Influence of Chronic Exposure to High Concentrations of D-Glucose and Long-Term β-Blocker Treatment on Intracellular Calcium Concentrations of Porcine Aortic Endothelial Cells

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Clinical observations indicate that diabetes leads to micro- and macroangiopathy involving endothelial dysfunction. Because recent studies indicate an antiangiopathic effect of celiprolol, but not of metoprolol, in type 1 diabetes, we investigated the direct influence of exposure to high D-glucose concentrations on endothelial cells and the possible effects of both β-blockers. Nine different chronic treatments were carried out on cultured porcine aortic endothelial cells: *1*) 5 mmol/l D-glucose ("normoglycemic" cells), *2*) 5 mmol/l D-glucose plus 15 mmol/l L-glucose (osmotic control), *3*) 5 mmol/l D-glucose plus 0.5 µmol/l celiprolol, *4*) 5 mmol/l D-glucose plus 0.05 µmol/l metoprolol, *5*) 5 mmol/l D-glucose plus 0.5 µmol/l celiprolol plus 5 µmol/l propranolol, *6*) 20 mmol/l D-glucose ("hyperglycemic" cells), *7*) 20 mmol/l D-glucose plus 0.5 µmol/l celiprolol, *8*) 20 mmol/l D-glucose plus 0.05 µmol/l metoprolol, and *9*) 20 mmol/l D-glucose plus 0.5 µmol/l celiprolol plus 5 µmol/l propranolol. Using the Fura-2 technique, application of either 1 nmol/l bradykinin or 1 µmol/l ATP to the normoglycemic endothelial cells led to a significant increase in intracellular calcium, whereas the hyperglycemic cells showed significantly less reactivity to both agents. Exposure of endothelial cells to L-glucose did not show any difference to normoglycemic controls. Coadministration of 20 mmol/l glucose and celiprolol demonstrated that the alteration of the calcium signal induced by high D-glucose concentrations could be significantly antagonized with celiprolol. In contrast, coincubation with metoprolol failed to normalize the calcium signal. This effect of celiprolol was completely abolished in the presence of propranolol. In normoglycemic cells, none of the β-blockers influenced the intracellular calcium response to bradykinin or ATP. These results indicate that chronic treatment with high D-glucose concentrations leads to an impairment of calcium signaling, which might be ameliorated by celiprolol. *Diabetes* 47:407–413, 1998

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ANOVA, analysis of variance; cGMP, cyclic guanosine monophosphate; Dil-Ac-LDL, 1,1′ dioctadecyl 3,3,3′,33′-tetramethylindo-carbocyanine-acetylated low-density lipoprotein; FITC, fluorescein isothiocyanate; Fura-2/AM, Fura-2 acetoxymethylester; MAPK, mitogen-activated protein kinase; PAEC, porcine aortic endothelial cell.

Diabetes, a disease that affects the metabolism not only of carbohydrates but also of lipids and protein, leads to micro- and macroangiopathy, the well-known complications of which include retinopathy, nephropathy, neuropathy, and intermittent claudication (1). The molecular mechanisms underlying this process are still unclear. However, there are several lines of evidence that endothelial dysfunction plays a central role in the development of micro- and macroangiopathy (2–4). The authors of these studies reported significant alterations in proliferation capacity, with an increase of cell growth and enhanced synthesis of fibronectin, type IV collagen, and laminin. Endothelial cell hyperplasia and concomitant thickening of the basement membrane then lead to circulatory disorders in the affected organ.

Furthermore, it is known that an impairment of NO and prostaglandin I_2 production may occur in diabetes, with a subsequent decrease in endothelium-dependent relaxation of vascular smooth muscle cells (5,6). Thus, in STZ-induced diabetic rats, Kamata et al. (7) found decreases in relaxation and cyclic guanosine monophosphate (cGMP) production in aortic rings after the administration of acetylcholine. In agreement with these results, Calver et al. (8) found a reduced response of blood vessels in the forearm to vasodilating substances administered to humans with diabetes.

Olbrich et al. (9), who examined isolated mesenteric beds of Wistar rats with diabetes of 6 months' duration, also found a reduction in endothelium-dependent relaxation and pointed out that long-term diabetes leads to thickening of the vessel walls and reduction in blood flow. In the same model, Olbrich et al. (10,11) tested the selective β1-blocker celiprolol (with β2-agonistic activity) for 6 months and demonstrated a significant improvement in endothelium-dependent vasodilation and a reduction in vessel wall thickening in diabetic subjects, claiming that celiprolol has an antiangiopathic effect in diabetic rats. It was not clear, however, whether the high blood glucose and celiprolol directly influenced the endothelial cells.

Because it is well known that endothelial constitutive NO synthase depends on intracellular calcium (12) and because some reports indirectly suggest a decrease of calcium influx in endothelial cells from diabetic animals, with subsequent impairment of endothelium-dependent relaxation (13), we wanted to investigate whether D-glucose itself, in high con-

centrations, impairs intracellular calcium concentration in endothelial cells and whether simultaneous treatment with celiprolol or metoprolol would be beneficial.

