Elevated Glucose Impairs Endothelium-dependent Relaxation by Activating Protein Kinase C

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Abstract

A possible relationship between protein kinase C activation and impaired receptor-mediated endothelium-dependent relaxation in diabetes mellitus was examined in isolated aorta from normal rabbit exposed to elevated glucose. Aorta treated for 10 min with 4-phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator, showed decreased relaxations to the endothelium-dependent vasodilator, acetylcholine, similar to normal aorta exposed to elevated glucose (22 and 44 mM) for 6 h. Relaxations to the receptor-independent endothelium-dependent vasodilator, A23187, and those caused by the direct smooth muscle vasodilator, sodium nitroprusside, were unaffected by treatment with PMA or exposure to elevated glucose. Indomethacin increased relaxations to acetylcholine of aorta treated with PMA indicating a role for vasoconstrictor prostanoids. PMA caused a significant increase in basal and acetylcholine-stimulated release of vasoconstrictor prostanoids including thromboxane A_2 from aortic segments with, but not without endothelium. Protein kinase C inhibitors, H-7 or sphingosine, restored the abnormal acetylcholine-induced relaxations as well as suppressed the abnormal release of prostanoids in aorta exposed to elevated glucose. These findings suggest that the dysfunction of receptor-mediated endothelium-dependent relaxation associated with exposure to elevated glucose is due to increased production of vasoconstrictor prostanoids by the endothelium as a consequence of protein kinase C activation. (*J. Clin. Invest.* 1991. 87:1643–1648.) Key words: endothelium • acetylcholine • aorta • rabbit

Introduction

Relaxation of the rabbit aorta to acetylcholine is mediated by the release of endothelium-derived relaxing factor(s) (EDRF)[1] (1). In aorta from diabetic rabbits or in those from normal rabbits exposed to elevated glucose for 6 h the relaxation to acetylcholine is impaired (2, 3). The impaired relaxations in both diabetic models are restored by cyclooxygenase and prostaglandin endoperoxide/thromboxane A_2 receptor blockade. Acetylcholine also causes an augmented release of vasoconstrictor prostanoids including thromboxane A_2 from aortic segments with, but not without endothelium in both the diabetic and elevated glucose models, consistent with a role of vasoconstrictor prostanoids in causing the abnormal vascular relaxation (2, 3).

Agonists like acetylcholine initiate their endothelial cell response by muscarinic receptor-mediated activation of phospholipase C which causes phosphatidylinositol breakdown forming inositol triphosphate, which releases intracellular calcium, and diacylglycerol which activates protein kinase C (4, 5). Exposure to elevated glucose has been reported to stimulate de novo diacylglycerol production in various cells, including endothelial cells, by providing substrate for phosphatidic acid synthesis, and this is associated with activation of protein kinase C (6, 7). In so far as the response to acetylcholine is modulated by diacylglycerol-activated protein kinase C, one might expect that acetylcholine-induced relaxations would be altered in arteries exposed to elevated glucose. These studies were undertaken to examine the role of protein kinase C in the abnormal endothelium-dependent responses and generation of vasoactive prostanoids in the rabbit aorta exposed to elevated glucose.

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.

Organ chamber studies. Rings of aorta (5 mm long) were suspended from strain gauges for measurement of isometric force. The rings were placed in organ baths (25 ml) filled with PSS of the following composition (in mM): NaCl, 118.3; KCl, 4.7; $MgSO_4$, 0.6; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; and calcium ethylenediamine tetraacetic acid 0.026. The solutions were maintained at 37°C and gassed with 95% O_2-5% CO_2 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 contractions in rabbit aorta by testing repeated contractions to potassium (80 mM). Thereafter, length was not altered. Aortic rings were then incubated with control (11 mM) or elevated (22 or 44 mM) glucose for 6 h. Since the relaxations caused by acetylcholine of aorta incubated in either 5.5 or 11 mM glucose for 6 h were not different (3), these studies were conducted with 11 mM glucose as control. After, the incubation rings were contracted with phenylephrine to 50% of their maximal contraction and then exposed to half-log increments in concentration of acetylcholine, adenosine diphosphate, A23187, or sodium nitroprusside. In some rings, a subthreshold concentration of phorbol ester (10^{-8} M), which does not affect the tone, was added 10 min before the relaxant agonist. Antagonists were added throughout the 6-h incubation period and physiological response.

