In diabetic patients, elevated plasma levels of t-PA and PAI-1 accompany impaired fibrinolysis. To identify mechanisms for these abnormalities, we examined whether vascular endothelial cells exposed to high glucose upregulate t-PA and PAI-1 production and whether ambient PA activity is decreased concomitantly. In 17 cultures of human umbilical vein endothelial cells grown to confluency in 30 mM glucose, the t-PA antigen released to the medium in 24 h was (median) 52 ng/10^6 cells (range 10–384) and the PAI-1 antigen was 872 ng/10^6 cells (range 217–2074)—both greater (P < 0.02) than the amounts released by paired control cultures grown in 5 mM glucose—29 ng/10^6 cells (range 7.5–216) and 461 ng/10^6 cells (range 230–3215), respectively. In the presence of high glucose, the steady-state levels of t-PA and PAI-1 mRNAs were increased correspondingly (median 142 and 183% of control, respectively, P < 0.05); high glucose per se and hypertonicity contributed to the upregulation in additive fashion. The PA activity of conditioned medium from cultures exposed to high glucose was 0.4 lU/ml (range 0.2–0.6), which was significantly lower (P < 0.02) than the PA activity of control medium (0.5 lU/ml, range 0.2–0.9). No difference was observed when comparing the PA activities of acidified conditioned media, expected to be depleted of inhibitors. Thus, high glucose coordinately upregulates endothelial t-PA and PAI-1 expression through effects exerted at the pretranslational level and enhanced by even mild degrees of hypertonicity. The decrease in ambient fibrinolytic potential may reflect an overwhelming effect of the increased availability of PAI-1. These findings propose a contributory mechanism for the fibrinolytic abnormalities of diabetes and the thrombotic tendency of the hyperglycemic hyperosmolar state. Diabetes 41: 1009–15, 1992
This pathogenetic construct is worthy because it provides a possible mechanism for the greater elevation in circulating levels of t-PA and PAI observed in NIDDM compared with IDDM patients (4, 7). It does not, however, readily explain the increased t-PA antigen levels (4, 7, 10), PAI activity (4, 12), and decreased fibrinolysis (4) documented also in IDDM patients (who do not exhibit portal hyperinsulinemia); nor does it account for increased levels of PAI-1 mRNA observed in retinal microvessels isolated from NIDDM patients (14).

The latter observations expand the focus to encompass vascular endothelium and suggest that disturbed endothelial synthesis of t-PA and PAI-1 may occur in diabetes, contributing to altered fibrinolytic activity locally and in the systemic circulation. Having observed that glucose concentrations mimicking diabetic hyperglycemia selectively alter endothelial cell gene expression (15–17), we examined the effects of high glucose on endothelial t-PA and PAI-1 production and on the functional balance of the secreted molecules.

**RESEARCH DESIGN AND METHODS**

Primary cultures of human endothelial cells harvested from individual umbilical veins (18) were plated in 60 mm tissue culture dishes at a cell density of 1.3 x 10^5 for controls and 1.7 x 10^5 for cells destined for high glucose treatment. The differential plating density was implemented to study experimental and control cells in the same assay at the same final density. This was otherwise unachievable owing to the replicative delay induced by high glucose (19), but desirable because both PA and PAI activity are highly dependent on the growth state of endothelial cells (20). Cells were cultured in medium 199 (Gibco, Grand Island, NY) supplemented with 14% heat-inactivated pooled human serum, 20 μg/ml ECGS (Sigma, St. Louis, MO), 90 μg/ml heparin (Gibco), 2 mM glutamine, and 17.5 mM HEPES buffer. The medium in experimental dishes was supplemented with D-glucose (Fisher, Fair Lawn, NJ) from the day after plating to achieve a final concentration of 30 mM, whereas control cultures were grown in 5 mM glucose. When subcultured, cells were maintained in their respective high or physiological glucose treatment. Cells derived from high-glucose cultures were seeded at ~120% the density of their paired controls. Of the 17 isolates studied, 11 were tested in primary culture and 6 in subculture (1st or 2nd passage).