RESEARCH DESIGN AND METHODS

Cell isolation and culture. Porcine aortic endothelial cells (PAECs) were isolated and cultured according to Rosenthal and Gotlieb (14). Briefly, the endothelial cells were harvested from porcine thoracic aorta (10 aortas per preparation) by treatment with 1 mg/ml dispase for 15 min at 37°C, rinsed with Medium 199 (containing 10% fetal calf serum, 5 mmol/l D-glucose, 3 mmol/l glutamine, 30 mmol/l HEPES, 100 mg/l penicillin G, and 100 mg/l streptomycin), centrifuged (100*g* for 10 min), and after resuspension with Medium 199, seeded in plastic 9.6-cm^2 Petri dishes (Nunc, Wiesbaden, Germany) coated with 0.2% gelatin. The cells were kept in a type B5060 EC/CO_2 incubator (Heraeus, Düsseödorf, Germany) at 37°C, saturated humidity, and 5% CO_2. After reaching confluence (5–8 days), the endothelial cells were passaged by incubation with a solution of trypsin (0.05%) and EGTA (0.02%) and subcultured in 25-cm^2 plastic flasks coated with 0.2% gelatin, with a split ratio of 1:5 (first passage). Thereafter, for the calcium experiments, the cells were again subcultured (second passage) and seeded in glass Petri dishes containing a gelatin-coated glass coverslip (50 × 50 mm). The endothelial cells were fed three times per week with Medium 199. At the start of the second passage, the cells were submitted to the various treatments (see below). After the cells reached confluence again (3–6 days), the coverslip with the endothelial cell monolayer was transferred to a heatable organ bath installed on an inverse microscope (Nikon, Tokyo, Japan) for the calcium measurements (see below).

Purity of the cell culture was tested by uptake of Dil-Ac-LDL (1,1′ dioctadecyl 3,3,3′,33′-tetramethylindo-carbocyanine-acetylated low-density lipoprotein) (15) and, for detection of contaminating smooth muscle cells, by staining of α–smooth muscle actin (14).

Treatment. To examine the effect of high glucose concentrations with and without the addition of celiprolol (a β_1-selective β-blocker with β_2-agonistic activity), metoprolol (a β_1-selective β-blocker without β_2-agonistic activity), or a combination of celiprolol and propranolol (an unselective β-blocker) (16) on endothelial cells, nine different experimental protocols were carried out at the moment the cells were seeded onto the glass coverslip. For a normal glucose concentration, we used 5 mmol/l D-glucose, which corresponds to a normal blood glucose level of 100 mg/dl; for hyperglycemic conditions, we used 20 mmol/l D-glucose, which corresponds to a blood glucose level of 400 mg/dl; and for osmotic control, we used a combination of 5 mmol/l D-glucose and 15 mmol/l L-glucose. β-Blockers were added in concentrations considered to be in the middle of the therapeutic range of 0.1–1.0 µmol/l celiprolol or of 0.01–0.1 µmol/l metoprolol (17,18), taking the plasma protein binding into account. For the combined treatment with celiprolol and propranolol, we chose a propranolol concentration that was 10 times higher.

Thus, we used five control groups with one of the following:

1. 5 mmol/l D-glucose alone
2. 5 mmol/l D-glucose plus 15 mmol/l L-glucose (for osmotic control)
3. 5 mmol/l D-glucose plus 0.5 µmol/l celiprolol (+Cp)
4. 5 mmol/l D-glucose plus 0.05 µmol/l metoprolol (+Mp)
5. 5 mmol/l D-glucose plus 0.5 µmol/l celiprolol plus 5 µmol/l propranolol (+Cp/Pp)

and four experimental groups with one of the following:

6. 20 mmol/l D-glucose (high D-glucose) alone
7. 20 mmol/l D-glucose plus 0.5 µmol/l celiprolol (+Cp)
8. 20 mmol/l D-glucose plus 0.05 µmol/l metoprolol (+Mp)
9. 20 mmol/l D-glucose plus 0.5 µmol/l celiprolol plus 5 µmol/l propranolol (+Cp/Pp)

The specific treatments of the different control or experimental groups started at the second passage and lasted until the cells had reached confluence (3–6 days). As done previously, the medium, which was supplemented with the corresponding glucose and β-blocker concentration, was changed three times a week.

