Differential changes of adrenoceptor- and muscarinic receptor-linked prostacyclin synthesis by the aorta and urinary bladder of the diabetic rat

¹J.Y. Jeremy, C.S. Thompson & D.P. Mikhailidis

Department of Chemical Pathology and Human Metabolism, Royal Free Hospital and School of Medicine, University of London, Pond Street, London NW3 2QG

1 The effect of experimental diabetes mellitus (DM; hyperglycaemic, non-ketototic; 2 months duration) in the rat on receptor-linked prostacyclin (PGI₂) synthesis (measured as 6-oxo-PGF_{1α} by radio-immunoassay) was studied in the aorta and urinary bladder using adrenaline, angiotensin II (AII) and acetylcholine (ACh). Signal transduction systems were studied via stimulation of PGI₂ synthesis with phorbol ester dibutyrate (PDBU; a protein kinase C activator [PKC]), Ca²⁺ ionophore A23187 (A23187) and thapsigargin (both elevate intracellular Ca²⁺, activating phospholipase A₂ [PLA₂]) and arachidonate (AA; substrate for PGI₂ synthesis).

2 In response to adrenaline, AII and phorbol ester, aortic PGI₂ release was markedly reduced (all > 75%) in diabetic rats compared to controls. EC₅₀s of the dose-response curves for adrenaline, AII and PDBU were also markedly increased in aortae from DM rats compared to controls. Although there was decreased output of PGI₂ in response to A23187 by aortae from diabetic rats compared to controls, there was no difference in the EC₅₀s (mean ± s.e.mean: diabetic, $2.7 \pm 0.2 \times 10^{-6}$ M; controls $2 \pm 0.18 \times 10^{-6}$ M). There were no differences in PGI₂ release (or in the EC₅₀s) in response to thapsigargin or AA between aortae from diabetic and control rats.

3 In the urinary bladder, there was a marked increase in PGI₂ output in response to ACh and a marked decrease in EC₅₀s for the ACh-PGI₂ dose-response curves in diabetic rats (EC₅₀ = $5.8 \pm 0.32 \times 10^{-7}$ M) compared to controls (EC₅₀ = $2.2 \pm 0.15 \times 10^{-6}$ M). Although there was an increase in PGI₂ output in the urinary bladders from diabetic rats in response to A23187, there were no differences in the EC₅₀s (control, $1.8 \pm 0.2 \times 10^{-6}$ M; diabetic, $1.1 \pm 0.15 \times 10^{-6}$ M). In the urinary bladders, there were no differences in PGI₂ output (or the EC₅₀s) in response to PDBU, thapsigargin or AA between diabetic or control rats.

4 These data indicate that: (i) reduced PGI₂ synthesis coupled to adrenoceptors and AII receptors in the aortae of diabetic rats may be due to diminished PKC activity and not to changes in receptor density and/or affinity, Ca²⁺ stores, PLA₂, cyclo-oxygenase or PGI₂ synthase; (ii) the diametrically opposite effect of DM on ACh-stimulated PGI₂ synthesis is not due to an increase in PKC activity, but possibly to an increase in muscarine receptor number and/or affinity; (iii) changes in receptor-linked PGI₂ synthesis are not ubiquitous in experimental DM and may be organ-specific.

Keywords: Prostacyclin; diabetes mellitus; rat aorta; rat urinary bladder; adrenoceptors; muscarinic receptors

Introduction

Patients with diabetes mellitus (DM) are at greater risk than the healthy population of developing cardiovascular disease (CVD; Banga & Sixma, 1986; Mikhailidis *et al.*, 1988). Of the many aetiological factors associating CVD with DM, a marked attenuation of prostacyclin (PGI₂) synthesis by blood vessels from diabetic laboratory animals have been reported (Harrison *et al.*, 1978; Rogers *et al.*, 1981; Jeremy *et al.*, 1987a). Apart from being a vasodilator and inhibitor of platelet aggregation (Moncada & Vane, 1979), PGI₂ modulates other vascular functions including cell proliferation (Huttner *et al.*, 1977; Marui *et al.*, 1990; Pomerantz & Hajjar, 1989) and metabolism of cholesterol (Hajjar *et al.*, 1982). In turn, disruption of these processes have all been implicated in the aetiology of CVD, in particular, atherogenesis and thrombosis (Jeremy & Mikhailidis, 1990).

