Assessment of vascular reactivity at different time-course on streptozotocin-induced diabetic rats

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Summary

Objective: Impairment of vascular reactivity in diabetes may be due to endothelium and/or vascular smooth muscle dysfunction. This study aimed to verify the role of endothelium and vascular smooth muscle in diabetes at different time-course after streptozotocin (STZ) administration.

Method: Endothelium-intact and denuded aortic rings obtained from non-diabetic and diabetic rats at different time-course (1-4 weeks) were subjected to graded concentrations of potassium chloride (KCl) and phenylephrine (PE), L-NAME and indomethacin, respectively.

Results: Vasoconstrictor responses (maximum contractility, \(E_{\text{max}}\)) to KCl in endothelium-intact and denuded aortic rings were significantly influenced at week 4 (\(P<0.05\)). In response to PE, \(E_{\text{max}}\) values of endothelium-intact and denuded rings significantly decrease as diabetes progresses (\(P<0.05\)). The \(pD_2\) values were significantly greater in endothelium-denuded than endothelium-intact diabetic aortic rings starting from 2 weeks onwards (\(P<0.05\)). In the endothelium intact, PE-induced contractile responses (\(E_{\text{max}}\) and \(pD_2\)) in the presence of L-NAME and indomethacin were significantly greater than PE alone (\(P<0.05\)). It was found that the percentage of calcium released from intracellular stores sensitive to PE decreased, due to diabetes.

Conclusion: This study clearly demonstrated that the alterations in vascular reactivity to vasoconstrictor phenylephrine were significantly influenced by the duration of diabetes.

Key words: Aortic rings; Indomethacin; L-NAME; Phenylephrine; Vascular reactivity; STZ-induced diabetes

Introduction

Endothelial dysfunction is a critical and initiating factor in developing vascular disease in diabetic state. Abnormalities in vascular reactivity have been implicated in the pathophysiology of different forms of cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes and chronic renal failure [1]. In patients with diabetes, there is an increase in vasoconstrictors and decrease in vasodilators [2]. The level of secretion of nitric oxide (NO) and prostacyclin is diminished in these individuals [3, 4].

The results of vascular reactivity studies in animal models of diabetes have reported conflicting findings. Some investigators found that contraction of the thoracic aorta in response to vasoconstricting agents was impaired [5, 6] whereas others indicated enhanced vascular reactivity to vasoconstricting agents involving diabetic and non-diabetic animals [7, 8]. The contractile dysfunctions might be due to defective in \(Ca^{2+}\) signaling mechanisms, including reductions in L-type \(Ca^{2+}\) channel current, depressed sarcoplasmic reticulum \(Ca^{2+}\) uptake and release mechanisms, and reduced rate of \(Ca^{2+}\) efflux on the Na\(^+\)-\(Ca^{2+}\) exchange [9].

The effects of diabetes on agonist-induced as well as endothelium-dependent reactivity in most cases were performed using the aorta from diabetic animals. There is not satisfactory explanation for the variable responses of diabetic blood vessels to vasoconstrictor and vasodilator agents. There is evidence to indicate that the effect of diabetes on vascular reactivity differs in different vessels and at different durations, from acute (1 day to 6 weeks) to chronic (10 weeks to 1 year) [10]. No conclusive findings were expounded on the intraindividual variations within the week of diabetic rats. To address this issue, we investigate the time-dependent relationship in vascular diabetic animals and endothelial function at different stages of diabetes using the streptozotocin (STZ)-induced diabetic rat model. We hypothesized that the impairment in vasoconstriction starts as early as 1 week and becomes more prominent at later stages of the disease. This scenario may be mediated at the level of NO and prostanoids production from endothelium and/or impaired intracellular \(Ca^{2+}\) signaling at vascular smooth muscle.

The present study focuses on issues regarding endothelial cell function in STZ-induced diabetic...
rats, including the role of endothelium in vascular reactivity and potential cellular mechanisms that may contribute to vascular dysfunction during diabetes. The contractile responses to Phenylephrine (PE) in endothelial-intact and denuded aortic rings from non-diabetic and STZ-induced diabetic rats were studied. Assessment of NO or prostanoids contribution was done by inhibiting nitric oxide synthase (NOS) or cyclooxygenase (COX) with L-NAME and indomethacin, respectively. The influence of the duration of diabetes was studied at 1 to 4 weeks after STZ injection.