Calcium measurements. The calcium measurements obtained with the Fura-2 dye were carried out according to Grynkiewicz et al. (19). Briefly, endothelial cell monolayers grown on glass coverslips were incubated with 1 µmol/l Fura-2 acetoxymethylester (Fura-2/AM) for 30 min at 37°C. After a postincubation period of another 30 min at 37°C to facilitate the Fura-2 ester hydrolysis, the monolayer was placed under an inverse microscope (Nikon) at a magnification ×400 using a ×40 oil-immersion objective (Fluor objective, Nikon) and was superfused with a HEPES-buffered saline solution containing 145 mmol/l NaCl; 5 mmol/l KCl; 2.5 mmol/l $CaCl_2$; 1 mmol/l $MgCl_2$; 5 or 20 mmol/l D-glucose, or 5 mmol/l D-glucose plus 15 mmol/l L-glucose; and 10 mmol/l HEPES, buffered at pH 7.4. All experiments were performed at 37°C, at pH 7.4, and at the glucose concentration to which the cells were exposed during culture (e.g., cells grown at 20 mmol/l glucose were investigated at 20 mmol/l). For excitation of the Fura-2 dye, we used a xenon lamp (type XBO 75W/2) equipped with a computer-controlled chopper and 340/380-nm monochromators. Excitation light was diverted by a 400-nm dichroic mirror onto the specimen. The resulting fluorescent light passed the dichroic mirror and a band-pass filter (510/20) for detection at an emission wavelength of 510 nm. The intensity of fluorescence was measured by means of a type SN 92-021 photomultiplier (Amko, Hamburg, Germany) linked to the PC system (Amko LTI Deltascan) for data acquisition. The sampling rate could be varied between 0.1 and 500 Hz and was normally adjusted to one sample per second. Details of the setup have been published previously (20).

The ratio of fluorescence of the Fura-2 dye excited with 340 and 380 nm and measured at 510 nm (emission wavelength) is known to reflect the intracellular free calcium concentration (21). Because of the generally known uncertainties in Fura-2 calibration techniques, it is now a common practice to express changes in intracellular free calcium concentration as changes in the ratio (22). In our experiments, all ratio values were registered after subtraction of the autofluorescence of the endothelial cells, and in the bar graphs, the change in ratio between the control level and the peak ratio after agonist application is given.

The experiments started at stable ratio values after an equilibration period of 10–15 min. Fluorescence was measured for a period of 5 min after addition of either 1 µmol/l ATP or 1 nmol/l bradykinin, both of which are substances that stimulate an intracellular calcium increase via an inositol triphosphate–dependent release of calcium from intracellular stores, thus acting as calcium agonists.

Statistical analysis. For statistical analysis, a two-factorial analysis of variance (ANOVA) was performed. If ANOVA indicated significant differences or significant interactions between disease and treatment, the data were further analyzed with a Tukey-HSD test. For the statistical analysis, we used Systat for Windows software, version 5.02 (Systat, Evanston, IL). Differences were considered significant when $P < 0.05$.

Materials. The following materials were obtained from Sigma (Deisenhofen, Germany): HEPES, trypsin, glutamine, gelatin, penicillin G (1650 U/mg), streptomycin, Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}), Triton X-100, metoprolol, propranolol, antibody to α–smooth muscle actin (mouse), anti–mouse IgG fluorescein isothiocyanate (FITC)-linked antibody, FITC-labeled phalloidin, Fura-2/AM, ATP, and bradykinin. Dil-Ac-LDL was purchased from Paesel + Lorei (Frankfurt, Germany), Medium 199 (with 0.05 mg/l glutathione, 20 mg/ml L-cysteine, 0.05 mg/l vitamin C, and α-tocopherol phosphate 0.01 mg/l) and fetal calf serum were purchased from Gibco BRL (Eggenstein, Germany), and dispase was obtained from Boehringer (Mannheim, Germany). Celiprolol was supplied by Upjohn (Heppenheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). The cell culture material was purchased from Nunc (Wiesbaden, Germany).

RESULTS

In all cell lines investigated, >99% of the tested cells incorporated Dil-Ac-LDL with no significant differences among the nine experimental groups. The content of contaminating smooth muscle cells was <0.1%. The cell lines treated with high concentrations of D-glucose did not show any promotion of smooth muscle cell growth in culture. All cells were investigated in the same proliferation phase, i.e., after reaching confluence. Confluence was observed after 5.2 ± 0.3 days in 5 mmol/l glucose–treated cells and 5.3 ± 0.3 days in 20 mmol/l glucose–treated cells.

In good accordance with the literature, application of either 1 nmol/l bradykinin or 1 µmol/l ATP to endothelial cell monolayers grown under 5 mmol/l D-glucose (control group treated without β-blockers) resulted in a rapid increase in ratio, reflecting a peak increase in intracellular calcium: for bradykinin, the control ratio of 1.045 ± 0.0013 increased by 0.198 ± 0.0248 ($P < 0.05$), and for ATP, the control ratio of 1.016 ± 0.0169 increased by 0.188 ± 0.015 ($P < 0.05$). In contrast, endothelial cells grown under a high concentration (20 mmol/l) of D-glucose exhibited significantly less reactivity to bradykinin or ATP: for bradykinin, the control ratio of 1.015 ± 0.024 increased by 0.0506 ± 0.0041, and for ATP, the control ratio of 0.995 ± 0.0089 increased by 0.06 ± 0.008. Figures 1 and