Radioimmunoassay. Segments of aorta (3 cm long) were incubated in PSS (1 ml) and gently bubbled with 95% O_2 - 5% CO_2 at 37°C for 6 h in control (11 mM) or elevated (44 mM) glucose. The PSS was changed

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1. *Abbreviations used in this paper:* EDRF, endothelium-dependent relaxing factor; PDD, phorbol didecanoate.

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every 1 h. At the end of the 6-h period, the segments were incubated in PSS (1 ml) first in the absence and then in the presence of acetylcholine (10^{-6} M) for 30 min. H-7 was added throughout the 6-h incubation. In some cases phorbol ester was added during the 30-min incubation period. After the incubations, the tissues were blotted dry and weighed. The incubates were frozen at −20°C until analyzed. Radioimmunoassay was used to quantify the release of thromboxane B_2 (the stable hydrolytic product of thromboxane A_2), 6-keto-$PGF_{1\alpha}$ (the stable hydrolytic product of PGI_2), $PGF_{2\alpha}$, and PGE_2 in the incubation buffers. Radioimmunoassays were performed using specific antisera of thromboxane B_2, $PGF_{1\alpha}$ and PGE_2 (courtesy of Dr. Lawrence Levine, Brandeis University, Waltham, MA), 6-keto-$PGF_{1\alpha}$ (Biomol Research Laboratory, Inc., Plymouth, PA), tritiated standards (DuPont-NEN, Boston, MA), and unlabeled standards (UpJohn, 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 prostanoids was < 5%. The limits of sensitivity for the radioimmunoassay with the experimental conditions described for thromboxane B_2, 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$ were 1 pg/ml, and for PGE_2, was 10 pg/ml. Standard curves performed with the addition of glucose (44 mM) were identical to those performed in control glucose.

Drugs. Agents used were A23187, acetylcholine chloride, adenosine diphosphate, indomethacin, phenylephrine, 4-phorbol 12-myristate 13-acetate (PMA), phorbol-12,13-didecanoate (PDD) and sodium nitroprusside (Sigma Chemical Co., St. Louis, MO), H-7 [1-(5-isoquinolinylsulfonyl)-2-methylpiperazine], and sphingosine (Biomol Research Laboratory). Concentrations were expressed as final molar 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% Na_2CO_3 immediately before use. Stock solutions of PMA and PDD were made in dimethylsulfoxide and further dilutions were made in PSS.

Data analysis. Relaxation at the steady state is expressed as a percentage of change in the level of tone induced by phenylephrine. The IC_{50} was estimated graphically as the concentration causing 50% relaxation of the induced tone. Data are expressed as means±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 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.

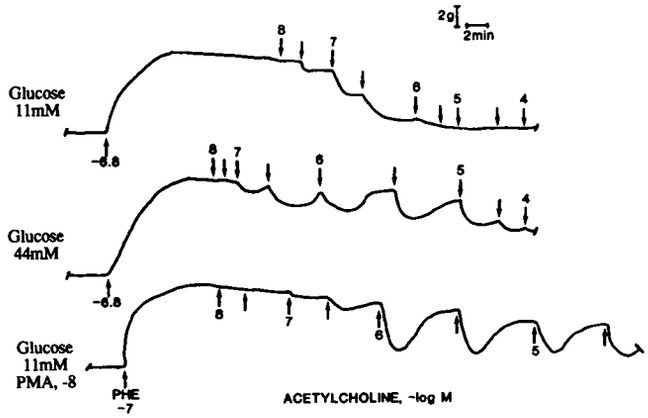

Figure 1. Isometric tension recordings of rings of aorta contracted with phenylephrine (*PHE*) and then exposed to increasing concentrations of acetylcholine. Aorta incubated in: (*A*) control glucose (11 mM), (*B*) elevated glucose (44 mM) for 6 h, or (*C*) PMA (10^{-8} M) for 10 min in control glucose before contraction with phenylephrine. Aorta incubated in elevated glucose for 6 h and those treated with PMA showed abnormal relaxations characterized by contractions after relaxation to each increment in acetylcholine concentration. Numbers with arrows indicate the negative logarithm of the molar concentration of acetylcholine. Arrows without numbers indicate half-log increments in concentration.

