 (PGH2). Under normal conditions, PGI2 generation is the major pathway of AA metabolism during functional hyperemia, and TXA2 production has been found to decrease during exercise in an exercise intensity-dependent manner (17). However, studies have shown elevated TXA2 and decreased PGI2 levels in urine and plasma of patients and animals with diabetes (13, 21), suggesting an altered AA metabolism in hyperglycemic states. Furthermore, we have shown that, in hyperglycemic obese Zucker rats, the thromboxane-receptor (TP) antagonist SQ-29548 partially restored the vasodilator response to muscle contraction, suggesting that an enhanced TP-mediated vasoconstriction is important for the impaired functional dilation (37). In addition to hyperglycemia, the obese Zucker rat also exhibits hyperlipidemia, hyperinsulinemia, and hyperleptinemia, conditions that could affect the functional hyperemia. Therefore, in the present study, we used the streptozotocin (STZ)-induced model of hyperglycemia to test the hypothesis that chronic hyperglycemia impairs functional dilation through an increased TP-mediated vasoconstriction.

METHODS

Animals

Male lean Zucker rats were acquired from Harlan Laboratories (n = 31). The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out according to both the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act. All of the rats were housed two to three animals per cage at 22°C (12-h:12-h light-dark cycle) with free access to food and water.

Blood Glucose and Insulin Measurements

For blood glucose levels, blood samples were collected from tail vein in conscious animals and analyzed with a Beckman clinical chemistry analyzer (Beckman Instruments, Fullerton, CA). After in vivo experiments, blood samples were collected via cardiac injection from unconscious animals for insulin measurements. Plasma insulin levels were analyzed with radiomimunoassay kits (Linco Research, St. Louis, MO).

Microcirculatory Surgical Preparation

The right spinotrapezius muscle was prepared for experimental observation as previously described (36, 37). In brief, rats were...
anesthetized with pentobarbital sodium (65 mg/kg ip), and the trachea was intubated. Animals spontaneously breathed a gas mixture containing 30% oxygen-70% nitrogen. The left jugular vein was cannulated for supplemental addition of anesthetic. At all times during the surgery and subsequent experiments, the spinotrapezius muscle was kept in situ and continuously superfused with a physiological salt solution of the following composition (in mM): 118.07 NaCl, 6.17 KCl, 2.55 CaCl₂, and 25 NaHCO₃, aerated with a 5% CO₂-95% N₂ gas mixture (pH 7.4, 37°C). Unless otherwise specified, all chemicals were purchased from Sigma (St. Louis, MO). At the completion of the study, animals were euthanized by a cardiac injection of pentobarbital sodium. Death was confirmed by a lack of heart beat and spontaneous breathing.

Experimental Measurements

Animals were allowed to stabilize for 15–30 min after completion of the surgical procedure. Segments of arteriolar arcades with similar diameters were selected for analyses. The microcirculation of the spinotrapezius muscle was transilluminated and observed with a Nikon microscope fitted with a ×10 water immersion objective (numerical aperture = 0.30). The microscopic image was televised with a Dage closed-circuit television camera and displayed on a Sony monitor. The magnification of the image was ×660 from the tissue to the monitor screen. Vessel diameter was measured by a Texas A&M video analyzer modified to function as a video micrometer. The resolution of this system was ±1 μm.

Muscle Stimulation

Two hooked silver-silver chloride electrodes (Grass Instruments) were placed at each end of the spinotrapezius and connected to a Grass S44 stimulator. Diameters of the vessels were obtained in the resting muscle and immediately after 2 min of electrical stimulation (4–5 V, 1 Hz).

Drugs and vasoactive agents. STZ was dissolved in 0.3 ml citrate buffer (pH 4.5; Sigma). Sustained release insulin implants (2 mm × 7 mm, 2 U/day, >40 days) were obtained from Linshin Canada. AA and SQ-29548 (TP-receptor antagonist; Cayman Chemical) were stored in ethanol as stock solutions. During the experiments, the final concentration of ethanol in the superfusion solution was <0.1%.