Materials and methods

Animals

Male Sprague Dawley (SD) rats with body weight between 200-250 g were used in this investigation. The rats were housed in individual cages in standard environmental conditions with 12 h day/night cycle and free access to tap water and standard rat chow (Gold Coin Feedmills, Malaysia). The rats were maintained at the Animal House of Universiti Sains Malaysia. All procedures involving rats were conducted according to the ethical guidelines approved by the Animal Ethics Committee, Universiti Sains Malaysia.

Induction of diabetes and physiological data collection

Diabetes was induced by intraperitoneal (i.p.) injection of STZ (Sigma, USA) (60 mg/kg body weight in 0.9% NaCl, pH 4.5) to rats fasted for 16 hours. Their diabetic conditions were confirmed by the high fasting blood glucose concentration 72 hours after injection of STZ. Rats with a fasting blood glucose concentration more than 15 mmol/L were considered diabetic and used in the experiment. Blood samples were collected from the tail vein and the blood glucose concentrations were tested using a blood glucose meter (Accu-Check Advantage, Roche, Germany). Metabolic control was evaluated on the basis of plasma glucose concentration and animal body weight before and at 1, 2, 3 and 4 weeks after STZ administration without insulin supplements.

In vitro studies of isolated aorta [11]

The rats were anesthetized with sodium pentobarbitone (60 mg/kg of body weight, i.p.). The chest and the abdomen were dissected through a medial sternotomy, and the thoracic aorta was excised and immediately placed into cold oxygenated Krebs solution, pH 4.5 (in mmol/L: NaCl, 118.6; KCl, 4.8; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.1; and glucose, 11). The thoracic aorta was then cleaned of excess connective tissue and fat, and sliced into rings with approximately over distending the vessel. The aortic rings were mounted horizontally between two stirrups in the organ chambers to be filled with 10 mL of Krebs solution (37°C; 95% O2 and 5% CO2). One stirrup was connected to an anchor and the other to an isometric force transducer (Grass FT03D) connected to a computerized data acquisition system (PowerLab; ADInstruments Pty Ltd, Australia). The rings were equilibrated for 60 minutes under a resting tension of 1 g before experiments work begun. During the equilibrium period, the rings were washed every 30 minutes.

Once the rings were stabilized, the presence of functional endothelium was assessed by the ability of acetylcholine (ACh, 0.1 µM) to induce more than 60% of relaxation of rings precontracted submaximally with PE (10-6 M) [5]. Denudation was achieved by gently rubbing the intimal layer of the vessel with a wire. Aortic rings were considered denuded when there was less than 10% relaxation to ACh.

After the functional endothelium was tested, the aortic rings were washed three times with the Krebs-Ringer bicarbonate solution and adjusted to 1 g tension before subjected to different drugs. Once the tension had stabilized, the aortic rings taken from non-diabetic and diabetic rats of different weeks after diabetic induction were used to run the following protocols.

To assess the role of non-receptor-mediated vasoconstrictor, the aortic rings either in the presence or absence of endothelium were subjected to cumulative concentration-response curves of KCl (10-50 mM). Then, the aortic rings with or without endothelium were subjected to cumulative concentrations-response curve of PE (1 nM – 10 µM). Thirdly, the aortic rings with the presence of endothelium were incubated for 30 minutes with 100 µM of L-nitroarginine methyl ester (L-NAME) to inhibit NO production [8]. After incubation, the rings were subjected to cumulative concentration-response curves of PE (1 nM – 10 µM). Indomethacin (10 µM) was used to inhibit prostanooid synthesis (these experiments were run in different rings). Indomethacin was incubated for 30 minutes followed by cumulative concentration-response curves of PE (1 nM – 10 µM) [12].

To assess the vascular reactivity of smooth muscle cells on intracellular Ca2+ release sensitive to PE, the rings were exposed to Ca2+-free solution that contained 0.1 mM ethylene glycol tetraacetic
acid (EGTA) for 15 minutes before application of 1 μM PE to induce the first transient contraction. The rings were then washed with normal Krebs solution containing CaCl$_2$ three times and incubated for at least 40 minutes for refilling of the intracellular Ca$^{2+}$ stores. Subsequently, the medium was rapidly replaced with Ca$^{2+}$-free solution and the rings were incubated for 15 minutes. The second contraction was then induced by PE. The contraction in Ca$^{2+}$-free solution was calculated as percentage of the maximal contraction in the absence of extracellular Ca$^{2+}$ relative to PE in Ca$^{2+}$-containing medium. Similarly, the contractile responses to PE in the normal Ca$^{2+}$-containing medium was also calculated [13].