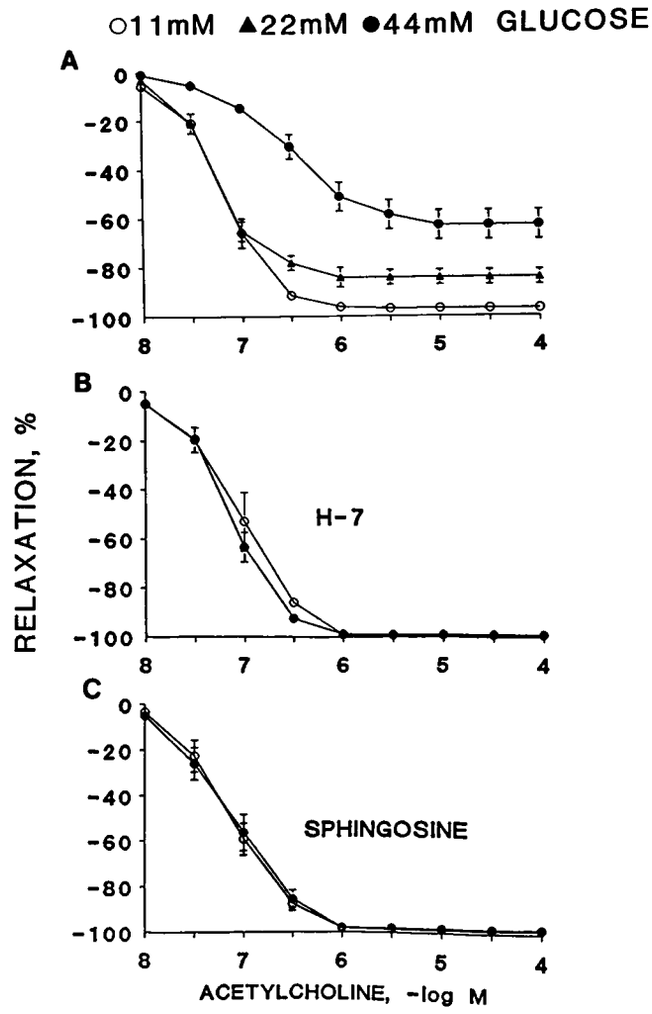

Figure 2. (*A*) Relaxations caused by acetylcholine of aortic rings incubated in control glucose (11 mM, *n* = 7) or elevated glucose (22 mM, *n* = 5, and 44 mM, *n* = 7) for 6 h. Relaxations were significantly less in aorta incubated with either 22 or 44 mM glucose compared with those in control glucose. (*B* and *C*) Effects of H-7 (10^{-5} M, *n* = 5) or sphingosine (10^{-5} M, *n* = 6), respectively, on relaxations induced by acetylcholine in aortic rings incubated in control or elevated (44 mM) glucose. H-7 or sphingosine had no significant effect on relaxation of rings incubated in control glucose, but restored those of rings incubated in elevated glucose (44 mM) such that the relaxations were not significantly different. Values are means±SE.

Results

Endothelium-dependent relaxation. Rings of aorta with intact endothelium incubated with 11 and 44 mM glucose for 6 h were contracted with phenylephrine (−log M: 6.7±0.2 and 6.8±0.2, *n* = 7, respectively), which caused contractions of 8.9±0.6 and 8.7±0.4 g, respectively. The relaxations induced by acetylcholine (10^{-8}–10^{-4} M) were significantly decreased in aortic rings incubated with elevated (44 mM) glucose com-

pared with those in control (11 mM) glucose (IC_{50}, −log M: 6.0±0.2 vs. 7.1±0.1, $n = 7$, respectively, $P < 0.05$; Figs. 1 and 2). Relaxations caused by addition of concentrations of acetylcholine between 3×10^{-7} and 10^{-4} M were followed by contractions in aorta incubated in elevated glucose, but not in control glucose (Fig. 1). Incubation of aortic rings in 22 mM glucose for 6 h caused a smaller but significant decrease in acetylcholine relaxation at concentrations from 3×10^{-6} to 10^{-4} M. For instance, the relaxation caused by acetylcholine (10^{-6} M) was significantly less in aorta incubated in glucose (22 mM) compared with those incubated in control glucose (84±3 vs. 98±1%, respectively, $n = 5$, $P < 0.05$).

