Mechanisms underlining gender differences in Phenylephrine contraction of normoglycaemic and short-term Streptozotocin-induced diabetic WKY rat aorta

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Abstract

The female gender reduces the risk, but succumbs more to cardiovascular disease. The hypothesis that short-term (8 weeks) Streptozotocin-induced diabetes could produce greater female than male vascular tissue reactivity and the mechanistic basis were explored. Aortic ring responses to Phenylephrine were examined in age- and sex-matched normoglycaemic/diabetic rats. The normoglycaemic male tissue contracted significantly more than the normoglycaemic female and the male/female diabetic tissues. Endothelial-denudation, l-NAME or MB reversed these differences suggesting an EDNO-cGMP dependence. 17β-oestradiol exerted relaxant effect on all endothelium-denuded (and normoglycaemic endothelium-intact male) tissues, but not endothelium-intact normoglycaemic female. The greater male tissue contraction is attributable to absent 17β-oestradiol-modulated relaxation. Indomethacin blockade of COX attenuated male normoglycaemic and female diabetic tissue contraction (both reversed by l-NAME), but augmented diabetic male tissue contraction. These data are consistent with the raised contractile TXA2 and PGE2 in normoglycaemic male and diabetic female tissues, and the relaxant PGI2 in diabetic male (and female). The higher levels of PGI2 in the normoglycaemic and diabetic female perhaps explain their greater relaxant response to Acetylcholine compared to the respective male. In conclusion, there is an endothelium-dependent gender difference in the effect of short term diabetes on vascular tissue reactivity which is COX mediated.

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1. Introduction

Gender (Pinna et al., 2001; Sanz et al., 2003) and diabetes mellitus (Pieper, 1998) influence vascular tissue reactivity to various contractile agonists. Diabetes-induced hyperglycaemia modifies the function of the l-arginine nitric oxide and/or cyclooxygenase pathways by increasing production of reactive oxygen species (ROS) which incapacitate the ability of the endothelium to secret antiatherosclerotic endothelium-derived relaxing factors (EDRFs) such as nitric oxide (NO) and prostaglandin I2 (PGI2; prostacyclin). Depending on the duration of disease, diabetes-induced ROS ultimately impair vasodilator and/or vasoconstrictor function (Pieper, 1999).

In short-term diabetes (up to 12 weeks), endothelium-dependent vasorelaxation is enhanced, whereas at latter stages, vasodilatation is diminished (Pieper, 1999). However, the data from several studies show inconsistencies with regards to the influence of diabetes mellitus on vascular contraction. Attenuation (Head et al., 1987; Myers and Messina, 1996; Misurski et al., 2001), enhancement (Abebe et al., 1990; Dresner et al., 1997) or unchanged contractile responses (Mulhern and Dorchety, 1989; Chang and Stevens, 1992) have all been observed. Differences in experimental conditions (type of vessels studied, age of the rats, rat strain, diabeticogenic agent employed and duration of diabetes) have been suggested to contribute to these discrepancies (Bell and Hye, 1983; Pieper, 1999). However, increasing evidence suggests that during short-term diabetes, these discrepancies arise from variations in the mobilization of the l-arginine nitric oxide and/or cyclooxygenase pathway under the various experimental conditions (Pieper, 1998; Browne et al., 2007).

The mechanism involved in the short-term diabetes-induced alteration of aortic contraction has been mostly demonstrated and understood in male animal models which may differ from the female animals. To the best of our knowledge, only few studies in this field have focused on the female gender (Pinna et al., 2001). We previously observed that short-term (8 weeks) diabetes attenuated agonist-evoked contraction of mesentery vessels isolated from male WKY rat (Chin et al., 2007). Since diabetes has been shown to be a stronger risk factor in the development of cardiovascular disease in the female gender than in the male (Barrett-Conner et al., 1991), we tested the hypothesis that short-term diabetes may produce greater diabetic female tissue reactivity (contractility) to Phenylephrine-compared to the male. Further, given that oestradiol promotes endothelial...
relaxant function (Mendelsohn and Karas, 1999) and has been reputed to reduce the rate of cardiovascular events (Stampfer and Colditz, 1991), the current study examined the effect of oestradiol on short-term diabetes-induced alterations in vascular contraction. The findings in this study may have implications for further understanding of the gender-related differences in cardiovascular disease outcome.

