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Impairment of endothelium-dependent but not of endothelium-independent dilatation in guinea-pig aorta rings incubated in the presence of elevated glucose

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1 Purine compounds such as ATP and adenosine, respectively endothelium-dependent and -independent vasodilators, are largely involved in the control of vascular tone and vascular reactivity to contracting stimuli. We investigated the relaxing activity of ATP and adenosine in guinea-pig aorta rings exposed for 6 h to elevated glucose concentration (50 mM), in order to mimic hyperglycaemic conditions. Guinea-pigs were reserpine-treated (2 mg kg^{-1}, i.p., 48 and 24 h before death).

2 Rings of aortae incubated in 50 mM glucose, contracted submaximally by 1 μM noradrenaline, lost endothelium-dependent relaxation in response to acetylcholine (10 nM to 10 μM). Aortae incubated with 50 mM mannose, as a hyperosmotic control, relaxed to acetylcholine normally. Rings of aortae incubated in 50 mM glucose, contracted submaximally by 3 mM 4-aminopyridine, lost endothelium-dependent relaxation in response to ATP (30 μM) whereas endothelium-independent relaxation in response to adenosine (0.3 mM) was well preserved.

4 The relaxation induced by A23187 or sodium nitroprusside (10 nM to 0.1 μM) did not differ between rings exposed to control (5.5 mM) or elevated glucose (50 mM) and contracted submaximally by 3 mM 4-aminopyridine.

5 When incubated with aortic tissue in the presence of elevated glucose, the cyclo-oxygenase inhibitors, indomethacin (10 μM) and mefenamic acid (30 μM), or the scavenger of superoxide anions, superoxide dismutase (150 u ml^{-1}), prevented the impairment of ATP-mediated relaxation.

6 The present results indicate that endothelium-dependent, receptor-induced relaxation in response to acetylcholine and ATP is impaired in guinea-pig aorta rings exposed to elevated glucose. The endothelial dysfunction caused by glucose might be located at a step between receptor activation and intracellular calcium increase, and might be related to an increased metabolism of arachidonic acid coupled to an increased production, or to a reduced inactivation of superoxide anions.

Keywords: Adenosine; ATP; endothelium; glucose; vasodilatation

Introduction

Numerous disease processes including hypercholesterolaemia (Jayakody *et al*., 1985), atherosclerosis (Freiman *et al*., 1986), ischaemia and reperfusion (Ku, 1982), acute or chronic hypertension (Lamping & Dole, 1987; Panza *et al*., 1990), congestive heart failure (Kubo *et al*., 1991) and diabetes (Oyama *et al*., 1986), are associated with abnormal endothelium-dependent vascular relaxation and increased vascular reactivity to different vasoactive substances leading to vasospasm and tissue ischaemia. Thus, in pathological conditions characterized by impaired endothelial integrity and function, it becomes essential to know which vasodilator mechanisms are compromised and which are preserved to defend vascular tissue against contracting and spasmogenic stimuli.

In the present study, guinea-pig aorta rings were perfused by a medium containing elevated concentrations of glucose, in order to evoke an impairment of endothelium-dependent relaxation (Valentovic & Lubay, 1985; Tesfamariam *et al*., 1990; Bohlen & Lash, 1993; Weisbrod *et al*., 1993) similar to that observed *in vivo*, in diabetic patients (Saenz de Tajada *et al*., 1989; Calver *et al*., 1992; Mc Veigh *et al*., 1992; Hsueh & Anderson, 1992).

Initially, noradrenaline was used as a contracting agent, to test the functional integrity of vascular preparations and the responsiveness to contracting stimuli. Subsequently, contractions were evoked by means of the K^+ channel blocker, 4-aminopyridine, which is known to induce arterial spasm *in vitro* and is used in experimental models of vasospatic angina (Ross *et al*., 1980; Lambert & Pepine, 1983; Uchida & Sugimoto, 1984; Uchida, 1985; Iwaki *et al*., 1988). Adenosine and ATP were used to evoke vascular relaxation. These purine compounds are released from vascular innervation (White, 1988), circulating platelets, or vascular tissue metabolism (Born & Kratzer, 1984), and sustain a prominent role among the natural factors, controlling the vascular tone. In vessels with intact endothelium, the vascular response to adenosine 5′-triphosphate (ATP) is generally vasodilatation, mediated by endothelial P_{2Y} purinoceptors through the release of prostacyclin and/or endothelium-derived relaxing factor (EDRF) (De Mey & Vanhoutte, 1981; Gordon & Martin, 1983; Needham *et al*., 1987; Boeynaems & Pearson, 1990), whereas the endothelium does not play an obligatory role in the vasodilator action of adenosine (De Mey & Vanhoutte, 1981; Furchgott, 1983).