Treatment with H-7 (10^{-5} M) or sphingosine (10^{-5} M) restored to normal the abnormal acetylcholine-induced relaxations of rings incubated with 44 mM glucose (IC_{50}, −log M: 7.1±0.1, $n = 5$, and 7.0±0.2, $n = 6$, respectively). Neither protein kinase C inhibitor had a significant effect on the relaxation to acetylcholine of rings incubated with control glucose (Fig. 2).

The relaxations caused by adenosine diphosphate were significantly decreased in aorta incubated in elevated glucose (44 mM) compared with those in control glucose (11 mM) (Fig. 3). Treatment with sphingosine (10^{-5} M) restored the abnormal

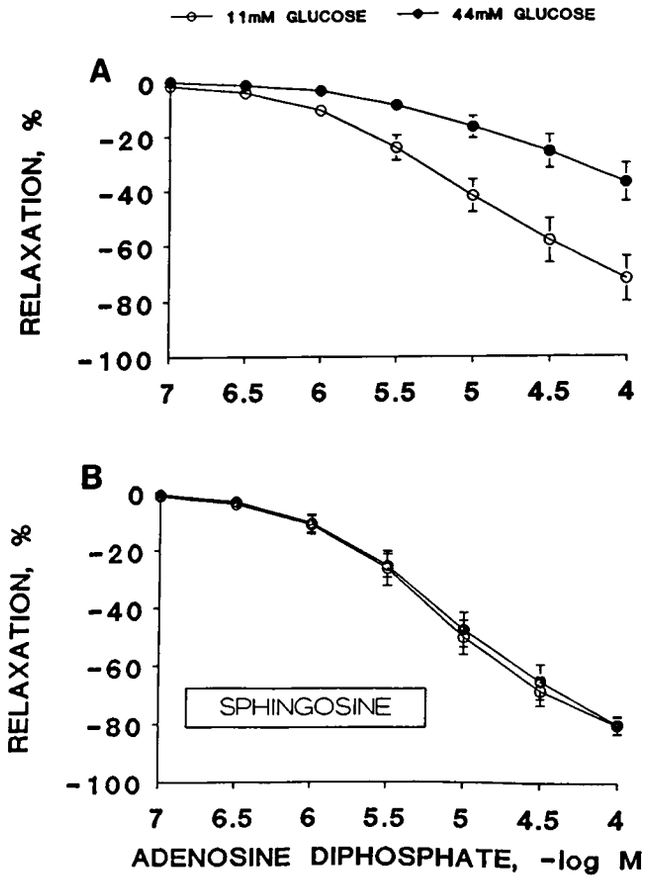

Figure 3. (*A*) Relaxations caused by adenosine diphosphate of aortic rings incubated in control (11 mM, $n = 8$) and elevated (44 mM, $n = 8$) glucose for 6 h. Relaxations were significantly less in aorta incubated in elevated glucose compared with those in control glucose. (*B*) Treatment with sphingosine (10^{-5} M, $n = 4$) restored the abnormal adenosine diphosphate relaxation of aorta incubated in elevated glucose such that the relaxations were not significantly different.

