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Effect of protein kinase C and phospholipase A_2 inhibitors on the impaired ability of human diabetic platelets to cause vasodilation

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1 The aim of this study was to examine the mechanism of impaired platelet-mediated endothelium-dependent vasodilation in diabetes. Exposure of human platelets to high glucose *in vivo* or *in vitro* impairs their ability to cause endothelium-dependent vasodilation. While previous data suggest that the mechanism for this involves increased activity of the cyclo-oxygenase pathway, the signal transduction pathway mediating this effect is unknown.

2 Platelets from diabetic patients as well as normal platelets and normal platelets exposed to high glucose concentrations were used to determine the role of the polyol pathway, diacylglycerol (DAG) production, protein kinase C (PKC) activity and phospholipase A_2 (PLA_2) activity on vasodilation in rabbit carotid arteries.

3 We found that two aldose-reductase inhibitors, tolrestat and sorbinil, caused only a modest improvement in the impairment of vasodilation by glucose exposed platelets. However, sorbitol and fructose could not be detected in the platelets, at either normal or hyperglycaemic conditions. We found that incubation in 17 mM glucose caused a significant increase in DAG levels in platelets. Furthermore, the DAG analog 1-oleoyl-2-acetyl-sn-glycerol (OAG) caused significant impairment of platelet-mediated vasodilation. The PKC inhibitors calphostin C and H7 as well as inhibitors of PLA_2 activity normalized the ability of platelets from diabetic patients to cause vasodilation and prevented glucose-induced impairment of platelet-mediated vasodilation *in vitro*.

4 These results suggest that the impairment of platelet-mediated vasodilation caused by high glucose concentrations is mediated by increased DAG levels and stimulation of PKC and PLA_2 activity.

Keywords: Glucose; signal-transduction; platelet; vasodilation; diabetes

Abbreviations: ADP, adenosine diphosphate; DAG, diacyglycerol; DEDA, dimethyleicosadienoic acid; EDNO, endothelium-derived nitric oxide; OAG, 1-oleoyl-2-acetyl-sn-glycerol; PKC, protein kinase C; PLA_2, phospholipase A_2; PMA, phorbol 12-myristate 13-acetate

Introduction

Activated normal platelets produce vasodilation *via* release of platelet-derived adenosine diphosphate (ADP), which in turn stimulates the release of endothelium-derived nitric oxide (EDNO) (Föstermann *et al*., 1988; Oskarsson & Hofmeyer, 1996). EDNO causes vascular smooth muscle relaxation and inhibits platelet aggregation and excessive thrombus formation (Moncada *et al*., 1991; Kaul *et al*., 1995). Recent reports suggest that platelets from patients with diabetes mellitus lack the ability to produce EDNO-dependent vasodilation (Oskarsson & Hofmeyer, 1996; 1997). This platelet defect can be reproduced *in vitro* by exposure of normal human platelets to high glucose concentrations, in a time and concentration dependent manner (Oskarsson & Hofmeyer, 1996). This glucose-induced platelet defect appears to involve activation of the cyclo-oxygenase pathway, including thromboxane synthase (Oskarsson *et al*., 1997). However, it remains unknown how exposure of platelets to high concentrations of glucose *in vivo* or *in vitro*, leads to increased activity of these enzymes.

Previous studies indicate that high glucose concentrations mediate some of their adverse biologic effects *via* the polyol pathway (Hawthorne *et al*., 1989; Cohen, 1986; Llewelyn *et al*., 1986; Tesfamariam *et al*., 1992; 1993; Greene, *et al*., 1987). Furthermore, high glucose increases intracellular diacylglycerol (DAG) levels (Ayo *et al*., 1991; Craven & Derubertis, 1990; Xia *et al*., 1994), upregulates protein kinase C (PKC) activity (Ayo *et al*., 1991; Craven *et al*., 1990; Xia *et al*., 1994; Craven & Derubertis, 1990; Tesfamariam *et al*., 1991; Lee *et al*., 1989) and can lead to increased arachidonic acid release *via* PKC-mediated increase in phospholipase A_2 activity (Williams & Schrier, 1993; Craven *et al*., 1988; Xia *et al*., 1995), which in turn increases activity of cyclo-oxygenase. In this study we explore the possible role of these metabolic pathways in mediating the inability of diabetic and hyperglycaemia-induced platelets to produce vasodilation.

