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Protein kinase C-mediated contractile responses of arteries from diabetic rats

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1 The role of protein kinase C (PKC) in mediating enhanced contractile responses of aortae and mesenteric arteries from male rats with 12–14 week streptozotocin-induced diabetes to noradrenaline (NA) was investigated using the PKC activator, phorbol 12,13-dibutyrate (PDB), and the PKC inhibitor, staurosporine.

2 Maximum contractile responses of aortae and mesenteric arteries from diabetic rats to NA were significantly enhanced compared with responses of arteries from age-matched control animals. The maximum NA responses were increased by 59.6 ± 7.9% in aortae and by 54.9 ± 7.4% in mesenteric arteries from diabetic animals, compared to their respective controls.

3 Pretreatment of aortae and mesenteric arteries from both control and diabetic animals with staurosporine (5×10^{-8} M) caused marked inhibition of contractile responses to a maximum concentration of NA (10^{-5} M in aortae; 3×10^{-5} M in mesenteric arteries). In the presence of staurosporine, no difference was observed in the magnitude of contractile responses of arteries from control and diabetic rats to NA.

4 Maximum contractile responses of mesenteric arteries from diabetic rats to PDB were significantly increased (by 45.0 ± 4.9%) compared to responses of arteries from control animals. In contrast, no significant difference was found in the magnitude of contractile responses of aortae from control and diabetic rats to PDB.

5 Staurosporine (5×10^{-8} M) caused marked attenuation of contractile responses of arteries from control and diabetic rats to a maximum concentration of PDB (3×10^{-6} M). In the presence of staurosporine, the difference in magnitude of contractile responses of mesenteric arteries from control and diabetic rats to PDB was abolished.

6 Contractile responses of aortae and mesenteric arteries from control and diabetic rats to PDB were reduced in the absence of extracellular Ca^{2+}, and in the presence of the Ca^{2+} channel blockers, nifedipine (3×10^{-6} M) or verapamil (3×10^{-6} M). Under these conditions, no difference was found in the magnitude of contractile responses of mesenteric arteries from control and diabetic rats to PDB.

7 These data suggest that enhanced contractile responses of aortae and mesenteric arteries from streptozotocin-induced diabetic rats to NA may result, at least in part, from increased activation of PKC. In addition, increased activation of PKC-mediated processes, which are dependent on the presence of extracellular Ca^{2+}, may further contribute to the enhanced contractile responses of diabetic mesenteric arteries to NA.

Introduction

It is now well known that vascular deterioration is one of the complicating features of diabetes mellitus (Garcia *et al.*, 1974; Christlieb *et al.*, 1976). It has been proposed that this is in part due to altered reactivity of vascular smooth muscle to neurotransmitters and circulating hormones (Weidmann *et al.*, 1979). In an attempt to investigate this hypothesis, the responsiveness of vascular preparations from animals with chemically-induced diabetes to various vasoactive agents has been extensively studied (Brody & Dixon, 1964; Ramanadhan & Tenner, 1984; MacLeod & McNeill, 1985; Agrawal *et al.*, 1987; White & Carrier, 1988). Although the results of these investigations appear to be controversial, previous studies from our laboratory have demonstrated that aortae and mesenteric arteries from male rats with streptozotocin (STZ)-induced diabetes of 12 weeks duration are more responsive to the contractile effects of noradrenaline (NA) than are the corresponding arteries from age-matched control rats (MacLeod, 1985; Harris & MacLeod, 1988). The increased responsiveness of the diabetic vessels to NA did not appear to result from a generalized increase in the contractility of the tissues (MacLeod, 1985), or to a decrease in the release of endothelium-derived relaxing factor (Harris & MacLeod, 1988), but was a consequence of stimulation of α_1-adrenoceptors (Abebe *et al.*, 1990). It could be prevented from occurring, or reversed once established, by treatment of the diabetic animals with insulin, indicating that it is a result of the diabetic state (MacLeod, 1985).