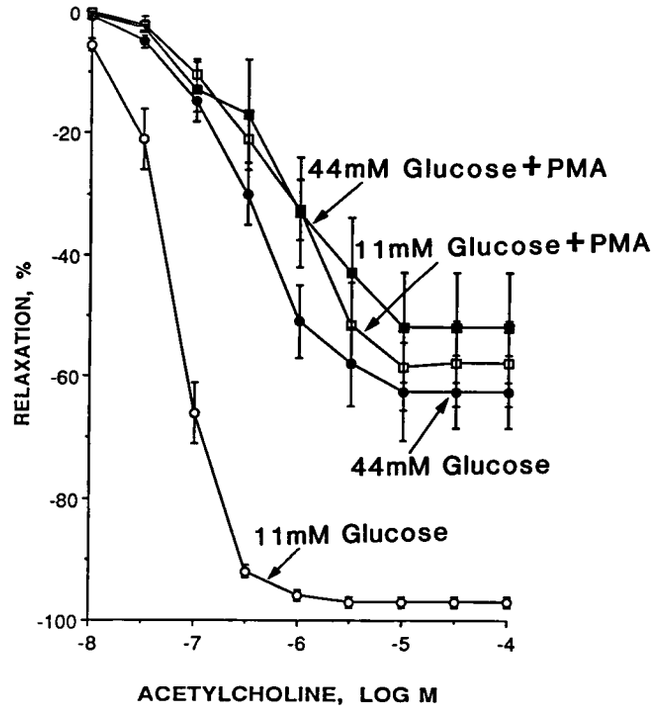

Figure 4. Effects of PMA (10^{-8} M) on the relaxation caused by acetylcholine of aortic rings incubated in control (11 mM) or elevated glucose (44 mM) for 6 h. Treatment with PMA for 10 min caused a significant rightward shift and reduction in maximal relaxation to acetylcholine in aorta incubated in control glucose ($n = 5$) which was similar to the inhibition caused by incubation in elevated glucose (44 mM) for 6 h alone. In rings incubated in elevated glucose, PMA had no further effect.

relaxation to adenosine diphosphate caused by elevated glucose (Fig. 3).

10-min exposure to PMA (10^{-8} M) inhibited acetylcholine-induced relaxations in aorta incubated in control glucose (Figs. 1 and 4). Similar to rings exposed to elevated glucose, rings treated with PMA contracted after relaxation to each concentration of acetylcholine (3×10^{-7} to 10^{-4} M; Fig. 1). The relaxation to acetylcholine in rings treated with PMA was not significantly different from that in rings incubated in elevated glucose (IC_{50}, −log M: 5.8±0.2, $n = 7$; Fig. 4). The addition of PMA had no significant further inhibitory effect in aorta incubated in elevated glucose (44 mM, IC_{50}, −log M: 5.8±0.4, $n = 3$; Fig. 4). PDD had no significant effect on acetylcholine-induced relaxations in aorta incubated in control glucose (IC_{50} −log M: 7.2±0.1, $n = 3$).

Indomethacin (10^{-5} M) significantly increased the sensitivity (IC_{50}, −log M: 6.6±0.1, $n = 5$) and maximal relaxation (95±1.3%, $n = 5$) to acetylcholine of aortic rings in control glucose treated with PMA.

PMA (10^{-8} M) had no significant effect on the relaxations caused by A23187 ($10^{-8} - 3 \times 10^{-6}$ M) in rings incubated in control glucose. The maximal relaxation occurred at 3×10^{-6} M and was 77±10.5% vs. 75±12.4% in control and PMA-treated rings, respectively. The IC_{50} was also not significantly different (−log M: 6.6±0.1 vs. 6.7±0.1, respectively, $n = 4$). The relaxations caused by A23187 also were not significantly different in rings incubated in elevated glucose treated with PMA (IC_{50}, −log M: 6.5±0.1, $n = 3$).

Table I. Basal Release of Immunoreactive Prostanoids from Aortic Segments Incubated for 6 h in Control (11 mM) or Elevated (44 mM) Glucose in the Presence and Absence of H-7 and PMA

	Untreated		H-7		PMA	
	Control	Elevated	Control	Elevated	Control	Elevated
Glucose:						
Thromboxane B_2:	4.0±0.1	4.0±1.0	3.5±0.7	3.7±0.9	6.5±1.0*	7.0±1.8
$PGF_{2\alpha}$:	22±30	34±20	31±9.0	33±10	37±8.0	39±9.0
6-Keto-$PGF_{1\alpha}$:	136±31	210±70	191±60	210±51	169±30	195±24
PGE_2:	87±20	94±22	90±30	101±40	127±41	126±25

Values are expressed as means±SE (pg/mg tissue per 30 min). The weight of the aorta segments used in the untreated, H-7, and PMA-treated groups for control versus elevated glucose incubation, respectively, were 70±10.5, 65±5.0, and 72±5.6 vs. 64±4.5, 75±4.7, and 69±5.8 mg, respectively, $n = 6$ each. Segments were treated with H-7 throughout the 6-h incubation period. PMA was present in a subsequent 30-min incubation. * Significant increase by PMA.