The precise mechanisms that determine reduced vascular PGI₂ synthesis are not clearly defined, although a reduction in substrate (arachidonate) levels in blood vessels has been suggested (Holman *et al.*, 1983; Jeremy *et al.*, 1987a; Dang *et al.*, 1988). It has also been established that vascular PGI₂ synthesis is stimulated by vasoactive agonists (e.g. noradrenaline, histamine, acetylcholine, angiotensin II;

Jeremy *et al.*, 1988a) an effect which in turn is mediated by G proteins, protein kinase C (PKC) and inositol trisphosphate (IP₃; Griendling *et al.*, 1986; Hallam *et al.*, 1988; Jeremy *et al.*, 1988a). The net result of activation of these systems is transmembrane Ca²⁺ influx and an increase in intracellular Ca²⁺ ([Ca²⁺]_i). Ca²⁺ then activates phospholipase A₂ (PLA₂) which in turn liberates PGI₂ substrate, arachidonic acid (AA), from phospholipid stores (Irvine, 1982). In this context, experimental DM in the rat is associated with altered receptor-linked functions in vascular tissue (Tomlinson *et al.*, 1992).

Thus, in order to elucidate further the mechanisms that determine diminished vascular PGI₂ synthesis, the effects of a range of stimulators that act at different sites in the receptor activation-PGI₂ synthesis pathway were studied on PGI₂ synthesis by the streptozotocin-induced diabetic rat: adrenaline (mediated by heterogeneous α-adrenoceptors; Jeremy *et al.*, 1985a) and angiotensin II (AII; Hassid & Williams, 1983; Stevens *et al.*, 1992), phorbol ester dibutyrate (PDBU; a PKC activator: Nishizuka, 1984; Jeremy & Dandona, 1986), thapsigargin (elevates intracellular Ca²⁺; Thastrup *et al.*, 1990; Levine & Watanabe, 1991), Ca²⁺ ionophore A23187 (creates artificial Ca²⁺ channels; Reed & Lardy, 1972) and arachidonic acid (AA; substrate for PGI₂ synthesis). In order to examine whether other non-vascular smooth muscle tissues

¹ Author for correspondence.

are affected in a similar or disparate manner to the aorta, PGI₂ synthesis by urinary bladder was investigated. It is also notable that the urinary bladder of the diabetic rat releases more PGI₂ than controls (Jeremy *et al.*, 1986a). Bladder PGI₂ synthesis was stimulated with acetylcholine (PGI₂ synthesis in the urinary bladder is mediated by muscarinic receptors; Jeremy *et al.*, 1986b) as well as PDBU, thapsigargin, A23187 and AA.

Methods

Induction of diabetes

All experiments were carried out on male Sprague Dawley rats of initial body weight of 250 g. Non-ketonic, hyperglycaemic diabetes was induced by injecting streptozotocin (65 mg kg⁻¹ body wt., i.v.). These rats developed glycosuria but not ketonuria or haematuria. Diabetic rats were fed either *ad libitum* with Diet 41B (Grain Harvester, Kent) and allowed free access to water. The study also included seven age-matched controls. Urine was monitored over the duration of diabetes for glucose, ketone bodies and proteins with Multistix (Ames Division, Miles Laboratories Ltd., Stoke Poges, Bucks). After 8 weeks, rats were anaesthetized with pentobarbitone (90 mg kg⁻¹ intraperitoneally; Sagatal: May and Baker Ltd., Dagenham, Essex) and blood was collected by cardiac puncture for measurement of blood glucose. Thoracic aortae and urinary bladders were rapidly excised and placed in Dulbecco's Minimum Essential Medium (MEM), pregassed with 95% O₂/5% CO₂.

Preparation and incubation of aortic and urinary bladder tissue

After removal of adventitia and fatty tissue, the aortae were cut into 2 mm rings with a scalpel blade on a Teflon block. The bladders were cut longitudinally into four strips and each strip into six further segments. Tissues were then pooled and randomised in Dulbecco's Minimum Essential Medium (MEM: pregassed with 95% O₂/5% CO₂) and incubated for 6 h in MEM, at 37°C, with changes of medium every 30 min, to allow prostanoïd release elicited by preparative handling to subside (Jeremy *et al.*, 1985a, 1987a). Following pre-incubation, tissues were placed in 1 ml MEM (per tube: 2 aortic rings, or 2 bladder segments, in duplicate [from each animal]). PGI₂ synthesis was then elicited in aortic tissue by the addition of varying concentrations of adrenaline, AII, PDBU, thapsigargin, AA and A23187 and in the bladder with ACh, PDBU, thapsigargin, AA and A23187. Tissues were then further incubated for 1 h at 37°C and aliquots of supernatant taken for estimation of 6-oxo-PGF_{1α} (the stable spontaneous hydrolysate of PGI₂; Moncada & Vane, 1979) concentrations by radioimmunoassay as previously described (Jeremy *et al.*, 1985a; 1987a).