Methods

Dunkin-Hartley guinea-pigs of either sex (300–350 g) were treated with reserpine (2 mg kg^{-1}, i.p.) 48 and 24 h before death, in order to eliminate the influence of noradrenaline, which might be released from sympathetic nerve terminals (Temma *et al*., 1977) during the experiment. The animals were then killed by cervical dislocation and exsanguinated and the thoracic aorta was removed and placed in a physiological salt solution of the following composition (mM): NaCl 120, KCl 2.7, $MgCl_2$ 0.9, NaH_2PO_4 0.4, $CaCl_2$ 1.37, $NaHCO_3$ 11.9 and glucose 5.5. The solution was bubbled vigorously with a mixture of 95% O_2 and 5% CO_2 which produced a pH of 7.5.

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The aorta was dissected free from connective tissue and cut into rings that were mounted vertically by means of stainless steel hooks in 10 ml organ baths containing the physiological solution, aerated as described above and maintained at $37 \pm 0.3^{\circ}$C. Changes in tension were recorded by means of an E.C.T.A. isotonic transducer - Linearcorder (Mark III, Watanabe, Japan). An initial tension of 0.8 g was applied to the rings, which were then allowed to equilibrate for 2 h. Initially, contractions were evoked by exposure to 1 μM noradrenaline (5 min contact time) at 30 min intervals, until three responses of equal amplitude were obtained (these responses corresponded to 80–90% of the maximal contractions induced by 120 mM KCl). The effect of acetylcholine (10 nM–10 μM) was tested on the contraction induced by the last addition of noradrenaline and the relaxing response to acetylcholine was considered as an index of functional integrity of the endothelium (Furchgott, 1983). In experiments performed on denuded guinea-pig aorta rings, the endothelium was removed by gently rubbing the intimal surface with polyether string. The denuded vessel preparations did not respond to acetylcholine.

Contractile responses to 1 μM noradrenaline and subsequent relaxing responses to 0.1 mM sodium nitroprusside were also compared in preparations with and without endothelium to ensure that the smooth muscle was not damaged during removal of the endothelium (mean tension generated by noradrenaline in preparations respectively with and without endothelium was 5.76 ± 0.09 g and 5.95 ± 0.25 g, $n=9$. Relaxation by sodium nitroprusside in preparations respectively with and without endothelium was 93.20 ± 2.18% and 95.31 ± 3.22%, $n=9$).

Where indicated, aortic rings were incubated with 50 mM glucose for 6 h. Lesser concentrations of glucose (44 mM) did not impair vascular reactivity to acetylcholine in a reproducible manner. Mannose (50 mM) was used as a hyperosmotic control. After the 6 h incubation, the arteries were contracted with noradrenaline (1 μM) until three responses of equal amplitude were obtained. The effect of acetylcholine (10 nM–10 μM) was tested on the contraction induced by the last addition of noradrenaline, to evaluate endothelium functional integrity.

In the experiments with 4-aminopyridine, the K^+ channel blocker was added at a concentration of 3 mM, which evoked 80–90% of the maximal contraction induced by 120 mM KCl, similar to that induced by 1 μM noradrenaline.

The responses to acetylcholine, A23187 and sodium nitroprusside were evaluated by increasing their bath concentrations in cumulative increments. Where indicated, the cyclooxygenase inhibitors indomethacin (10 μM) and mefenamic acid (30 μM), or the scavenger of superoxide anions, superoxide dismutase (SOD, 150 u ml^{-1}), were present during the 6 h incubation and during subsequent concentration-response experiments. Indomethacin, mefenamic acid and A23187 were dissolved in ethanol. The final concentration of ethanol in the bath medium did not exceed 0.3% and did not affect the basal tone or the reactivity of aorta rings to contracting or relaxing agents.

Data analysis

Relaxation is expressed as % change from the level of contraction induced by noradrenaline or 4-aminopyridine. The half-maximal inhibitory concentration (IC_{50}) was estimated graphically as the concentration causing 50% relaxation. Data are expressed as mean ± s.e.

Statistical comparison between concentration-response curves was performed by means of repeated measures analysis of variance, followed by Newman-Keuls multiple-range test. Responses of rings from the same animal were compared by means of Student's t test for paired comparisons. P values <0.05 were regarded as significant. In all experiments, n refers to the number of guinea-pigs from which the rings were obtained.