To examine whether the effects of high glucose were dose-related, 3 additional primary cultures were studied after exposure to 30, 15, and 5 mM glucose for 10 days. One of these cultures also was tested after acute exposure to high glucose (24 h prior to harvest). The role of hypertonicity was evaluated by comparing the effects of 30 mM glucose with those of isotonic 30 mM glucose. Isotonicity was maintained by adding D-glucose to custom-made NaCl-free medium 199 (Gibco) supplemented with NaCl to achieve a final concentration of 130 mM (instead of the standard 142 mM) to compensate for the 25 mM excess D-glucose (17).

To measure t-PA and PAI-1 antigens and PA activity, confluent monolayers that had been exposed to 30 or 5 mM glucose since the inception of culture were incubated for 24 h with fresh complete medium 199, maintaining the respective glucose concentrations. The conditioned media (4 ml/dish) were collected and centrifuged immediately at 400 g for 5 min at 4°C to remove detached cells and debris; the supernatants were frozen at −80°C until used for determining t-PA and PAI-1 antigens and PA activity. The monolayers were washed twice with cold PBS, scraped off the dish with a rubber policeman, and centrifuged at 400 g for 5 min at 4°C. The pellets were lysed in 1 ml of PBS containing 0.25% Triton X100 for 15 min at 37°C, then frozen at −80°C until used for determination of t-PA and PAI-1 antigens in cell extracts.

The t-PA and PAI-1 antigens in conditioned media and in cellular extracts were measured by ELISA (American Diagnostica, New York, NY). The Imubind-5 t-PA ELISA kit measures the t-PA antigen present in both its free and bound form. Heparin in low concentration does not influence the determination; no cross-reaction with urokinase is described (21). The Imubind plasma PAI-1 ELISA kit detects active and latent (inactive) forms of the protein, whereas the t-PA/PAI complexes are recovered with low efficiency (22). This may result in underestimation of the amount of PAI-1 relative to the t-PA secreted to the medium because the t-PA antigen is detected efficiently by the ELISA assay whencomplexed with PAI-1. The fact, however, that PAI-1 is secreted in a 10-fold molar excess over t-PA renders the magnitude of any underestimation negligible. Both t-PA and PAI-1 antigens were measured in ng/ml and are expressed in ng/10^6 cells.

The functional balance of the t-PA and PAI-1 secreted to the medium (PA activity) was determined with a spectrophotometric assay (PA Colorimetric test, Boehringer Mannheim, Mannheim, Germany). Samples of conditioned medium (unprocessed or acidified with acetic acid as in preparation of plasma euglobulin fraction) were incubated with an excess of plasminogen in the presence of fibrin equivalents following manufacturer's specifications. The unbound PA converts the plasminogen into plasmin. Plasmin releases p-nitroaniline from the chromogenic substrate CBS 10:66, whose concentration is measured at 405 nm. Quantitation of PA activity in the sample was obtained via a calibration curve constructed using t-PA standards. The PA activity was measured in IU/ml.

For RNA studies, total RNA was extracted from confluent experimental and control cultures by the guanidine isothiocyanate method (23), and the RNA samples (10 μg) were electrophoresed on 1% agarose gel containing 2.2 M formaldehyde. Northern blots (15) were hybridized to 32p-labeled probes (Multiprime DNA labeling systems, Amersham, Arlington Heights, IL) for human t-PA, PAI-1, and γ-actin. The 475-nucleotide t-PA cDNA was the EcoRI fragment of the coding sequence of the human t-PA cDNA (24). The 436-nucleotide PAI-1 cDNA was the Sal I-Bgl II fragment of the human PAI-1 cDNA (9). Hybridization to a γ-actin cDNA was used as internal control to correct for loading inequalities—because we...
TABLE 1
Cell density and thymidine incorporation of human endothelial cell cultures grown to confluency in physiological (5 mM) or high-glucose (30 mM) concentrations