Methods

Diabetic patients and normal subjects

After receipt of informed consent, blood samples (90 ml) were collected from healthy volunteers (both genders, age 18–55) and patients with the diagnosis of diabetes mellitus (type I or II) for at least one year (both genders, age 18–55) and platelets were isolated and washed as described previously (Oskarsson & Hofmeyer, 1996). Exclusion criteria for study subjects included hypercholesterolaemia (total cholesterol >5.7 mM [220 mg dl^{-1}]), cigarette smoking, use of medications that

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affect platelet function and diagnosis of kidney disease or other disorders that affect platelet function. The study protocol was reviewed and approved by the Human Investigations Committee, University of Iowa.

Preparation of arteries

Normal male New Zealand white rabbits (weighing 2.5–3.0 kg) were given heparin (150 U kg^{-1} i.v.) and sacrificed by an overdose of sodium pentobarbital (50 mg kg^{-1} i.v.). Common carotid arteries were excised and prepared as previously described (Oskarsson & Hofmeyer, 1996; 1997). Arterial segments were imaged by a video camera, which projected vessel images onto a monitor that was coupled to a digital image acquisition system (Oskarsson & Hofmeyer, 1996; 1997). Vessel responses to agonists or activated platelets were allowed to reach a stable plateau (3–4 min), then digitally acquired, and archived for subsequent analysis with a quantitative edge detection program (NIH Image, NIH, Bethesda, MD, U.S.A.). Vascular responses are reported as per cent change in vessel diameter.

Platelet-mediated vasodilation

As described before (Oskarsson & Hofmeyer, 1996; 1997), arterial segments were perfused with Krebs buffer at a constant flow rate of 1.0 ml min^{-1} at an intraluminal distending pressure of 60 mmHg. To ensure normal constrictor and dilator responses, repeated doses of abluminally suprafused phenylephrine (1 μM) and intraluminally perfused acetylcholine (1 μM) were applied until reproducible results were obtained.

After achieving stable resting baseline, an image was acquired for baseline quiescent arterial diameter. The arterial segments were then preconstricted *via* abluminal administration of phenylephrine (10 μM). Upon reaching a stable preconstricted diameter, the intraluminal perfusate was switched from aerated Krebs buffer through both perfusion arms, to thrombin (0.2 Units ml^{-1}) in Krebs buffer through one perfusion arm, and normal Tyrode's buffer (containing 6.6 mM [118 mg dl^{-1}] D-glucose) through the other. The artery was allowed to equilibrate while being perfused with this solution for 4 min, after which a reference diameter image of the preconstricted vessel was acquired for use in succeeding analysis of change in vessel diameter. When platelets incubated in high glucose were going to be tested, Tyrode's buffer containing 17 mM [300 mg dl^{-1}] D-glucose was preperfused along with thrombin and Krebs buffer to obtain baseline diameter, in order to correct for any possible direct effect of the high glucose concentration on vasodilation. Subsequently, the arm perfused with Tyrode's (normal or high glucose) alone was changed to platelets suspended in Tyrode's buffer (normal or high glucose). The thrombin and platelet perfusates mix 1 : 1 at the Y-connector, giving a final platelet concentration of 1×10^8 platelets ml^{-1} and a final thrombin concentration of 0.1 Units ml^{-1}. Each dose of platelets was perfused for 3–4 min, at which time the artery had reached a stable plateau in response to the platelets. An image of the artery was acquired and the diameter compared to the quiescent and preconstricted baseline images.

The effect of aldose reductase inhibitors

After isolation, platelets were incubated in normal or high glucose Tyrode's buffer, as indicated, with or without tolrestat (50 μM) or sorbinil (400 μM), gifts from Ayerst Laboratories (New York, NY, U.S.A.) and Pfizer, Inc. (Groton, CT, USA.), respectively, for 4 h. Baseline diameter before perfusion of activated platelets was obtained with normal or high glucose Tyrode's buffer, with or without the drug, as indicated, to correct for any intrinsic effects of the drugs or glucose on the arterial diameter. In the concentrations used in this study, tolrestat or sorbinil had no direct affect on the vessel diameter. Subsequently the platelets, in the appropriate Tyrode's solutions with or without the drugs, were activated with thrombin and perfused as described above and the change in vessel diameter analysed.

