Effect of Elevated Glucose on Cyclic GMP and Eicosanoids Produced by Porcine Aortic Endothelium

Robert M. Weisbrod, Michael L. Brown, and Richard A. Cohen

The short-term effects of elevated glucose on cyclic GMP (cGMP) and eicosanoid production in pig aortic endothelial cell monolayers was determined by incubating cells in 5.5 mM or 44 mM glucose for 6 hours. Bradykinin- or A23187-stimulated cGMP production was significantly reduced in cells incubated in 44 mM glucose compared with 5.5 mM glucose. Stimulation of cGMP levels with exogenously added nitric oxide (NO) was also decreased to a similar extent in cells exposed to 44 mM glucose. These data suggest that NO production stimulated by bradykinin or A23187 was unchanged by elevated glucose. Assayed eicosanoids, including 6-ketoprostaglandin (PG) Fα, PGEα, PGFαα, and 15(S)-hydroxy-(5Z, 8Z, 11Z, 13E)-eicosatetraenoic acid, stimulated by bradykinin or A23187, were increased in cells exposed to 44 mM glucose. These eicosanoid products formed from exogenously added arachidonic acid did not differ between cells incubated in 5.5 mM or 44 mM glucose. Hyperosmolar concentrations of mannose or sucrose had no effect on cGMP levels but did mimic the effect of elevated glucose on eicosanoid production. These data suggest that hyperglycemia in diabetes may interfere with NO-induced guanylate cyclase activation but not NO production in the endothelium and that increased phospholipase activity, secondary to hyperosmolarity, may account for elevated eicosanoid levels. (Arteriosclerosis and Thrombosis 1993;13:915–923)

KEY WORDS • pig aortic endothelial cells • nitric oxide • cyclic GMP • eicosanoids • glucose • diabetes mellitus

The endothelium is an important source of vasoactive substances that regulate vascular tone. Endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO), stimulates smooth muscle guanylate cyclase to produce cyclic GMP (cGMP) and initiate relaxation. Eicosanoids also produced in the endothelium may act as vasodilators or vasoconstrictors, depending on the vascular bed and species. Alterations in the production or action of these substances may explain abnormal endothelium-dependent vasomotor responses in several vascular diseases, including diabetes mellitus, hypertension, and hypercholesterolemia.

Studies on diabetic rabbit and rat models and human vascular tissue have shown decreased endothelium-dependent relaxations. It is possible that the abnormal relaxation seen in diabetes is due to decreased levels of NO, increased vasoconstrictor eicosanoids, or a combination of both. Tesfamariam et al6 have shown that acetylcholine-induced relaxations of the aorta are reduced in the alloxan-induced diabetic rabbit and that there is a development of dose-dependent contractions that occurs concomitantly with increased production of vasoconstrictor eicosanoids. Since the abnormal relaxations are restored and the contractions are blocked by cyclooxygenase inhibitors or the prostaglandin (PG) H2/thromboxane A2 receptor antagonist SQ29548, increased activity of the cyclooxygenase pathway has been implicated in the abnormal vasomotor response. Alternatively, Abiru et al8 have suggested that the abnormal relaxation of diabetic arteries is due to decreased NO production, because decreased cGMP levels were measured in diabetic rabbit aorta. The abnormal production of vasoactive substances by the endothelium that is observed in diabetic rabbit aorta is believed to be directly caused by exposure of the endothelial cells to hyperglycemia, because following a 6-hour exposure to 44 mM glucose, normal rabbit aortic rings show reduced endothelium-dependent relaxation and increased eicosanoid production quite similar to those seen in the diabetic rabbit aorta.11 To better understand the mechanism by which elevated glucose level alters the function of the endothelium in intact arteries in the short-term, the present studies were designed to determine the effects of exposure of cultured endothelial cells for 6 hours to elevated glucose levels under conditions similar to those used for the studies of intact arteries. Pig aortic endothelial cells (PAECs) are an apt model with which to study the
effects of elevated glucose levels, as Brown et al have demonstrated that exposure of PAECs to elevated glucose levels for several days causes a decreased production of 15(S)-hydroxy-(5Z, 8Z, 11Z, 13E)-eicosatetraenoic acid (15-HETE). In the present studies NO was assessed by measuring the accumulation of endothelial cell cGMP that occurs during NO production. Eicosanoids were measured by radioimmunoassay. A preliminary report of some of these observations has been made.