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Glucose concentration</th>
<th>Cell number × 10^6</th>
<th>^3H thymidine incorporation (cpm × 10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary cultures (n, 11)</td>
<td>5 mM</td>
<td>3.5 (1.7–4.5)</td>
<td>10,000 (3,294–29,825)</td>
</tr>
<tr>
<td></td>
<td>30 mM</td>
<td>3.1 (1.8–3.9)</td>
<td>7,839 (1,975–43,107)</td>
</tr>
<tr>
<td>Subcultures (n, 6)</td>
<td>5 mM</td>
<td>2.5 (2.0–4.5)</td>
<td>2,135 (871–12,712)</td>
</tr>
<tr>
<td></td>
<td>30 mM</td>
<td>2.4 (2.0–3.3)</td>
<td>2,430 (436–8,294)</td>
</tr>
<tr>
<td>Primary plus subcultures (n, 17)</td>
<td>5 mM</td>
<td>2.8 (1.7–4.5)</td>
<td>5,764 (871–29,825)</td>
</tr>
<tr>
<td></td>
<td>30 mM</td>
<td>2.7 (1.8–3.9)</td>
<td>6,856 (436–43,107)</td>
</tr>
</tbody>
</table>

Values represent the median and (range) for the indicated number of observations. Seeding density for cells destined for high glucose treatment was higher than for the paired controls (see METHODS).

documented in this study and in a previous one (17) that high glucose does not alter the levels of actin mRNA in endothelial cells. Hybridization and washing conditions were described previously (15). The membranes were exposed to Kodak XAR-5 film at −80°C for 3–48 h (PAI-1 and γ-actin) or 3–7 days (t-PA). Densitometric analysis of autoradiograms was performed at nonsaturating exposures with a Zeineh soft laser scanning densitometer. Both bands of the PAI-1 mRNA were analyzed, and the sum of their densities was used for quantitation. High-glucose–induced differences in signal intensity are expressed as percent of the paired controls.

On the day of cell harvest for the various determinations, cell number and pulse thymidine incorporation (25) were measured in 2–3 dishes from each treatment to assess the replicative activity of the cultures.

Because the replicative and biosynthetic activity of the different isolates showed substantial variability and yielded some extreme values, the data are summarized with the median and range of the observations. Statistical analysis was done using the Wilcoxon signed rank test.

RESULTS

Exposure to high glucose was 13 ± 4 (mean ± SD) days for primary cultures and 31 ± 7 days for subcultures. At harvest, both primary cultures and subcultures grown in high glucose exhibited cell density and pulse thymidine incorporation similar to their paired control cultures (Table 1). For all 17 isolates studied, cell density was 2.7 × 10^6 cells (range 1.8–3.9) in high glucose and 2.8 × 10^6 cells (range 1.7–4.5) in physiological glucose; and the respective thymidine incorporation values were 6856 cpm/10^6 cells (range 436–43,107) and 5764 cpm/10^6 cells (range 871–29,825). The pattern of biosynthetic response to high glucose was similar in primary cultures and subcultures, except that isolates unresponsive to high glucose were found only among the primary cultures.

The amount of t-PA and PAI-1 antigens released to the medium in 24 h by the 17 individual isolates is reported in Table 2. Exposure to high glucose resulted in increased t-PA release by 13 isolates; for all 17 isolates, the t-PA released to the medium was 52 ng/10^6 cells (range 10–384) vs. 29 ng/10^6 cells (range 7.5–216) under control conditions (P < 0.02). Cell-bound t-PA antigen was not different between control and high-glucose cultures, neither in the primaries nor in the subcultures. For all 17 isolates, cell-bound t-PA was 7.2 ng/10^6 cells (range 0.4–10.5) in cells exposed to high glucose and 5.7 ng/10^6 cells (range 0.6–12.5) in control cells. PAI-1 release was increased by high glucose in all cultures except 3 primaries. The PAI-1 released to the medium by the 17 isolates was 872 ng/10^6 cells (range 217–2074) vs. 461 ng/10^6 cells (range 230–3215) released by control cells (P < 0.02). Cell-bound PAI-1 antigen was not different between control and high-glucose cultures, neither in the primaries nor in the subcultures. For all 17 isolates, cell-bound PAI-1 antigen was 19 ng/10^6 cells (range 5–73) in cells exposed to high glucose and 18 ng/10^6 cells (range 5–73) in control cells.

