Elevated Glucose Promotes Generation of Endothelium-derived Vasoconstrictor Prostanoids in Rabbit Aorta

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Abstract

The effects of glucose on endothelium-dependent responses and vasoactive prostanoid production were determined by incubating isolated rabbit aortae in control (5.5 or 11 mM) or elevated (44 mM) glucose for 6 h to mimic euglycemic and hyperglycemic conditions. Rings of aortae incubated in elevated glucose, contracted submaximally by phenylephrine, showed significantly decreased endothelium-dependent relaxations induced by acetylcholine compared with the aortae incubated in control glucose. Treatment with indomethacin, a cyclooxygenase inhibitor, or SQ29548, a prostaglandin H2/thromboxane A2 receptor antagonist, restored acetylcholine relaxation of rings in elevated glucose to normal, while these agents had no effect on the relaxation of rings incubated in control glucose. Aortae incubated with mannose (44 mM) as a hyperosmotic control relaxed to acetylcholine normally. The relaxations in response to A23187 and sodium nitroprusside were not different between rings exposed to control and elevated glucose. Radioimmunoassay measurements showed a significant increase in acetylcholine-stimulated release of thromboxane A2 and prostaglandin F2alpha in aortae with, but not without endothelium incubated with elevated, but not with control glucose. Thus a possible mechanism for endothelium dysfunction in diabetes mellitus is the hyperglycemia-induced increased generation of endothelium-derived vasoconstrictor prostanoids. (J. Clin. Invest. 1990. 85:929–932.)

Introduction

The endothelium contributes to the local regulation of vascular smooth muscle function by releasing endothelium-derived relaxing factors (EDRF), prostaglandins (PG) and enzymes that activate or degrade vasoactive hormones (1, 2). The integrity and function of the endothelial cell layer are profoundly altered in diabetic animals and man (3). Altered prostanoid production is among the many factors implicated in the pathogenesis of diabetic vascular disease (4–7). Recent evidence indicates that aortic rings from alloxan-induced diabetic rabbits with a mean plasma glucose of 20 mM demonstrate an abnormal cholinergic receptor-mediated endothelium-dependent relaxation. The impaired relaxation is mediated by an increased production of vasoconstrictr prostanoids including thromboxane A2 by the diabetic endothelium (4). Whether this abnormality results from hyperglycemia or hyperlipidemia associated with the diabetic experimental model is not known. These studies were undertaken to examine the direct effects of an elevated glucose milieu per se, on endothelium-dependent responses and prostanoid production by incubating isolated rabbit aortic rings in control or elevated glucose media. Our results indicate that exposure to an increased glucose concentration for 6 h can impair cholinergic endothelium-dependent relaxations by augmenting the production of vasoconstrictor prostanoids from the endothelium.