The effect of PKC and PLA_2 inhibitors

A similar procedure was used to test the effect of PKC and PLA_2 inhibition on glucose-induced inhibition of platelet mediated vasodilation. Platelets were incubated in Tyrode's buffer containing either low or high glucose for 4 h, with or without the PKC-inhibitor calphostin C (50 nM) or the PLA_2 inhibitors manoalide (50 μM) or dimethyleicosadienoic acid (DEDA) (50 μM). Similarly, platelets isolated from patients with diabetes mellitus were treated for 2 h with or without calphostin C or manoalide. Subsequently these different groups of platelets were thrombin (0.1 U ml^{-1}) activated and perfused through a phenylephrine (10 μM) preconstricted normal rabbit carotid artery and the change in vessel diameter measured. Calphostin C or manoalide in Tyrode's buffer alone did not elicit vessel diameter changes at the concentration used.

PKC priming of platelets

Platelets were incubated with the desired concentration of PKC agonist (phorbol 12-myristate 13-acetate (PMA), 80 nM or 1-oleoyl-2-acetyl-sn-glycerol (OAG), 5 μM) for 20 min. The platelets were then washed three times to remove the agonist from the solution and were resuspended in Tyrode's buffer. This procedure usually resulted in some loss of platelets, since some platelet activation and aggregation occurred, but only to a limited extent. Platelet counts were performed after the PKC priming and the final concentration adjusted accordingly. These platelets were then examined for their ability to induce vasodilation as described above.

Biochemical determinations

Sorbitol and fructose content of human platelets was analysed by gas liquid chromatography following derivatization as described previously (Yorek *et al*., 1987). Diacylglycerol content was determined by the method described by Preiss *et al*. (1986) and the amount of DAG was calculated from a standard curve using pure *sn*-1,2-diolein and reported as pmol 10^8/platelets.

Data analysis

All data are presented as mean value $\pm$ s.e.mean. The changes in vessel diameter are expressed as per cent change in diameter (a negative value representing vasoconstriction). The number of experiments (n) for each data point refers to the number of platelet donors. Statistical analysis was performed with unpaired t-test and with one or two way analysis of variance (ANOVA) with correction for repeated measures or by pairwise multiple comparisons (Bonferroni's method) where appropriate. A P value <0.05 was considered significant.

Results

The polyol pathway

We hypothesized that high concentration of glucose causes impairment of platelet-mediated vasodilation by metabolism of glucose *via* the polyol pathway. The aldose-reductase inhibitor tolrestat attenuated to a very modest degree the glucose-induced impairment of the ability of platelets to cause vasorelaxation: 32 ± 4 vs -3 ± 4 vs $7\pm5\%$ for normal platelets, high glucose treated platelets and tolrestat- and high glucose-treated platelets, respectively ($n=6$, $P<0.05$). Similar results were obtained with sorbinil (results not shown). When intracellular platelet sorbitol level was measured directly in normal human platelets after a 4 h incubation in Tyrode's buffer with either normal or high glucose, glucose levels were 259 ± 25 vs 591 ± 61 nmol 10^8 platelets^{-1}, respectively ($n=5$, $P<0.01$), while sorbitol and fructose, the downstream glucose metabolites of the polyol pathway, could not be detected under either set of conditions.

Glucose-mediated increase in DAG

In this study we show that *in vitro* incubation of normal human platelets in high glucose causes a significant increase in platelet DAG levels, which is evident after 30 min (Figure 1).

The role of protein kinase-C (PKC)

DAG and OAG are known activators of PKC (Go *et al*., 1987). Data in Figure 2 show that normal human platelets incubated with the DAG analogue, (OAG), in order to mimic the effect of increased intracellular DAG, lost their ability to cause vasodilation.

Next we tested whether enhanced PKC activity plays a role in the signalling pathway leading to impaired ability of diabetic platelets to cause vasodilation. We found that platelets from patients with diabetes mellitus that were treated with the PKC-inhibitor calphostin-C produced normal vasodilation, while untreated platelets from the same patients lacked the ability to cause vasorelaxation (Figure 3A). Similarly, while normal platelets incubated in high glucose lost their ability to cause vasorelaxation, co-incubation with calphostin-C prevented the glucose-mediated impairment of platelet-mediated vasodilation (Figure 3B). Calphostin-C did not affect the ability of normal platelets to mediate vasodilation: 35 ± 3 vs $37\pm4\%$ increase in vessel diameter, with or without the inhibitor ($n=5$), respectively. Similar results were obtained with the PKC-inhibitor H7 (50 μM) (results not shown).

To test whether activation of PKC activity can mimic the high glucose mediated effect, normal platelets were 'primed' by a 20 min incubation in Tyrode's buffer containing PMA (80 nM). These platelets completely lost their ability to produce vasorelaxation (Figure 4).