**Drugs**
Phenylephrine hydrochloride, L-NAME, indomethacin and ACh hydrochloride were obtained from Sigma Aldrich (St. Louis, MO, USA). Indomethacin was dissolved in 5% sodium carbonate [14]. Other drugs were dissolved in normal saline (0.9% NaCl).

**Data analysis**
Concentration-response curves to KCl and PE are expressed in grams. The response was considered as the difference between absolute tension developed and baseline tension. All values are expressed as mean ± S.E.M (standard error of the mean). Concentration-response relationships were evaluated by comparing the tension achieved during maximum constriction (E$_{\text{max}}$) and the negative logarithm of the concentration producing 50% of maximum responses (-log EC$_{50}$). The sensitivity of the agonists is expressed as pD$_2$; pD$_2$ values were derived from nonlinear regression analysis, using the computer software GraphPad Prism 5.0 for Windows (GraphPad Software Inc., USA). Results were analysed using Student’s t test and analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. P value less than 0.05 was considered significant.

**Results**
**Characteristics of diabetic animals**
All rats that received STZ became diabetic. The blood glucose levels of non-diabetic and diabetic rats remained between 4-7 mmol/L and above 15 mmol/L, respectively, throughout the study (Table 1). There is a tendency to reduce the blood glucose levels after 3 weeks of induction. On the other hand, the body weights of diabetic rats were significantly lower than those of non-diabetic rats and start to stabilize after 3 weeks of induction. The body weights of non-diabetic rats significantly increased every week compared to baseline of non-diabetic rats (Table 2).

**Vascular contraction to KCl and PE**
KCl, a non-receptor-mediated vasoconstrictor, produced concentration-dependent contractions of aortic rings obtained from non-diabetic and different weeks of STZ-induced diabetic rats (Fig.1). The functional removal of the endothelium did not affect the concentration dependent contractions in all groups. The lowest tension was significantly developed in the rings of diabetic rats at 4 weeks as reflected by t values between non-diabetic and diabetic aortic rings, either with or without the presence of endothelium.

Phenylephrine induced a concentration-dependent contraction of aortic rings either in the presence or absence of an intact endothelium obtained from non-diabetic and at different weeks of STZ-induced diabetic rats (Fig.2). In the non-diabetic aortic rings, the pD$_2$ value was unaltered but the maximum response found to be significantly higher after removal of endothelium. The lowest tension in endothelial-intact rings was significantly developed at 2 weeks whereas the lowest tension in denuded rings developed at 4 weeks after STZ induction (P<0.05, Table 4). The response to PE was higher in denuded aortic rings in both non-diabetic and diabetic groups. The concentration-dependent contractions of denuded diabetic aortic rings shifts to the left starting from week 2 onwards and

**Table 1.** Plasma glucose concentrations of non-diabetic and diabetic rats at the time the vascular reactivity experiments were conducted.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic (mmol/L)</th>
<th>Diabetic (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.31 ± 0.08</td>
<td>4.51 ± 0.34</td>
</tr>
<tr>
<td>1 wk</td>
<td>6.03 ± 0.18</td>
<td>30.84 ± 0.8*</td>
</tr>
<tr>
<td>2 wk</td>
<td>5.39 ± 0.18</td>
<td>30.49 ± 1.31*</td>
</tr>
<tr>
<td>3 wk</td>
<td>5.24 ± 0.18</td>
<td>25.04 ± 2.2*</td>
</tr>
<tr>
<td>4 wk</td>
<td>5.18 ± 0.09</td>
<td>27.59 ± 1.64*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M of glucose measurement in mmol/L. N=5 for each level. *P<0.05 vs. baseline levels.

**Table 2.** Average body weight of rats from non-diabetic and diabetic rats at the time the vascular reactivity experiments were conducted.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic (gram)</th>
<th>Diabetic (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>217.60 ± 5.57</td>
<td>217.25 ± 10.56</td>
</tr>
<tr>
<td>1 wk</td>
<td>249.80 ± 7.64*</td>
<td>193.50 ± 11.06</td>
</tr>
<tr>
<td>2 wk</td>
<td>271.20 ± 8.16*</td>
<td>187.75 ± 8.87*</td>
</tr>
<tr>
<td>3 wk</td>
<td>294.40 ± 6.17*</td>
<td>172.00 ± 5.99*</td>
</tr>
<tr>
<td>4 wk</td>
<td>304.40 ± 5.90*</td>
<td>171.50 ± 5.56*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M of the average body weight in gram. N=5 for each level. *P<0.05 vs. baseline levels.
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Figure 1. Aortic rings with intact (●) or denude (■) endothelium were stimulated with increasing concentration of KCl (mM) in non-diabetic and diabetic rats. Vertical bars represent S.E.M for 5 rats each. *P<0.05 between intact and denuded rings.