The PA activity of conditioned medium was measured in 13 cultures (7 primary and 6 subcultures) to assess the functional balance of secreted t-PA and PAI-1 antigens. Exposure to high glucose resulted in decreased PA activity in 5 of 7 primary cultures and in all subcultures (Fig. 1). For all 13 isolates tested, exposure to high glucose induced a decrease in PA activity in 10 cultures (P < 0.05) and did not change in 3 cultures.

Table 2. Amount of t-PA and PAI-1 antigens released to the medium by human endothelial cells cultured in physiological (5 mM) or high-glucose (30 mM) concentrations

<table>
<thead>
<tr>
<th>Cell isolate</th>
<th>5 mM (ng/10^6 cells/24 h)</th>
<th>30 mM (ng/10^6 cells/24 h)</th>
<th>5 mM (ng/10^6 cells/24 h)</th>
<th>30 mM (ng/10^6 cells/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>69.2</td>
<td>81.8</td>
<td>635</td>
<td>1040</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>17.4</td>
<td>238</td>
<td>442</td>
</tr>
<tr>
<td>3</td>
<td>29.1</td>
<td>20.7</td>
<td>368</td>
<td>268</td>
</tr>
<tr>
<td>4</td>
<td>22.4</td>
<td>10.6</td>
<td>353</td>
<td>368</td>
</tr>
<tr>
<td>5</td>
<td>12.7</td>
<td>21.8</td>
<td>265</td>
<td>217</td>
</tr>
<tr>
<td>6</td>
<td>35.4</td>
<td>49.7</td>
<td>467</td>
<td>605</td>
</tr>
<tr>
<td>7</td>
<td>22.6</td>
<td>25.5</td>
<td>314</td>
<td>329</td>
</tr>
<tr>
<td>8</td>
<td>69.5</td>
<td>85.9</td>
<td>3215</td>
<td>2074</td>
</tr>
<tr>
<td>9</td>
<td>44.1</td>
<td>32.4</td>
<td>461</td>
<td>1676</td>
</tr>
<tr>
<td>10</td>
<td>15.6</td>
<td>125.1</td>
<td>810</td>
<td>903</td>
</tr>
<tr>
<td>11</td>
<td>27.7</td>
<td>16.0</td>
<td>631</td>
<td>954</td>
</tr>
<tr>
<td>S</td>
<td>12</td>
<td>43.7</td>
<td>52.0</td>
<td>736</td>
</tr>
<tr>
<td>13</td>
<td>173.3</td>
<td>384.0</td>
<td>839</td>
<td>1340</td>
</tr>
<tr>
<td>14</td>
<td>216.0</td>
<td>236.0</td>
<td>852</td>
<td>1470</td>
</tr>
<tr>
<td>15</td>
<td>26.7</td>
<td>78.8</td>
<td>320</td>
<td>873</td>
</tr>
<tr>
<td>16</td>
<td>47.7</td>
<td>118.8</td>
<td>231</td>
<td>451</td>
</tr>
<tr>
<td>17</td>
<td>22.3</td>
<td>40.0</td>
<td>321</td>
<td>993</td>
</tr>
</tbody>
</table>

Isolates 1-11 were studied in primary culture (P); isolates 12-17 were studied in subculture (S).
FIG. 1. Effect of high glucose on PA activity of the conditioned medium of human umbilical vein endothelial cells. Cultures grown to confluency in high (30 mM) or physiological (5 mM) glucose were incubated for 24 h with fresh complete medium 199 maintaining the respective glucose concentrations. The conditioned media (4 ml/dish) were collected, processed, and analyzed for PA activity as described in METHODS. Each symbol represents an isolate from a single umbilical cord, and the data from the same isolate studied under control and high-glucose conditions are connected by a line. ●●●●, isolates studied in primary culture; ○○○○, isolates studied in subculture.

