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Protein kinase C (PKC) involved in enhancement of α_1 -adrenoceptor-mediated responses of the main pulmonary artery in rats with diabetes mellitus

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Diabetes mellitus (DM) causes multiple dysfunctions including circulatory disorders such as cardiomyopathy, angiopathy, atherosclerosis and arterial hypertension. Moreover, DM can strongly affect pulmonary circulation, enhancing the wall thickness of the pulmonary arteries, changing their tone and contractility, and gas exchange in the lungs. It can lead to marked loss of lung function and respiratory efficiency. It is also known that protein kinase C (PKC) activity increases in DM and that PKC is involved in the mechanisms of DM-associated vascular complications. However, the effect of DM on pulmonary artery tone has been poorly investigated and the role of PKC in this remains unknown. The aim of this study was to investigate changes in contractility of pulmonary arteries in rats with DM and to determine the possible role of PKC in this process. Experimental type 1 DM was elicited in male Wistar rats by single streptozotocin (STZ, 65 mg/kg) injection. DM was verified by the presence of hyperglycaemia. The investigation was performed on the isolated rings of the main pulmonary arteries using the method of vascular tone registration. Phenylephrine (PhE, 0.1 nM - 1 mM) caused dose-dependent constriction of the pulmonary arteries. The pD₂ (negative logarithm of the agonist concentration required for half-maximum response) of this constriction increased in rats with DM, however significant changes in amplitude of PhE-induced constriction were not observed. PKC inhibition with chelerythrine and staurosporine (1 µM) significantly shifted PhE the concentration-response curve to the right in intact diabetic vessels but had no effect on sensitivity to PhE in deendothelised diabetic vessels. Our data suggest that type 1 DM leads to enhancement in pulmonary artery an-adrenoceptor-mediated contractility and PKC activity in the endothelium rather than in vascular SMCs is involved in this process.

Keywords: diabetes mellitus; protein kinase C; vascular tone; vascular smooth muscle; endothelium; pulmonary artery

Introduction

Diabetes mellitus (DM) is a complex syndrome that is rapidly rising in incidence throughout the world. Hyperglycemia and alterations in metabolism are the most severe components of DM (Zimmet, 2011). The adverse long-term effects of DM involve many organ systems and are associated with a complex pathology leading to a large number of secondary cellular and subcellular changes.

DM leads to multiple dysfunctions including cardiovascular diseases, one of the major causes of morbidity, mortality, end-stage renal disease, and blindness (Madonna and de Caterina, 2011). The macrovascular manifestations of DM include angiopathy, atherosclerosis, medial calcification, and arterial hypertension mostly located in the coronary and carotid arteries (Whiteley et al., 2005; Cosson et al., 2006), cerebral vessels, and the large peripheral arteries of the lower extremities (Funk et al., 2012). Increased blood flow and vascular tone elevation have been documented in diabetes (Madonna and de Caterina, 2011).

Hyperglycaemia is a key factor responsible for the development of vascular complications in diabetes (Boussageon et al., 2011; Madonna and de Caterina, 2011). Several hyperglycaemia-associated mechanisms have been identified as contributing to the development of vascular dysfunction associated with DM. One of such mechanisms may involve activation of protein kinase C (PKC) pathways (Kizub et al., 2014). PKC is a family of regulatory enzymes (serine/threonine kinases) (Cosentino-Gomes et al., 2012) that plays a prominent role in the signal transduction of several vascular functions including regulation of vascular smooth muscle contractility (Somlyo and Somlyo, 2003; Cosentino-Gomes et al., 2012). It has been shown that dysfunctions of these systems are associated with the diabetic state and involve PKC-dependent mechanisms which are implicated as an important players in the pathogenesis of diabetic microangiopathy (Clarke and Dodson, 2007; Kizub et al., 2014) and macroangiopathy (Geraldes and King, 2010; Kizub et al., 2014). Numerous PKC isozymes (α , β_1 , β_2 , γ , ε , η , ζ , δ , and $\nu\lambda$) have been shown to be activated or overexpressed in vascular smooth muscle cells (SMCs) and the endothelium of different vascular regions in subjects with diabetes (Ramana et al., 2005; Klymenko et al., 2014).