The relaxations caused by sodium nitroprusside (10^{-9}–10^{-5} M) were not significantly different between rings incubated in control glucose in the presence or absence of PMA (IC_{50}; −log M: 8.0±0.2 vs. 8.0±0.1, respectively, $n = 3$).

Prostanoid production. Under basal conditions, the release of thromboxane B_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and PGE_2 was not significantly different between aorta with intact endothelium incubated in elevated (44 mM) or control (11 mM) glucose (Table I). Treatment with H-7 (10^{-5} M) had no significant effect on the basal release of any of the prostanoids. PMA (10^{-8} M) caused a significant increase in the basal release of thromboxane B_2 from aorta in control glucose, but the increase in aorta exposed to elevated glucose (44 mM) did not reach statistical significance (Table I). The phorbol ester had no significant effects on basal release of the other prostanoids measured.

Acetylcholine (10^{-6} M) significantly increased release of all the measured prostanoids in segments of aorta incubated in either control or elevated glucose (Fig. 5). The acetylcholine-stimulated release of thromboxane B_2 and $PGF_{2\alpha}$ was significantly greater from aorta incubated in elevated glucose than from those incubated in control glucose. The increase of thromboxane B_2 caused by acetylcholine (10^{-6} M) from aorta incubated in elevated glucose was 3.9±1.0-fold compared with a 1.6±0.1-fold increase from aorta incubated in control glucose ($P < 0.01$, $n = 6$).

H-7 had no significant effect on the acetylcholine-stimulated release of thromboxane B_2, $PGF_{2\alpha}$, or 6-keto $PGF_{1\alpha}$ from aorta incubated in control glucose, but significantly decreased that of PGE_2 (Fig. 5). Treatment with H-7 prevented the increase in acetylcholine-stimulated thromboxane B_2 and $PGF_{2\alpha}$ from aorta incubated in elevated glucose. H-7 had no significant effect on the release of 6-keto-$PGF_{1\alpha}$ from aorta incubated in elevated glucose.

Treatment with PMA (10^{-8} M) caused a significant increase in acetylcholine-stimulated release of thromboxane B_2 and PGE_2 from aorta incubated in control glucose (Fig. 5). In aorta incubated in elevated glucose, treatment with PMA did not cause a significant change in the acetylcholine-stimulated release of any of the measured prostanoids such that there was no significant difference between release from aorta in control or elevated glucose. There was no significant difference between the increased acetylcholine-stimulated thromboxane B_2 and $PGF_{2\alpha}$ release caused by elevated glucose in the absence of PMA and that caused by PMA in control glucose.

In segments without endothelium incubated in control or elevated glucose, PMA did not significantly increase acetylcholine-stimulated thromboxane B_2 release (4.7±0.5 vs. 5.1±0.2 pg/mg tissue, respectively, $n = 3$).

A23187 (10^{-6} M) significantly increased release of all the prostanoids in aortic segments with intact endothelium incubated in either control or elevated glucose (Table II). The A23187-stimulated increase of the measured prostanoids was not significantly different between segments incubated in control or elevated glucose.

Discussion

PMA, which mimics the stimulant action of diacylglycerol on protein kinase C (9), inhibited endothelium-dependent relaxation caused by acetylcholine in aorta incubated in control