FIG. 2. Effect of high glucose on t-PA, PAI-1, and γ-actin mRNA levels in 3 cultures of umbilical vein endothelial cells. Cultures (1 primary and 2 subcultures) were exposed to 30 mM glucose for 16 ± 3 days; paired control cultures were exposed to 5 mM glucose. Northern analysis (10 μg of total RNA per lane) was performed as described in METHODS; each pair of samples (5 and 30 mM glucose) was studied in the same blot. The figure presents a composite of 3 northern blots.

Glucose decreased (P < 0.02) PA activity from 0.5 IU/ml (range 0.2–0.9) to 0.4 IU/ml (range 0.2–0.6). An inverse (r = −0.36) correlation between the glucose-induced decrease in medium PA activity and the increase in PAI-1 release, albeit not statistically significant, suggested the occurrence of reciprocal changes. To gain further insight into the role played by inhibitors in determining medium PA activity, the PA activity of the unprocessed conditioned medium for 8 cultures was compared with the respective acetic acid-precipitated fraction. The latter, being analogous to the euglobulin fraction of plasma, is expected to be relatively free of inhibitors of the fibrinolytic system (26); indeed, it exhibited PA activity for control cultures (1.55 IU/ml [range 1.15–2.3]) that was greater than the corresponding unprocessed conditioned medium (0.5 IU/ml [range 0.35–0.8]). Interestingly, the high-glucose cultures exhibited PA activity of the acidified medium identical to control (1.55 IU/ml [range 0.7–1.7]), whereas the PA activity of the paired unprocessed medium was significantly lower than control (0.4 IU/ml [range 0.2–0.6], P < 0.05).

To investigate whether the increased t-PA and PAI-1 secretion by cells exposed to high glucose reflected increased synthesis, we measured the steady-state levels of the respective mRNAs (Fig. 2). In cultures exposed to 30 mM glucose, t-PA mRNA was 142% of control (range 117–226%), (P < 0.05), and PAI-1 mRNA was 183% of control (range 66–283%), (P < 0.05). In accordance with previous observations (17), γ-actin mRNA levels were unchanged by high glucose (108% of control [range 78–140%]). The two PAI-1 mRNA species—both of which direct the synthesis of the PAI-1 protein and differ by 1 kb of sequence in the 3' untranslated region as a result of alternative polyadenylation (27)—are affected differentially by some perturbations (28). We thus examined whether cells exposed to high glucose exhibited an altered ratio of the 3.2 to the 2.3 kb band. In 14 autoradiograms at nonsaturating exposure, the ratio of the two mRNA species in high-glucose cells was 3.8 ± 2.3, which was not different from that observed in control cells (4.4 ± 1.8).

The effect of high glucose on PAI-1 expression appeared to be dose-related, as in 3 primary isolates exposed to 30 mM glucose for 10 days PAI-1 mRNA levels were 176, 186, and 277% of control, whereas in the paired cultures exposed to 15 mM glucose they were 60, 140, and 246% of control, respectively. In one of the isolates, PAI-1 mRNA levels also were measured after acute exposure to high glucose. In cells exposed to 30 mM glucose for 24 h, the PAI-1 transcript increased to 210% of control (the value after 10 days of exposure was 186% of control); and in companion cells exposed to 15 mM glucose, PAI-1 mRNA levels increased to 125% of controls (140% after 10 days). The contribution of hypertonicity (323 ± 1.9 vs. 299 ± 4 mOsm/kg in control medium) to the glucose-induced overexpression of PAI-1 and t-PA was quantitated in two highly responsive cultures. In these two cultures, PAI-1 mRNA levels increased to 655 and 645% of control, respectively, in response to hypertonic glucose, but only to 377 and...
379% of control in response to isotonic high glucose (Fig. 3). The same pattern was observed for the t-PA transcript, which was increased to 290% of control in one of the cultures exposed to hypertonic glucose, and to 126% of control in the companion cultures grown in isotonic high glucose. The second culture showed a small t-PA response to both hypertonic and isotonic high glucose (125% of control).