Despite a number of studies addressed to vascular complications in DM, there is little known about the effect of DM on pulmonary circulation. The present study has been devoted to an investigation of alterations in pulmonary artery tone associated with DM and the possible role of PKC in this process.

Material and methods

Experiments were performed on isolated vascular rings obtained from the main pulmonary artery of male Wistar rats

weighing 190-210 g. All experimental procedures conformed to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and were approved by the Ethics Committee of the Educational and Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Ukraine and State Institution "Institute of Pharmacology and Toxicology of NAMS of Ukraine", Kyiv, Ukraine. Two groups of animals were used: diabetic rats injected with streptozotocin (STZ, 60-65 mg/kg, i.p.) and maintained for 9 weeks, and age-matched controls. STZ was dissolved in buffer solution containing 0.9% NaCl and 10 mM citrate (pH = 4.6). DM development was verified by the presence of hyperglycaemia (plasma glucose higher than 20 mM) every 4 weeks after streptozotocin injection, and on the day of experimentation. The diabetic group of animals (blood glucose 26.02 ± 5.04 mM, n = 8) was compared with the control rats (blood glucose 6.74 ± 0.12 mM, n = 8; two-tailed t-test P < 0.05). Blood samples were obtained from the tail tip and analyzed using a glucose meter Bionime Rightest GS300 (Bionime Gmbh, Switzerland).

The animals were killed by cervical dislocation. The heart and lungs were rapidly removed and placed into Krebs' physiological salt solution (composition in mM: 133.00 NaCl, 16.30 NaHCO₃, 1.05 MgCl₂, 1.38 NaH₂PO₄, 4.70 KCl, 7.00 D-glucose, 2.75 CaCl₂ and 10 HEPES, pH 7.4). For rings from diabetic animals, the concentration of glucose was increased to 25 mM. Vessels were dissected free, cleaned of connective and adipose tissue, cut into 2–3 mm rings and mounted on fine stainless steel hooks in organ baths perfused with warmed to 37 °C Krebs' solution. For vascular tone measurement capacitive tension detectors (Danish Myo Technology, Aarhus, Denmark) and LabScribe 2 (World Precession Instrument Inc., USA) software were used. The resting tension of the vessels was gradually increased to 0.7–0.8 g. After 1 hour equilibration, each vascular ring was exposed twice to 60 mM KCl exposure to assess its viability and reproducibility of contractions.

In experiments with deendothlised vessels the endothelium was disrupted chemically with 15 min incubation of the vascular rings in Krebs' solution containing 0.1 mg/ml of saponine. Successful endothelium removal was determined by absence of 10 μ M acetylchole-induced relaxation following the contraction in response to 1 mM phenylephrine (PhE) application.

Data are presented as mean \pm SE with n indicating the number of vascular preparations tested. Contractile responses were expressed as a percentage of the maximum response produced by 60 mM KCl or 1 mM PhE. The sensitivity to the agonist was expressed as pD₂ (negative logarithm of the agonist concentration required for half-maximum response). In order to determine this parameter, data points were calculated using the Origin 8.1 software (OriginLab Corporation, Northampton, MA, USA) with the 'DoseResp' function in the following form:

$$T = \frac{100}{1 + 10^{\log(x0 - x) \cdot p}},$$

where *T* is normalized tension (expressed as % of maximal contraction) at the agonist concentration *x* (expressed as negative logarithm), x_0 is the midpoint of the concentration-effect curve (e.g. T = 50%) and p is the slope factor of the curve. Mean values were obtained by averaging pD₂ values obtained from individual preparations. Statistical comparisons were made using Student's unpaired two-tailed t-test for two groups. Differences for P < 0.05 were considered to be statistically significant.