Figure 2. Aortic rings with intact (●) or denude (■) endothelium were stimulated with increasing concentration of PE (M) in non-diabetic and diabetic rats. Vertical bars represent S.E.M for 5 rats each. *P<0.05 between intact and denuded rings.

Table 3. The effects of endothelial-intact and denuded aortic rings on the sensitivity (pD2) and the maximal effect (E_max) of KCl-induced contraction in isolated rat aortic ring preparations from non-diabetic and diabetic rats.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>KCl</th>
<th>Endothelium intact</th>
<th>Denuded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E_max (g)</td>
<td>pD2 (-log EC50)</td>
<td>E_max (g)</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>0.51 ± 0.04</td>
<td>-15.22 ± 2.92</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>Diabetic 1 wk</td>
<td>0.56 ± 0.03</td>
<td>-14.73 ± 3.29</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>Diabetic 2 wk</td>
<td>0.54 ± 0.03</td>
<td>-14.95 ± 2.09</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>Diabetic 3 wk</td>
<td>0.44 ± 0.02</td>
<td>-14.59 ± 1.67</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>Diabetic 4 wk</td>
<td>0.35 ± 0.02</td>
<td>-14.34 ± 2.32</td>
<td>0.29 ± 0.02*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M of 5 rats used in the present study. *P<0.05 compared to non-diabetic rats; †P<0.05 compared to intact aortic rings of the same group.

Table 4. The effects of endothelial-intact and denuded aortic rings on the sensitivity (pD2) and the maximal effect (E_max) of PE-induced contraction in isolated rat aortic ring preparations from non-diabetic and diabetic rats.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endothelium intact</td>
</tr>
<tr>
<td></td>
<td>E_max (g)</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>Diabetic 1 wk</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>Diabetic 2 wk</td>
<td>0.53 ± 0.02*</td>
</tr>
<tr>
<td>Diabetic 3 wk</td>
<td>0.56 ± 0.02*</td>
</tr>
<tr>
<td>Diabetic 4 wk</td>
<td>0.53 ± 0.03*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M of 5 rats used in the present study. *P<0.05 compared to non-diabetic rats; †P<0.05 compared to intact aortic rings of the same group.
showed significantly higher pD₂ values compared than those of endothelial-intact rings. In summary, increased sensitivity is shown by higher pD₂ values in denuded diabetic rings, but endothelium-intact rings shows opposite behaviour as diabetic progress.

To assess the contribution of the endothelium-derived relaxing factor (i.e., NO) in response to PE, the aortic rings of both non-diabetic and diabetic rats were incubated for 30 minutes in NOS inhibitor, L-NAME. The contractile responses of both non-diabetic and diabetic aortic rings responded to L-NAME were found to show greater tension development (Fig.3). The E_max values in the presence of L-NAME showed significantly more tension as compared to aortic rings without L-NAME (P<0.05, Table 5), presumably due to increase of NO production before L-NAME treatment. In terms of pD₂ values, treatment with L-NAME showed the lowest pD₂ values at 2 and 3 weeks compared to non-diabetic rats.

In order to determine the role of the vasodilator prostacyclin in the endothelium-dependent modulation of contractile responses, the effects of prostaglandin synthesis inhibitor indomethacin (10 µM) were tested in non-diabetic and diabetic aortic rings. The maximal contractile response to PE was lower with indomethacin in non-diabetic rats. Indomethacin increased PE-induced contractions in diabetic aortic rings at 2 and 4 weeks after diabetic induction (Fig.4). There was no difference in sensitivity (pD₂) or maximal contractility (E_max) between non-diabetic and different weeks of diabetic rats except for higher E_max value at 4 weeks diabetes with indomethacin. In contrast, PE dose-response curves were shifted to the left in the presence of indomethacin in all groups as compared to without indomethacin, thus demonstrating an increased sensitivity to PE as reflected by pD₂ values (P<0.05, Fig.4).

**Figure 3.** Contractile response to an increasing concentration of PE in the aortic rings with the absence (●) or presence (■) of L-NAME from non-diabetic and diabetic rats. The response to PE is expressed as absolute tension. Vertical bars represent S.E.M for 5 rats. *P<0.05 compared to aortic rings without L-NAME.