Materials and drugs

Adrenaline bitartrate, angiotensin II, acetylcholine chloride, arachidonic acid (calcium salt; 99.9% pure), phorbol ester dibutyrate, Ca²⁺ ionophore A23187, Dulbecco's Minimum Essential Medium and streptozotocin were purchased from Sigma Chemical Co. (Poole, Dorset). Thapsigargin was purchased from Novabiochem UK (Nottingham). Antisera against 6-oxo-PGF_{1α} of high serological specificity was purchased from Capell Laboratories (West Chester, PA, U.S.A.). ³H-ligands were purchased from New England Nuclear (Dreieich, Germany) and unlabelled ligands from UpJohn Co. (Kalamazoo, MI, U.S.A.).

Statistical analysis

Values are expressed as mean ± s.d. of mean. A one-way analysis of variance was performed on the data and the

Figure 1 Effect of adrenaline on prostacyclin (PGI₂, as 6-oxo-PGF_{1α}) release by the aortae of diabetic (▲) and age-matched control rats (●). Each point is the mean ± s.d., *n* = 7. **P* < 0.01. EC₅₀s: control, $5.8 \pm 0.4 \times 10^{-7}$ M; diabetics, $4.1 \pm 0.3 \times 10^{-6}$ M.

Figure 2 Effect of angiotensin II on prostacyclin (PGI₂, as 6-oxo-PGF_{1α}) release by the aortae of diabetic (▲) and age-matched control rats (●). Each point is the mean ± s.d., *n* = 7. **P* < 0.01. EC₅₀s: control, $5.4 \pm 0.35 \times 10^{-7}$ M; diabetics, $4 \pm 0.28 \times 10^{-6}$ M.

significance determined by Student's *t* test. *P* values less than 0.001 were taken to be significant. Significant differences between responses are expressed graphically with an asterisk. EC₅₀s are expressed as mean ± s.e.mean, *n* = 7.

Results

Body weights (median [range], *n* = 35) of the animal groups at the time they were killed were: controls, 475 g (449–516); diabetic, 239 g (220–280). At death, blood glucose concentrations (mmol l⁻¹; median [range]) were: controls, 8.4 (6.8–10.3); diabetic, 30.3 (28.2–32.7).

Aortic PGI₂ release (expressed as pg 6-oxo-PGF_{1α} liberated mg⁻¹ tissue min⁻¹) was significantly reduced in diabetic rats compared to controls in response to adrenaline (Figure 1), AII (Figure 2) and PDBU (Figure 3). EC₅₀s of the dose-response curves for adrenaline, AII and PDBU were also

Figure 3 Effect of phorbol ester dibutyrate on prostacyclin (PGI₂ as 6-oxo-PGF_{1α}) release by the aortae of diabetic (▲) and age-matched control rats (●). Each point is the mean $\pm$ s.d., $n = 7$. * $P < 0.01$. EC₅₀s: control, $1.9 \pm 0.05 \times 10^{-8}$ M; diabetics, $1.4 \pm 0.09 \times 10^{-7}$ M.

Figure 4 Effect of A23187 on prostacyclin (PGI₂ as 6-oxo-PGF_{1α}) release by the aortae of diabetic (▲) and age-matched control rats (●). Each point is the mean $\pm$ s.d., $n = 7$. * $P < 0.01$.

markedly increased in aortae from diabetic rats compared to controls (see figure legends for values). Although there was decreased output of PGI₂ in response to A23187 by aortae from diabetic rats compared to controls (Figure 4), there was no marked difference in the EC₅₀s (diabetic, $2.7 \pm 0.2 \times 10^{-6}$ M; controls, $2 \pm 0.18 \times 10^{-6}$ M). There were no differences in PGI₂ release in response to thapsigargin between diabetic and control aortae (Figure 5) and a marginal increase in the EC₅₀s (control, $4.2 \pm 0.38 \times 10^{-7}$ M; diabetics, $6.2 \pm 0.48 \times 10^{-7}$ M). There were no differences in PGI₂ release in response to AA in aortae from control (maximal output, $118 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $3.4 \pm 0.22 \times 10^{-5}$ M) or diabetic rats (maximal output, $116 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $3.6 \pm 0.18 \times 10^{-5}$ M).