Results

Effect of DM on intact pulmonary artery contractility. In control intact pulmonary artery rings isolated from non-diabetic rats, phenylephrine (PhE), a selective α_1 -adrenoceptor agonist, applied at ascending concentrations (0.1 nM – 1 mM) evoked concentration-dependent contractions with a maximal response (E_{max}) 146.76 ± 18.92 % (n = 16) of constriction evoked by 5-minute application of

60 mM KCl (Fig. 1 and 2.4). A plateau response was obtained after the addition of each test PhE concentration before the addition of a subsequent dose. pD₂ value of PhE in control vessels consisted 7.49 ± 0.19 (n = 16) (Fig. 2*B*).

In intact pulmonary arteries isolated from diabetic rats, the responsiveness to PhE was enhanced since the diabetic vessels responded to lower concentrations of the agonist. pD₂ value of PhE in diabetic vessels was 8.02 ± 0.09 (n = 16, P < 0.05) (Fig. 2). In contrast, pulmonary artery rings from diabetic animals showed no significant difference in E_{max} between nondiabetic control and diabetic rats. It was $130.4 \pm 10.5\%$ (n = 16; P > 0.05) of constriction evoked by 60 mM KCl (Fig. 1).



Fig. 1. Mean data of relevant amplitude of the contractile responses to phenylephrine (PhE, 0.1 nM – 1 mM) in intact (E^+) pulmonary artery from control (Ctrl) and diabetic (DM) rats before and after PKC inhibition with chelerythrine (Chel, 1 μ M) and staurosporine (Stsp, 1 μ M); * – P < 0.05

Effect of DM on deendothelised pulmonary artery contractility. To evaluate the role of the endothelium in pulmonary artery contractility in DM experiments were performed on deendothelised vascular rings. In deendothelised pulmonary arteries from control rats, 0.1 nM - 1 mM PhE application led to vasoconstriction development with a maximal amplitude of $99.4 \pm 22.5\%$ (n = 8) of contraction evoked by 60 mM KCl (Fig. 3). pD₂ value of PhE-induced constriction in these vessels was 7.55 ± 0.16 (n = 8) (Fig. 4).

In the deendothelised pulmonary artery of animals with experimental DM, the dose-response curve of PhE was significantly shifted to the right as compared to the intact vessels of the control animals, showing decrease in vascular sensitivity to the agonist with pD₂ value of 7.09 ± 0.22 (n = 8, P < 0.05) (Fig. 4). In contrast to deendothelised control vessels, significant changes in the amplitude of PhE-evoked contraction in diabetic pulmonary artery rings were not observed (138.7 ± 24.0%; n = 8; P > 0.05; Fig. 3).

Effect of PKC inhibition on intact pulmonary artery contractility in norm and DM. To investigate the role of PKC in pulmonary artery tone alterations in DM, vascular tissues were pretreated for 20 min with combination of potent cell-permeable PKC inhibitors chelerythrine (1 μ M) and staurosporine (1 μ M). PKC inhibition had no significant effect on the amplitude of Phe-induced constriction in intact pulmonary arteries of control rats. Maximum contraction in response to 100 μ M PhE was 175.5 ± 20.8% (n = 12; P > 0.05 as compared to control) (Fig. 1). On the other hand, PKC inhibition resulted in an unexpected leftward shift of the concentration-response curve for PhE with pD₂ value 8.55 ± 0.49 (n = 8; P < 0.05) (Fig. 2).

In diabetic pulmonary arteries PKC inhibition significantly shifted the concentration-response curve for PhE to the right (Fig. 2). Mean pD_2 value in diabetic arteries was 6.67 ± 0.29 (n = 12;

P < 0.05) compared with the control tissue. PKC inhibition also significantly decreased amplitude of PhE-induced constriction in pulmonary arteries from diabetic animals (Fig. 2). In these conditions its maximum was $28.3 \pm 8.6\%$ (n = 12; P < 0.05) of constriction evoked by 60 mM KCl (Fig. 1).