Methods

Cell Culture

Pig thoracic aortas were obtained from a local abattoir and were aseptically prepared by removing the perivascular connective tissue and opening longitudinally to expose the luminal surface. The PAECs were obtained by scraping the luminal surface once with a razor blade. They were transferred to a 100-mm-diameter Petri dish (Costar, Cambridge, Mass.) containing 10 mL medium and dispersed by pipetting up and down several times. Cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO No. 430-1600, Grand Island, N.Y.) supplemented with 3.7 g/L NaHCO3, 10% fetal bovine serum (FBS; Hazelton or Sigma), 100 units/mL penicillin, and 100 μg/mL streptomycin (GIBCO). The media were changed every 2–3 days. Cell cultures were maintained in a humidified incubator at 37°C in the presence of 5% CO2 to keep the media saturated. NO was assessed by measuring the uptake of fluorescently labeled acetylated low density lipoprotein (Biomedical Technologies Inc., Stoughton, Mass.). Confluent monolayers in six-well plates were incubated for 6 hours with physiological salt solution (PSS) of the following composition (in mM): NaCl, 118.3; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 25.0; and Na2EDTA, 0.026. To mimic diabetes, paired monolayers were exposed to PSS containing either 5.5 or 44 mM glucose for 6 hours. The calculated osmolarity of the low- and high-glucose PSS was approximately 310 and 832 mOsm, respectively. PSS was replaced every 2 hours with fresh PSS. Radioimmunoassays were performed by using specific antisera for 6-keto-PGF1α, PGE2, PGF2α, thromboxane B2 (TXB2, the stable hydrolytic product of thromboxane A2), and 15-HETE in the PSS. Radioimmunoassays were performed by using specific antisera for 6-keto-PGF1α, PGE2, PGF2α, thromboxane B2, and 15-HETE (Advanced Magnetics, Cambridge, Mass.), tritiated standards (DuPont-Neuralg New England Nuclear, Boston), and unlabeled standards (UpJohn, Kalamazoo, Mich.). Cross-reactivity with other measured eicosanoids was ≤5%. The limits of sensitivity of the radioimmunoassay were 1 pg/mL for 6-keto-PGF1α, PGE2, PGF2α, thromboxane B2, and 15-HETE. Results are expressed as nanograms eicosanoid per well.

Drugs

Agents used were bradykinin, A23187, ATP, arachidonic acid, indo-methacin, IBMX, N-nitro-L-arginine (NNA), SOD (Sigma Chemical Co., St. Louis, Mo.), and NO (Matheson Gas, Gloucester, Mass.). Concentrations are expressed as final molar concentrations. Indomethacin was prepared in 2% Na2CO3 immediately before use. A23187 was prepared in 95% ethanol.
IBMX was prepared in dimethyl sulfoxide. NO was prepared in water (4°C) in air-tight Vacutainer tubes (Becton Dickinson, Rutherford, N.J.) that were purged of O₂ by vacuum for 20 minutes and bubbling with N₂ for 20 minutes. NO gas was bubbled in 8 mL water for 10 minutes to yield a saturated solution (approximately 10⁻³ M) and was serially diluted 1:10 so that a 100-fold dilution of the NO solution occurred when it was added to the cells. NO solutions were made up immediately before addition to the cells.

Data Analysis
Data are expressed as mean±SEM. Statistical evaluation of the data was achieved using Student’s t test for paired comparisons between cells incubated in 5.5 mM or 44 mM glucose PSS from the same plate or with unpaired comparisons between drug treatments in different plates. A value of p<0.05 was considered significant. In all experiments, n represents the number of 9.6-cm² wells used. On any one day, from one to three replicates of each experimental condition were performed.