Drugs

All the reagents used in this study: noradrenaline bitartrate, 4-aminopyridine, acetylcholine chloride, sodium nitroprusside, the calcium ionophore A23187, indomethacin, mefenamic acid, SOD from bovine erythrocytes, ATP (adenosine-5′-trisphosphate) disodium salt, adenosine free base, glucose, mannose, were obtained from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.).

Results

Relaxing effect of acetylcholine in aorta rings before and after 6 h incubation in the presence of 5.5 or 50 mM glucose or 50 mM mannose

Rings of guinea-pig aortae with intact endothelium before and after 6 h incubation in the presence of 5.5 mM glucose, or after 6 h incubation in the presence of 50 mM glucose or 50 mM mannose, treated with 1 μM noradrenaline produced similar contractions (5.76 ± 0.09 g, $n=8$; 5.83 ± 0.08 g, $n=8$; 5.08 ± 0.12 g, $n=9$; 6.03 ± 0.11 g, $n=9$, respectively). When the contractile effect of 1 μM noradrenaline had reached its maximum the rings were exposed to increasing concentrations of acetylcholine (10 nM to 10 μM). The concentration-dependent relaxing effect of acetylcholine was well preserved after 6 h incubation of aorta rings in the presence of 5.5 mM glucose or 50 mM mannose whereas it had disappeared in preparations incubated for 6 h with 50 mM glucose (Figure 1). The IC_{50} of acetylcholine in aorta rings before 6 h incubation in the presence of 5.5 mM glucose was 0.20 μM ($n=8$), after 6 h with 5.5 mM glucose was 0.26 μM ($n=8$), and after 6 h incubation in the presence of 50 mM mannose was 0.20 μM ($n=9$).

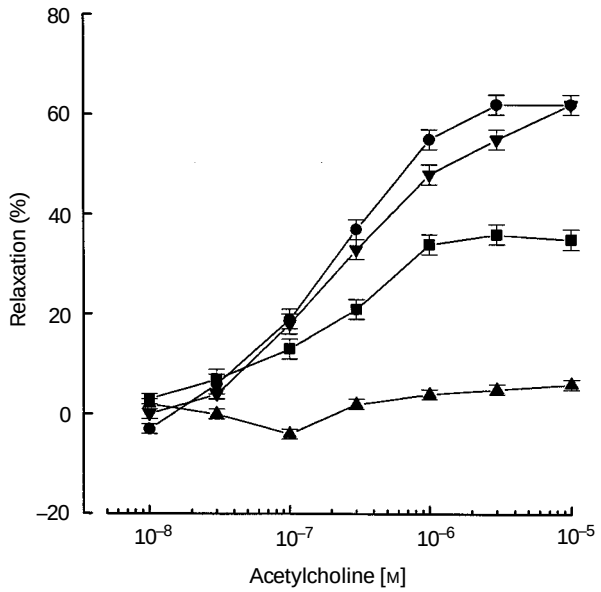

Figure 1 Comparison of relaxation induced by acetylcholine in aorta rings contracted by 1 μM noradrenaline before (■, $n=8$) and after 6 h incubation with 5.5 mM glucose (●, $n=8$), 50 mM glucose (▲, $n=8$) or 50 mM mannose (▼, $n=8$). Relaxation induced by acetylcholine (at 10^{-6} M, 3×10^{-6} M and 10^{-5} M) was significantly less after 50 mM glucose than either before incubation, after 5.5 mM glucose or after 50 mM mannose ($P<0.01$). At 3×10^{-7} M acetylcholine, relaxation was significantly less after 50 mM glucose than relaxation either before incubation ($P<0.05$), after 5.5 mM glucose or 50 mM mannose ($P<0.01$). At 10^{-7} M acetylcholine, relaxation was significantly less after 50 mM glucose than after 5.5 mM glucose ($P<0.05$).

Relaxing effect of ATP and adenosine in aorta rings with or without endothelium after 6 h incubation in the presence of 5.5 mM or 50 mM glucose

The K^+ channel blocker, 4-aminopyridine, was used at a concentration (3 mM) that evoked 80–90% of the maximal contraction induced by 120 mM KCl, similar to that induced by 1 μM noradrenaline. The contractions induced by 3 mM 4-aminopyridine in aorta rings with or without endothelium after 6 h incubation with 5.5 mM glucose, and in aorta rings with endothelium after 6 h incubation with 50 mM glucose, did not differ (5.88 ± 0.13 g, $n=8$; 6.34 ± 0.09 g, $n=9$; 6.23 ± 0.06 g, $n=8$, respectively). When the contractile effect of 4-aminopyridine had reached its maximum, the rings were exposed to 30 μM ATP or to 0.3 mM adenosine. The relaxing effect of adenosine was preserved in aorta rings deprived of endothelium or in aorta rings exposed to elevated glucose concentration. By contrast, the relaxing effect of ATP was absent in endothelium-denuded preparations or in aorta rings incubated for 6 h with 50 mM glucose (Figure 2).