Fig. 2. The changes in sensitivity to phenylephrine (PhE) in intact (E^+) pulmonary artery from control and diabetic rats: A – original traces illustrating agonist concentration-response in intact control (top) and diabetic (bottom) rat pulmonary artery preparations; B – mean data of normalized amplitude of the contractile responses plotted versus PhE concentration (0.1 nM – 1 mM) in intact (E^+) pulmonary artery from control (Ctrl) and diabetic (DM) rats before and after PKC inhibition with chelerythrine (Chel, 1 μ M) and staurosporine (Stsp, 1 μ M); vertical dotted lines represent pD₂ value of PhE-induced constriction dose-response curves fitted by equation 1 (see Methods section); it should be noted that dose-response curves

"DM" and "Ctrl + Chel + Stsp" are superposed; * - P < 0.05

Effect of PKC inhibition on deendothelised pulmonary artery contractility in norm and DM. PKC inhibition with chelerythrine and staurosporine had no effect on E_{max} of deendothelised control pulmonary artery contraction evoked by 100 μ M PhE with value 64.2 ± 14.9% (n = 9; P > 0.05) (Fig. 3). The sensitivity of deendothelised vessels from control animals to PhE also was not changed, and pD₂ value of PhE-induced constriction in these vessels was 7.77 ± 0.31 (n = 9; P > 0.05) (Fig. 4).

In diabetic endothelium-free vascular tissues PKC inhibition also had no effect on maximal amplitude of PhE-induced vasoconstriction. It was $103.0 \pm 27.3\%$ (n = 8; P > 0.05) (Fig. 3). PKC inhibition also did not affect concentration-response curve for PhE in deendothelised vessels from diabetic animals. pD₂ value of PhE-induced constriction in these vessels was 6.99 ± 0.32 (n = 8; P > 0.05) as compared to control (Fig. 4). Table 1 represents comparison of pD₂ values which characterize PhE-induced constriction dose-response curve in both intact and deendothelised pulmonary arteries from control and diabetic rats.



Fig. 3. Mean data of relevant amplitude of the contractile responses to phenylephrine (PhE, 0.1 nM – 1 mM) in deendothelised (E⁻) pulmonary artery from control (Ctrl) and diabetic (DM) rats before and after PKC inhibition with chelerythrine (Chel, 1 μ M) and staurosporine (Stsp, 1 μ M); * – P < 0.05



Fig. 4. Mean data of normalized amplitude of the contractile responses plotted versus phenylephrine (PhE) concentration (0.1 nM – 1 mM) reflecting the changes in sensitivity to PhE in deendothelised (E⁻) pulmonary artery from control (Ctrl) and diabetic (DM) rats before and after PKC inhibition with chelerythrine (Chel, 1 μ M) and staurosporine (Stsp, 1 μ M); vertical dotted lines represent pD₂ value of PhE-induced constriction dose-response curves fitted by equation 1 (see Methods section); it should be noted that dose-response curves for control and DM are superposed;* – P < 0.05

Table 1

Mean pD₂ for PhE-induced constriction dose-response curve in intact (E⁺) and deendothelised (E⁻) pulmonary artery from control and diabetic (DM) rats in the absence or presence of combined action of chelerythrine (1 μ M) and staurosporine (1 μ M)

	Control		Control + chelerythrine	DM	DM + chelerythrine
			and staurosporine		and staurosporine
pD ₂ ·	E+	7.49 ± 0.19	8.55 ± 0.49	8.02 ± 0.09	6.67 ± 0.29
		(n = 16)	(n=8)*	(n=16)*	(n = 12)*
	E-	7.55 ± 0.16	7.77 ± 0.31	7.09 ± 0.22	6.99 ± 0.32
		(n = 8)	(n = 9)#	(n=8)*#	(n = 8)

Note: * - P < 0.05 in comparison with pulmonary artery rings from control animals; # - P < 0.05 in comparison between E^+ and E^- pulmonary artery rings.