Results

cGMP Levels
After 30 minutes of equilibration in the presence of IBMX, basal cGMP levels in PAECs exposed to 5.5 mM glucose were 13.7±3.6 fmol/g. Bradykinin (10⁻⁷ M) increased cGMP to 348.9±51.2 fmol/g at 1 minute, and values had decreased to 51.1±9.7 after 10 minutes (Figure 1). Basal levels of cGMP were not significantly different in PAECs exposed to 44 mM glucose for 6 hours (12.2±2.4 fmol/g). cGMP levels at 1 minute after bradykinin stimulation were significantly decreased in cells incubated in 44 mM glucose compared with cells incubated in 5.5 mM glucose, but at 10 minutes there was no significant difference (Figure 1).

In cells incubated in 5.5 mM glucose for 6 hours, A23187- (10⁻⁶ M) stimulated cGMP levels at 1 minute were similar to those after addition of bradykinin, but cGMP increased further at 10 minutes (Figure 1). In PAECs exposed to 44 mM glucose for 6 hours, A23187-stimulated levels of cGMP were significantly less than control cells at 1 and 10 minutes (Figure 1). ATP (10⁻⁵ M) also stimulated cGMP levels at 1 minute (Figure 2) and returned to near basal levels at 10 minutes (data not shown). Although the average ATP-stimulated cGMP levels were less in cells exposed for 6 hours to 44 mM glucose, the differences were not significant (Figure 1).

In the presence of SOD (150 units/mL; n=6) added throughout the 6-hour period, elevated glucose still caused a statistically significant decrease in cGMP levels stimulated by bradykinin (10⁻⁷ M) (Figure 2). Exogenous NO (10⁻⁸ M) stimulated cGMP levels maximally at 1 minute, and levels decreased at 10 minutes (data not shown). Levels of cGMP 1 minute after NO stimulation were significantly less in cells exposed to 44 mM glucose for 6 hours compared with those exposed to 5.5 mM glucose (Figure 2).

In the absence of IBMX, basal cGMP levels (4.8±1.2 fmol/µg; n=3) were stimulated by A23187 (10⁻⁶ M) at 1 and 10 minutes (24±1.0 and 15±2.0 fmol/µg, respectively). In PAECs exposed to 44 mM glucose, basal levels of cGMP in the absence of IBMX were unchanged (4.2±0.8 fmol/µg), but A23187-stimulated levels were significantly less at 1 and 10 minutes (14±0.7 and 6.1±1.3 fmol/µg, respectively; n=3) compared with 5.5 mM glucose.

Eicosanoid Production
In unstimulated cells, 6-keto-PGF₁α levels were unchanged over the 30-minute period measured, and there was no significant difference in basal levels of 6-keto-PGF₁α between cells incubated in 5.5 mM or 44 mM glucose (Figure 3). 6-Keto-PGF₁α levels were stimulated by bradykinin (10⁻⁷ M), peaking at 10 minutes and unchanged at elevated levels at 30 minutes (Figure 3). The other eicosanoids measured showed similar time courses to that shown for 6-keto-PGF₁α (data not shown); in further studies, therefore, eicosanoids were...
measured after 10 minutes of agonist stimulation. Ten minutes after bradykinin stimulation, levels of 6-keto-PGF$_{1\alpha}$, PGE$_2$, and 15-HETE were significantly increased in cells exposed to 44 mM glucose compared with 5.5 mM glucose (Figure 4). The time course of eicosanoid production after addition of A23187 (10$^{-6}$ M) was similar to that after addition of bradykinin (data not shown). After 10 minutes of stimulation with A23187, levels of PGE$_2$ and PGF$_{2\alpha}$ were significantly increased in cells exposed to 44 mM glucose, but the levels of the other eicosanoids measured were not significantly different (Figure 4). TxB$_2$ levels did not increase after stimulation with bradykinin or A23187, and there was no significant difference between the levels produced by cells exposed to either 5.5 or 44 mM glucose.