Contractions of arteries in response to α_1-adrenoceptor stimulation are known to be associated with increased metabolism of sarcolemmal phosphoinositides (for review see Heagerty & Ollerenshaw, 1987). The hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C results in the generation of the second messengers, inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 has been shown to be involved in the release of Ca^{2+} from intracellular stores while DAG activates protein kinase C (PKC), resulting in protein phosphorylation and the generation of physiological responses. It has been suggested that the activation of PKC is associated with the tonic component (Campbell *et al.*, 1985; DeFeo & Morgan, 1985; Chatterjee & Tejada, 1986; Heagerty & Ollerenshaw, 1987), while IP_3 mediates the initial phasic component of the contractile responses of vascular smooth muscle to NA (Suematsu *et al.*, 1984; Hashimoto *et al.*, 1986). The tumour promoting phorbol esters have been shown to mimic the actions of DAG in activating PKC and causing contraction of vascular tissues (Castagna *et al.*, 1982; Danthaluri & Deth, 1984; Nishizuka, 1984; Rasmussen *et al.*, 1984; Gleason & Flaim, 1986; Miller *et al.*, 1986; Chiu *et al.*, 1987; Singer & Baker, 1987).

In view of the observed increases in contractile responsiveness of arteries from diabetic rats to NA (MacLeod, 1985; Harris & MacLeod, 1988), it is possible that the phosphoinositide messenger system is augmented in these tissues. The present study was undertaken to investigate whether

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PKC-mediated contractile responses are altered in aortae and mesenteric arteries from male rats with STZ-induced diabetes of 12–14 weeks duration. In this study, we used the phorbol ester, phorbol 12,13-dibutyrate (PDB) as a PKC activator (Castagna *et al.*, 1982), and the microbial alkaloid, staurosporine as a PKC inhibitor (Tamaoki *et al.*, 1986) to probe the status of PKC-mediated contractile mechanisms.

Methods

Induction of diabetes

Male Wistar rats weighing 190–220 g received a single injection of STZ (60 mg kg^{-1}) into the lateral tail vein. Age-matched control rats were injected with the citrate buffer vehicle (pH 4.5) used to dissolve the STZ. All injections were given to the animals under light ether anaesthesia. Three days after injection, rats were monitored for the development of glucosuria using Lilly Tes-tape. The control and diabetic rats were caged separately and housed in a similar environment. Both groups of animals were given the same diet (Purina Rat Chow) and water *ad libitum*.

Tissue preparation and contraction studies

Twelve to fourteen weeks after injection, diabetic and age-matched control rats were weighed and killed by stunning followed by decapitation. Blood was collected at the same time for serum glucose assay. The thoracic aorta and superior mesenteric artery were removed from each rat and placed in Krebs solution of composition (mM): NaCl 113, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2 and dextrose 11.5. After removal of adhering fat and connective tissues, ring preparations of aorta and mesenteric artery were prepared and placed individually in isolated tissue baths, containing 20 ml Krebs solutions maintained at 37°C and aerated with 95% O_2–5% CO_2, for isometric tension studies as described previously (MacLeod, 1985). Care was taken in these experiments not to damage the endothelium lining the vessels; the function of the endothelium was occasionally checked by monitoring responses to acetylcholine. The muscle preparations were equilibrated for 90 min under a resting tension of 2 g for aorta and 1 g for mesenteric artery. These resting tensions were found in preliminary experiments to be optimum for both control and diabetic arteries. During the equilibration period, the bathing solution was changed every 20–30 min. All experiments were carried out in the presence of 0.1 μM desipramine, 1 μM hydrocortisone, and 1 μM timolol to block neuronal and extraneuronal uptake, and β-adrenoceptors, respectively.

Following the equilibration period, cumulative concentration-response curves to NA and PDB were obtained in arterial rings from control and diabetic rats, in Krebs solution containing 2.5 mM Ca^{2+}. For determination of the involvement of PKC in NA- and PDB-induced contractions, responses to a single maximum concentration of NA (10^{-5} M in aortae or 3×10^{-5} M in mesenteric arteries) or PDB (3×10^{-6} M) were obtained in arteries pretreated with staurosporine (5×10^{-8} M) for 25 min. Responses of arteries to 8×10^{-2} M KCl were also obtained in the presence of staurosporine (5×10^{-8} M). In preliminary concentration-response experiments, 8×10^{-2} M KCl was found to produce maximum contractile responses in arteries from both control and diabetic rats. Responses to KCl were measured in the presence of 10^{-7} M phentolamine to block the effects of any neurally released NA. Control responses to NA, PDB and KCl were obtained 60 min before incubation of the tissues with staurosporine. The specificity of the action of PDB was further established by treating tissues with the biologically inactive phorbol ester, 4α-phorbol (10^{-8}–10^{-5} M for 30 min) (Castagna *et al.*, 1982). To assess the relative importance of extracellular Ca^{2+} in PKC-mediated contractile responses in control and diabetic preparations, concentration-response curves to PDB were also obtained in Ca^{2+}-free Krebs solution containing 1 mM ethylene glycol bis (β-aminoethyl ether)-N,N,N',N'-tetra-acetic acid (EGTA) following a 30 min equilibration period in this solution. Calcium chloride was replaced by NaCl to maintain osmolarity. The effect of Ca^{2+} entry blockade on contractile responses to PDB was assessed by adding verapamil or nifedipine (3×10^{-6} M) to the tissue bath 15 min before a single maximum concentration (3×10^{-6} M) of PDB. In preliminary experiments, this concentration of each of the blockers was found to produce the maximum inhibitory effect on PDB-induced contractions. Control responses to PDB were obtained 60 min before addition of the blockers.