Relaxing effect of sodium nitroprusside and A23187 in aorta rings after 6 h incubation in the presence of 5.5 or 50 mM glucose

Sodium nitroprusside and the calcium ionophore, A23187 (10 nM–0.1 μM), were added cumulatively to aorta rings contracted with 3 mM 4-aminopyridine after 6 h incubation in the presence of 5.5 or 50 mM glucose. The relaxation caused by sodium nitroprusside and by A23187 was not significantly different between rings incubated in control (5.5 mM) or elevated (50 mM) glucose. The maximal relaxation and the IC_{50} of both the compounds are presented in Table 1.

Effect of indomethacin, mefenamic acid and SOD on relaxing effect induced by ATP in aorta rings after 6 h incubation in the presence of 50 mM glucose

ATP (30 μM) was added to aorta rings contracted with 3 mM 4-aminopyridine after 6 h incubation in the presence of 10 μM indomethacin or 30 μM mefenamic acid or 150 u ml^{-1} SOD. None of these agents modified significantly the contractile response to 4-aminopyridine, which was 5.96 ± 0.16 g ($n=4$) in rings exposed to 50 mM glucose, 6.04 ± 0.19 g ($n=5$) in rings exposed to 50 mM glucose plus indomethacin, 6.08 ± 0.12 g ($n=5$) in rings exposed to 50 mM glucose plus mefenamic acid, and 5.87 ± 0.09 g ($n=5$) in rings exposed to 50 mM glucose plus SOD. Neither the cyclo-oxygenase inhibitors nor SOD had a significant effect on the relaxation to ATP of rings incubated with control glucose (data not shown). As shown in Figure 3, the addition of indomethacin or mefenamic acid or SOD during the 6 h incubation with 50 mM glucose prevented the impairment of ATP-induced relaxation, so that the relaxing effect induced by the nucleotide did not differ statistically from that observed in rings incubated for 6 h in the presence of 5.5 mM glucose. In contrast, neither catalase (500 u ml^{-1}) nor allopurinol (1 mM) restored ATP-induced relaxation in aorta rings exposed for 6 h to 50 mM glucose (data not shown).

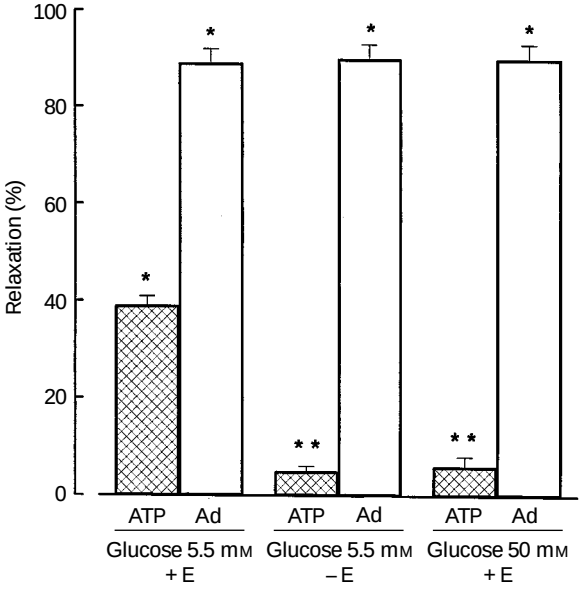

Figure 2 Relaxation induced by 30 μM ATP and 0.3 mM adenosine (Ad) in aorta rings contracted by 3 mM 4-aminopyridine, with (+E) or without (−E) endothelium, after 6 h incubation with 5.5 mM or 50 mM glucose. *$P<0.001$ vs 4-aminopyridine alone. **Non-significant vs 4-aminopyridine alone.