Arachidonic acid (10$^{-6}$ and 10$^{-5}$ M) stimulated production of eicosanoids at 10 minutes to levels similar to those caused by bradykinin or A23187 (Figure 5). There were no apparent differences in the levels of eicosanoids produced from exogenous arachidonic acid in cells exposed to 44 mM glucose compared with 5.5 mM glucose (Figure 5).

**Effects of Hyperosmolarity**

Supplementing 5.5 mM glucose for 6 hours with mannose (38.5 mM) or sucrose (38.5 mM) to achieve equivalent osmolarity to 44 mM glucose had no significant effect on cGMP levels under basal conditions or at 1 or
Physiological salt solution for 6 hours. Values are expressed as mean±SEM; n=4 wells performed on four separate days.

Note that the right y axis has a different scale for TxB2 and 15-HETE.

10 minutes after bradykinin stimulation (Figure 6). Exogenous NO (10^-6 M) stimulated levels of cGMP were also not significantly affected by mannose (Figure 6).

Levels of eicosanoids under basal conditions were unaffected by exposure to hyperosmolar mannose or sucrose for 6 hours compared with 5.5 mM glucose (data not shown). Bradykinin (10^-7 M) stimulated levels of eicosanoids after 10 minutes were significantly elevated in cells exposed to elevated mannose or sucrose compared with 5.5 mM glucose (Figure 6).

**Interactions Between cGMP and Eicosanoids**

Treatments with indomethacin (10^-6 M) markedly inhibited production of all prostanoids as well as 15-HETE (Table 1), but had no apparent effect on basal or A23187-stimulated eicosanoid levels (Table 1). IBMX (10^-4 M), which markedly increased cGMP levels at 1 or 10 minutes after stimulation with A23187, had no significant effect on levels of eicosanoids produced by PAECs (Table 1).

**Discussion**

PAECs have been used by others as a model to study the release of endothelium-derived vasoactive factors including NO and eicosanoids. White and Martin have studied production of NO and 6-keto-PGF1α simultaneously by methods similar to those used here. Intracellular levels of cGMP in the endothelial cells were used in this study as an indicator of NO production. This technique is validated by the fact that the NO synthase inhibitor NNA prevented the agonist-induced rise in cGMP, as previously reported with another NO synthase inhibitor. Measurement of the product of guanylate cyclase provides an assay that depends not only on NO production but also on guanylate cyclase stimulation and phosphodiesterase activity. Differences in guanylate cyclase sensitivity and phosphodiesterase activity may be inferred if any changes occur in cGMP levels in response to exogenous NO. The phosphodiesterase inhibitor IBMX was used to enhance the levels of cGMP and to exclude the contribution of phosphodiesterase to the differences observed.

The eicosanoids measured by radioimmunoassay in this study demonstrate that these endothelial cells produce large amounts of PGE2 and PGF2, in addition to 6-keto-PGF1α. PAECs also produce 15-HETE. In this study the release of 15-HETE was reduced significantly by indomethacin, which suggests, along with other studies, that it can arise as a result of cyclooxygenase activity. Thromboxane A2 levels did not significantly increase after agonist stimulation, raising doubt as to whether thromboxane A2 is made enzymatically in significant amounts by these cells. This is also suggested by the fact that TxB2 was the only metabolite that did not increase after the addition of exogenous arachidonic acid (Figure 5). Nevertheless, the levels detected were reduced by indomethacin (Table 1), and it is unlikely that the low levels represent cross-reactivity with other eicosanoids because an increase was not observed with elevated glucose, as seen for the bulk of eicosanoids.

IBMX greatly enhanced the levels of cGMP but did not influence the measurements of released eicosanoids (Table 1). In fact, entirely preventing the rise in cGMP with NNA did not influence eicosanoid release (Table 1). Also, inhibiting eicosanoid production with indomethacin had no apparent effect on cGMP levels (Table 2). These studies indicate that although NO and eicosanoids are released by the agonists simultaneously, there is no apparent influence of either product on the release of the other, as suggested previously.