At the end of each experiment, tissues were blotted dry, and their lengths and weights determined in order to calculate their cross-sectional areas, using the formula:

Cross-sectional area (mm^2) = weight (mg) (length (mm) × density (mg mm^{-3})$^{-1}$, where the density of vascular smooth muscle was assumed to be 1.05 mg mm^{-3} (Wyse, 1980). Contractile responses of each preparation to agonists were calculated as the increase in tension (g) per mm^2 in response to each concentration of agonist. Concentration-response curves were analysed by non-linear regression analysis using all the data points for determination of pD_2 ($-\log ED_{50}$) values.

Blood glucose determination

Serum glucose levels were monitored by the glucose oxidation method, employing the Boehringer periodochrome glucose kit.

Statistical analysis

All values are expressed as mean ± s.e.mean. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and considered to be significantly different if $P < 0.05$.

Drugs

Streptozotocin, (−)-noradrenaline hydrochloride, phorbol 12,13-dibutyrate, 4α-phorbol, verapamil and nifedipine were all obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Staurosporine was purchased from Calbiochem Corporation (La Jolla, CA, U.S.A.). A stock solution of noradrenaline was prepared daily in deionized water containing 1 mg ml^{-1} ascorbic acid. Phorbol 12,13-dibutyrate, 4α-phorbol and staurosporine were dissolved in dimethylsulphoxide, and verapamil and nifedipine in ethanol. Stock solutions of all drugs were diluted with deionized water to appropriate concentrations. The total volume of drug added to the tissue bath never exceeded 0.4 ml. The final dimethylsulphoxide or ethanol concentration in the bathing medium was without effect on contractile responses. Experiments involving PDB, 4α-phorbol, verapamil, nifedipine and staurosporine were performed in tissue baths protected from light.

Results

General characteristics of diabetic rats

Twelve to fourteen weeks after injection, the diabetic rats had significantly decreased body weights and elevated serum glucose levels compared to age-matched control animals (Table 1). Arterial preparations from diabetic rats were smaller in cross-sectional areas than those from control animals. The diabetic rats also manifested other symptoms of the disease including glucosuria, diarrhoea, polyuria, polydipsia and cataracts.

Table 1 Characteristics of diabetic and control rats

	n	*Body weight* (g)	*Serum glucose* (mg dl^{-1})	*Cross-sectional area* (mm^2) *Aortae*	*Mesenteric arteries*
Control	23	521 ± 6	94.7 ± 3.9	1.43 ± 0.04	0.39 ± 0.02
Diabetic	23	341 ± 8*	337.0 ± 13.8*	1.00 ± 0.03*	0.30 ± 0.02*

Values are mean ± s.e.mean.
* $P < 0.05$ with respect to corresponding control values.

Contractile responses to NA and PDB

As previously found (MacLeod, 1985; Harris & MacLeod, 1988), contractile responses to NA were significantly greater in aortae and mesenteric arteries from diabetic rats compared to control arteries (Figure 1). In aortic preparations, the maximum response to NA was increased from 2.12 ± 0.18 g mm^{-2} (mean ± s.e.mean, $n = 12$) in control vessels to 3.21 ± 0.38 g mm^{-2} ($n = 12$) in diabetic vessels. The maximum increase in tension in response to NA in diabetic mesenteric arteries was 5.28 ± 0.53 g mm^{-2} ($n = 10$) which was significantly greater than the corresponding value of 3.60 ± 0.45 g mm^{-2} ($n = 10$) obtained in control preparations. However, diabetes did not alter the sensitivities (as reflected by pD_2 values) of these arteries to NA (pD_2 values in diabetic aortae = 6.65 ± 0.14, mean ± s.e.mean; pD_2 value in control aortae = 6.88 ± 0.06; pD_2 value in diabetic mesenteric arteries = 6.19 ± 0.11; pD_2 value in control mesenteric arteries = 6.40 ± 0.11).