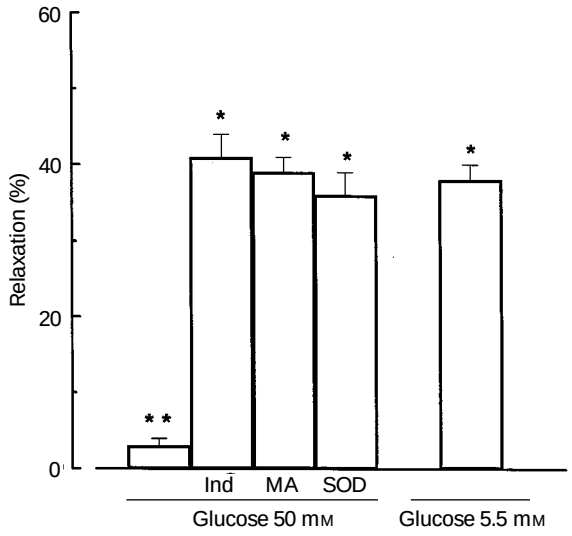

Figure 3 Effect of 10 μM indomethacin (Ind), 30 μM mefenamic acid (MA) and 150 u ml^{-1} superoxide dismutase (SOD) on relaxation induced by 30 μM ATP in aorta rings contracted by 3 mM 4-aminopyridine after 6 h incubation with 5.5 mM or 50 mM glucose. *$P<0.001$ vs 4-aminopyridine alone. **Non-significant vs 4-aminopyridine alone.

Table 1 Relaxation induced by Na-nitroprusside and the calcium ionophore, A23187, in aorta rings precontracted by 4-aminopyridine after 6 h incubation with 5.5 mM or 50 mM glucose

Drugs	*Incubation*	*Maximal relaxation* (%)	*IC_{50}* (nM)
Na-nitroprusside	5.5 mM glucose ($n=8$)	98.07 ± 2.31	25.30 ± 0.81
Na-nitroprusside	50 mM glucose ($n=8$)	96.41 ± 3.72*	26.00 ± 0.62
A23187	5.5 mM glucose ($n=8$)	95.00 ± 3.42	19.00 ± 0.50
A23187	50 mM glucose ($n=8$)	93.73 ± 4.28*	18.50 ± 0.42

*Differences in maximal relaxation between 5.5 mM glucose and 50 mM glucose were not statistically significant.

Discussion

In the present study, a 6 h incubation of guinea-pig aorta rings with 50 mM glucose, to mimic hyperglycaemic conditions, abolished endothelium-dependent relaxation induced by cholinoceptor stimulation. The alteration caused by 50 mM glucose was not due to hyperosmotic effect because the same concentration of mannose had no effect on the relaxation induced by acetylcholine. Moreover, it was not due to an interaction between reserpine and glucose, inasmuch as high glucose was equally effective in aortic rings from non reserpine-treated guinea-pigs (data not shown). These results are in accord with data obtained by Tesfamariam *et al.* (1990) in rabbit aorta exposed to elevated glucose concentrations.

Guinea-pig aorta rings incubated for 6 h with control glucose (5.5 mM) and contracted by the K^+ channel blocker, 4-aminopyridine, were highly sensitive to the relaxing effect of ATP and even more to that of adenosine. However, the vascular response to ATP disappeared in aorta rings incubated with elevated glucose concentration (50 mM) or in aorta rings deprived of endothelium, whereas vascular relaxation evoked by adenosine remained unmodified in both the experimental conditions. These data agree with previous observations (Headrick & Berne, 1990; Collis & Brown, 1993), which showed that in guinea-pig aorta the relaxing activity of adenosine is mainly endothelium-independent, whereas that evoked by ATP requires endothelium integrity, and indicate that only the latter is impaired by elevated glucose. Inasmuch as guinea-pig aorta relaxation in response to ATP is mediated by P_{2Y} purinoceptors leading to endothelium-derived relaxing factor (EDRF) release (Dorigo *et al.*, unpublished observations), it can be concluded that in the tissue exposed to elevated glucose concentration the endothelium-dependent relaxation caused by purinoceptor and cholinoceptor stimulation is impaired. This is in accord with previous findings showing that P_{2Y}-mediated, endothelium-dependent dilatation in response to ATP is also impaired in cerebral arterioles (Mayhan, 1989; Mayhan *et al.*, 1991) and in thoracic aorta (Pieper & Gross, 1988; Hattori *et al.*, 1991; Karasu & Altan, 1993).