**Effect of Elevated Glucose on cGMP**

A 6-hour exposure to elevated glucose significantly decreased agonist-stimulated cGMP levels; the lower levels were noted during the peak stimulation by bradykinin but persisted throughout the stimulation by A23187. The difference caused by glucose is unlikely to be due to a change in phosphodiesterase activity because the difference was noted both in the presence and
absence of IBMX. The fact that exogenous NO-stimulated levels were decreased similarly to those elicited by the agonists indicates that the effect of elevated glucose on the cGMP rise in response to the agonists is not due to decreased NO production but rather to impaired stimulation of PAEC guanylate cyclase. This result may imply that agonist-induced, receptor-mediated transmembrane signaling, which leads to a rise in intracellular calcium levels and activation of NO synthase, is also normal in those PAECs exposed to elevated glucose.

Superoxide anion interferes with guanylate cyclase stimulation by NO, so that increased levels of the free radical in elevated glucose could explain the decrease in cyclic nucleotide observed. This is less likely, given that SOD did not prevent the decrease in cGMP. The effect of elevated glucose on guanylate cyclase stimulation was not due to hyperosmolarity, because increasing the osmolality to the same levels with mannose or sucrose had no significant effect on the bradykinin- or NO-stimulated levels of cGMP.

Effect of Elevated Glucose on Eicosanoid Production

Although basal levels of eicosanoids were unaffected by exposure to elevated glucose, there was a generalized increase when production was stimulated by either bradykinin or A23187 in cells exposed to elevated glucose. The effect of elevated glucose on the response to the two agonists was similar, with the notable exception of 15-HETE, which was increased when cells exposed to elevated glucose were stimulated with bradykinin but not when they were stimulated with A23187. The increased eicosanoid production caused by expo-
Glucose and Endothelial Vasoactive Products

Weisbrod et al

Table 1. Effects of Cyclooxygenase, NO Synthase, and Phosphodiesterase Inhibition on A23187-Stimulated Eicosanoid Formation

<table>
<thead>
<tr>
<th>Agent</th>
<th>Glucose concentration</th>
<th>6-Keto-PGF$_{2a}$</th>
<th>PGE$_{2a}$</th>
<th>PGF$_{2a}$</th>
<th>TxB$_2$</th>
<th>15-HETE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.5 mM</td>
<td>5.5 mM</td>
<td>44 mM</td>
<td>44 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin (10$^{-6}$ M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>26±8.3</td>
<td>1.6±0.5*</td>
<td>35±12</td>
<td>0.5±0.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>9.8±2.7</td>
<td>11±0.5*</td>
<td>13±3.3</td>
<td>0.8±0.2*</td>
<td>0.09±0.04</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>NNA (3×10$^{-4}$ M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>14±2.3</td>
<td>6.2±0.8</td>
<td>11±6.9</td>
<td>17±5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>15±3.4</td>
<td>6.0±2.5</td>
<td>18±4.9</td>
<td>10±6.4</td>
<td>0.05±0.01</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>IBMX (10$^{-4}$ M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>9.5±1.3</td>
<td>6.6±0.7</td>
<td>10±3.1</td>
<td>17±5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>11.0±1.2</td>
<td>6.7±1.2</td>
<td>8±6.9</td>
<td>12±1.5</td>
<td>0.08±0.02</td>
<td>1.0±0.2</td>
</tr>
</tbody>
</table>

NO, nitric oxide; PG, prostaglandin; TxB$_2$, thromboxane B$_2$; 15-HETE, 15(S)-hydroxy-(5Z, 8Z, 11Z, 13E)-eicosatetraenoic acid; nd, not detectable; -, in the absence of; +, in the presence of. Data are expressed as mean±SEM in nanograms per well; n=4, 2, and 9 for indomethacin, N-nitro-L-arginine (NNA), and 3-isobutyl-1-methylxanthine (IBMX), respectively. Cells used in each of the three treatments are from different primary cultures. Pig aortic endothelial cells were incubated for 6 hours in physiological salt solution containing 5.5 mM or 44 mM glucose. Indomethacin was present throughout the 6-hour incubation, and NNA and IBMX were added during the last 30 minutes. Eicosanoid levels were measured after stimulation with A23187 (10$^{-6}$ M) for 10 minutes.