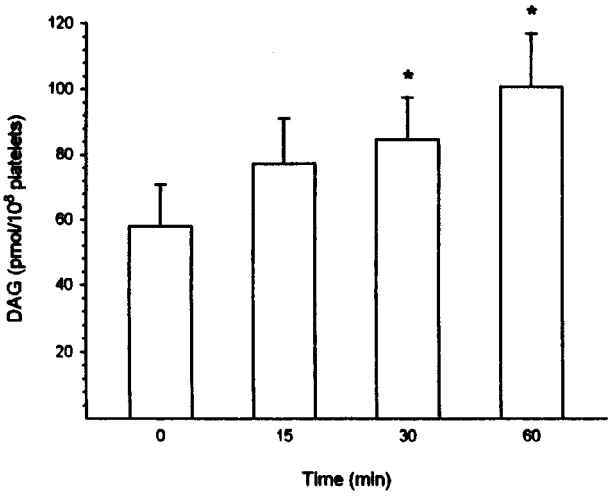

Figure 1 Platelets isolated from healthy donors ($n=6$) were incubated in Tyrode's buffer containing 17 mM (300 mg dl^{-1}) glucose for 15, 30 and 60 min and DAG levels measured. The amount of DAG is expressed as pmol 10^8 platelets^{-1}. *$P<0.01$.

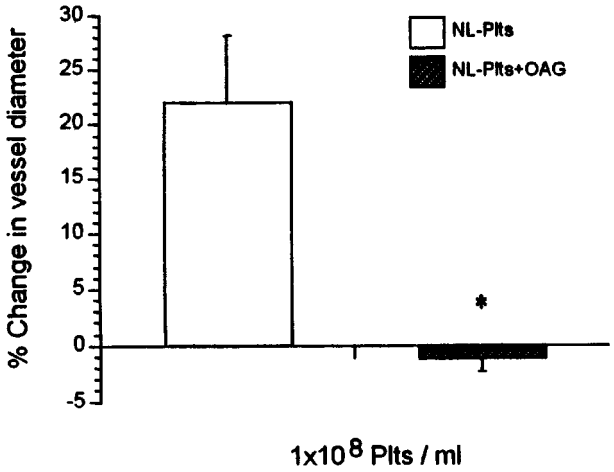

Figure 2 Platelets from healthy donors ($n=6$) were isolated and treated with or without the diacylglycerol (DAG) analogue 1-oleoyl-2-acetyl-glycerol (OAG, 2.5 μg ml^{-1}) for 20 min. After a washout period, treated and untreated platelets were thrombin activated (0.1 U ml^{-1}) and perfused through a phenylephrine (10 μM) preconstricted rabbit carotid artery, and the change in vessel diameter measured. *$P<0.01$.

The role of phospholipase A_2

Enhanced PKC activity can lead to increased activity of the cyclooxygenase pathway *via* activation of phospholipase A_2 (PLA_2) (Williams & Schrier 1993; Craven *et al*., 1988; Nemenoff *et al*., 1993). Data in Table 1 show that the PLA_2 inhibitors manoalide and DEDA significantly improved the ability of high glucose incubated platelets to produce vasodilation. In addition we found that the PLA_2 inhibitor manoalide improved the ability of platelets isolated from patients with diabetes mellitus to cause vasorelaxation: 0 ± 2 vs $17\pm5\%$ increase in vessel diameter for untreated vs treated diabetic platelets, respectively ($n=6$, $P<0.05$).

Discussion

Metabolism of glucose *via* the polyol pathway may play a role in the pathophysiology of various diabetes-related complications (Greene *et al*., 1987), including endothelial dysfunction (Hawthorne *et al*., 1989; Tesfamariam *et al*., 1992; Greene *et al*., 1987). Increased metabolism of glucose through this pathway increases the intracellular ratio of NADH/NAD^+, which may influence the activity of various enzymes, including prostaglandin hydroperoxidases in the cyclo-oxygenase pathway for which NADH can serve as a cofactor (Kukreja *et al*., 1986). This could, in part, explain the increased production of vasoconstrictive prostanoids in diabetic vessels (Tesfamariam *et al*., 1990; 1993) and increased thromboxane A_2 synthesis by