Experimental Protocols

Effects of chronic hyperglycemia on vascular function in STZ rats. Twenty-one lean Zucker rats (8 wk old) were used for this set of experiments (Fig. 1). On day −7, blood samples were collected from the tail vein to measure 8-h fasting glucose levels. On day 0, diabetes was induced in 15 of the lean animals with a single intraperitoneal injection of STZ (70 mg/kg). Six lean rats were injected with citrate buffer vehicle. On day 7, eight STZ-induced diabetic rats were randomly chosen to serve as the insulin-treated group, and fasting blood glucose was measured in these animals. On day 8, animals in the insulin-treatment groups were anesthetized with 2% isoflurane, and insulin pellets (7 mm long) were implanted underneath the abdominal skin. Fasting blood glucose levels were also measured on days 14 and 28. Two STZ rats died 1 wk after STZ injection. Two STZ + insulin rats died during the experiment. Four weeks after STZ injection, vehicle control, STZ, and STZ + insulin rats were prepared for microcirculatory observation and studied as described above.

Effects of acute hyperglycemia on vascular function. Ten lean Zucker rats (12 wk old) were used for this set of experiments. Diameters of the arterioles were obtained in the resting muscle and immediately after 2 min of electrical stimulation and AA (10 μM) administration. After the arteriole had returned to its resting diameter, the spinotrapezius muscle was treated with 20 mM D-glucose or mannitol for 1 h, and the muscle stimulation and AA protocol were repeated. The spinotrapezius muscles were then treated with the TP-receptor antagonist SQ-29548 (1 μM) for 30 min. The muscle stimulation and AA protocol were repeated. At the end of the experiment, adenosine (10 μM) was added to the superfusion solution to determine the maximal luminal diameter.

Data analysis and statistical methods. Arteriolar diameter data were collected at 1 Hz by a computer equipped with a Computer Boards eight-bit analog-to-digital converter and stored to disk for later analysis. The body weight, blood glucose levels, and vasodilatory responses to normal, STZ, and STZ + insulin rats were compared by two-way repeated-measures ANOVA. The effects of SQ-29548 on vasodilatory responses were analyzed by repeated-measures ANOVA. Where significant main effects occurred, individual groups were compared by using the Holm-Sidak method. All data are means ± SD. The basal diameter, the functional or AA-induced dilation in response to acute hyperglycemia or mannitol, and the maximal dilation to adenosine were compared using t-test. A probability of P < 0.05 was accepted as statistically significant for all comparisons.

RESULTS

Effects of Chronic Hyperglycemia in STZ Rats

Body weight and glucose levels in normal, STZ, and STZ + insulin rats. Figure 2 presents the effects of STZ-induced diabetes and insulin treatment on body weight and 8-h fasting glucose levels and insulin-treatment groups were anesthetized with 2% isoflurane, and insulin pellets (7 mm long) were implanted underneath the abdominal skin. Fasting blood glucose levels were also measured on days 14 and 28. Two STZ rats died 1 wk after STZ injection. Two STZ + insulin rats died during the experiment. Four weeks after STZ injection, vehicle control, STZ, and STZ + insulin rats were prepared for microcirculatory observation and studied as described above.

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glucose levels. There was a significant decrease in body weight in STZ rats compared with controls, and insulin treatment partially restored the body weight in STZ rats (Fig. 2A). STZ rats exhibit higher fasting glucose levels than controls, whereas the blood glucose levels in STZ + insulin rats were normalized (Fig. 2B).

**Vasodilatory responses.** Basal diameters of the selected arterioles before and after SQ-29548 treatment are shown in Table 1. The vasodilatory responses to muscle stimulation and AA administration in each group are presented in Fig. 3. Functional (Fig. 3A) and AA-induced (Fig. 3B) vasodilations were significantly attenuated in STZ rats compared with normal rats. Insulin treatment in STZ rats resulted in a normalization in both vasodilatory responses. SQ-29548 treatment also restored the vasodilatory responses to muscle contraction or AA administration in STZ rats but had no effect in normal or insulin-treated animals. Superfusion with 10 μM adenosine resulted in similar increases in diameter in all of the groups (Table 1), which were greater than the vasodilatory responses to muscle contraction and AA administration.