Phorbol 12,13-dibutyrate caused relatively slowly developing, concentration-dependent contractions of arterial rings. The magnitude of the maximum contractions and sensitivities of aortae from control and diabetic rats to PDB were similar (Figure 2; Table 2). In contrast to the aortae, maximum contractile responses of mesenteric arteries to PDB were enhanced in diabetic vessels compared to control tissues in the presence of 2.5 mM Ca^{2+} (Figure 2; Table 2). However, as with the aortae, sensitivities of the mesenteric preparations from the two groups of animals to PDB did not differ from each other (Table 2). Arteries from both control and diabetic rats did not contract in response to the inactive phorbol ester, 4α-phorbol (10^{-8}–10^{-5} M, $n = 3$) (data not shown).

Effects of staurosporine

The effect of the PKC inhibitor, staurospórine, on maximum contractile responses of arteries to NA (10^{-5} M in aortae or 3×10^{-5} M in mesenteric arteries) was investigated (Figure 3). In the absence of staurosporine, NA induced greater maximum contractile responses in both aortae and mesenteric arteries from diabetic rats than in control preparations. Pretreatment of arteries from both groups of animals with staurosporine (5×10^{-8} M) caused marked inhibition of the maximum contractile responses to NA. Staurosporine

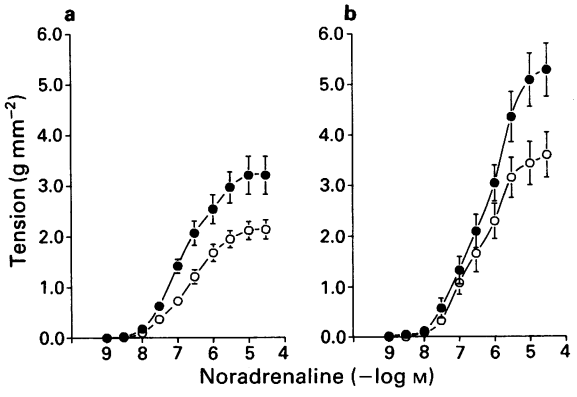

Figure 1 Concentration-response curves for noradrenaline-induced contractions of (a) aortae and (b) mesenteric arteries from control (○) and diabetic (●) rats. Each point represents the mean and vertical lines indicate s.e.mean of 10–12 observations.

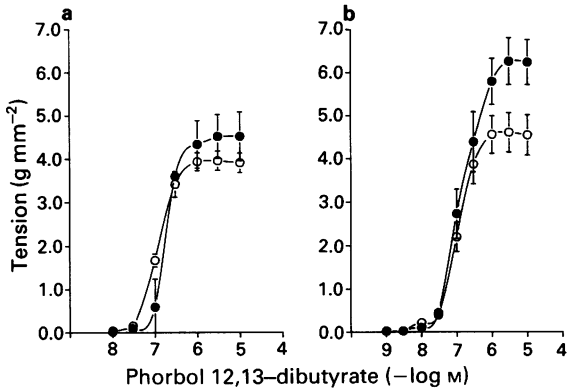

Figure 2 Concentration-response curves for phorbol 12,13-dibutyrate-induced contractions of (a) aortae and (b) mesenteric arteries from control (○) and diabetic (●) rats in Krebs solution containing 2.5 mM Ca^{2+}. Each point represents the mean and vertical lines show s.e.mean of 9–10 observations.

Table 2 Phorbol 12,13-dibutyrate pD_2 values and maximum contractile responses in the presence and absence of Ca^{2+} in aortae and mesenteric arteries from diabetic and control rats

	pD_2 values Control	Diabetic	*Contractile response* (g mm^{-2}) Control	Diabetic
Aorta				
2.5 mM Ca^{2+}	6.90 ± 0.06	6.73 ± 0.04	3.96 ± 0.23	4.50 ± 0.59
0 Ca^{2+}	6.03 ± 0.07	6.05 ± 0.08	1.24 ± 0.81	1.15 ± 0.22
Mesenteric artery				
2.5 mM Ca^{2+}	6.91 ± 0.09	6.77 ± 0.12	4.61 ± 0.46	6.26 ± 0.58*
0 Ca^{2+}	6.09 ± 0.20	5.83 ± 0.09	1.45 ± 0.11	1.30 ± 0.14

Values are mean ± s.e.mean of 6–10 observations.
* $P < 0.05$ with respect to corresponding control value.