Methods

The abdominal aorta was dissected from male New Zealand white rabbits (2.2–2.5 kg) killed by exsanguination after anesthesia with pentobarbital sodium (30 mg/kg i.v.) and anticoagulation with heparin sodium (150 U/kg i.v.). The adhering perivascular tissue was carefully removed. Rings of aortae (5 mm long) were suspended from strain gauges for measurement of isometric circumferential force. The rings were placed in organ baths (25 ml) filled with physiological salt solution (PSS) of the following composition (in mM): NaCl 118.3, KCl 4.7, MgSO4 0.6, K2HPO4 1.2, CaCl2 2.5, NaHCO3 25.0, and calcium ethylene-diamine tetraacetic acid 0.026. The solutions were maintained at 37°C and gassed with 95% O2, 5% CO2 to maintain pH at 7.4. Length of the smooth muscle was increased stepwise over 90 min to adjust basal tension to 6 g. This was found to be optimal for contraction by testing repeated contractions to potassium (80 mM). Thereafter, length was not altered. Aortic rings were then incubated in 5.5, 11, or 44 mM glucose for 6 h. Mannose (44 mM) was used as a hyperosmotic control. After the 6-h incubation the arteries were contracted with phenylephrine to 40–50% of their maximal contraction induced by potassium (120 mM). When the contraction stabilized the responses to acetylcholine, A23187, and sodium nitroprusside were obtained by increasing bath concentration in half-log cumulative increments. Inhibitors were present during the 6-h incubations and during subsequent concentration-responses. Radioimmunoassay: Segments of aortae (2.5 cm) were incubated in PSS and gently bubbled with 95% O2, 5% CO2 at 37°C for 6 h in control (11 mM) or elevated (44 mM) glucose. The PSS was changed every hour. Segments were prepared in which the endothelium was left intact or removed mechanically by gently rolling the segment on wet filter paper using forceps inserted into the lumen. At the end of the 6-h period the segments were incubated in PSS (1 ml) sequentially in the absence and presence of acetylcholine (10–5 M) for 30 min each. The tissues were blotted dry and weighed. The incubates were frozen at −80°C until analyzed. Radioimmunoassays were used to quantify the release of thromboxane B2 (the stable hydrolytic product of thromboxane A2), 6-keto-PGF1alpha (the stable hydrolytic product of prostacy-
clin), PGF₂α, and PGE₁ in the incubation buffers. Radioimmunoassays were performed using specific antisera of thromboxane B₂, PGF₂α, and PGE₁ (courtesy of Dr. Lawrence Levine, Brandeis University, Waltham, MA), 6-keto-PGF₁α (Biomol Research Laboratories, Inc., Plymouth, PA), tritiated standards (DuPont-NEN, Boston, MA), and unlabeled standards (UpJohn Co., Kalamazoo, MI; 8). Standard curves contained an equal volume of PSS to that being assayed and all dilutions were made with PSS. Cross-reactivity with other measured prostanooids was < 5%. The limits of sensitivity for the radioimmunoassay with the experimental conditions described for thromboxane B₂, 6-keto-PGF₁α, and PGF₂α were 1 pg/ml and for PGE₁ was 10 pg/ml. Standard curves performed with the addition of glucose (44 mM) were identical to those performed in control glucose.

**Drugs.** The pharmacological agents used were the following: acetylcholine chloride, calcium ionophore A23187, indomethacin, mannose, phenylephrine, and sodium nitroprusside (Sigma Chemical Co., St. Louis, MO), dazmegrel (Pfizer Inc., Groton, CT), ibuprofen and meclofenamate (Biomol Research Laboratories, Inc.), and SQ29548, a gift from Schubb Pharmaceuticals (Princeton, NJ). Concentrations were expressed as final molar bath concentrations. Unless otherwise specified, drugs were dissolved in distilled water such that volumes of 0.1 ml were added to the organ bath. A23187 was prepared in ethanol (95%). Indomethacin was prepared in 2% Na₂CO₃ immediately before use. Stock solutions of SQ29548 were made in 95% ethanol and further dilutions were made in PSS. Ibuprofen and meclofenamate were prepared in 0.1 N NaOH.

**Data analysis.** Maximal relaxation obtained in response to each concentration of agonist is expressed as percent change in the level of tone induced by phenylephrine. The IC₅₀ was estimated graphically as the concentration causing 50% relaxation of the induced tone. Data are expressed as mean±SE. Statistical evaluation of the data was made using repeated measures of analysis of variance for concentration-response curves or Student's t test for paired comparisons of responses of rings or release of prostanooids from arterial segments from the same animal. P values < 0.05 were regarded as significant. In all experiments, n equals the number of rabbits from which rings were taken.

**Results**

**Endothelium-dependent relaxations.** Rings of aortae with intact endothelium incubated with 5.5, 11, or 44 mM glucose for 6 h were contracted with phenylephrine (concentration, −log M: 6.5±0.5, n = 5; 6.8±0.2, n = 10; and 6.7±0.1, n = 10; respectively) which caused similar contractions of 7.9±0.8, 7.7±0.4, and 7.2±0.4 g, respectively. The rings were then exposed to increasing concentrations of acetylcholine (10⁻⁵–10⁻⁴ M). The relaxations induced by acetylcholine were significantly decreased in aortic rings incubated with elevated (44 mM) glucose compared with those in control (5.5 and 11 mM) glucose. Relaxations caused by acetylcholine (3 × 10⁻⁷–10⁻⁴ M) were followed by contractions of aortae incubated in elevated, but not in control glucose (Fig. 1). Aortae incubated with mannose (44 mM) for 6 h relaxed to acetylcholine normally (IC₅₀ −log M: 7.2±0.1, n = 4). Aortae incubated in elevated glucose contracted with PGF₂α instead of phenylephrine showed similar impaired acetylcholine-induced relaxations (data not shown).