In contrast to the aorta there was a marked increase in PGI₂ release in response to ACh in the urinary bladder of diabetic rats compared to controls (Figure 6) and a marked decrease in EC₅₀s (diabetic, $5.8 \pm 0.32 \times 10^{-7}$ M; controls, $2.2 \pm 0.15 \times 10^{-6}$ M). Although there was an increase in PGI₂

output in the urinary bladders from diabetic rats in response to A23187 (Figure 7), there were no differences in the EC₅₀s (control, $1.8 \pm 0.2 \times 10^{-6}$ M; diabetic, $1.1 \pm 0.15 \times 10^{-6}$ M). There were no differences in PGI₂ release in response to PDBU in bladders from control (maximal output, $48 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $1 \pm 0.08 \times 10^{-6}$ M) and diabetic rats (maximal output, $52 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $1 \pm 0.09 \times 10^{-6}$ M). There were also no differences in PGI₂ release in response to thapsigargin in bladders from control (maximal output, $21 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $2 \pm 0.14 \times 10^{-6}$ M) or diabetic rats (maximal output, $23 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $2.1 \pm 0.05 \times 10^{-6}$ M). There were no differences in PGI₂ release in response to AA in bladders from control (maximal output, $62 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $1 \pm 0.1 \times 10^{-5}$ M) or diabetic rats (maximal output, $66 \text{ pg mg}^{-1} \text{ min}^{-1}$; EC₅₀, $8.5 \pm 0.92 \times 10^{-6}$ M).

Figure 5 Effect of thapsigargin on prostacyclin (PGI₂ as 6-oxo-PGF_{1α}) release by the aortae of diabetic (▲) and age-matched control rats (●). Each point is the mean $\pm$ s.d., $n = 7$. * $P < 0.01$.

Figure 6 Effect of acetylcholine on prostacyclin (PGI₂ as 6-oxo-PGF_{1α}) release by the urinary bladder of diabetic (▲) and age-matched control rats (●). Each point is the mean $\pm$ s.d., $n = 7$. * $P < 0.01$.

Figure 7 Effect of A23187 on prostacyclin (PGI₂ as 6-oxo-PGF_{1α}) release by the urinary bladder of diabetic (▲) and age-matched control rats (●). Each point is the mean $\pm$ s.d., $n = 7$. * $P < 0.01$.

Discussion

Although it is well established that PGI₂ synthesis by the rat aorta is diminished in experimental DM (Harrison *et al.*, 1978; Rogers *et al.*, 1981; Jeremy *et al.*, 1987a), the mechanisms that determine this reduction are not clearly defined. In the present study there was a marked reduction in PGI₂ release in aortae from diabetic rats in response to adrenaline, AII and phorbol ester. Since receptor-linked PGI₂ synthesis in the rat aorta is mediated by PKC (Jeremy *et al.*, 1988a) and the reduction in PGI₂ release stimulated by agonists and phorbol ester were almost identical, these data indicate that the defect in receptor-linked PGI₂ synthesis in the aortae of diabetic rats may be due to alterations of PKC activity rather than to changes of affinity and/or number of receptors linked to PGI₂ synthesis (viz. α -adrenoceptor and AII receptors). It should be noted that in the rat aorta, the endothelium contributes negligible amounts of the total PGI₂ generated by this vessel in response to agonists, phorbol ester and ionophores (Jeremy & Dandona, 1989). The present data therefore reflect PGI₂ released by the vascular smooth muscle component of the aorta rather than that generated by the endothelium.

Recent data consolidate the proposal that vascular PKC activity is altered in DM. Lee *et al.* (1989) demonstrated a marked reduction in cytosolic (but an increase in membrane-associated) PKC in aortae of diabetic rats, an effect mimicked *in vitro* by high concentrations of glucose. Under normal conditions, receptor activation by agonists elicits the activation of cytosolic PKC (Iwamoto *et al.*, 1992). Activated PKC phosphorylates effector proteins and also moves into the membrane where it down-regulates receptor activity (Iwamoto *et al.*, 1992). The data of Lee *et al.* (1989) therefore points to a generalized movement of PKC into the plasma membrane of vascular cells in diabetic rats which in turn would serve to diminish receptor-linked responses, including PGI₂ synthesis. A reduction in cytosolic PKC would also account for the diminished response to phorbol ester reported in this study, since phorbol esters elicit responses by activating cytosolic PKC (Nishizuka, 1984; Iwamoto *et al.*, 1992). In terms of other receptor-linked functions, both attenuation and potentiation of receptor-linked contraction of aortae from diabetic rats has been reported (for review see Tomlinson *et al.*, 1992). How PKC relates to these variable alterations of contractility warrants consideration.