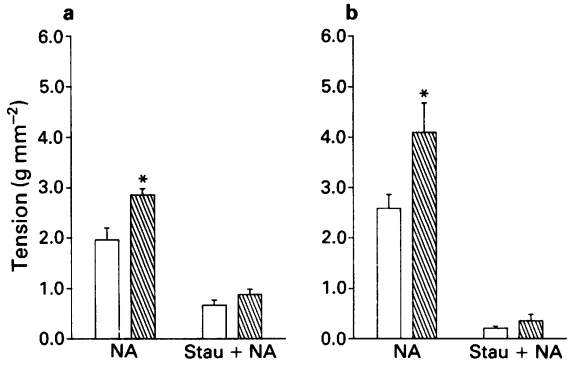

Figure 3 Effects of staurosporine (Stau) on noradrenaline-induced contractions of (a) aortae and (b) mesenteric arteries from control (open columns) and diabetic (hatched columns) rats. Tissues were incubated with 5×10^{-8} M staurosporine for 25 min before the addition of noradrenaline (10^{-5} M in aortae or 3×10^{-5} M in mesenteric arteries). Each column represents the mean and bars show s.e.mean of 8–9 observations. * $P < 0.05$ with respect to corresponding control values.

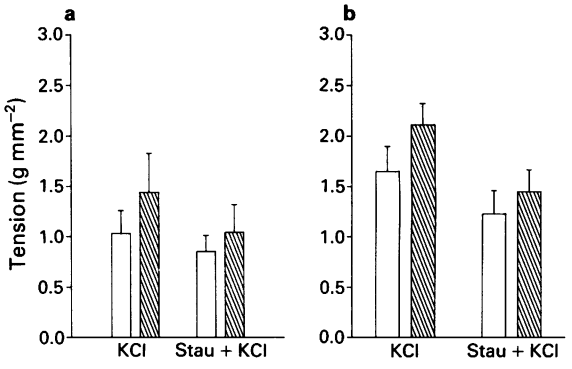

Figure 5 Effects of staurosporine (Stau) on KCl-induced contractions of (a) aortae and (b) mesenteric arteries from control (open columns) and diabetic (hatched columns) rats. Tissues were incubated with 5×10^{-8} M staurosporine for 25 min before the addition of 8×10^{-2} M KCl. Each point represents the mean and bars show s.e.mean of 5–10 observations.

appeared to exert a greater inhibitory effect on responses of mesenteric arteries. In the presence of staurosporine, the difference in contractile responses observed between control and diabetic arteries to NA was abolished.

Responses of arteries to PDB were also measured after pretreatment with staurosporine (Figure 4). In the absence of staurosporine, contractile responses of mesenteric arteries, but not aortae, from diabetic rats to 3×10^{-6} M PDB were significantly greater than control. Staurosporine (5×10^{-8} M) also produced marked inhibition of contractions of both arteries from control and diabetic rats to PDB, with greater antagonism of responses of mesenteric arteries. The inhibitory effect of staurosporine abolished the difference in contractile responses to PDB found between control and diabetic mesenteric arteries.

Contractions of arteries induced by KCl were also measured in the presence of staurosporine in order to assess the specificity of the action of the inhibitor in antagonizing PKC-mediated responses to NA and PDB (Figure 5). Consistent with our previous results (MacLeod, 1985), the magnitudes of contractions of untreated arteries from control and diabetic rats to KCl were similar. In contrast to its action against NA and PDB, staurosporine (5×10^{-8} M) caused relatively less inhibition of contractions induced by KCl (8×10^{-2} M) in both aortae and mesenteric arteries from control and diabetic animals. In the presence of staurosporine, no differences were observed in contractile responses of arteries from control and diabetic rats to KCl.

Effects of extracellular Ca^{2+} removal on PDB responses

Phorbol 12,13-dibutyrate produced concentration-dependent contractions of aortae and mesenteric arteries incubated in Ca^{2+}-free solution containing 1 mM EGTA. However, the magnitude of these responses was markedly reduced compared to the responses observed in the presence of 2.5 mM Ca^{2+} (Figure 6; Table 2). In Ca^{2+}-free solution, no differences were observed in the magnitude of contractile responses or sensitivities of control and diabetic aortae to PDB. Similarly, contractile responses and sensitivities of diabetic mesenteric arteries to PDB in Ca^{2+}-free solution did not differ from those in control vessels (Figure 6; Table 2).