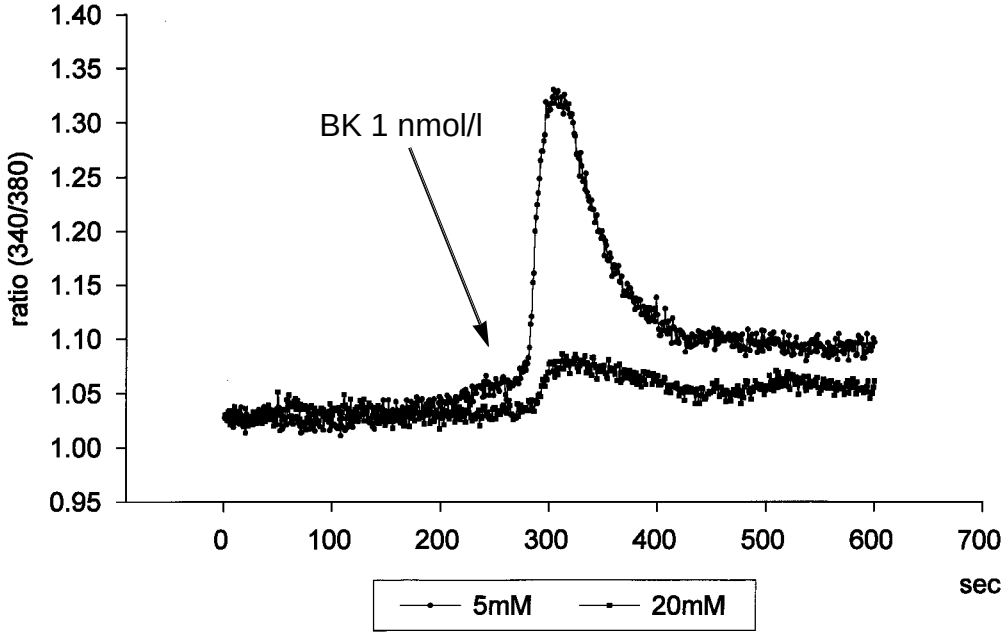

FIG. 1. Original trace of the influence of long-term exposure to normal (5 mmol/l) and high (20 mmol/l) D-glucose concentrations on intracellular calcium, measured as an increase in the 340/380 ratio of fluorescence of Fura-2–loaded PAECs after the application of 1 nmol/l bradykinin.

2 show original registrations of the ratio of PAECs after application of bradykinin and ATP, respectively, to cells grown under 5 and 20 mmol/l D-glucose. In Figs. 3 and 4, the statistical analysis of six experiments is demonstrated: incubation of the endothelial cells to 20 mmol/l D-glucose resulted in a significantly ($P < 0.001$) smaller increase in ratio after application of bradykinin or ATP compared with the control cells grown under normal glucose concentrations. Because it is well known that mammalian cells do not metabolize L-glucose (23), we used a combination of 5 mmol/l D-glucose and 15 mmol/l L-glucose for osmotic control. Endothelial cells grown under 15 mmol/l L-glucose and 5 mmol/l D-glucose showed no significant differences regarding their calcium response to bradykinin or ATP compared with cells grown under 5 mmol/l D-glucose alone (Figs. 3 and 4).

To examine the effects of β-blockers on endothelial calcium response, we treated a control group of endothelial cells grown under normal d-glucose concentrations (5 mmol/l) with 0.5 μmol/l celiprolol or 0.05 μmol/l metoprolol, or with a combination of 0.5 μmol/l celiprolol and 5 μmol/l propranolol. Neither celiprolol nor metoprolol nor the combination of celiprolol and propranolol altered the bradykinin- or ATP-induced increase in intracellular calcium in these control cells, measured as an increase in ratio, and similar changes were observed for the calcium plateau ratio (Figs. 5 and 6).

Endothelial cells grown under parallel exposure to high D-glucose concentrations (20 mmol/l) and celiprolol did not show the reduction in calcium signal associated with high D-glucose concentrations after application of bradykinin or ATP, and these cells differed significantly ($P = 0.033$ for bradykinin; $P = 0.008$ for ATP) from untreated cells exposed to high D-glucose concentrations. In contrast, coincubation