**Effect of Acute Hyperglycemia**

Figure 4 shows the effects of acute hyperglycemia on both basal arteriolar diameter and vasodilatory responses to muscle contraction or AA treatment. Treatment with 20 mM d-glucose significantly decreased basal diameter, whereas the same concentration of mannitol had no effect. In all cases, muscle contraction and AA treatment resulted in a significant increase in luminal diameter. Neither glucose nor mannitol treatment affected the increases in diameter in response to muscle contraction or AA. SQ-29548 treatment did not affect the vasodilatory responses (data not shown). Maximal vasodilatory responses induced by adenosine were not different between glucose and mannitol treatment (data not shown).

**DISCUSSION**

We tested the hypothesis that hyperglycemia impairs functional vasodilation via an increased TP-mediated vasoconstriction. The major findings in the present study are as follows. 1) In STZ rats, vasodilatory responses to muscle contraction and AA were significantly attenuated compared with that shown in controls, and TP-receptor blockade with SQ-29548 normalized the vasodilatory responses. 2) Blood glucose levels were normalized after insulin treatment in STZ rats. 3) Normalization of blood glucose restored vasodilatory responses in STZ rats. 4) SQ-29548 treatment had no effect in insulin-treated animals.

<table>
<thead>
<tr>
<th>Basal Diameter, μm</th>
<th>Basal Diameter After SQ-29548, μm</th>
<th>Maximal Diameter, μm</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>9 (SD 1)</td>
<td>9 (SD 1)</td>
</tr>
<tr>
<td>STZ</td>
<td>9 (SD 2)</td>
<td>9 (SD 2)</td>
</tr>
<tr>
<td>STZ + insulin</td>
<td>11 (SD 1)</td>
<td>11 (SD 1)</td>
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Values are means (SD). SQ-29548 does not affect the basal diameter in each group. Adenosine induces a significant increase in arteriolar diameters in control, streptozotocin (STZ), and STZ + insulin groups. Neither the basal nor the maximal diameters are significantly different between groups.

![Fig. 3. A: functional vasodilation is blunted in STZ rats, and insulin treatment restores the dilatory response. SQ-29548 restores functional dilation in STZ rats but has no effect in normal or insulin-treated rats. *P = 0.001 STZ vs. normal or STZ + insulin (n = 5 for STZ and n = 6 for normal and STZ + insulin). B: arachidonic acid (AA)-induced dilation is significantly impaired in STZ rats but is normalized in insulin-treated rats. SQ-29548 restores AA-induced dilation in STZ rats but has no effect in normal or insulin-treated rats. *P = 0.001 STZ vs. normal or STZ + insulin (n = 5 for STZ and n = 6 for normal and STZ + insulin).](http://ajpheart.physiology.org.org)

![Fig. 4. A: when compared with control diameters, the basal arteriolar diameters are decreased during exposure to topical hyperglycemia, whereas functional and AA-induced dilations are not significantly affected by glucose treatment. *P = 0.040 glucose vs. control (n = 5 for each group). B: when compared with control diameters, mannitol treatment does not affect the basal arteriolar diameters or the vasodilatory responses to muscle contraction and AA.](http://ajpheart.physiology.org.org)
Finally, 5) acute hyperglycemia decreased basal diameter, whereas the functional and AA-induced dilations were not impaired. These findings support our hypothesis that impaired functional dilation is related to a chronic hyperglycemia-mediated increase in TP-mediated vasoconstriction.