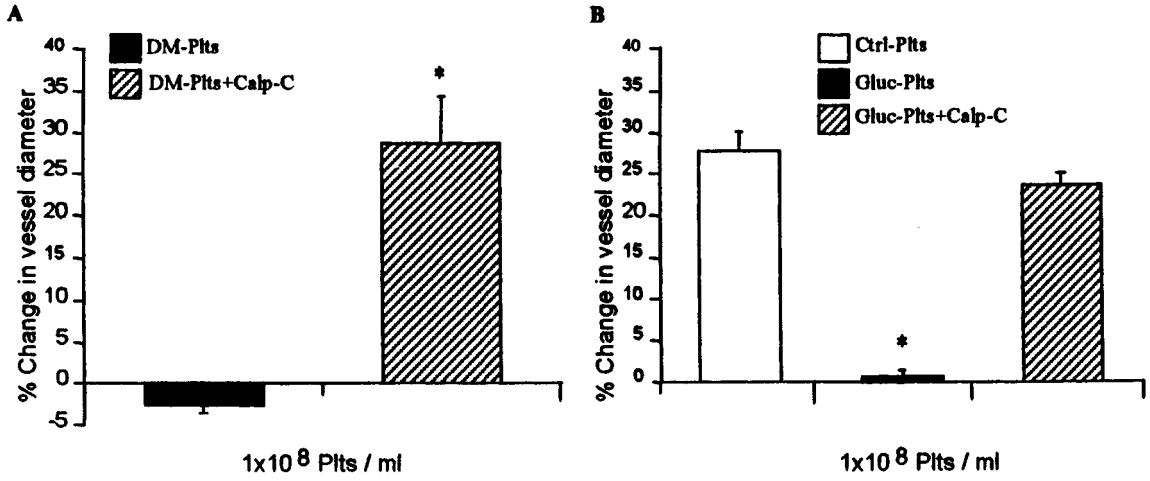

Figure 3 (A) Platelets were isolated from patients with diabetes mellitus ($n=6$). Platelets were incubated in Tyrode's buffer for 2 h with or without calphostin-C (50 nM). Subsequently the platelets were thrombin (0.1 U ml^{-1}) activated and perfused through a phenylephrine (10 μM) preconstricted normal rabbit carotid artery, and the change in vessel diameter measured. *$P<0.01$. (B) Platelets isolated from healthy donors ($n=6$) were incubated in Tyrode's buffer containing either 6.6 mM (118 mg dl^{-1}) [NL Plts] or 17 mM (300 mg dl^{-1}) [Glucose Plts] glucose for 4 h. For the last 2 h the PKC-inhibitor calphostin-C (50 nM) was added to some of the high glucose treated platelets. Subsequently the three groups of platelets were thrombin (0.1 U ml^{-1}) activated and perfused through a phenylephrine (10 μM) preconstricted normal rabbit carotid artery, and the change in vessel diameter measured. *$P<0.01$ vs NL-Plts and Gluc-Plts + Calp-C.

Table 1 Normal platelets were incubated in buffer containing 6.6 or 17 mM glucose with or without the PLA2-inhibitors manoalide (1 μM) or DEDA (50 μM) for 4 h. Afterwards, the effect on vasodilation was determined

	Normal glucose buffer	*High glucose buffer*	*High glucose buffer and drug*
% Vasodilation (6)	25.9 ± 2.8	1.9 ± 1.7*	20.6 ± 3.8 (Manoalide)
% Vasodilation (4)	35.5 ± 6.7	0.9 ± 1.7*	27.5 ± 6.4 (DEDA)

Results are mean ± s.e.mean; ( ) = number of platelet donors. *$P<0.01$.