Discussion

The data obtained clearly indicate that type 1 DM development leads to increase in sensitivity to PhE in rats' pulmonary arteries reflecting enhancement in vascular α_1 -adrenoceptor-mediated contractility. However, PKC inhibition in deendothelised vessels from diabetic animals had no effect on the sensitivity to PhE. It may suggest that mechanisms of pulmonary artery contractility enhancement in DM are associated with activity of PKC in the endothelial cells rather than in vascular SMC. There is a variety of evidence to indicate that sensitivity to α_l -adrenoceptors-mediated stimulation is markedly elevated in STZ-diabetic rats' systemic arteries: tail artery (Kizub et al., 2010), mesenteric artery (White and Carrier, 1990; Mueed et al., 2005; Kizub et al., 2010), and aorta (Xavier et al., 2003) whereas other studies have shown an increase in amplitude of α_1 -adrenoceptors-mediated vasoconstriction in these arteries (Abebe and McLeod, 1991; Chow et al., 2001; Lee et al., 2011). Similar results showing enhanced noradrenaline-induced vasoconstriction have been obtained in high glucose concentration in the aorta of OLETF (Otsuka Long-Evans Tokushima Fatty) rat, an experimental model of insulin-independent DM (Nobe et al., 2003). On the other hand, a few studies have shown that the aortas of STZ-diabetic rats exhibited no changes in sensitivity to PhE (Chang and Stevens, 1992; Kizub et al., 2010).

In contrast to our data, Gurney and coauthors have shown that STZ-induced diabetes significantly blunted the maximum response of rats' conduits, but not resistance pulmonary arteries to PhE, without changes in the sensitivity to PhE. Endothelium-dependent vasodilatation of these vessels was also unaffected in DM (Gurney and Howarth, 2009). Other researchers have demonstrated that in the intrapulmonary arteries of STZ-diabetic rats the concentration-response curve PhE was characterized by an increased maximal response in the diabetic group compared with the control one without changes in pD₂ value. Endothelium-dependent relaxant response was dramatically reduced in pulmonary arteries from diabetic rats (Lopez–Lopez et al., 2008).

Our data indicate that PKC activity is involved in elevated α_1 adrenoceptors-mediated vasoconstriction in the pulmonary arteries of STZ-diabetic rats. It has been shown by us previously that PKC inhibition suppressed elevated sensitivity to PhE in the smooth muscle of the tail artery of STZ-diabetic rats (Kizub et al., 2010). In our present study two potent inhibitors of PKC, chelerythrine and staurosporine were both used to achieve complete inhibition of PKC. Chelerythrine is a specific inhibitor of PKC substrate-binding site (Herbert et al., 1990), whereas staurosporine (or antibiotic AM-2282) is a selective inhibitor of ATP-binding site of PKC (Ishii et al., 1996). We have previously shown that changes in sensitivity of systemic vessels (aorta and tail artery) to α_1 -adrenoceptors agonists in PKC inhibition in healthy rats differ. It has been demonstrated that PKC inhibition had no significant effect on the contractile responses to PhE of the tail artery of healthy rats but evoked decrease in sensitivity to PhE in aorta (Kizub et al., 2010). On the other hand, there is evidence that PKC inhibition with other potent PKC inhibitors Ro-318220 and calfostin C in mesenteric arteries of healthy rats had no effect on maximum amplitude of norepinephrine (NE)-evoked contraction but significantly elevated its sensitivity to NE (Mueed et al., 2005).

The role of PKC in enhancement in contractility of the pulmonary artery has not been demonstrated before and is clearly shown in the present study for the first time. It has only been demonstrated previously by Yamada and Yokota (Yamada and Yokota, 1997) that in human pulmonary arteries endothelial cell culture PKC activators stimulated endothelin-1 (ET-1) release, whereas PKC inhibition with staurosporine led to decrease in both basal and stimulated levels of ET-1. These authors have also demonstrated that PKC activators stimulated prostacyclin (or prostaglandin I₂, PGI₂) release (Yamada and Yokota, 1997).