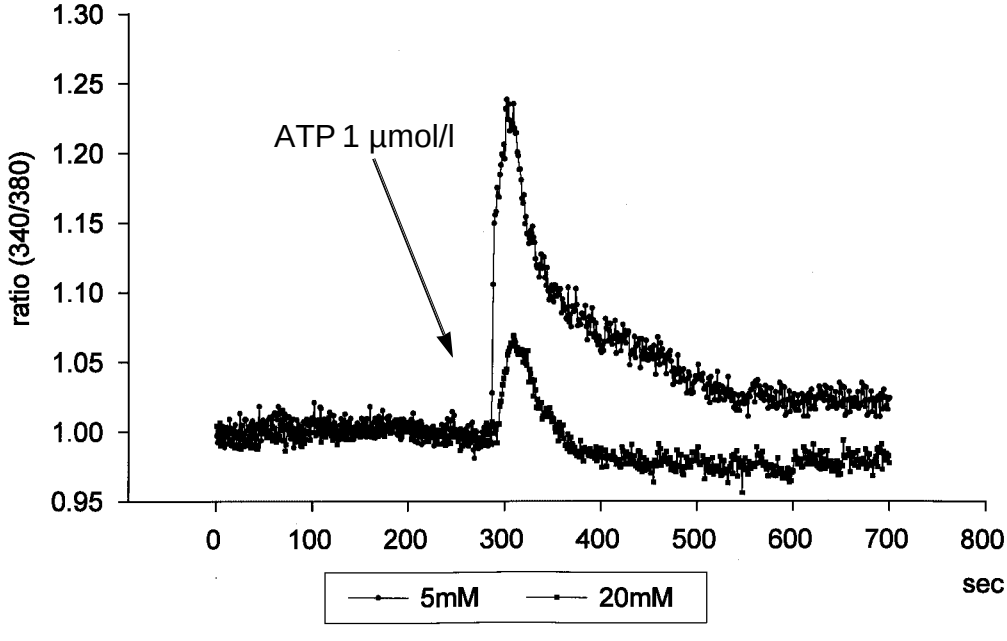

FIG. 2. Original trace of the influence of long-term exposure to normal (5 mmol/l) and high (20 mmol/l) D-glucose concentrations on intracellular calcium, measured as an increase in the 340/380 ratio of fluorescence of Fura-2–loaded PAECs after the application of 1 μmol/l ATP.

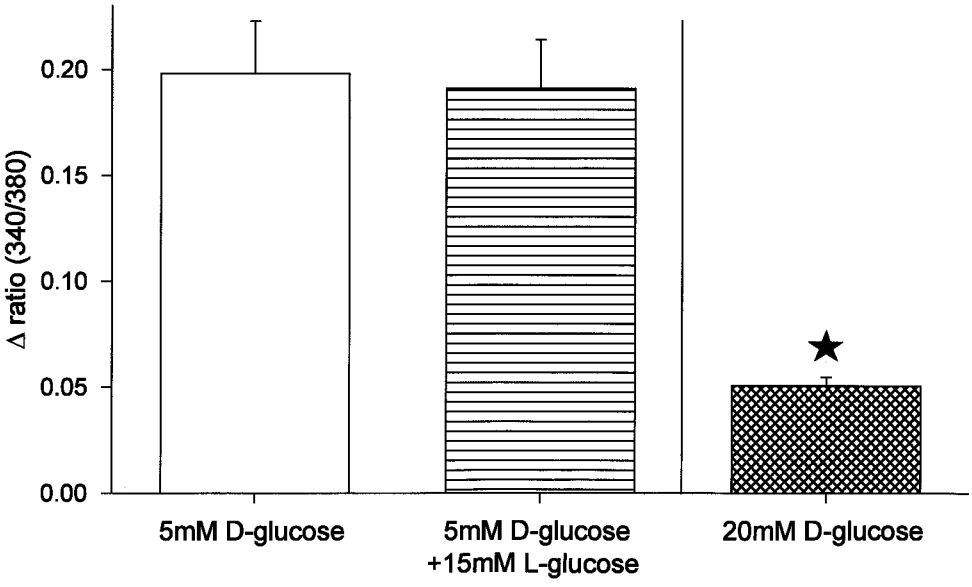

FIG. 3. Influence of long-term exposure to normal (5 mmol/l) and high (20 mmol/l) D-glucose concentrations on changes in intracellular calcium (peak calcium), measured as change (Δ) in ratio after application of 1 nmol/l bradykinin. For osmotic control, the change in ratio of cells exposed to 5 mmol/l D-glucose plus 15 mmol/l L-glucose is given. All values are means ± SE of six experiments. Significant changes from 5 mmol/l D-glucose are marked with a solid star (★) ($P < 0.001$).

with metoprolol after treatment with bradykinin or ATP failed to improve the calcium signal in incubated cells that were exposed to high D-glucose concentrations.

To find out whether a β_2-receptor agonistic activity of celiprolol might be responsible for the positive effect on the calcium signal, we used a combined treatment of 0.5 µmol/l celiprolol and a tenfold higher dose of propranolol (5 µmol/l), which is an unselective β-blocker that blocks both β_1- and β_2-receptors (16,24). This parallel treatment of the endothelial cells grown under 20 mmol/l D-glucose with celiprolol plus propranolol produced no improvement in the calcium signal when bradykinin or ATP was applied (Figs. 5 and 6), i.e., the protective effect seen with celiprolol alone was abolished.