Effects of Ca^{2+} channel blockers on PDB responses

The effects of the Ca^{2+} channel blockers, verapamil and nifedipine, on PDB responses were evaluated in Krebs solution containing 2.5 mM Ca^{2+} (Figure 7). Contractile responses of mesenteric arteries, but not aortae, from diabetic rats to 3×10^{-6} M PDB were significantly enhanced compared with those from controls in the absence of the Ca^{2+} channel blockers. Pretreatment of arteries with 3×10^{-6} M verapamil or nifedipine resulted in inhibition of responses of both arteries from diabetic and control rats to 3×10^{-6} M PDB, although these effects were greater in diabetic mesenteric preparations. The inhibitory effects of the Ca^{2+} channel blockers

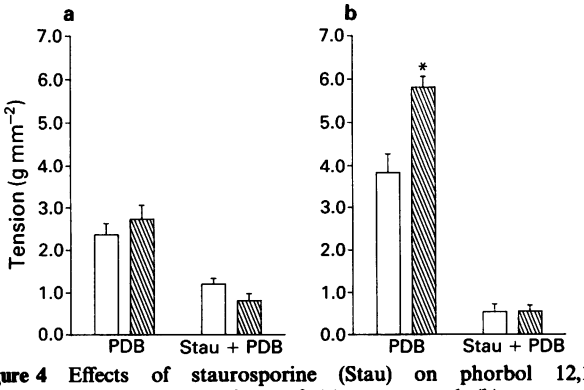

Figure 4 Effects of staurosporine (Stau) on phorbol 12,13-dibutyrate-induced contractions of (a) aortae and (b) mesenteric arteries from control (open columns) and diabetic (hatched columns) rats. Tissues were incubated with 5×10^{-8} M staurosporine for 25 min before the addition of 3×10^{-6} M phorbol 12,13-dibutyrate. Each column represents the mean and bars show s.e.mean of 5–10 observations. * $P < 0.05$ with respect to corresponding control values.

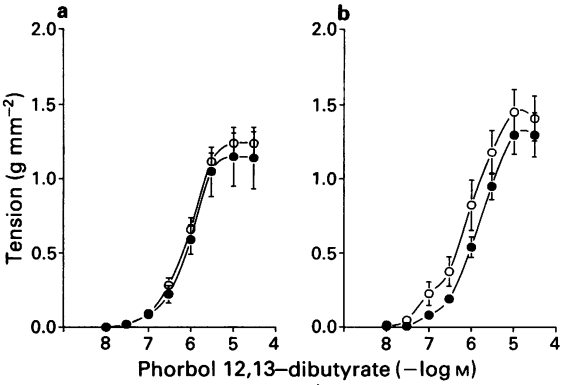

Figure 6 Concentration-response curves for phorbol 12,13-dibutyrate-induced contractions of (a) aortae and (b) mesenteric arteries from control (○) and diabetic (●) rats in Ca^{2+}-free solution containing 1 mM EGTA. Each point represents the mean and vertical lines show s.e.mean of 6 observations.

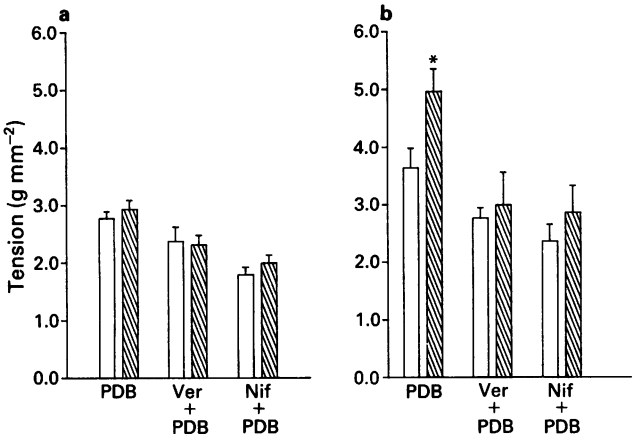

Figure 7 Effects of verapamil (Ver) and nifedipine (Nif) on phorbol 12,13-dibutyrate-induced contractions of (a) aortae and (b) mesenteric arteries from control (open columns) and diabetic (hatched columns) rats. Tissues were incubated with 3×10^{-6} M of the blockers for 15 min before the addition of 3×10^{-6} M phorbol 12,13-dibutyrate (PDB). Each column represents the mean and bars show s.e.mean of 8–16 observations. * $P < 0.05$ with respect to corresponding control values.

abolished the difference between control and diabetic mesenteric arteries in contractile responses to PDB.