DISCUSSION

This study demonstrates that in the presence of high glucose, human vascular endothelial cells upregulate the expression and secretion of both t-PA and PAI-1, in part in response to high glucose per se and in part in response to hypertonicity, and that the phenomenon is accompanied by a decrease in ambient fibrinolytic potential.

In inducing coordinate overexpression of t-PA and PAI-1, the effects of high glucose resemble those of thrombin (29,30) and basic fibroblast growth factor (31); and differ from the effects of other physiopathological stimuli, such as cytokines (28) and transforming growth factor β (31) that induce a divergent upregulation of the two products; or endotoxin (29) and shear stress (32) that only affect either PAI-1 or t-PA. The possibility that the effect of high glucose on endothelial cell t-PA and PAI-1 may be mediated through enhanced production of molecules with autocrine activity is unlikely. Cytokines (28) and transforming growth factor β (31) stimulate only PAI-1 production, and enhanced availability of basic fibroblast growth factor would be expected to potentiate endothelial cell proliferation (33) which is instead impeded by high glucose (19). Endothelial cell growth factor and heparin in the culture medium actually might have hampered by high glucose (19). Endothelial cell growth factor and heparin in the culture medium actually might have decreased PAI-1 synthesis (34), but because the agents were added to both control and high-glucose cultures, their effect should not be relevant to the differences observed. In this study, as in previous ones (15,17,35), we encountered substantial variability in constitutive gene expression and responsiveness to high glucose among individual endothelial cell isolates. The former cannot be attributed solely to the experimental procedure (35) nor to the effect of time in culture (36) because it was observed within primary cultures or early passages. Among the determinants of variable response to high glucose, time of exposure appears to be one (15), but not the only one (17,37), at least for the upregulation of fibronectin and collagen IV expression. Although the primary cultures that failed to show a t-PA and PAI-1 response to high glucose in this study might have exhibited a response after longer exposure, it must be noted that PAI-1 upregulation by high glucose can occur within 24 h (this study, 17). These considerations leave open the possibility that individual genetic characteristics may play a role in modulating basal gene expression and responsiveness to high glucose.

Should future studies find that the effects of high glucose on t-PA and PAI-1 expression result from increased gene transcription (akin to those on expression of basement membrane components) (17), it would be pertinent to explore whether they are exerted through a common regulatory sequence. Although the human t-PA promoter has a phorbol ester-responsive element (38), and phorbol esters increase the transcription of the PAI-1 gene (39), the effects of high glucose are unlikely to be mediated by protein kinase C activation, the principal target of the phorbol esters. We have observed that human umbilical vein endothelial cells exposed to high glucose do not exhibit protein kinase C translocation (35). Whether the extensive region of shared nucleotides present in the 5’ flanking sequences of the human t-PA and PAI-1 genes (40) harbors elements important in their coordinate regulation by high glucose (or other stimuli) would deserve investigation.