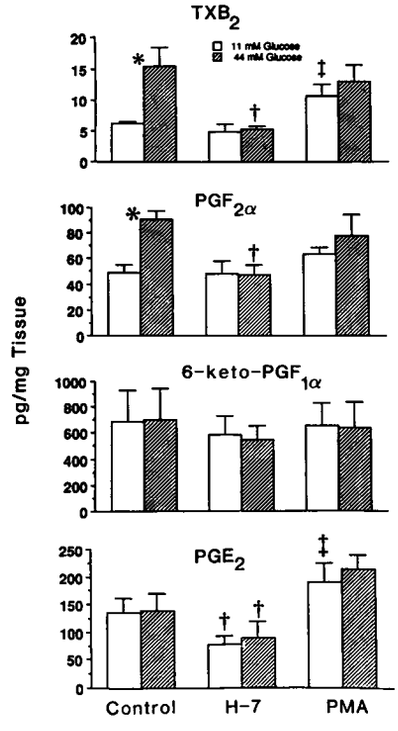

Figure 5. Acetylcholine (10^{-6} M) stimulated release of immunoreactive prostanoids from aortic segments with endothelium incubated in control (11 mM) or elevated (44 mM) glucose for 6 h. Values are means±SE (pg/mg tissue per 30 min), $n = 6$. *Significant difference between prostanoid production in control and elevated glucose. †Significantly decreased release caused by H-7 (10^{-5} M) added throughout the 6-h incubation. ‡Significantly increased release caused by PMA (10^{-8} M) added for 30 min before and during the 30-min stimulation with acetylcholine.

Table II. Basal and A23187 (10^{-6} M) Stimulated Release of Immunoreactive Prostanoids from Aortic Segments Incubated for 6 h in Control (11 mM) or Elevated (44 mM) Glucose

	Basal		A23187	
	Control	Elevated	Control	Elevated
Glucose:				
Thomboxane B_2:	3.8±1.3	2.7±1.1	28.1±4.7	36±8.8
$PGF_{2\alpha}$:	19.1±2.6	42.2±4.2*	205±38.4	268±39.8
6-Keto-$PGF_{1\alpha}$:	153±12.8	193±6.9	2639±337	2928±278
PGE_2:	63±12.7	69±6.9	440±77	402±38

Values are expressed as means±SE (pg/mg tissue per 30 min). The weight of the aortic segments used for control and elevated glucose incubation were 72±11 vs. 72±11, respectively, $n = 4$. * Significantly different from control.

glucose. It has been reported that PMA inhibits receptor-mediated release of EDRF from cultured endothelial cells (10) and from the intact endothelium of rabbit aorta, canine coronary artery, and rabbit pulmonary artery (11–13). The impairment is likely due to protein kinase C activation rather than to a nonspecific effect of the phorbol ester because in these and previous studies 4-phorbol didecanoate, which does not stimulate protein kinase C (9), was without effect on acetylcholine-induced relaxations. The similarity in the contractions, which follow the relaxation caused by each concentration of acetylcholine observed in diabetic rabbit aorta (2) or normal aorta after 6 h exposure to elevated glucose to those observed after 10-min treatment with PMA, suggests that protein kinase C activation may explain the abnormal relaxation in aorta exposed to elevated glucose. The lack of additive inhibitory effects on acetylcholine relaxations in aorta exposed to elevated glucose treated with PMA is also consistent with protein kinase C activation as a common mechanism.

The impairment of endothelium-dependent relaxation caused by elevated glucose (3) and diabetes (2, 14–16) is specific for receptor-mediated responses, because relaxations to acetylcholine and adenosine diphosphate are impaired, but not relaxations to A23187. The impaired relaxation caused by PMA is specific for receptor-mediated relaxation since endothelium-dependent relaxation stimulated by the calcium ionophore, A23187, is unaffected. Direct smooth muscle relaxation caused by sodium nitroprusside also was not affected by PMA. Since the mode of action of sodium nitroprusside and EDRF is similar (17), this would suggest that the responsiveness of vascular smooth muscle to EDRF is not altered by PMA. Since both acetylcholine and A23187 increase endothelial cell calcium to release EDRF, PMA may affect the formation or release of EDRF elicited by acetylcholine at a stage proximal to increases in intracellular free calcium. Because the calcium ionophore increases calcium independently of receptors, it likely bypasses the site of action of PMA on protein kinase C. Thus, inhibition of endothelium-dependent relaxation can be explained by the fact that activation of protein kinase C interferes with receptors or disturbs signal transduction distal to the receptor (12, 13, 18). Since activation of protein kinase C inhibits phospholipase C, the release of calcium mobilizing inositol triphosphate in response to receptor stimulation may be limited (9, 19). Although no attempts were made in these experiments to directly assess the activation by PMA of protein kinase C in intact aortic endothelial cells, previous studies in cultured endothelial cells have demonstrated protein kinase C activation by phorbol ester (6, 20).