It is well established that the increase in blood flow in skeletal muscle during exercise is reduced in humans and animals with diabetes (30, 36). However, the mechanisms responsible for the impaired hyperemia are unclear. Previous studies have supported the critical role of prostaglandins(s) in mediating functional vasodilation (11, 26, 35) and the impairment in AA metabolism in response to hyperglycemia. Therefore, it is possible that hyperglycemia may impair the functional vasodilation through alteration of AA metabolism. Consistent with the results of our previous work in hyperglycemic obese Zucker rats (36, 37), the present study shows that the arteriolar dilations in response to muscle contraction and AA were significantly blunted in STZ rats (Fig. 3). Moreover, SQ-29548 completely restored the impaired dilatory responses in STZ rats in contrast to the partial restoration observed in obese Zucker rats (37), implying an obligatory role for an elevated TP-mediated constriction in this hyperglycemic model. A similar study by Mayhan et al. showed that SQ-29548 restored the impaired acetylcholine-induced cerebral arteriolar dilation in STZ rats (25), whereas U-46619, a thromboxane analog, produced a similar vasooconstriction in control and diabetic rats (24). On the basis of these findings, it was suggested that the impaired vasodilatory response in diabetic rats was most likely due to an increased production of PGH2 and/or TXA2 (25).

The reasons for the differences between obese Zucker rats and STZ rats, a more serious hyperglycemic model, in the ability of SQ-29548 treatment to completely restore the functional and AA-induced dilation are not clear. When compared with STZ diabetic rats, obese Zucker rats have other cardiovascular disease risk factors, such as hyperinsulinemia and dyslipidemia, which may impair AA metabolism or functional dilation via different mechanism(s). Indeed, we have previously demonstrated an impaired iloprost-mediated vasodilation in spinotrapezius muscle from obese Zucker rats (37). In the present study, the TP-receptor antagonist restored both the functional and AA-induced vasodilation, suggesting that the PG12-mediated vasodilation may not be altered in this experimental model. The alterations in prostaglandin productions in STZ rats may be time dependent. There is evidence that the production of PG12 in the rat mesenteric bed was unchanged 1 wk after STZ injection but was reduced to 50% of control values 4–8 wk later (29). The available data suggest that PG12 release is decreased in STZ rats; however, because the AA-induced vasodilation is normalized following TP-receptor inhibition, we would hypothesize that metabolites of AA, other than PG12, are responsible for the normalized vasodilatory responses. Future experiments are needed to determine which, if any, metabolite of AA is involved in the normalized vasodilatory responses. Together, these results suggest that the impaired functional dilation in STZ rats is associated with increased TP activation.

In the present study, the impaired vasodilatory responses to muscle contraction and AA were restored after blood glucose levels improved (Fig. 3), and SQ-29548 showed no effect in insulin-treated animals. These results suggest that the increased TP-mediated vasoconstriction and attenuated functional dilation are probably due to chronic hyperglycemia. Indeed, several studies have suggested that a possible mechanism for the endothelial dysfunction in diabetes is hyperglycemia-induced increase in endothelium-derived vasoconstrictor prostanooids. For example, Tesfamariam et al. (33) showed a reduced vasodilatory response to acetylcholine in isolated rabbit aorta in hyperglycemic conditions and showed that SQ-29548 restored the dilatory response. Additional studies have also suggested an enhanced TP-mediated vasoconstriction in response to chronic hyperglycemia (3, 38).

Evidence has accumulated indicating that the generation of reactive oxygen species (oxidative stress) may play an important role in impaired vasodilatory response in individuals and animals with diabetes (8, 10). Dai et al. (9) showed that either SQ-29548 or a free radical scavenger would restore the endothelium-dependent vasodilation in diabetic rats, suggesting a possible relationship between the enhanced oxidative stress and TP activation. Increased formation of ONOO− in cultured human aortic endothelial cells exposed to high glucose results in a shift in AA metabolism to the PG12 precursor PGH2 or other TP-receptor agonists (38). Therefore, it is possible that the increased TP-mediated vasoconstriction observed in the present study is secondary to an enhanced superoxide anion production. Alternatively, it is well established that advanced glycation end products are involved in the pathogenesis of diabetic complications (28). A possible mechanism by which advanced glycation end products elicit toxic effects is the generation of reactive oxygen species (16). In addition, hyperglycemia is proposed to modulate NO synthase activity and increase vasoconstrictor eicosanoid in endothelial cells through nonenzymatic glycation of serum albumin (2). Moreover, other evidence suggests that there is upregulation of cyclooxygenase-2 in diabetic humans and animals (7, 23), which may contribute to the generation of vasoconstrictor metabolites from AA (31). However, more studies are needed to elucidate the link between chronic hyperglycemia and altered AA metabolism.