How a mild degree of medium hypertonicity contributes to overexpression of t-PA and PAI-1 is not clear. Increased medium osmolarity could potentiate the effects of ambient glucose by increasing cellular glucose transport activity, although such an effect has been reported only in tissues that are insulin dependent for glucose transport and upon the addition of at least 100 mM excess osmolyte (41). If this were the operative mechanism, then it should be argued that the pathway mediating the glucose-induced upregulation of t-PA and PAI-1 differs from that mediating upregulation of basement membrane components, because the latter is independent of hypertonicity (17). Alternatively, osmotic changes may modify the expression of t-PA and PAI-1 themselves, as it has been reported to occur for other proteins (42). Time-course and dose-response studies performed with osmolytes other than glucose should help define whether t-PA and PAI-1 overexpression is part of the adaptive response of endothelial cells to even mild degrees of ambient hypertonicity.

The amount of t-PA and PAI-1 proteins synthesized and secreted by our cultures paralleled that reported in other studies for the same cell type (28,43), and the enhancement induced by high glucose quantitatively reflected the increase in the respective mRNA levels. This agrees with our previous findings relative to tissue factor (16) and basement membrane components (17), and indicates that the modulatory effects of high glucose on the synthesis of a cluster of endothelial cell proteins is primarily, if not solely, exerted at the pretranslational level. How a combined and proportionally similar augmentation of t-PA and PAI-1 may lead to a net decrease.
in ambient fibrinolytic potential remains to be explained. This phenomenon occurs in vitro with thrombin (29) and in several clinical situations (21,44); and because both in vitro (45) and in vivo (46) modifications in PAI-1 activity alter fibrinolysis in a reciprocal fashion, it has been explained on the basis of an overwhelming effect of the inhibitor. This is plausible insofar as PAI-1 is secreted in large molar excess over t-PA and binds to available t-PA in preference to becoming inactivated (47). Although we cannot exclude that decreased PA activity of cultures exposed to high glucose might reflect a functionally compromised t-PA molecule (reported in uncontrolled diabetic patients) (48), we favor the interpretation based on an overwhelming role of the increased availability of PAI-1. This is because the difference in PA activity between normal and high glucose cultures was abolished in acidified medium, which is depleted of inhibitors (26).

Our findings have possible clinical implications. The effects of hyperglycemia on vascular endothelium may be a determinant in the increased plasma levels of t-PA and PAI-1 and decreased fibrinolysis observed in both IDDM and NIDDM patients. Degrees of hyperoncoticity as encountered in HNAD may magnify the phenomenon and thus contribute to the vascular occlusions often complicating the clinical course of HNAD (49). The finding that levels of PAI-1 mRNA are increased in retinal microvessels obtained from NIDDM patients (14) supports a vascular contribution to excess PAI-1 in diabetes. In addition, retinal microvascular endothelial cells isolated from IDDM patients, but not from nondiabetic subjects, upregulate the synthesis and secretion of t-PA and PAI-1 in response to some growth factors in vitro (50). Less supportive of our findings are the decreased immunostaining for t-PA found in retinal vessels of diabetic patients (51) and the weak inverse correlation between glycemic control and fibrinolysis (52,53). However, it is reasonable to argue that immunolocalization cannot provide assessment of synthesis and secretion (indeed, decreased endothelial cell deposits of t-PA are a postulated counterpart to chronically enhanced secretion [44]) and that poor correlation between glycemic control and fibrinolysis may indicate simply that high glucose is not the only or overwhelming factor modulating fibrinolysis in vivo. It is to be expected that at the level of t-PA and PAI-1 secretion, additional influences (e.g. 28–32) are likely to be operative in any given patient at any given time to amplify, distort, or negate the effect of hyperglycemia.

Overall, the effects of high glucose on vascular endothelium are remarkably compatible with the nature and epidemiology of vascular disease in diabetes. On the one hand, the aberrations in endothelial cell biosynthetic activity induced by high glucose are in the direction of thrombogenicity: altered functional balance of t-PA/PAI-1, increased procoagulant response to thrombin (16), decreased prostacyclin production (54). On the other hand, because the effects of high glucose are relatively small and probably variable among individuals, the expected consequence is a lowered threshold for thrombotic events rather than a florid thrombotic disorder.

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