DISCUSSION

In our study, we could demonstrate that PAECs grown under high D-glucose concentrations (20 mmol/l) over several days showed a significantly reduced responsiveness to bradykinin or ATP, both substances that increase the intracellular calcium concentration. This impairment of the stimulated calcium signal could be antagonized by coincubation with celiprolol but not with coincubation with metoprolol, and the celiprolol effect on this D-glucose–induced alteration of the calcium signal could be completely abolished by additional treatment with propranolol.

The reduction of the calcium signal by application of high concentrations of D-glucose is obviously not due to an elevation of the osmotic pressure: no significant changes in calcium signal could be shown in experiments with additional application of 15 mmol/l D-glucose compared with experiments in which cells were grown under normal (5 mmol/l) D-glucose conditions.

Furthermore, in our experiments, we found that the cells of all experimental groups reached confluence after the same time interval (3–6 days), indicating that all cells were approximately in the same proliferation phase, so that the differences seen under the various treatments cannot be ascribed to differences in proliferation-dependent alterations of signal

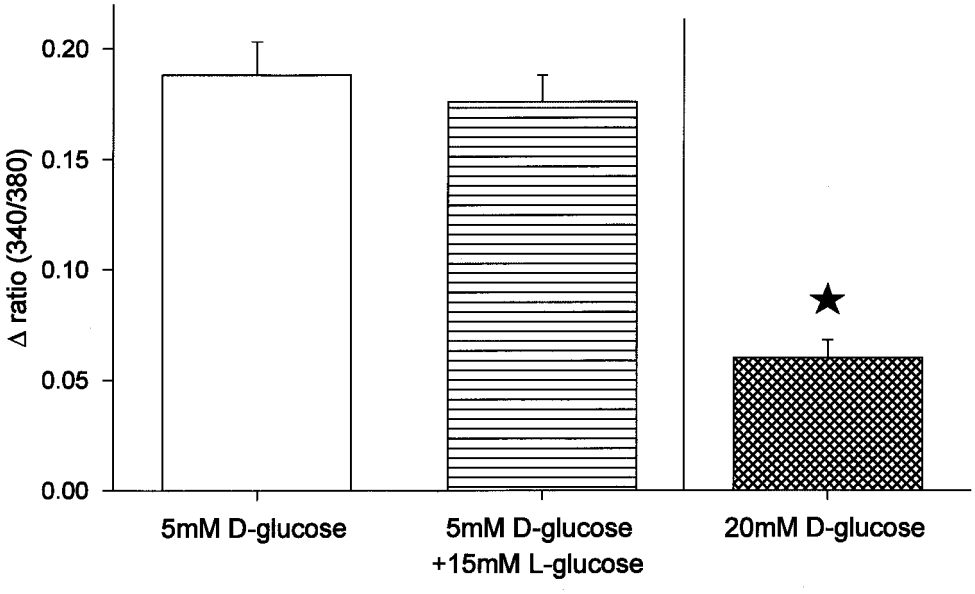

FIG. 4. Influence of long-term exposure to normal (5 mmol/l) and high (20 mmol/l) D-glucose concentrations on changes in intracellular calcium (peak calcium), measured as change (Δ) in ratio after application of 1 µmol/l ATP. For osmotic control, the change in ratio of cells exposed to 5 mmol/l D-glucose plus 15 mmol/l L-glucose is given. All values are means ± SE of six experiments. Significant changes from 5 mmol/l D-glucose are marked with a solid star (★) ($P < 0.001$).