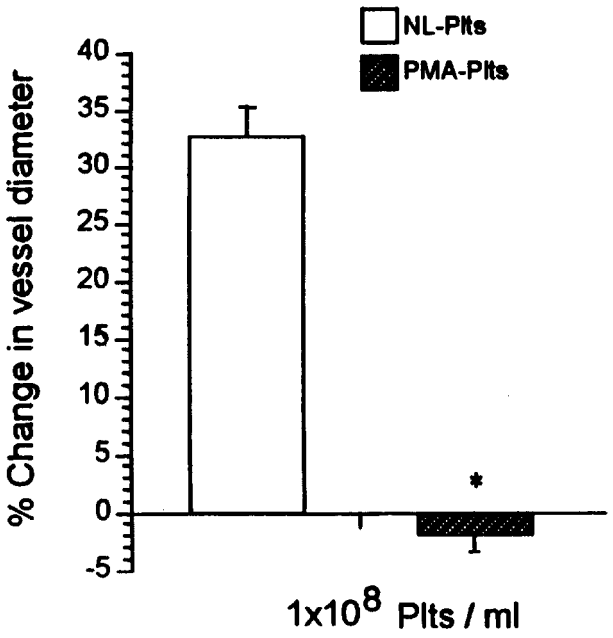

Figure 4 Platelets from healthy donors ($n=8$) were isolated separated into two groups and treated with or without phorbol 12-myristate 13-acetate (PMA) (80 nM) for 20 min. After a washout period, treated and untreated platelets were thrombin (0.1 U ml^{-1}) activated and perfused through a phenylephrine (10 μM) preconstricted rabbit carotid artery, and the change in vessel diameter measured. *$P<0.01$ for PMA-Plts vs NL-Plts.

platelets from patients with diabetes mellitus (Catalano *et al.*, 1990). Therefore, since our previous data indicate that the inability of glucose exposed platelets to cause vasodilation is associated with increased activity of the cyclo-oxygenase pathway (Oskarsson *et al.*, 1997), we proposed that the polyol pathway might play an important role.

To test this hypothesis we treated platelets with two structurally different aldose-reductase inhibitors in an attempt to block the first step of the polyol pathway that converts glucose to sorbitol. We found that both inhibitors caused similar, statistically significant, yet very modest and probably not biologically significant improvement in the ability of glucose treated normal platelets to produce vasodilation. Secondly, we measured sorbitol and fructose levels in platelets after 4 h of incubation in buffer containing either normal (6.6 mM) or high glucose (17 mM). We observed an expected rise in glucose concentration in the high glucose treated platelets, but despite applying a very sensitive assay we could not detect any sorbitol or fructose in either condition. This suggests that human platelets do not have an active polyol pathway, at least under the conditions tested or that sorbitol and fructose are rapidly metabolized by platelets when exposed to a high glucose concentration. Therefore, the modest restoration of the impaired vasodilation by glucose exposed platelets mediated by the aldose-reductase inhibitors is probably a non-specific effect. We conclude that enhanced metabolism of glucose by the polyol pathway in platelets is not likely to be the underlying cause for glucose induced impairment of platelet-mediated vasodilation.

DAG levels have previously been shown to be increased by high glucose conditions *in vitro* (Ayo *et al.*, 1991; Craven *et al.*,

1990), as well as in tissue from diabetic animals (Tesfamariam *et al*., 1993; Xia *et al*., 1994). In this study, we observed for the first time an increase in DAG levels in human platelets after exposure to high glucose levels *in vitro*. The same concentration of glucose also impairs the ability of the platelets to mediate vasodilation. The mechanism responsible for the glucose-induced elevation in intracellular DAG levels is still unclear, but may be both *via* increased *de novo* synthesis (Craven *et al*., 1990), as well as by increased phospholipid turnover (Zawalich & Zawalich, 1988). To test whether the observed glucose-induced increase in DAG levels might be causally related to the glucose-mediated impairment of vasodilation caused by platelets, we used the DAG analogue OAG. We observed that platelets incubated with OAG for 20 min totally lost their ability to cause vasodilation. This strongly supports the hypothesis that the glucose-induced increase in DAG represents a step in the signalling pathway leading to this glucose-induced platelet defect.

Previously, hyperglycaemic conditions have been shown to increase PKC activity (Ayo *et al*., 1991; Craven *et al*., 1990; Xia *et al*., 1994; Craven & Derubertis, 1990; Tesfamariam *et al*., 1991; Lee *et al*., 1989). This may, at least in part, occur *via* a glucose-mediated increase in DAG, a well-known agonist for PKC (Ayo *et al*., 1991; Xia *et al*., 1994; Go *et al*., 1987). Therefore, we proposed that PKC activation might be an important step in mediating the glucose-induced platelet defect. Our studies show that the PKC inhibitors calphostin C and H7 completely prevented any glucose-induced impairment of platelet-mediated vasodilation. Calphostin C also normalized the ability of platelets isolated from diabetic patients to cause vasodilation. Furthermore, priming normal platelets with a low concentration of the phorbol ester PMA, an activator of PKC, caused impairment in platelet-mediated vasorelaxation similar to the effect observed after incubating normal platelets in high glucose or OAG. The data obtained with PKC inhibitors and PMA suggest that the activation of PKC contribute to the diabetes- and glucose-induced defect in platelet-mediated vasodilation.