The step at which elevated glucose impairs the endothelium-dependent relaxation induced by cholinoceptor or by purinoceptor stimulation remains to be elucidated. In the endothelial cell, EDRF is synthesized through oxidation of L-arginine by a calcium-activated NADPH-dependent enzyme. Released EDRF causes relaxation of smooth muscle by activation of soluble guanylyl cyclase to raise guanosine 3′:5′-cyclic monophosphate (cyclic GMP) levels (Rappoport & Murad, 1983; Moncada *et al.*, 1991). In guinea-pig aorta rings exposed to elevated glucose concentration the relaxing response to the calcium inophore A23187 (which is known to cause endothelium-dependent relaxation by raising the intracellular calcium concentration in a manner unrelated to any receptor mechanism), as well as the relaxing response to sodium nitroprusside (an endothelium-independent vasodilator which relaxes smooth muscle by a mechanism similar to that of EDRF) (Rappoport & Murad, 1983), were well preserved. These results support the notion that in guinea-pig aorta the EDRF release in response to an increase of calcium content in endothelial cell and the guanylyl cyclase activity in smooth muscle are not altered by hyperglycaemic conditions (Tesfamariam *et al.*, 1989, 1990; Karasu & Altan, 1993). Thus, endothelial dysfunction induced by elevated glucose seems to be located in a step between receptor activation and intracellular calcium increase. Both acetylcholine and ATP hyperpolarize the endothelial cell, thus providing an electrochemical gradient for maintained calcium entry during receptor stimulation (Adams, 1994). Furthermore, receptor-linked EDRF release is a phenomenon involving G-protein activation associated with calcium mobilization in the endothelial cell (Wennalm, 1994) and there is evidence that in guinea-pig aorta both cholinoceptors and P_{2Y} purinoceptors are linked to G-proteins (unpublished observation). The probability thus arises that, as suggested in early atherosclerosis and in hypercholesterolaemia (Flavahan, 1992), G-proteins linked to receptor-dependent EDRF release may also be inactivated in hyperglycaemic conditions. This hypothesis is substantiated by the observation that elevated glucose affects vascular cell functions by changing signal transduction through receptors (Brownlee *et al.*, 1988). Also, in diabetic animals a dysfunction in the G-protein system has been found in different tissues (Gawler *et al.*, 1987; Green & Johnson, 1991).

In the present study the mechanism responsible for endothelial dysfunction appears to be related to arachidonic acid metabolism and the generation of free radicals. In fact indomethacin and mefenamic acid, two cyclo-oxygenase inhibitors, and SOD, a specific scavenger of superoxide anions (McCord & Fridovich, 1969), prevented glucose-induced impairment of endothelium-dependent relaxation in response to ATP. It is therefore possible that the impairment of endothelial cell responses observed with elevated glucose concentration was related to an augmented flux of arachidonic acid coupled to an increased generation and/or to a reduced inactivation of superoxide anions. In accordance with this interpretation, previous observations have shown that diabetes promotes the metabolism of arachidonic acid and that cyclo-oxygenase catalysis involves free radical intermediates which, in turn, activate arachidonic acid metabolism. Because catalase and allopurinol did not prevent the impairment caused by elevated glucose it can be concluded that hydrogen peroxide or superoxide anions produced by xanthine oxidase activity are not involved.

In guinea-pig aorta rings exposed to elevated glucose concentration the increased metabolism of arachidonic acid and the increased levels of free radicals might be due to an increased activation of protein kinase C. Previous studies in several different blood vessels have provided evidence that protein kinase C activation can inhibit receptor-operated endothelium-dependent vasorelaxation, but not the relaxation to agents which bypass membrane receptors and release nitric oxide directly, such as the calcium ionophore A23187 or sodium nitroprusside (Cherry & Gillis, 1988; Morrison & Pollock, 1990). It has been suggested that an increased activation of protein kinase C could selectively inhibit the pertussis toxin-sensitive G-protein involved in EDRF relaxing pathway (Flavahan *et al.*, 1991). Further studies are needed in order to substantiate this hypothesis.

The present results indicate that in guinea-pig aorta exposed to an elevated glucose concentration the receptor-operated endothelium-dependent control of vascular reactivity is impaired, so that only direct muscle relaxing agents may protect vascular tissue against spasmogenic stimuli.

References

ADAMS, D.J. (1994). Ionic channels in vascular endothelial cells. *Trends Cardiovasc. Med.*, **4,** 18–26.

BOEYNAEMS, J.M. & PEARSON, J.D. (1990). P2 purinoceptors on vascular endothelial cell: Physiological significance and transduction mechanisms. *Trends Pharmacol. Sci.*, **II,** 34–37.

BOHLEN, H.G. & LASH, J.M. (1993). Topical hyperglycemia rapidly suppresses EDRF-mediated vasodilation of normal rat arterioles. *Am. J. Physiol.*, **265** (*Heart Circ. Physiol.*, 34), H219–H225.

BORN, G.V. & KRATZER, M.A.A. (1984). Source and concentration of extracellular adenosine triphosphate during haemostasis in rats, rabbits and man. *J. Physiol.*, **354,** 419.

BROWNLEE, M., CERAMI, A. & VILASSARA, H. (1988). Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *New Engl. J. Med.*, **318,** 1315–1321.

CALVER, A., COLLIER, J. & VALLANCE, P. (1992). Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J. Clin. Invest.*, **90,** 2548 – 2554.