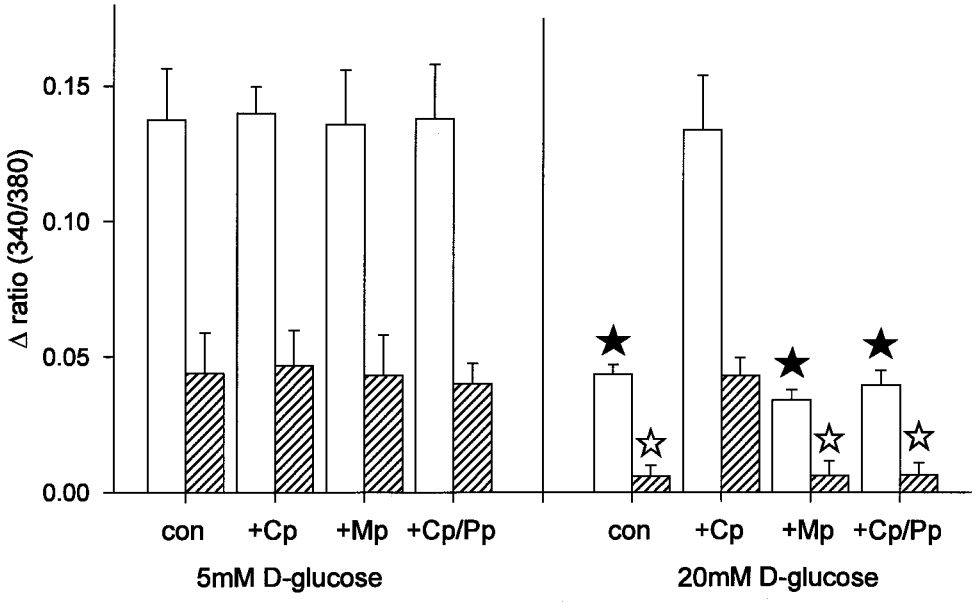

FIG. 5. Influence of long-term exposure to normal (5 mmol/l) and high (20 mmol/l) D-glucose concentrations with or without additional treatment with either 0.5 µmol/l celiprolol (+Cp) or 0.05 µmol/l metoprolol (+Mp), or a combination of 0.5 µmol/l celiprolol with 5 µmol/l propranolol (+Cp/Pp), (control [con] = no drug treatment) on changes in intracellular calcium (calcium peak, left column [□] of each pair, and calcium plateau, right column [▨] of each pair), measured as changes in Δ ratio after application of 1 nmol/l bradykinin. All values are given as means ± SE of six experiments. Significant changes of peak calcium (20 vs. 5 mmol/l D-glucose) are marked with a solid star (★) ($P < 0.05$); significant changes of plateau calcium (20 vs. 5 mmol/l D-glucose) are marked with an open star (☆) ($P < 0.05$).

transduction. This observation seems contradictory to the findings of Curcio and Ceriello (25) and Graier et al. (26), who stated that hyperglycemia reduced endothelial cell proliferation. Interestingly, Curcio and Ceriello found that the hyperglycemia-induced reduction in growth could be prevented by antioxidants such as glutathione and radical scavengers. This finding might explain the missing reduction in cell growth in our study because in the cell culture medium used, 0.05 mg/l glutathione (reduced form), 20 mg/l L-cysteine, 0.05 mg/l vitamin C, and 0.01 mg/l α-tocopherol were present.

In accordance with our finding of a reduction in calcium signal dependent on high D-glucose concentrations, Pieper and Dondlinger (27) reported on an attenuation of the increase in intracellular calcium in response to bradykinin in endothelial cells cultured in 25 mmol/l D-glucose. In addition to these experiments, several experimental data demonstrate that an endothelial dysfunction with an impairment of endothelium-dependent relaxation on the basis of impaired release of NO can be found to occur in diabetes (9,28–31). Because it is well known that the endothelial constitutive NO synthase depends on intracellular calcium (12), the impaired responsiveness to calcium-mobilizing agents in endothelial cells grown under hyperglycemic conditions may contribute, at least in part, to the worsening of endothelium-dependent relaxation that is documented in the literature. The reduced plateau calcium rather than the peak calcium could especially lead to reduced NO release. Thus, a decreased NO/cGMP production in endothelial cells exposed to elevated glucose concentrations has been reported by Weisbrod et al. (32) in cultured PAECs, Magill and Danaberg (33) in rat aortic endothelial cells, and Pieper and Dondlinger (34) in cultured bovine aortic endothelial cells.

In contrast to these findings, however, Graier et al. (22,35) and Wascher et al. (36) demonstrated that short-term (24-h)