Previous studies have shown that enhanced activity of the cyclo-oxygenase pathway, including thromboxane-synthase, is involved in the glucose-induced impairment of platelet-mediated vasodilation (Oskarsson & Hofmeyer 1997). One of the most important rate limiting factors for the activity of the cyclo-oxygenase pathway is the availability of its main substrate, arachidonic acid, which in turn is mainly controlled by the activity of phospholipase A_2 (Williams, *et al*., 1993). Since PLA_2 can be activated by PKC (Williams *et al*., 1993; Craven *et al*., 1988; Nemenoff *et al*., 1993), and because evidence provided in this study suggest that PKC activation plays a role in the signalling mechanism leading to an impairment in platelet-mediated vasodilation, PLA_2 is a likely candidate as an intermediate step in the signal transduction pathway. Our studies demonstrate that two different inhibitors of PLA_2 prevented the glucose-induced impairment of platelet-mediated vasorelaxation. Moreover, manoalide normalized the ability of diabetic platelets to cause vasodilation. This result supports the hypothesis of PLA_2 involvement in the glucose-mediated impairment of platelet-mediated vasodilation.

In summary, the results of this study along with recently published data (Oskarsson & Hofmeyer 1997; Oskarsson *et al*., 1997) suggest that high glucose levels cause an increase in platelet DAG that upregulates the activity of PKC, which in turn increases the activity of phospholipase A_2 that causes release of arachidonic acid which leads to increased activity of cyclo-oxygenase and thromboxane synthase in platelets (Oskarsson *et al*., 1997). From a clinical perspective this pathway is of considerable interest since it lends itself to therapeutic interventions with inhibitors both at the level of cyclo-oxygenase and the thromboxane-synthase.

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References

AYO, S.H., RADNIK, R., GARONI, J.A., TROYER, D.A. & KREISBERG, J.I. (1991). High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. *Am. J. Physiol.,* **1,** F571 – F577.

CATALANO, D.G., AVERNA, M., NOTARBARTOLO, A., STRANO, A., CIABATTONI, G. & PATRONO, C. (1990). Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N. Engl. J. Med.,* **322,** 1769 – 1774.

COHEN, M. (1986). Aldose reductase, glomerular metabolism, and diabetic nephropathy. *Metabolism,* **35** (suppl I): 55 – 59.

CRAVEN, P.A., DAVIDSON, C.M. & DERUBERTIS, F.R. (1990). Increase in diacylglycerol mass in isolated glomeruli by glucose from de novo synthesis of glycerolipids. *Diabetes,* **39,** 667 – 674.

CRAVEN, P.A. & DERUBERTIS, F.R. (1990). Protein kinase C is activated in glomeruli from streptozotocin diabetic rats. *J. Clin. Invest.,* **83,** 1667 – 1675.

CRAVEN, P.A., PATTERSON, C.M. & DERUBERTIS, F.R. (1988). Role of enhanced arachidonate availability through phospholipase A2 pathway in metabolism of increased prostaglandin synthesis by glomeruli from diabetic rats. *Diabetes,* **37,** 429 – 435.

FÖSTERMANN, U., MÜGGE, A., BODE, S.M. & FRÖLICH, J.C. (1988). Response of human coronary arteries to aggregating platelets: Importance of endothelium-derived relaxing factor and prostanoids. *Circ. Res.,* **63,** 306 – 312.

GO, M., SEKIGUCHI, K., NOMURA, H., KIKAWA, N. & NISHISUKA, Y. (1987). Further studies on the specificity of diacylgycerol for protein kinase C activation. *Biochem. Biophys. Res. Commun.,* **144,** 598 – 605.

GREENE, D.A., LATTIMER, S.A. & SIMA, A.A.D. (1987). Sorbitol, phospho-inositides and sodium-potasium-ATPase in the pathogenesis of diabetic complications. *N. Engl. J. Med.,* **316,** 599 – 606.

HAWTHORNE, G.C., BARTLETT, K., HETHERINGTON, C.S. & ALDERTI, K.G.M.M. (1989). The effect of high glucose on polyol pathway activity and myo-inositol metabolism in cultured human endothelial cells. *Diabetologia,* **32,** 163 – 166.

KAUL, S., NAQVI, T.Z., FISHBEIN, M.C., CERCEK, B., FORRESTER, J.S., MOLLOY, M.D., BADIMON, J.J., HUTSELL, T.C. & SHAH, P.K. (1995). Profound inhibition of arterial thrombosis by a novel nitric oxide donor. *J. Am. Coll. Cardiol.* Issue (Feb) 34A, (Abstract).