**Figure 4.** Contractile response to an increasing concentration of PE in the aortic rings with the absence (●) or presence (■) of indomethacin from non-diabetic and diabetic rats. The response to phenylephrine is expressed as absolute tension. Vertical bars represent S.E.M for 5 rats. *P<0.05 compared to aortic rings without indomethacin.
Role of intracellular and extracellular calcium mobilization on the PE-induced contraction

To assess the contribution of extracellular calcium mobilization, the aortic rings were incubated in Ca\(^{2+}\)-free medium containing 0.1 mM EGTA. In endothelium-denuded aortic rings, a transient contractile response in Ca\(^{2+}\)-free medium was elicited by 10^{-6} M PE. A second contraction known as sustained contraction was then induced again by PE. The percentage contractile response seems to significantly reduced in diabetic rats as early as 1 week in responses to PE in Ca\(^{2+}\)-free medium (P<0.05, Fig. 5). When the same protocol was repeated in normal Ca\(^{2+}\)-containing medium, no significant difference was seen in diabetic aortic rings.

Figure 5. Histograms showing the mean of the response induced by 10^{-6} M PE without endothelium in Ca\(^{2+}\)-containing (2.5 mM CaCl\(_2\)) or Ca\(^{2+}\)-free medium. Results are expressed as mean ± S.E.M. (N=8). *P<0.05 between diabetic groups compared with the non-diabetic group.

Discussion

STZ has been shown to cause direct irreversible damage to \(\beta\)-cells of pancreatic islets of Langerhans, resulting in degranulation and loss of insulin secretion. Our previous studies demonstrated that the number of insulin-positive cells using immunohistochemical methods decreased markedly 72 hours after STZ injection compared with normal rats [15]. However, the percentage of insulin-positive cells per islet was increased after 14 days of STZ induction. In the present study, the blood glucose levels have a tendency to reduce after 3 weeks of induction. The possible explanation for the finding is that the pancreatic endocrine cells have the potential to proliferate the partially destroyed \(\beta\)-cells after induction of diabetes with STZ. In contrast to blood glucose, body weight in STZ-induced diabetic rats decreases every week and stabilizes 3 weeks after STZ. Non-diabetic rats gain weight throughout the 4 weeks.

Our study shows no significant differences in KCl mediated vasoconstriction between endothelial intact and denuded aortic rings in diabetic or non diabetic rats. This suggests no endothelial influence on the contraction to the non-receptor mediated agonist [16]. The study also reveals that 4-week STZ-induced diabetes inhibits the contractions induced by KCl in both endothelium-intact and denuded aortic rings in a dose-dependent manner (Fig. 1). The present results suggest that the effectiveness of potassium channels decrease with progression of diabetes, which is independent of endothelium, which in turn reduce the calcium influx through voltage-dependent calcium channels. It is well known that KCl-induced contraction mainly results from Ca\(^{2+}\) influx upon depolarization of the cell membrane, which activates voltage-dependent L-type Ca\(^{2+}\) channels [17-19], which further activate Ca\(^{2+}\)-induced Ca\(^{2+}\)-release through a ryanodine receptor at sarcoplasmic reticulum [20]. A decreased sensitivity to Ca\(^{2+}\) under depolarizing conditions suggests that there is either a suppression of Ca\(^{2+}\) channels or a decreased sensitivity to Ca\(^{2+}\) at the level of the contractile proteins [13].

The contractile response to PE is significantly higher in non-diabetic aortic rings after removal of the endothelium. This suggests the possibility that the release of endothelium-derived relaxing factors (EDRF) such as NO and prostacyclin contribute to resting basal tone and its inhibition by removing the endothelium induces an increase in tension and enhances the effect of vasoconstrictors which normally induce NO release. Another important facet of this investigation is the evaluation of possible factors or lack of factors responsible for altered responses in vasoconstrictions at various stages of disease. For example, the E\(_{max}\) value for both agonists (KCl and PE) of the non-diabetic rats is higher compared to E\(_{max}\) values of diabetic rats. We hypothesized that an increase in tension in the denuded non-diabetic rats may produce excess
vasoconstrictor prostaglandins (e.g., TXA₂) since inhibition of prostanoid synthesis with indomethacin reduces the response in the non-diabetic rats (Table 5).