CHERRY, P.D. & GILLIS, C.N. (1988). Antagonism of acetylcholine-mediated relaxation of rabbit pulmonary arteries by phorbol myristate acetate. *J. Pharmacol. Exp. Ther.*, **247,** 542 – 549.

COLLIS, M.G. & BROWN, C.M. (1983). Adenosine relaxes the aorta by interacting with an A2 receptor and an intracellular site. *Eur. J. Pharmacol.*, **96,** 61 – 69.

DE MEY, J.G. & VANHOUTTE, P.M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.*, **316,** 347 – 355.

FLAVAHAN, N.A. (1992). Atherosclerosis or lipoprotein-induced endothelial dysfunction. Potential mechanisms underlying reduction in EDRF/nitric oxide activity. *Circulation*, **85,** 1927 – 1938.

FLAVAHAN, N.A., SHIMOKAWA, H. & VANHOUTTE, P.M. (1991). Inhibition of endothelium-dependent relaxations by phorbol myristate acetate in canine coronary arteries: role of a pertussis toxin-sensitive G protein. *J. Pharmacol. Exp. Ther.*, **256,** 50 – 55.

FREIMAN, P.C., MITCHELL, G.G., HEISTAD, D.D., ARMSTRONG, M.L. & HARRISON, D.G. (1986). Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. *Circ. Res.*, **58,** 783 – 789.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53,** 557 – 573.

GAWLER, D., MILLIGAN, G., SPIEGEL, A.M., UNSON, C.G. & HOUSLAY, M. (1987). Abolition of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes. *Nature*, **327,** 229 – 232.

GORDON, J.L. & MARTIN, W. (1983). Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of 86Rb efflux from isolated endothelial cells. *Br. J. Pharmacol.*, **79,** 531 – 541.

GREEN, A. & JOHNSON, J.L. (1991). Evidence for impaired coupling of receptors to Gi protein in adipocytes from streptozocin-induced diabetic rats. *Diabetes*, **40,** 88 – 94.

HATTORI, Y., KAWASAKI, H., ABE, K. & KANNO, M. (1991). Superoxide dismutase recovers altered endothelium-dependent relaxation in diabetic rat aorta. *Am. J. Physiol.*, **261** (*Heart Circ. Physiol.*, 30), H1086 – H1094.

HEADRICK, J.P. & BERNE, R.M. (1990). Endothelium-dependent and -independent relaxations to adenosine in guinea-pig aorta. *Am. J. Physiol.*, **259** (*Heart Circ. Physiol.*, 28), H62 – H67.

HSUEH, W.A. & ANDERSON, P.W. (1992). Hypertension, the endothelial cell and the vascular complications of diabetes mellitus. *Hypertension*, **20,** 253 – 263.

IWAKI, M., NAKAYA, Y., KAWANO, K., MIZOBUCHI, S., NAKAYA, S. & MORI, N. (1988). Tetraethylammonium-induced contraction of rabbit coronary artery. *Heart Vessels*, **4,** 141.

JAYAKODY, R.L., SENARATNE, M.P., THOMSON, A.B.R. & KAPPAGODA, C.T. (1985). Cholesterol feeding impairs endothelium-dependent relaxation of rabbit aorta. *Can. J. Physiol. Pharmacol.* **63,** 1206 – 1209.

KARASU, Ç. & ALTAN, M. (1993). The role of the endothelial cells on the alterations in vascular reactivity induced by insulin-dependent diabetes mellitus: effect of insulin treatment. *Gen. Pharmacol.*, **24,** 743 – 755.

KU, D.D. (1982). Coronary vascular reactivity after acute myocardial ischemia. *Science* (Wash. DC), **218,** 576 – 578.

KUBO, S.H., RECTOR, T.S., BANK, A.J., WILLIAMS, R.E. & HEIFETZ, S.M. (1991). Endothelium-dependent vasodilatation is attenuated in patients with heart failure. *Circulation*, **84,** 1589 – 1596.

LAMBERT, C.R. & PEPINE, C. (1983). A new model for coronary artery spasm. *Clin. Res.*, **31,** 866.

LAMPING, K.G. & DOLE, P. (1987). Acute hypertension selectively potentiates constrictor responses of large coronary arteries to serotonin by altering endothelial function in vivo. *Circ. Res.*, **61,** 904 – 913.

MAYHAN, W.G. (1989). Impairment of endothelium-dependent dilatation of cerebral arterioles during diabetes mellitus. *Am. J. Physiol.*, **256** (*Heart Circ. Physiol.*, 25), H621 – H625.