With regard to Ca²⁺ mobilization, no differences were found in the output of PGI₂ in response to thapsigargin, between diabetic and control aortae. Although PGI₂ output by diabetic aortae was diminished when stimulated with A23187, this reduction was far less than that seen with

agonists and phorbol ester. Furthermore, the EC₅₀s for A23187 in both the diabetic and control aortae were similar. It has been shown that AA stores in vascular tissue, as well as circulating AA, are reduced in experimental DM (Thomson, 1980; Holman *et al.*, 1983; Dang *et al.*, 1988). This probably accounts for the reduction of PGI₂ release in response to A23187 by aortae from diabetic rats. Since thapsigargin mobilizes intracellular Ca²⁺ (Tharstrup, 1990; Levine & Watanabe, 1991) and A23187 elicits entry of extracellular Ca²⁺ via creation of artificial Ca²⁺ channels (Reed & Lardy, 1972), these data indicate that Ca²⁺ pools and/or PLA₂ activity in the aorta are unaltered by DM in the rat. It is also notable that in response to thapsigargin, the absolute output of PGI₂ was markedly less than that derived from A23187, adrenaline, AII or phorbol ester. This may indicate that intracellular Ca²⁺ elevation liberates AA from distinctly different phospholipid stores from other stimulators.

With AA-stimulated PGI₂ synthesis, no difference in the output of PGI₂ was seen between control and diabetic aortae. This consolidates previous conclusions that there is no alteration of either cyclo-oxygenase or PGI₂ synthase activity in blood vessels from diabetic rats (Jeremy *et al.*, 1987a).

In contrast to the aorta, there was a marked increase in PGI₂ output in response to ACh, as well as a profound decrease in the EC₅₀ for ACh, in urinary bladders from diabetic rats when compared with controls. Although there was a significant increase in PGI₂ output in response to A23187 by bladders from diabetic animals there was no marked differences in the EC₅₀s of A23187 in diabetic and control bladders. There were also no differences in the EC₅₀s or output of PGI₂ in response to phorbol ester, thapsigargin, A23187 or AA. Thus, in the case of the bladder, there appears to be a specific increase in muscarine receptor number/activity linked to PGI₂ but no changes in PKC, Ca²⁺ stores, PLA₂, cyclo-oxygenase or synthase enzymes. These conclusions are in agreement with a recent study in which marked left shifts of the dose-response curves for ACh-stimulated contraction were found in urinary bladders from diabetic rats (Latifpour *et al.*, 1992). The diametrically opposite pattern of receptor-linked PGI₂ synthesis by the urinary bladder and the aorta can be ascribed to the particular stresses imposed on the bladder in experimental DM. In diabetic rats, the urinary bladder is markedly enlarged, a result of chronic distension caused by polydipsia and polyuria (Lincoln *et al.*, 1984). Thus, the up-regulation of muscarinic receptors (and the associated increase in PGI₂ [a contractor of urinary bladder smooth muscle; Mikhailidis *et al.*, 1987]) in the urinary bladder of diabetic rats is probably a specific adaptive event relating to increased micturition rate. It is notable that distension (and attendant hyperplasia) elicits an increase in PGI₂ synthesis in other tissues (e.g. human urinary bladder – Jeremy *et al.*, 1987c, human aorta – Tsang *et al.*, 1987; and portal vein – Ritter *et al.*, 1986).

Changes of PGI₂ synthesis in DM appear to be organ-specific. For example, there are no changes in 'spontaneous' (non-stimulated) PGI₂ synthesis in any region of the gastrointestinal tract (also markedly enlarged due to hyperphagia; Schedl *et al.*, 1982), whereas there is a three-fold increase in PGI₂ synthesis by the splanchnic vasculature and hepatic portal vein of the diabetic rat (Jeremy *et al.*, 1988b). There is also a marked output of PGI₂ from perfused mesenteric vessels from diabetic rats (Fujii *et al.*, 1986). Decreases in PGI₂ have been reported in all regions of the brain and the penis (Jeremy *et al.*, 1985b; 1987b). In contrast, Stevens *et al.* (1992) found no significant differences in PGI₂ release stimulated by AII from perfused lungs from diabetic rats. Taken together, these experimental findings indicate that large arteries appear to be more susceptible to DM than microvasculature. This in turn may be of importance from a clinical point of view since vascular complications (viz. atherosclerosis in large arteries) are the predominant cause of death in DM. Thus, apart from mediating PGI₂ release, PKC also controls other vascular functions including contractility