Discussion

The results of the present study confirm previous findings from this laboratory that aortae and mesenteric arteries from rats with STZ-induced diabetes are more responsive to the contractile effects of NA than are the corresponding arteries from age-matched control rats (MacLeod, 1985; Harris & MacLeod, 1988). This observation is also consistent with the results of other investigators who used intact animals as well as isolated tissues from various experimental diabetic models (Brody & Dixon, 1964; Agrawal *et al.*, 1987; White & Carrier, 1988). However, the mechanism underlying this observation is not well understood. The current study has examined the possibility that diabetes induces changes in contractile mechanisms resulting from activation of PKC.

Our finding that contractile responses of arteries to NA were inhibited by the putative PKC inhibitor, staurosporine (Tamaoki *et al.*, 1986) suggests that at least part of the NA response of these arteries is mediated via activation of PKC. This observation is consistent with the view that PKC activation is involved in the tonic contractile responses of vascular smooth muscle to agents like NA which stimulate the phosphoinositide messenger system (Campbell *et al.*, 1985; DeFeo & Morgan, 1985; Chatterjee & Tejada, 1986; Heagerty & Ollerenshaw, 1987). Recently, it was suggested that staurosporine may block contractile responses of arteries to agonists, including those causing PKC activation, by a PKC-independent mechanism, possibly involving inhibition of myosin light chain kinase (Ruegg & Burgess, 1989). If this were the case, staurosporine would be expected to block KCl-induced contractions, which are believed to be associated with activation of myosin light chain kinase, and occur independently of PKC activation (Nakaki *et al.*, 1985; Turla & Webb, 1990). However, the concentration of staurosporine used in the present investigation caused much greater inhibition of contractile responses of arteries to NA and the PKC activator, PDB, than to KCl. This suggests that the inhibitor was relatively more selective in inhibiting PKC-mediated responses. Therefore, it seems likely that the contractile responses of arteries to NA measured in the present study are mediated, at least partly, by PKC activation. The fact that the enhanced contractile responses of arteries from diabetic rats to NA were abolished in the presence of staurosporine suggests that at least part of the increased responsiveness is the result of enhanced activation of PKC-mediated mechanisms in these tissues. However, these data do not demonstrate whether this results from increased activation of PKC by increased production of DAG, and/or from increased responsiveness of contractile processes to activation of PKC.

Phorbol 12,13-dibutyrate was used in the present investigation to probe further the status of PKC-mediated contractile mechanisms because of its greater water solubility than the commonly used analogue, 12-O-tetradecanoyl-phorbol 13-acetate (TPA). Contraction of vascular smooth muscle by active phorbol esters such as PDB or TPA, in the absence and presence of extracellular Ca^{2+}, is a well documented phenomenon (Danthaluri & Deth, 1984; Kojima *et al.*, 1984; Rasmussen *et al.*, 1984; Gleason & Flaim, 1986; Miller *et al.*, 1986; Chiu *et al.*, 1987; Singer & Baker, 1987; Swamura *et al.*, 1987). The assumption that the contractions induced by these agents are mediated by PKC is supported by a variety of evidence including the lack of contractile effect in response to inactive phorbol analogues, blockade of the action of phorbol esters by PKC inhibitors and a lack of reported non-specific actions of phorbol esters (Miller *et al.*, 1986; Singer & Baker, 1987). Moreover, there are studies showing that the phorbol ester receptor and PKC are the same protein (Kikkawa *et al.*, 1983; Niedel *et al.*, 1983). In the present study, we have shown that the biologically inactive phorbol ester, 4α-phorbol, (Castagna *et al.*, 1982) did not cause contractions of arteries. In addition, contractile responses of arteries from both control and diabetic rats to PDB were inhibited by staurosporine. Staurosporine also abolished the difference in contractile responses observed between diabetic and control mesenteric arteries to PDB. These data suggest that the contractile responses of arteries to PDB observed in the present investigation are also mediated by PKC activation, and that the increased responsiveness of mesenteric arteries from diabetic rats to this agent is the result of enhanced activation of PKC-mediated mechanisms in these tissues.