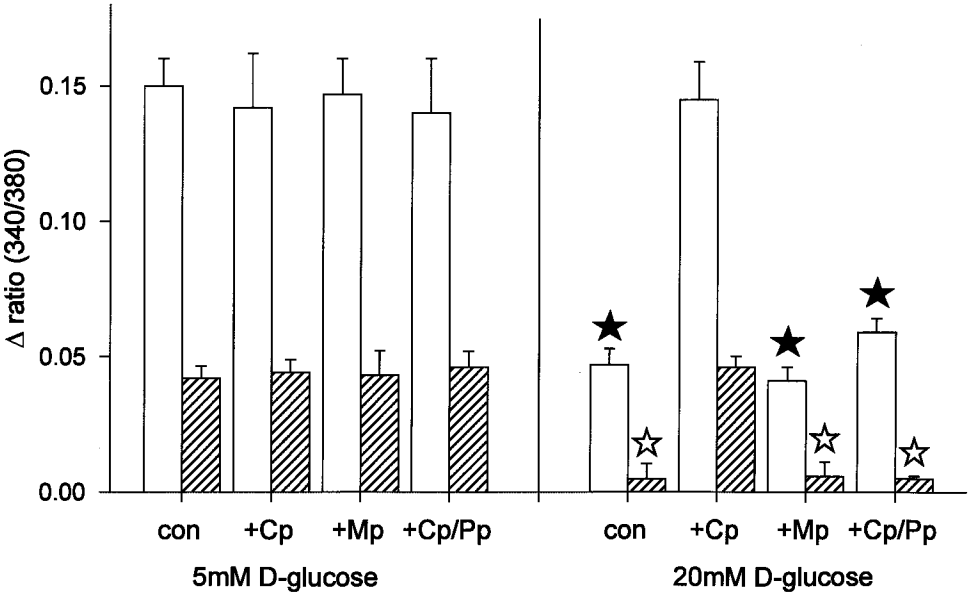

FIG. 6. Influence of long-term exposure to normal (5 mmol/l) and high (20 mmol/l) D-glucose concentrations with or without additional treatment with either 0.5 µmol/l celiprolol (+Cp) or 0.05 µmol/l metoprolol (+Mp), or a combination of 0.5 µmol/l celiprolol and 5 µmol/l propranolol (+Cp/Pp), (control [con] = no drug treatment) on changes in intracellular calcium (calcium peak, left column [□] of each pair, and calcium plateau, right column [▨] of each pair), measured as changes in Δ ratio after application of 1 µmol/l ATP. All values are given as means ± SE of six experiments. Significant changes of peak calcium (20 vs. 5 mmol/l D-glucose) are marked with a solid star (★) ($P < 0.05$); significant changes of plateau calcium (20 vs. 5 mmol/l D-glucose) are marked with an open star (☆) ($P < 0.05$).

incubation of endothelial cells with high (44 mmol/l) D-glucose concentrations resulted in an amplification of agonist-induced calcium response. Thus, it may be possible that endothelial cells react in a biphasic way to hyperglycemic conditions: in a first phase, short-term exposure to elevated D-glucose may activate the endothelial cell with an increase in calcium response to calcium-mobilizing agents and an increase in NO formation (22,35), and in a second phase, prolonged administration of high concentrations of D-glucose may result in an impairment of the endothelial cells with a reduced responsiveness to calcium-mobilizing agents such as bradykinin or ATP. Several reports support this hypothesis: Mogensen (37), Mathiesen et al. (38), and Thuesen et al. (39) found increased blood flow and reduced peripheral resistance in early stages of diabetes. In contrast, long-term diabetes leads to macro- and microangiopathy with reduced vascular reactivity (3,40,41).

Interestingly in our experiments, celiprolol, but not metoprolol, was able to antagonize the impairment caused by chronic application of high D-glucose concentration. In clinical studies, Malminiemi et al. (42) could show that celiprolol had a positive effect on insulin sensitivity and glucose tolerance in patients with a pathological glucose tolerance test result and Olbrich et al. (11,31,43) demonstrated an antiangiopathic effect of celiprolol that was not seen with metoprolol in an in vivo model of streptozotocin-induced diabetes in rats. Hence, we investigated the direct effect on cultured endothelial cells of a therapeutic concentration of celiprolol (a β_1-selective blocker with β_2-agonistic activity) (44) compared with metoprolol, another commonly used β_1-selective β-blocker without β_2-agonistic activity (18). The presence of β-adrenoceptors on endothelial cells has been demonstrated by several authors (45–47). Stimulation of β_1-receptors resulted in an increase of NO formation (45), whereas stimulation of β_2-receptors resulted in a decrease of endothelial permeability (47) and an increase of cell growth (46).

The fact that celiprolol, but not metoprolol, antagonized the worsening effect of high concentrations of D-glucose on endothelial cells may indicate an involvement of the β_2-receptor, because in contrast to celiprolol, metoprolol (a selective β_1-receptor antagonist) has no intrinsic sympathomimetic activity. This possibility of β_2-receptor involvement is further supported by the experiments with propranolol, an unselective (i.e., β_1- and β_2-receptor) β-blocker, which itself antagonized the positive celiprolol effect. Thus, it could be speculated that not the β_1-blocking activity of celiprolol but rather its agonistic effects on the β_2-receptor might be responsible for the improvement of the calcium signaling in cells incubated with high concentrations of D-glucose. It has been shown that celiprolol can stimulate cAMP accumulation in S49 lymphoma cells, but only when adenylate cyclase was simultaneously stimulated with forskolin (48). In L6 myoblasts, however, celiprolol induced β-receptor downregulation but failed to increase cAMP accumulation (49). Thus, celiprolol may exert its effects via increased cAMP, but at present, there is no clear experimental evidence. On the other hand, complex effects of the β_2-adrenoceptor stimulation on cell growth, differentiation, and proliferation must also be taken into account, since increase in cAMP after β2-stimulation may inhibit the mitogen-activated protein kinase (MAPK) pathway (50,51). The MAPK pathway can be activated via protein kinase C, e.g., in mesangial cells of diabetic subjects (52). It has been shown that hyperglycemia can enhance protein kinase C activity leading to MAPK activation (52,53). In addition, it should also be considered that high glucose concentrations can impair endothelium-dependent relaxation (54) and inhibit both calcium signal and NO production (34) via a process that is reversed by oxygen radical scavengers. Furthermore, at least some β_2-adrenoceptor agonists and some β-blockers exhibit additional radical scavenging activity (55–57). Other factors, such as activation of the polyol pathway, are also known to play a role in impairment of endothelial cells associated with high glucose concentrations (58). It is uncertain at present whether substances acting on β_2-adrenoceptors may interfere with that pathway. The exact mechanism underlying this phenomenon remains unclear and should be elucidated in more detail in future studies.

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