Although it has been shown in systemic vessels (aorta, mesenteric and tail artery) from STZ-diabetic rats that PKC inhibition with chelerythrine had no effect on sensitivity of these arteries to PhE (Kizub et al., 2010), studies on the deendothelised mesenteric artery of diabetic rats have demonstrated that PKC inhibition suppressed elevation in NE-induced contractile response (Mueed et al., 2005). In contrast to this, other authors have shown that PKC inhibition did not affect hyperreactivity to prostaglandin

 E_2 (PGE₂) in the mesenteric artery of Goto-Kakizaki rats with 2-type DM (Ishida et al., 2012).

Our results showed that PKC inhibition in the deendothelised pulmonary artery from diabetic animals had no effect on sensitivity to PhE. It may allow us to suggest that mechanisms of pulmonary artery contractility enhancement in DM can be associated with activity of PKC in the endothelium. PKC involvement in vascular contractile abnormalities in DM can be mediated by a few mechanisms described. PKC activation in vascular endothelium in DM can result in endothelium-dependent vasodilator dysfunction via inhibition of the pathways associated with nitric oxide (NO) (Ishii et al., 1996; Beckman et al., 2002; Pitocco et al., 2010; Kizub et al., 2014), endothelium-derived hyperpolarizing factor (EDHF) (Gao et al., 2011), and PGI₂ (Cosentino et al., 2003). Secondly, PKC activation in the endothelium in DM can enhance endotheliumdependent vasoconstriction mediated by ET-1 (Matsumoto et al., 2009), PGE₂ and thromboxane A₂ (TXA₂) (Cosentino et al., 2003). Activation of nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidase or Nox) have also been shown to be involved in PKC-mediated endothelial dysfunction in DM via reactive oxygen species (ROS) formation (Gao et al., 2011; Kolluru et al., 2012; Kizub et al., 2014).

NO-associated mechanisms underlying diabetes-associated vascular dysfunction may include decreased endothelial nitric oxide synthase (eNOS) activity and expression (Hirata et al., 1995), uncoupling of eNOS and degradation of NO secondary to enhanced superoxide production (Cosentino et al., 2003; Pitocco et al., 2010), attenuation of NO signaling and decreased NO bioavailability (Matsumoto et al., 2009). In diabetes PKC may affect NO bioavailability not only via intracellular accumulation of ROS (Pitocco et al., 2010) but also by decreasing eNOS activity (Hirata et al., 1995). It is established that dysfunction of eNOS in DM can be associated with its suppression by some PKC isoforms (Hirata et al., 1995; Ishii et al., 1996; Bohlen and Nase, 2001; Mehta et al., 2009). As has been shown in DM, PKC can phosphorylate eNOS (Hirata et al., 1995) on its inhibitory site Thr495 (Fleming et al., 2001) and reduces eNOS phosphorylation on the activating Ser1177 site (Michell et al., 2001) blunting eNOS activity. On the other hand, PKC-mediated inhibition in eNOS activity is linked to the ability of PKC to phosphorylate another inhibitory phosphorylation site Thr497 of eNOS reducing its affinity for calmodulin and, hence, the generation of NO (Matsubara et al., 2003). Alternatively, PKC-dependent reduction in eNOS expression has been shown in retinal (Suzuma et al., 2002) and aortic (Hink et al., 2001) endothelial cells in diabetes. Decreased eNOS expression in diabetes can occur through PKCmediated activation of vascular Nox by inducing ROS-dependent scavenging and reducing NO level (Inoguchi et al., 2000).