A part of the contractile response of both arteries to PDB was dependent on the presence of extracellular Ca^{2+}, since Ca^{2+}-free solution containing EGTA or the Ca^{2+} channel blockers verapamil or nifedipine caused a reduction in the magnitude of the PDB contractions. The observation that the extent of reduction of the PDB response by verapamil or nifedipine was not as great as that observed in Ca^{2+}-free, EGTA solution is in agreement with previous findings that a substantial component of the PDB response is resistant to Ca^{2+} channel blockers (Sybertz *et al.*, 1986; Chiu *et al.*, 1987). Similar Ca^{2+}-dependencies of contractile responses to PDB and TPA have been observed by a number of other investigators in rat aortae and mesenteric arteries as well as in other types of blood vessels (Rasmussen *et al.*, 1984; Baraban, 1985; Gleason & Flaim, 1986; Chiu *et al.*, 1987; Singer & Baker, 1987; Turla & Webb, 1987). Phorbol 12,13-dibutyrate has also been shown to stimulate the influx of [$^{45}Ca^{2+}$] in rabbit and rat aortae (Gleason & Flaim, 1986; Chiu *et al.*, 1987). These data suggest that activation of PKC with phorbol esters leads to opening of Ca^{2+} channels allowing the influx of extracellular Ca^{2+} into smooth muscle cells. The results of the present investigation demonstrate that the enhanced responsiveness of mesenteric arteries from diabetic rats to PDB was dependent on the availability or entry of extracellular Ca^{2+}, since in the absence of Ca^{2+} or in the presence of verapamil or nifidepine, the magnitudes of the PDB-induced contractions were similar in control and diabetic mesenteric arteries. Further evidence supporting this observation was obtained from experiments showing that upon the re-addition of Ca^{2+} to Ca^{2+}-free medium, diabetic mesenteric arteries pretreated with PDB contracted more than control arteries (data not shown). These observations suggest that PDB-mediated activation of PKC in mesenteric arteries from STZ-diabetic rats may result in increased influx of Ca^{2+} through cell membrane Ca^{2+} channels, thereby leading to increased tension development. In this

regard, it is possible that the increased Ca^{2+} influx in response to PDB is due to increased activation of Ca^{2+} channels by PKC-dependent phosphorylation (Berridge *et al.*, 1987; Heagerty & Ollerenshaw, 1987; Fish *et al.*, 1988) and/or an increased number of Ca^{2+} channels available for activation by PKC, in the diabetic vessels. However, other explanations which include (a) that the Ca^{2+} sensitivity of PKC may be increased in the presence of PDB to a greater extent in the diabetic vessels, and/or (b) augmentation of other Ca^{2+}-dependent cellular events occurs subsequent to PKC activation in diabetes, cannot be excluded.

It is not clear why diabetes induced enhanced contractile responses of mesenteric arteries to direct activation of PKC by PDB in the presence of extracellular Ca^{2+} without affecting the responses in aortae. One possible explanation for this variation in tissue responsiveness is that different types of PKC may be present in the two types of blood vessels, with differential responsiveness to diabetes. Alternatively, the number and/or nature of Ca^{2+} channels activated by PKC may be affected by diabetes in a different manner in the two kinds of vascular tissues, and/or other events occurring subsequent to PKC activation may be influenced differently by diabetes in the two types of vessels. These possibilities appear to be partly supported by the results of other investigators who have shown that differences in PKC activity exist in different vascular beds (Wagner *et al.*, 1987; Silver *et al.*, 1988).

Our data indicate that contractile responses to NA mediated via PKC activation are enhanced, although those resulting from direct activation of PKC are unaltered, in aortae from diabetic rats. This suggests that the increased contractions observed in aortae from diabetic rats in response to NA may result, at least in part, from increased activation of PKC by enhanced production of DAG in these tissues. In support of this, recently we have found greater phosphoinositide turnover in diabetic aortae than in control preparations in response to NA (Abebe & MacLeod, unpublished observations). On the other hand, contractile responses to both direct activation of PKC, and to activation of PKC in response to NA, were enhanced in mesenteric arteries from diabetic rats. Enhancement of NA-induced phosphoinositide turnover was also observed in mesenteric arteries from diabetic rats (Abebe & MacLeod, unpublished observations). Therefore, both increased activation of PKC by enhanced production of DAG, and increased responsiveness of contractile mechanisms to PKC activation, may contribute to the increased contractile responses of diabetic mesenteric arteries to NA.

In conclusion, the results of the present study provide evidence that enhanced contractile responses of aortae and mesenteric arteries from STZ-induced diabetic rats to NA may result, at least in part, from increased activation of PKC. In addition, increased activation of PKC-mediated processes, which are dependent on the presence of extracellular Ca^{2+}, may further contribute to the enhanced contractile responses of diabetic mesenteric arteries to NA.

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