(Danthaluri & Deth, 1984; Rasmussen *et al.*, 1984) and cell proliferation, the pathognomonic lesion of atherosclerosis (Berridge, 1984). PKC also modulates the generation of vascular nitric oxide (NO; Lewis & Henderson, 1987; Smith & Lang, 1990), also an important vasodilator and inhibitor of

platelet aggregation (Palmer *et al.*, 1987; Radomski *et al.*, 1987). Thus, the mechanisms and consequences of changes in PKC in vascular tissue from diabetic animals warrants further investigation.

References

- BANGA, J.D. & SIXMA, J.J. (1986). Diabetes mellitus, vascular disease and thrombosis. *Clinics Haematol.*, **15**, 465–470.
- BERRIDGE, M.J. (1984). Inositol triphosphate and diacyl glycerol as second messengers. *Biochem. J.*, **220**, 345–360.
- DANG, A.Q., FAAS, F.H., LEE, J.A. & CARTER, W.J. (1988). Altered fatty acid composition in the plasma, platelets and aorta of streptozotocin-induced diabetic rat. *Metabolism*, **37**, 1065–1072.
- DANTHALURI, N.R. & DETH, R.C. (1984). Phorbol ester-induced contraction of arterial smooth muscle and inhibition of alpha-adrenergic response. *Biochem. Biophys. Res. Commun.*, **125**, 1103–1112.
- FUJII, K., SOMA, M., HUANG, Y.-S., MANKU, M.S. & HORROBIN, D.F. (1986). Increased release of prostaglandins from the mesenteric vascular bed of diabetic animals: the effects of glucose and insulin. *Prostagl. Leuk. Med.*, **24**, 151–162.
- GRIENDLING, K.K., RITTENHOUSE, S.E., BROCK, T.A., EKSTEIN, L.S., GIMBRONE, M.A. & ALEXANDER, R.W. (1986). Sustained diacylglycerol formation from inositol phospholipids in angiotensin II-stimulated vascular smooth muscle cells. *J. Biol. Chem.*, **261**, 5901–5906.
- HAIJAR, D.P., WEKSLER, B.B., FALCONE, D.J., HEPTON, J.M., TACK-GOLDMAN, K. & MINICK, C.R. (1982). Prostacyclin modulates cholesteryl ester hydrolytic activity by its effect on cyclic adenosine monophosphate in rabbit aortic smooth muscle. *J. Clin. Invest.*, **70**, 70479–70488.
- HALLAM, T.J., PEARSON, J.D. & NEEDHAM, L.A. (1988). Thrombin-stimulated elevation of human endothelial cell cytoplasmic free calcium concentrations causes prostacyclin production. *Biochem. J.*, **251**, 243–249.
- HARRISON, H.E., REECE, A.H. & JOHNSON, M. (1978). Decreased vascular prostacyclin in experimental diabetes. *Life Sci.*, **33**, 351–356.
- HASSID, A. & WILLIAMS, C. (1983). Vasoconstrictor evoked prostaglandin synthesis in cultured vascular smooth muscle. *Am. J. Physiol.*, **245**, C278–C284.
- HOLMAN, R.T., JOHNSON, S.B., GERRARD, J.M., MAUER, S.M., KUPCHO-SANBERG, S. & BROWN, D. (1983). Arachidonic acid deficiency in streptozotocin induced diabetes. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 2375–2379.
- HUTTNER, I.I., GWEBE, E.T., PANGANAMALA, R.V., SHARMA, H.M. & GEER, J.C. (1977). Fatty acids and their prostaglandin derivatives: inhibitors of proliferation in aortic smooth muscle cells. *Science*, **197**, 289–291.
- IRVINE, R.F. (1982). How are the levels of arachidonic acid controlled in mammalian cells? *Biochem. J.*, **204**, 3–16.
- IWAMOTO, Y., KOIDE, H., OGITA, K. & NISHIZUKA, Y. (1992). The protein kinase C family for the regulation of cellular functions. *Bio. Med. Rev.*, **1**, 1–6.
- JEREMY, J.Y. & DANDONA, P. (1986). Role of the diacyl glycerol protein kinase C system in mediating adrenoceptor-prostacyclin synthesis coupling in the rat aorta. *Eur. J. Pharmacol.*, **136**, 311–316.
- JEREMY, J.Y. & DANDONA, P. (1989). Effect of endothelium removal on stimulatory and inhibitory modulation of rat aortic prostacyclin synthesis. *Br. J. Pharmacol.*, **96**, 243–250.
- JEREMY, J.Y. & MIKHAILIDIS, D.P. (1990). Vascular and platelet eicosanoids, smoking and atherosclerosis. In *Tobacco Smoking and Atherosclerosis*. ed. Diana, J. *Adv. Exp. Med. Biol.*, Vol 273, pp. 135–146. New York, London: Plenum Press.
- JEREMY, J.Y., MIKHAILIDIS, D.P. & DANDONA, P. (1985a). Adrenergic modulation of vascular prostacyclin synthesis. *Eur. J. Pharmacol.*, **114**, 33–40.
- JEREMY, J.Y., MIKHAILIDIS, D.P., THOMPSON, C.S. & DANDONA, P. (1986a). The effect of streptozotocin-induced diabetes on PGI₂ synthesis by the rat urinary bladder. *J. Urol.*, **135**, 1290–1292.