EDHF also can be involved in endothelial dysfunction development in DM. It has been established that EDHF-type vasorelaxation is impaired in DM (Matsumoto et al., 2006; Gao et al., 2011; Leo et al., 2011). The relaxant pathway associated with SMCs hyperpolarization is thought to be independent of NO and prostacyclin production by the endothelial cells and has been attributed to the release of EDHF (Feletou and Vanhoutte, 2010). The identity of EDHF remains controversial, but rather than a true chemical mediator of endothelium-dependent hyperpolarization, there is considerable support for the view that EDHF reflects a hyperpolarization signal that is mediated from endothelial cells to vascular SMCs via myoendothelial gap junctions (MEGJs) (Matsumoto et al., 2006; Figueroa and Duling, 2009; Feletou and Vanhoutte, 2010). Direct communication within and between endothelial cells and SMCs through gap junctions (GJs) is an important modulator of vascular tone and essential in the control and coordination of vascular function (Figueroa and Duling, 2009; Feletou and Vanhoutte, 2010). The vascular GJs are composed of intercellular channels clusters allowing the direct passage of electrical current and small signaling molecules between adjacent cells (Figueroa and Duling, 2009). In vascular tissues GJs consist of connexin proteins (Cx37, Cx40, Cx43 and Cx45) (Brisset et al., 2009).

Connexin phosphorylation is highly sensitive to glucose concentration through the regulation via PKC dependent signaling pathway (Lin and Takemoto, 2005). It has been demonstrated that diabetes affects MEGJs function in resistance arteries (Lin and Takemoto, 2005; Georgescu et al., 2006). Endothelial Cx37 and Cx40 protein expression levels and endothelial GJs consisting of these connexins have been reported to be reduced in different vascular regions of STZ-induced type 1 diabetic mice (Hou et al., 2008; Makino et al., 2008). A few studies have reported that exposure to high glucose results in a down-regulation of Cx43 in vascular endothelial cells (Sato et al., 2002; Chen et al., 2008; Li and Roy, 2009).

Inoguchi and coauthors have reported in bovine aortic endothelial cells, that hyperglycemia inhibited gap junctional intercellular communication via PKC (Inoguchi et al., 1995; Inoguchi et al., 2001). Similar results have been demonstrated in retinal microvessels of rats with STZ-induced diabetes (Oku et al., 2001). It has been also demonstrated that high glucose levels, via PKC-mediated phosphorylation of Cx43, can reduce gap junctional intercellular communication activity in bovine aortic SMCs (Kuroki et al., 1998).

Diabetes and hyperglycemia may induce an increase in expression end secretion of ET-1 in vascular tissues as well (Kalani, 2008). Investigation of ET-1 in the development of abnormal retinal hemodynamics in DM showed that overexpression of ET-1 is associated with PKC activation, presumably PKC- β_2 and PKC- δ isoforms (Park et al., 2000). It has been also reported that increase in contraction in response to ET-1 in the aorta and coronary arteries of rats is associated with augmented PKC activation in type 1 DM (Hattori et al., 1999; Tickerhoof et al., 2003).

Cyclooxygenases (COX)-derived prostanoids, which are arachidonic acid metabolites, also can play a significant role in diabetic vascular complications and have been implicated in hyperglycemia-induced endothelial dysfunction (Cosentino et al., 2003; Aljofan and Ding, 2010; Ishida et al., 2012). As has been shown in human aortic endothelial cells, hyperglycemia causes PKC-dependent increase in expression of inducible COX-2 isoform associated with an increase in TXA₂ and a reduction of PGI₂ release (Cosentino et al., 2003). In this mechanism glucose-induced activation of PKC may result in eNOS-dependent formation of peroxynitrite and tyrosine nitration and inactivation of PGI₂ synthase (PGIS) (Cosentino et al., 2003). Production of vasoactive eicosanoid PGE₂ can be also increased in diabetic vessels (Xia et al., 1996).

Conclusions

The present study shows that type 1 DM leads to increase in sensitivity to PhE in the pulmonary artery of rats reflecting enhancement in vascular α_1 -adrenoceptor-mediated contractility and that mechanisms of such enhancement in DM are associated with activity of PKC in the endothelium rather than in vascular SMCs. These mechanisms and the role of endothelium-mediated signaling in this process remain to be investigated in future studies.

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