Treatment with indomethacin, meclofenamate or ibuprofen (10⁻⁵ M) restored acetylcholine relaxations of rings incubated with elevated glucose (IC₅₀ −log M: 7.3±0.1, n = 6, 7.2±0.1, n = 4 and 7.2±0.1, n = 4, respectively), such that the relaxations did not differ statistically from those observed in rings incubated in control glucose. Similarly, treatment with SQ29548 (3 × 10⁻⁶ M) restored acetylcholine relaxations of rings incubated in elevated glucose to normal (IC₅₀ −log M: 7.0±0.1, n = 4). Neither the cyclooxygenase inhibitors nor SQ29548 had a significant effect on the relaxation to acetylcholine of rings incubated with control glucose (Fig. 2). In rings of aortae incubated in elevated glucose, treatment with dazmegrel (3 × 10⁻⁶ M) did not significantly affect the abnormal relaxations caused by acetylcholine (IC₅₀ −log M: 6.2±0.2, n = 3).

The relaxations caused by A23187 (10⁻⁵–3 × 10⁻⁶ M) were not significantly different between rings incubated in control (11 mM) or elevated (44 mM) glucose. The maximal relaxation caused by A23187 (3 × 10⁻⁶ M) was 34±5.6 vs. 37±5.4%, respectively, (n = 5).

**Endothelium-independent relaxations.** The relaxations caused by sodium nitroprusside (10⁻⁶–10⁻³ M) were not significantly different between rings incubated in control (11 mM) or elevated (44 mM) glucose. The maximal relaxation caused by A23187 (3 × 10⁻⁶ M) was 34±5.6 vs. 37±5.4%, respectively, (n = 5).

**Prostanoid production.** Under basal conditions or in the presence of acetylcholine (10⁻⁶ M), the production of the prostanooids, thromboxane B₂, 6-keto-PGF₁α, PGF₂α, and PGE₂, was significantly greater in segments with, than in seg-
The effect of incubation on the response of the aorta to acetylcholine in the presence of endothelium was significant (Figure 2A). Similar observations were also made in the absence of endothelium (Figure 2B). Incubation with elevated glucose (44 mM) in the presence of endothelium enhanced the relaxations to acetylcholine, while in the absence of endothelium, incubation with glucose did not significantly alter the relaxations to acetylcholine (Figure 2C). These results suggest that the enhanced relaxation response to acetylcholine in the presence of endothelium may be due to the production of endothelium-derived mediators, such as nitric oxide, that are not produced under control conditions.

Table I. Basal and Acetylcholine-stimulated Release of Immunoreactive Prostanoids from Aortic Segments with and without Endothelium Incubated in Control and Elevated Glucose

<table>
<thead>
<tr>
<th>Glucose</th>
<th>With endothelium</th>
<th>Without endothelium</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Elevated</td>
</tr>
<tr>
<td>Thromboxane B2</td>
<td>8.5±1.1†</td>
<td>8.2±1.4†</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>14±1.8†</td>
<td>24.9±3.4†</td>
</tr>
<tr>
<td>PGF2α</td>
<td>61±6.2†</td>
<td>94±18†</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>152±26†</td>
<td>193±29†</td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
<td>201±67†</td>
<td>273±54†</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>937±164†</td>
<td>963±88†</td>
</tr>
</tbody>
</table>

Values are expressed as means±SE (pg/mg tissue per 30 min). The weights of the rabbit aortae used for control and elevated glucose incubations were 44±5.1 and 49±6.8 mg (with endothelium, n = 6) and 46±3.1 and 44±3.8 (without endothelium, n = 3), respectively. * Indicates significant difference between prostanoid production in control and elevated glucose. † Indicates significantly greater release from segments with endothelium compared to those from those without endothelium. ‡ Indicates significant increase caused by acetylcholine (10−6 M) compared with basal.