- JEREMY, J.Y., MIKHAILIDIS, D.P. & DANDONA, P. (1986b). Prostanoid synthesis by the rat urinary bladder: evidence for stimulation through muscarinic receptor-linked calcium channels. *Naunyn Schmiedebergs Arch Pharmacol.*, **334**, 463–467.
- JEREMY, J.Y., MIKHAILIDIS, D.P. & DANDONA, P. (1988a). Excitatory receptor linked prostanoid synthesis in mammalian smooth muscle: the role of calcium, protein kinase C and G proteins. *Prostagl. Leuk. Essential Fatty Acids Rev.*, **34**, 215–228.
- JEREMY, J.Y., THOMPSON, C.S., MIKHAILIDIS, D.P. & DANDONA, P. (1985b). Experimental diabetes inhibits prostacyclin synthesis by the rat penis: pathological implications. *Diabetologia*, **28**, 365–368.
- JEREMY, J.Y., THOMPSON, C.S. & DANDONA, P. (1988b). Prostanoid synthesis by the gastrointestinal tract, mesenteric vasculature and hepatic portal vein of diabetic rat. *Diabetes*, **37**, 196A.
- JEREMY, J.Y., THOMPSON, C.S., MIKHAILIDIS, D.P., OWEN, R.H. & DANDONA, P. (1987a). Fasting and diabetes mellitus elicit opposite effects on agonist-stimulated prostacyclin synthesis by the rat aorta. *Metabolism*, **36**, 616–620.
- JEREMY, J.Y., THOMPSON, C.S., MIKHAILIDIS, D.P. & DANDONA, P. (1987b). Changes in prostanoid synthesis in the cerebrum, cerebellum and brain stem of the diabetic rat. *Diab. Res.*, **6**, 95–98.
- JEREMY, J.Y., TSANG, V., MIKHAILIDIS, D.P., ROGERS, H., MORGAN, R.J. & DANDONA, P. (1987c). Eicosanoid synthesis by human urinary bladder mucosa: pathological implications. *Br. J. Urol.*, **59**, 36–39.
- LATIFPOUR, J., GOUSSE, A., YOSHIDA, M. & WEISS, R.M. (1992). Effect of insulin and myoinositol on muscarinic receptor: alterations in diabetic rat bladder. *J. Urol.*, **147**, 760–763.
- LEE, T.-S., SALTSMAN, K.A., OHASHI, H. & KING, G.L. (1988). Activation of protein kinase C by elevation of glucose concentration: proposal for a mechanism in the development of diabetic vascular complications. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 5141–5145.
- LEVINE, L. & WATANABE, M. (1991). Amplification of prostaglandin I₂ production by thapsigargin. *Prostaglandins*, **42**, 291–302.
- LEWIS, M.J. & HENDERSON, A.H. (1987). A phorbol ester inhibits the release of endothelium-derived relaxing factor. *Eur. J. Pharmacol.*, **137**, 167–171.
- LINCOLN, J., HAVEN, A.J., SAWYER, M. & BURNSTOCK, G. (1984). The smooth muscle of rat bladder in the early stages of streptozotocin-induced diabetes. *Br. J. Urol.*, **56**, 24–27.
- MARUI, N., SAKAI, T., HOSOKAWA, N., YOSHIDA, M., AOIKE, A., KAWAI, K., NISHINO, H. & FUKUSHIMA, M. (1990). N-myc suppression and cell cycle arrest at G₁ phase by prostaglandins. *FEBS Lett.*, **66**, 15–18.
- MIKHAILIDIS, D.P., JEREMY, J.Y. & DANDONA, P. (1987). Urinary bladder prostanoids: their synthesis, function and possible role in the pathogenesis and treatment of disease. *J. Urol.*, **137**, 1–6.
- MIKHAILIDIS, D.P., JEREMY, J.Y. & DANDONA, P. (1988). The role of prostaglandins, leukotrienes and essential fatty acids in the pathogenesis of the complications associated with diabetes mellitus. *Prostagl. Leuk. Essential Fatty Acids Rev.*, **33**, 205–206.
- MONCADA, S. & VANE, J.R. (1978). Prostacyclin formation and effects. In *Chemistry, Biochemistry and Pharmacology of Prostacyclin*. ed. Roberts, S.M. & Scheinman, F. pp. 258–273. Oxford: Pergamon Press.
- NISHIZUKA, Y. (1984). The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature*, **308**, 693–698.
- PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.
- POMERANTZ, K.B. & HAIJAR, D.P. (1984). Eicosanoid effects on cell proliferation, in vitro: relevance to atherosclerosis. *Prostagl Leuk Med.*, **16**, 1–10.
- RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987). Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br. J. Pharmacol.*, **92**, 181–187.
- RASMUSSEN, H., FORDER, J., KOJIMA, I. & SCRIBANINE, A. (1984). TPA induced contraction of isolated rabbit vascular smooth muscle. *Biochem Biophys. Res. Commun.*, **122**, 776–786.