MAYHAN, W.G., SIMMONS, L.K. & SHARPE, G.M. (1991). Mechanism of impaired responses of cerebral arterioles during diabetes mellitus. *Am. J. Physiol.*, **260** (*Heart Circ. Physiol.*, 29), H319 – H326.

MCCORD, J.M. & FRIDOVICH, I. (1969). Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). *J. Biol. Chem.*, **244,** 6049 – 6055.

MCVEIGH, G.E., BRENNAN, G.M., JOHNSTON, G.D., MCDERMOTT, B.J., GRATH, L.T., HENRY, W.R., ANDREWS, J.W. & HAYES, J.R. (1992). Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*, **35,** 771 – 776.

MONCADA, S., PALMER, R.M. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43,** 109 – 141.

MORRISON, K.J. & POLLOCK, D. (1990). Impairment of relaxations to acetylcholine and nitric acid by a phorbol ester in rat isolated aorta. *Br. J. Pharmacol.*, **101,** 432 – 437.

NEEDHAM, L., CUSACK, N.J., PEARSON, J.D. & GORDON, J.L. (1987). Characteristic of the P2 purinoceptor that mediates prostacyclin production by pig aortic endothelial cells. *Eur. J. Pharmacol.*, **134,** 199 – 209.

OYAMA, Y., KAWASAKI, H., HATTORI, Y. & KANNO, M. (1986). Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. *Eur. J. Pharmacol.*, **132,** 75 – 78.

PANZA, J.A., QUYYUMI, A.A., BRUSH, J.E. & EPSTEIN, S.E. (1990). Abnormal endothelium-dependent relaxation in patients with essential hypertension. *N. Engl. J. Med.*, **323,** 22 – 27.

PIEPER, G.M. & GROSS, G.J. (1988). Oxygen free radicals abolish endothelium-dependent relaxation in diabetic rat aorta. *Am. J. Physiol.*, **255** (*Heart Circ. Physiol.*, 24), H825 – H833.

RAPPOPORT, R.M. & MURAD, F. (1983). Agonist-induced endothelial-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.*, **52,** 353 – 357.

ROSS, G., STINSON, E., SCHROEDER, J. & GINSBURG, R. (1980). Spontaneous phasic activity of isolated human coronary arteries. *Cardiovasc. Res.*, **14,** 613 – 618.

SAENZ DE TEJADA, I., GOLDSTEIN, I., AZADZOI, K. & COHEN, R.A. (1989). Impaired neurogenic and endothelium dependent relaxation of human penile smooth muscle: The pathophysiological basis for impotence in diabetes mellitus. *N. Engl. J. Med.*, **320,** 1025 – 1030.

TEMMA, K., AKERA, T., BRODY, T.M. & MANNIAN, A.A. (1977). Hydroxylated chlorpromazine metabolites: Positive inotropic action and the release of catecholamines. *Mol. Pharmacol.*, **13,** 1076 – 1085.

TESFAMARIAM, B. (1994). Free radicals in diabetic endothelial cell dysfunction. *Free Radical Biol. Med.*, **16,** 383 – 391.

TESFAMARIAM, B., BROWN, M.L., DEYKIN, D. & COHEN, R.A. (1990). Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. *J. Clin. Invest.*, **85,** 929 – 932.

TESFAMARIAM, B., JAKUBOWSKI, J.A. & COHEN, R.A. (1989). Contraction of diabetic rabbit aorta caused by endothelium-derived PGH2-TXA2. *Am. J. Physiol.*, **257** (*Heart Circ. Physiol.*, 26), H1327 – H1333.

UCHIDA, Y. & SUGIMOTO, T. (1984). Decreasing potassium conductance: a possible mechanism of the development of coronary vasospasm. *Fed. Proc.*, **43,** 429.

UCHIDA, Y. (1985). Decreased potassium conductance. A possible mechanism of phasic coronary vasospasm. *Jap. Circ. J.*, **49,** 128 – 139.

VALENTOVIC, M.A. & LUBAY, W.C. (1985). Elevated glucose in vivo and in vitro adversely alters prostaglandin generation in rat aortae and platelets. *Prostaglandins Leukotrienes Med.*, **19,** 271 – 277.

WEISBROD, R.M., BROWN, M.L. & COHEN, R.A. (1993). Effect of elevated glucose on cyclic GMP and eicosanoids produced by porcine aortic endothelium. *Arterioscler. Tromb.*, **13,** 915 – 923.

WENNALM, Å. (1994). Endothelial nitric oxide and cardiovascular disease. *J. Int. Med.*, **235,** 317 – 327.

WHITE, T.D. (1988). Role of adenine compounds in autonomic neurotransmission. *Pharmacol. Ther.*, **38,** 129 – 168.

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