*Indomethacin significantly decreased all eicosanoids; NNA or IBMX had no apparent effect.

Table 2. Effects of Cyclooxygenase or NO Synthase Inhibition on A23187-Stimulated cGMP Formation in Pig Aortic Endothelial Cells

<table>
<thead>
<tr>
<th>Agent</th>
<th>Glucose concentration</th>
<th>Basal</th>
<th>A23187 (10$^{-6}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Minute</td>
</tr>
<tr>
<td>Indomethacin (10$^{-6}$ M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5.5 mM</td>
<td>11±4.4</td>
<td>430±110</td>
</tr>
<tr>
<td>+</td>
<td>5.5 mM</td>
<td>7.9±1.6</td>
<td>450±0.0</td>
</tr>
<tr>
<td>NNA (3×10$^{-4}$ M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5.5 mM</td>
<td>5.6±1.5</td>
<td>440</td>
</tr>
<tr>
<td>+</td>
<td>5.5 mM</td>
<td>4.6±0.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

NO, nitric oxide; cGMP, cyclic GMP. Data are expressed as mean±SEM in femtomoles cGMP per microgram protein; n=2–5 for indomethacin and 1–2 for N-nitro-L-arginine (NNA). After a 6-hour incubation in physiological salt solution containing 5.5 mM or 44 mM glucose, pig aortic endothelial cells were equilibrated for 30 minutes with 3-isobutyl-1-methylxanthine (10$^{-4}$ M). Indomethacin was present throughout the 6-hour incubation and NNA was added during the last 30 minutes. cGMP levels were measured in unstimulated cells (basal) and after 1 and 10 minutes of A23187 (10$^{-6}$ M). Indomethacin had no apparent effect on cGMP levels, whereas NNA abolished A23187-stimulated increases in cGMP levels.

Sure to elevated glucose apparently depends on the release of endogenous arachidonic acid substrate, because the production following the addition of exogenous substrate was unaffected. This suggests that the increased production in response to the two agonists depends on altered phospholipase activity. These results differ from the effect of culturing PAECs for two passages in 15.6 mM glucose, in which an overall decrease in arachidonic acid metabolites were produced, although interestingly, 15-HETE released in response to the two agonists or cells exposed to elevated glucose. The fact that cGMP levels in response to both bradykinin and A23187 were decreased by glucose also suggests that the increase observed in arachidonate metabolites is specific for that pathway, rather than a generalized increase in activation of cell signaling pathways. Interestingly, unlike the decreased NO responsiveness, the increase observed in eicosanoids after exposure to elevated glucose was apparently due to hyperosmolarity, as eicosanoids were also increased after exposure to elevated mannose and sucrose.

In contrast to our findings that glucose increased bradykinin-stimulated prostacyclin, measured as 6-keto-
PGF\textsubscript{2\alpha}, others have found that diabetic human and animal vascular tissue and endothelial cells cultured in elevated glucose produce less prostacyclin (see References 22 and 23). In our own studies, the aortas of 6-week-old diabetic rabbits\textsuperscript{5} and those of normal rabbits incubated for 6 hours in elevated glucose\textsuperscript{11} showed no significant change in this eicosanoid, although others, notably PGF\textsubscript{2\alpha} and thromboxane A\textsubscript{2} were increased. Ono et al\textsuperscript{23} found that cultured bovine endothelial cells grown for five to eight passages in elevated glucose had markedly decreased prostacyclin production. In addition to differences in species, the different results could be due to several factors. In cultured endothelial cells it has been demonstrated that arachidonic acid content can decrease as much as 66% over the first four passages, and this can markedly decrease eicosanoid production.\textsuperscript{24} This factor alone could explain the difference found in our cells, which were grown for only two passages. Also, tissues from diabetics and cells grown for several passages with serum, unlike our cells, which underwent a brief exposure to elevated glucose in PSS, are exposed to insulin, which can itself influence eicosanoid release.\textsuperscript{25} Several studies, including our own, have suggested that elevated glucose interferes with prostacyclin production at the level of phospholipase A\textsubscript{2}.\textsuperscript{1,26} Whether elevated glucose causes an increase or decrease in eicosanoids, perhaps the most important factor is the duration of exposure, because the decreases observed have occurred over a duration of days to weeks. The present study suggests that the early and perhaps most direct result of elevated glucose is an increased release of endothelial cell eicosanoids, including prostacyclin.