Discussion

The present experiments performed after exposure to conditions mimicking hyperglycemia demonstrate impairment of endothelium-dependent acetylcholine-induced relaxation by stimulated production of endothelium-derived vasconstrictor factor(s). The abnormal relaxations observed after incubation with elevated glucose for 6 h was a time-dependent effect because incubation for 2 or 3 h in elevated glucose caused a less pronounced abnormality (unpublished observations). The alterations caused by elevated glucose are not due to a hyperosmotic effect because the same concentration of mannose had no effect on the relaxations induced by acetylcholine. The cyclooxygenase inhibitors, indomethacin, meclofenamate, and ibuprofen, restored acetylcholine-induced relaxations suggesting that the inhibition was mediated by a cyclooxygenase product produced in the presence of elevated glucose. The restoration of acetylcholine-induced relaxation by the cyclooxygenase inhibitor, SQ29548 (9), is consistent with mediation by prostaglandin endoperoxides or their derivatives, including thromboxane A2 and PGF2α.

A role for these vasconstrictor prostaglandins is further supported by radioimmunoassay measurements. In these experiments elevated glucose induced rapid alterations in arachidonic metabolites produced during stimulation with acetylcholine yielding increased amounts of thromboxane A2 and PGF2α. Cholinergic agents have been shown to also stimulate prostaclin and PGE2 synthesis by the endothelium of rabbit aorta (10, 11). It is less likely that prostaclin or PGE2 contribute to the abnormal relaxation to acetylcholine because the stimulated release of these prostanoids was independent of glucose concentration in the medium, and both are less potent vasconstrictors of the rabbit aorta compared with the thromboxane A2 mimetic, U46619, or PGF2α (4).

Experiments with pharmacological antagonists as well as radioimmunoassay measurements point to vasconstrictor prostaglandins including thromboxane A2 and PGF2α as the mediators of the abnormal acetylcholine response. A major role for thromboxane A2 in the abnormal acetylcholine-mediated relaxation is less likely as suggested by the failure of the thromboxane synthase inhibitor, dazmegrel (12), to correct the...
response. Increased production of prostaglandin endoperoxides could cause the abnormal acetylcholine response of aorta exposed to elevated glucose because the vasconstriction which they cause is blocked by SQ29548 (9) and their formation is not prevented by dazmegrel (12). The preferential synthesis of more potent vasoconstrictor endoperoxide-derived prostanooids may also favor their direct role in the impaired relaxation to acetylcholine.

The relaxations induced by sodium nitroprusside, an endothelium-independent vasodilator which relaxes smooth muscle by a mechanism similar to that of EDRF (13), as well as to that to the calcium ionophore A23187, a non-receptor-mediated endothelium-dependent vasodilator, were not different between aortae in control or elevated glucose. This suggests that the release, or responsiveness of the smooth muscle to EDRF is not altered by elevated glucose. A major finding in this study is that elevated glucose enhances release of vasoconstrictor prostanooids following cholinergic stimulation, and the endothelium is its source. This is supported by the normal basal release of prostanooids in aortae with endothelium and by the normalization of acetylcholine-stimulated prostanooid production after removal of the endothelium.

The present findings complement the findings in isolated aortae of alloxan-induced diabetic rabbits, which showed impaired endothelium-dependent relaxations induced by acetylcholine that were corrected by cyclooxygenase inhibition or SQ29548 and were associated with increased thromboxane A2 production (4). Others have reported that endothelium-dependent relaxations to acetylcholine are impaired in aortae of streptozotocin-induced diabetic rat and in the spontaneously diabetic BB Wistar rat (14-16). Additionally, decreased endothelium-dependent relaxations to acetylcholine have been reported in isolated penile corpus cavernosum tissue of impotent diabetic men (17). Thus, the present studies suggest that by varying the glucose concentration in the medium, a useful in vitro model is achieved for studying the changes in endothelial cell vasodilator function as well as prostanooid production seen in diabetic animals and man. In experimental diabetes, hyperlipidemia and elevated cholesterol similar to that seen in diabetic patients have been reported to increase platelet thromboxane A2 generation (18, 19). The present study provides evidence that elevated plasma glucose per se may be a primary factor for the increased production of vasoconstrictor prostanooids by the endothelium. The observation that glucose can readily contribute to changes in the function of the endothelium by inducing generation of vasoconstrictor prostanoinds in response to cholinergic stimulation suggests that production of these prostanooids during hyperglycemia may contribute to vascular complications in diabetes mellitus.

Acknowledgments

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References


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