It has been reported that elevated glucose can increase protein kinase C activation by increasing de novo synthesis of diacylglycerol in cultured endothelial cells and adipocytes (6, 7). The protein kinase C inhibitors, H-7 or sphingosine, restored abnormal acetylcholine and adenosine diphosphate-induced relaxations caused by elevated glucose, consistent with the hypothesis that the abnormality is caused by activation of protein kinase C. Relaxations of aorta incubated in control glucose were unaffected by treatment with H-7 or sphingosine, indicating a specific action of the protein kinase C inhibitors with respect to the effects of elevated glucose. The similarity between the abnormal endothelium-dependent vascular relaxation observed in diabetes mellitus (2, 14–16) to those caused by elevated glucose or PMA suggests that activation of protein kinase C during hyperglycemia may be responsible. The in vitro effect of glucose is dependent on elevated concentrations between 22 and 44 mM for the relatively brief exposure of 6 h studied here, whereas the similar changes observed previously in diabetic rabbits followed 6 wk exposure to glucose levels of ~ 20 mM (2).

As it does in aorta exposed to elevated glucose (3), indomethacin increased toward control the abnormal acetylcholine-induced relaxations observed in aorta treated with PMA without having a significant effect on the response in control glucose. Thus, the abnormal acetylcholine-induced relaxation may be related to the ability of PMA to stimulate prostanoids which, by their own vasoconstrictor action or by interfering with the release and/or action of EDRF, may inhibit the relaxation caused by acetylcholine. In support of this suggestion, PMA caused a significantly greater acetylcholine-stimulated release of thromboxane B_2 and PGE_2 in aorta incubated in control glucose. Since all the measured prostanoids are vasoconstrictors in the rabbit abdominal aorta (2), the increased release may account for the reduced relaxations observed in aorta treated with PMA. Importantly, elevated glucose which increased acetylcholine-stimulated release of thromboxane A_2 and $PGF_{2\alpha}$ had no additional effect to that of PMA, again suggesting a common mechanism. Supporting this, H-7 suppressed the increase in acetylcholine-stimulated thromboxane A_2 and $PGF_{2\alpha}$ caused by elevated glucose, suggesting that activation of protein kinase C in the setting of elevated glucose leads to enhanced prostanoid synthesis. Previous studies have supported a role for protein kinase C in the stimulation of phospholipase A_2 and prostanoid synthesis (21–23). In this study the effect of PMA was likely exerted on the endothelium as reflected by the lack of increase in thromboxane A_2 in segments without endothelium. Thus, the similarity in the abnormal release of prostanoids in aorta treated with PMA for 30 min to that from diabetic aorta or normal aorta exposed to elevated glucose for 6 h, supports a role for activation of protein kinase C in intact endothelium exposed to elevated glucose in causing enhanced acetylcholine-stimulated release of prostanoids which are implicated in the abnormal relaxation of the aorta (2, 3). As for the abnormal endothelium-dependent relaxations caused by elevated glucose, the abnormal prostanoid production apparently is related to endothelial cell receptor activation, because prostanoids released in response to A23187

were unaffected by elevated glucose. This further suggests that the abnormality caused by elevated glucose results from dysfunction in endothelial cell receptor-mediated signal transduction which could be mediated by activation of endothelial cell protein kinase C.

As in the diabetic rabbit aorta, after a 6-h exposure of normal rabbit aorta to elevated glucose, there is selective impairment in receptor-mediated endothelium-dependent relaxation and enhanced generation of vasoconstrictor prostanoids. The effects of elevated glucose are mimicked by a brief treatment with PMA and are prevented by inhibitors of protein kinase C. The abnormal prostanoids released by diabetic blood vessels may have effects other than vasoconstriction. These include increased platelet aggregation and smooth muscle proliferation, actions which can be implicated in thrombotic and atherosclerotic vascular disease. Protein kinase C inhibitors might reverse hyperglycemia-induced vascular changes, because as demonstrated in this study they may prevent abnormal release of prostanoids, restore endothelium-dependent relaxation, and preserve the release of PGI_2 from endothelial cells exposed to elevated glucose. Since an increase in protein kinase C activity alters many cellular functions, the activation of protein kinase C and increased generation of vasoactive prostanoids by elevated glucose may be responsible for the development of diabetic vascular complications.

Acknowledgments

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