The present study demonstrates that time-dependent hyperreactivity in the PE-induced contraction starts at 2 weeks in endothelium-intact and 1 week in denuded aortic rings after induction of diabetes (Table 4). The contractility reduces in both endothelium-intact and denuded aortic rings as diabetes progresses. In endothelium-intact aortic rings, we cannot exclude the possible contribution of the superimposition of the variable EDRFs such as NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) in reducing the maximum tension (E\text{max}) in diabetes rats. Furthermore, there are reports of increased vasodilator eicosanoids production such as prostacyclin in aortic rings of early stages of diabetes (i.e., 4-week diabetic rats) [5]. Our results agree with the observation and demonstrate that endothelium-derived prostanooids (prostacyclin) may facilitate the vasodilating effects of PE in diabetic aortic rings, since enhanced contractile responses to PE after prostanoïd’s inhibitor with indomethacin is observed at 2 weeks onwards (Table 5).

Based on our observations, the contractility significantly decreases at 1 week after STZ induction in denuded aortic rings (Table 4). Another possibility that diminished contraction to PE in denuded aortic rings may be due to inhibition of the intracellular Ca\textsuperscript{2+} release from the sarcoplasmic reticulum at the level of vascular smooth muscle. To test this possibility, Ca\textsuperscript{2+}-free medium experiment is implemented in denuded aortic rings. Our results show that there is a markedly reduced PE-induced contraction in Ca\textsuperscript{2+}-free medium as early as 1 week after diabetic induction (Fig.5), indicating inhibition on intracellular Ca\textsuperscript{2+} release. The results of the present study are in accordance with the previous studies which stated that inhibited vessel contraction through blocking calcium influx and intracellular calcium release [21]. In recent year, the evidence suggests that the store-operated Ca\textsuperscript{2+} channels are altered in diabetes and contribute to the hyporesponsiveness of vascular smooth muscle [11, 22]. The result is in accordance with the finding reported by Choi et al stated that the defects in Ca\textsuperscript{2+} signaling mechanisms including reductions in L-type Ca\textsuperscript{2+} channel current, depressed sarcoplasmic reticulum Ca\textsuperscript{2+} uptake and release mechanisms, and reduced rate of Ca\textsuperscript{2+} efflux on the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange [9].

L-NAME treatment is conducted in the presence of endothelium-intact specifically to inhibit the synthesis of NO from the endothelial cells [23]. The results show the PE-induced contraction significantly enhances in the presence of L-NAME in both non-diabetic and STZ-induced diabetic rats. The results are in accordance with the previous studied by Sanchez et al suggested that NO release blunts the response of the aortic rings to PE [24]. It has been suggested that diminished contraction to agonists in arterial rings with endothelium is due to the basal release of NO [24-26]. Furthermore, it is well known that the endothelium not only mediates relaxation but is a source of contracting factors (EDCF); EDCF can profoundly affect vascular tone and counteract relaxing factors produced within the endothelium [27]. Therefore, the enhancement in contraction to PE in the presence of L-NAME may be also attributed to the EDCF. Our observations also show that denuded diabetic rings possess only slight increment in PE-induced contraction presumably due to the lack of EDCF since endothelium has been removed in denudation.

Moreover, removal of endothelium shifts the PE concentration response curve to the left as reflected by increased in pD\textsubscript{2} values in diabetic rats. Thus, the sensitivity of the denuded diabetic aortic rings
to PE was found to be enhanced as compared to endothelium-intact diabetic rings presumably due to the lack of NO. Vice versa, note that an increase in NO synthesis and release would appear to be responsible for the decreased sensitivity to PE in the endothelial-intact aortic rings.

The results clearly show that vasoconstriction in endothelium-intact aortic rings from STZ-induced diabetic rats varies with time. The results suggest a major role for changes in production of eicosanoids and/or NO in altering the responsiveness of aortic rings from diabetic rats after STZ injection. In addition, this time-dependent vascular reactivity of the STZ-induced diabetic rats in the PE-induced contraction seems to be associated with receptor-mediated agonist as early as one week after STZ whereas KCl, a non-receptor-mediated agonist, only starts at 4 weeks after STZ-induced diabetes. More importantly, our observations may help to reconcile the discrepant data in the literature which exhibits either increased [7, 8] or decreased vasoconstriction in diabetes [5, 6]. A limitation of previous studies is that these evaluations focused on single point in time following onset of disease and which cannot take into account any fluctuating changes during the course of disease. Further studies are under investigation to elucidate the mechanisms involved in producing impaired responses to relaxing agents in the diabetic vasculature.

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References