In the present study, the STZ rats exhibited a restricted weight gain, which was improved with insulin treatment (Fig. 2). Whether low body weight in STZ rats also contributed to the impaired functional dilation is unknown. For example, Higashi et al. (12) suggested that low body mass index is a risk factor for impaired endothelium-dependent vasodilation because of an impaired NO response. However, in the present study, the impaired functional dilation was associated with a blunted AA-induced dilation, both of which were completely restored by SQ-29548, suggesting that an abnormality of AA metabolism rather than NO is responsible for the impaired functional dilation. Future studies are needed to determine the contribution of low body mass to the altered AA metabolism.

The STZ rat is a model of Type 1 diabetes with deficient insulin levels. Treatment with exogenous insulin will not only improve hyperglycemia but also improve insulin levels. A previous study demonstrated that insulin treatment could improve the endothelial function in vessels in the absence of diabetes, most likely through an NO-dependent mechanism (1). In the present study, the contribution of TP-receptor blockade to the improved functional dilation does not support the role for NO or insulin. In addition, although STZ has been shown to cause inflammatory responses that could modify AA metabo-
lism, there is evidence that insulin treatment does not alter the inflammatory response (14). Therefore, the increased functional dilation and AA-induced dilation after insulin treatment are not likely because of improved inflammatory responses. Together, it is most likely the chronic hyperglycemia that impairs functional vasodilation through an enhanced TP-receptor-mediated vasconstriction.

The present study supports a role for acute hyperglycemia in the maintenance of basal vascular diameter but not the impairment of functional hyperemia. In the present study, acute topical hyperglycemia was used to prevent alterations in plasma insulin levels. Consistent with previous studies (5), Fig. 4A shows that acute hyperglycemia reduced basal arteriolar diameter, whereas an osmotic control had no effect. Many studies have demonstrated that endothelium-derived NO is important in maintaining the basal vascular tone and that NO inhibition results in a decrease in arteriolar diameter (34). We postulate that the reduction in resting diameter by acute hyperglycemia (Fig. 4) is due to a decreased NO production and/or bioavailability. Preliminary studies showed that acute glucose treatment failed to further decrease the basal diameter after pretreatment with nitro-1-arginine methyl ester, an NO synthase inhibitor (data not shown). There is evidence showing that NO concentration in arterioles significantly decreases during acute exposure to hyperglycemia (20). Various mechanisms have been proposed to be involved in the suppression of NO concentration, such as increased oxidant production and activation of protein kinase C, which may reduce NO availability and blunt NO synthase activity, respectively (6, 34). In the present study, although the same size arcade arterioles were chosen to be studied in all groups, it is not clear whether the basal diameter or basal blood flow was altered in STZ rats. However, SQ-29548 did not change the basal diameter in all of the groups, indicating that the TP-mediated constriction is not important in mediating basal vascular tone. Furthermore, the maximal vasodilation induced by adenosine was not different between normal and STZ rats, suggesting that the basal vascular tone of selected arterioles in the present study may not be altered in chronic hyperglycemic conditions.

In summary, in STZ rats, the functional and AA-induced vasodilatory responses are attenuated; these responses were normalized by treatment with the TP-receptor antagonist SQ-29548. Normalization of blood glucose level in STZ + insulin rats improves functional and AA-induced dilation. Treatment with the TP-receptor antagonist did not improve functional and AA-induced dilation in the STZ + insulin animals. One hour of acute exposure to high glucose resulted in a decreased basal diameter but did not impair functional and AA-induced dilation. These results suggest that the impaired functional vasodilation in STZ rats is due to a chronic hyperglycemia-induced increase in TP-mediated constriction. Further studies are needed to determine how chronic hyperglycemia increases TP-mediated vasoconstriction and other factor(s) that may contribute to the hyperglycemia-induced impairment in functional vasodilation.

ACKNOWLEDGMENTS

The authors thank Jennifer Dearman for technical help with these experiments.

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These studies were supported by National Heart, Lung, and Blood Institute Grants HL-51971 and HL-63958.


GRANTS

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