KUKREJA, R.C., KONTOS, H.A., HESS, M.Z. & ELLIS, E.F. (1986). PGH synthase and lipoxygenase generate superoxide in the presence of NADH and NADPH. *Circ. Res.,* **59,** 612 – 619.

LEE, T.S., MACGREGOR, L.C., FLUHARTY, S.J. & KING, G.L. (1989). Differential regulation of protein kinase C and (Na,K)-adenosine triphosphatase activities by elevated glucose levels in retinal capillary endothelial cells. *J. Clin. Invest.,* **83,** 90 – 94.

LLEWELYN, J.G., SIMPSON, C.M.F., THOMAS, P.K., KING, R.H.M. & HAWTHORNE, J.N. (1986). Changes in sorbitol, myo-inositol and lipid inositol in dorsal root and sympathetic ganglia from streptozotocin-diabetic rats. *Diabetologia,* **29,** 876 – 881.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.,* **43,** 109 – 142.

NEMENOFF, R.A., WINIZ, S., QIAN, N.X., PUTTEN, V.V., JOHNSON, G.L. & HEASLEY, L.E. (1993). Phosphorylation and activation of a high molecular weight form of phospholipase A_2 by p42 microtubule-associated protein 2 kinase and protein kinase C. *J. Biol. Chem.,* **268,** 1960 – 1964.

OSKARSSON, H.J. & HOFMEYER, T.G. (1996). Platelet-mediated endothelium-dependent vasodilation is impaired by platelets from patients with diabetes mellitus. *J. Am. Coll. Cardiol.,* **27,** 1464 – 1470.

OSKARSSON, H.J. & HOFMEYER, T.G. (1997). Diabetic human platelets release a substance which inhibits platelet-mediated vasodilation. *Am. J. Phys.,* **273,** H371 – H379.

OSKARSSON, H.J., HOFMEYER, T.G. & KNAPP, H.R. (1997). Malondialdehyde inhibits platelet-mediated vasodilation by interfering with platelet-derived ADP. *JACC,* **29** (Suppl A): 304A.

PREISS, J., LOOMIS, C.R., BISHOP, W.R., STEIN, R., NIEDEL, J.E. & BELL, R.M. (1986). Quantitative measurement of *sn*-1,2-diacylglcerols present in platelets, hepatocytes, and ras- and sis-transformed normal rat kidney cells. *J. Biol. Chem.,* **261,** 8547 – 8600.

TESFAMARIAM, B., BROWN, M.L. & COHEN, R.A. (1991). Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase. *C. J. Clin. Invest.,* **87,** 1643 – 1648.

TESFAMARIAM, B., BROWN, M.L. & COHEN, R.A. (1992). Aldose reductase and myo-inositol in endothelial cell dysfunction caused by elevated glucose. *J. Pharmacol. Exp. Ther.,* **263,** 153 – 157.

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., PALACINO, J.J., WEISBROD, R.M. & COHEN, R.A. (1993). Aldose reductase inhibition restores endothelial cell function in diabetic rabbit aorta. *J. Cardiovasc. Pharmacol.,* **21,** 205 – 211.

WILLIAMS, B. & SCHRIER, R.W. (1993). Glucose-induced protein kinase C activity regulates arachidonic acid release and eicosanoid production by cultured glomerular mesangial cells. *J. Clin. Invest.,* **92,** 2889 – 2896.

XIA, P., INOGUCHI, T., KERN, T.S., ENGERMAN, R.L., OATES, P.J. & KING, G.L. (1994). Characterization of the mechanism for the chromic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. *Diabetes,* **43,** 1122 – 1129.

XIA, P., KRAMER, R.M. & KING, G.L. (1995). Identification of the mechanism for the inhibition of Na+, K+-adenosine triphosphatase by hyperglycemia involving activation of protein kinase C and cytosolic phospholipase A2. *J. Clin. Invest.,* **96,** 733 – 740.

YOREK, M.A., DUNLAP, J.A. & GINSBERG, B.H. (1987). myo-Inositol metabolism in 41A3 neuroblastoma cells: effects of high glucose and sorbitol levels. *J. Neurochem.,* **B,** 53 – 61.

ZAWALICH, W.S. & ZAWALICH, K.C. (1988). Phosphoinositide hydrolysis and insulin release from isolated perfused rat islets: studies with glucose. *Diabetes,* **37,** 1294 – 1300.

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