- REED, P.W. & LARDY (1972). A23187: a divalent cation ionophore. *J. Biol. Chem.*, **247**, 6970–6978.
- RITTER, J.M., HAMILTON, D.P., BARROW, S.E., HEAVEY, D.J., HICKLING, N.E., TAYLOR, K.M., HOBBS, K.E. & DOLLERY, C.T. (1986). Prostacyclin in the circulation of patients with vascular disorders undergoing surgery. *Clin. Sci.*, **71**, 743–747.
- ROGERS, S.P. & LARKINS, R.G. (1981). Production of 6-oxo-PGF_{1α} by rat aorta: influence of diabetes, insulin treatment and caloric deprivation. *Diabetes*, **30**, 935–939.
- SCHEDL, H.P., WILSON, H.D., RAMASWAMY, K. & LICHTENBERGER, L. (1982). Gastrin and growth of the alimentary tract in the streptozotocin diabetic rat. *Am. J. Physiol.*, **242**, G460–G463.
- SMITH, J.A. & LANG, D. (1990). Release of endothelium-derived relaxing factor from pig cultured aortic endothelial cells, as assessed by changes in endothelial cell cyclic GMP content, is inhibited by phorbol ester. *Br. J. Pharmacol.*, **99**, 565–571.
- STEVENS, E.J., WILLARS, G.B. & TOMLINSON, D.R. (1992). Basal and angiotensin II-stimulated prostacyclin release by perfused rat lung in experimental diabetes: effects of insulin and aldose reductase inhibition. *Br. J. Pharmacol.*, **105**, 46P.
- THARSTRUP, O. (1990). Role of Ca²⁺ (+)-ATPase in the regulation of cellular Ca²⁺ signalling, as studied with the selective microsomal Ca²⁺ (+)-ATPase inhibitor, thapsigargin. *Agents Actions*, **29**, 8–15.
- THOMSON, A.B.R. (1980). Unidirectional flux rate of cholesterol and fatty acids into the intestine of rats with drug-induced diabetes mellitus. *J. Lipid Res.*, **21**, 687–698.
- TOMLINSON, K.C., GARDINER, S.M., HEBDEN, R.A. & BENNETT, T. (1992). Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. *Pharmacol. Rev.*, **44**, 103–149.
- TSANG, V., JEREMY, J.Y., MIKHAILIDIS, D.P., WALESBY, R.K., WRIGHT, J.C. & DANDONA, P. (1988). Release of prostacyclin from the human aorta. *Cardiovasc. Res.*, **22**, 489–493.

(Received August 12, 1992

Revised October 5, 1992

Accepted December 17, 1992)