**Comparisons With Blood Vessels Exposed to Elevated Glucose**

Aortas isolated from diabetic rabbits that have plasma glucose values averaging 20 mM or normal aortas exposed to 44 mM glucose for 6 hours release increased amounts of eicosanoids when stimulated by the endothelium-dependent agonist acetylcholine and demonstrate decreased relaxation.\textsuperscript{5,11} The increased eicosanoid release was ascribed to the endothelial cells because it was eliminated when the endothelium was removed. Although not all studies agree,\textsuperscript{27-29} abnormal endothelium-dependent relaxations in diabetes have been observed in the rat aorta,\textsuperscript{9,20-33} rabbit aorta,\textsuperscript{5,8,11} pig and dog coronary artery,\textsuperscript{24} and human penile corpus cavernosum.\textsuperscript{10} The reversal by cyclooxygenase inhibitors of reduced endothelium-dependent relaxations of rabbit aorta exposed to elevated glucose suggests that eicosanoids produced by the endothelium cause contraction that offsets the relaxation mediated by NO. Therefore, reduced NO production need not be invoked to explain the impaired endothelium-dependent relaxation, of which it is the primary mediator.\textsuperscript{5,11} This possibility is further supported by the apparent lack of any effect on NO release with increased eicosanoid production in PAECs exposed to elevated glucose.

The identity of the endothelium-derived eicosanoid vasoconstrictor is not known with certainty. Recent studies have suggested that the prostaglandin end products themselves, although they can all contract the rabbit aorta when applied exogenously,\textsuperscript{2} may not be released in sufficient quantities by the endothelium of the isolated rabbit aorta to contract the smooth muscle. Under normal-glucose conditions relatively large amounts of eicosanoids are produced, but there is no effect of cyclooxygenase blockade on endothelium-dependent relaxations.\textsuperscript{11} Also, aldose reductase inhibitors\textsuperscript{35} or free-radical scavengers\textsuperscript{36} both normalize the impaired endothelium-dependent relaxation of the diabetic rabbit aorta without affecting the increased production of eicosanoid metabolites. These findings suggest that the metabolism of glucose by the aldose reductase pathway may give rise to free radicals\textsuperscript{37,38} that can interfere with endothelium-dependent relaxation. Furthermore, superoxide anion was shown to be a key element in the contraction caused by exogenous prostaglandin endoperoxide (PGH\textsubscript{2}) but not the prostaglandin end products.\textsuperscript{29} A generalized increase in eicosanoid metabolites would be compatible, nevertheless, with an increase in PGH\textsubscript{2} formation by cyclooxygenase, which also generates free radicals.\textsuperscript{40,41} It has been shown that the levels of PGH\textsubscript{2} produced in the rabbit aortic endothelium are sufficient to contract the smooth muscle.\textsuperscript{42} It would appear then that the coincident production of free radicals and PGH\textsubscript{2} may account for the endothelium-derived vasoconstrictor in blood vessels exposed to elevated glucose. Of interest is the fact that exposure of the rabbit aorta to hyperosmolar mannose does not impair endothelium-dependent relaxation as does glucose,\textsuperscript{11} which suggests that the metabolism of glucose is required for free-radical production, whereas the exposure to hyperosmolality increases eicosanoid production.

In conclusion, various studies have shown that diabetes and exposure to elevated glucose can alter the levels of vasoactive substances, such as eicosanoids, released from the endothelium of blood vessels. These studies in PAECs may provide a model system in which to study the elaboration of vasoactive substances from endothelium exposed to elevated glucose.

**References**

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