2. Methods

2.1. Animals

Male and female normoglycaemic Wistar–Kyoto (WKY) rats, aged 12–13 weeks were obtained from the University of Malaya animal unit, and all the experimental procedures were subjected to the University of Malaya and the International Medical University Animal Experimentation Ethics Committee approval. The animals were maintained under room conditions (temperature: 22 ± 2 °C, humidity 30–40%) with free access to food and water. Diabetes was achieved by a single dose (65 mg/kg of body weight, i.p.) of Streptozotocin (STZ) dissolved in cold normal saline. Plasma glucose levels was measured in animals 3 days following STZ-injection and animals were considered diabetic only if their blood glucose levels was ≥ 17 mmol/L. Diabetic animals were kept for 8 weeks before sacrifice.

2.2. Preparation and mounting of aortic ring

The vascular function of aortic rings with or without endothelium was assessed using methods previously described (Ajay et al., 2006, 2007). Briefly, normoglycaemic and diabetic rats were sacrificed by cervical dislocation. The chest cavity was exposed and the descending aorta was cut into small (3–4 mm) transverse rings. For endothelium-denuded tissues, the endothelium was removed by gentle rotation of the rings on an appropriately sized forceps. Both endothelium-intact and -denuded tissues were suspended between two L-shaped stainless steel hooks in a 5 ml organ bath containing normal Krebs physiological salt solution (KPSS) of the following composition (mM): NaCl 118.2, KCl 4.7, CaCl2 2H2O 2.5, KH2PO4 1.2, MgCl2 1.2, glucose 11.7, and NaHCO3 25.0. The bath solution was maintained at 37 °C and gassed with a mixture of O2 (95%) and CO2 (5%) throughout the study. The rings were stretched to a preload of 1 g and allowed to equilibrate for 35–40 min in KPSS, following which rings were contracted thrice (each for 5 min) with isotonic high potassium chloride solution (high K+, 80 mM) each followed by a washout. The integrity of the endothelium was assessed by exposing Phenylephrine (1 μM)-contracted tissues to the endothelium-dependent relaxant, Acetylcholine (ACh, 10 μM). The tissue was considered endothelium-denuded or endothelium-intact if the relaxation to ACh was ≤ 5% or ≥ 50% of the peak Phenylephrine-induced contraction, respectively. Some segments of aorta isolated from normoglycaemic and diabetic male/female rats were snap-frozen in liquid nitrogen and stored at 80 °C for subsequent biochemical analysis.

3. Experimental protocol

3.1. Contractile function

To test the role of endothelium in modulating vascular contractile function, endothelium-intact and -denuded male and female tissues were exposed to cumulative concentrations of Phenylephrine (10^{-5}–10^{-2} M) at 4 min intervals following a 20–25 min incubation in KPSS. To assess the possible role of the nitric oxide-cyclic GMP and the cyclooxygenase pathways on contractile function in diabetic rats, concentration-response curves to Phenylephrine (10^{-11}–10^{-2} M) was recorded in aortic rings incubated with the nitric oxide synthase inhibitor – Nω-nitro-l-arginine methyl ester (L-NAME, 10 μM), cyclic GMP inhibitor – Methylene blue (MB, 10 μM) or the cyclooxygenase (COX) inhibitor – Indomethacin (10 μM), respectively. To determine the effect of oestradiol in vascular reactivity during diabetes, endothelium-intact and endothelium-denuded aortic rings from both genders were incubated in 17β-estradiol (0.1 μM) for 20–25 min, following which dose-dependent responses to Phenylephrine were recorded. The possibility exists that fluctuations in the levels of the female reproductive hormones during the oestrous cycle may affect vascular function. For this reason the stages of the oestrous cycle in female rats on the day of sacrifice were determined by microscopic examination of vaginal smears. In normoglycaemic female tissues, we found no significant differences in vascular responses to Phenylephrine between the oestradiol rich (proestrus/oestrus) and oestradiol deficient (metestrus/diestrus) phases consistent with the finding of earlier workers (Li et al., 1997; Sanz et al., 2003), but diabetics caused a cessation of normal oestrous cycle resulting in a permanent diestrous state (Kim et al., 2006). Responses to Phenylephrine in normoglycaemic female were, therefore, used as controls for responses in diabetic female rats, irrespective of the state of the oestrous cycle. The contractile responses of aortic rings to graded concentrations of Phenylephrine were expressed as percentages of the maximum contractile effect of high K+ in respective tissues.

3.2. Relaxant function

To determine if NO function is impaired in the 8-week diabetic model, we exposed endothelium-intact normoglycaemic or diabetic male and female aortic rings to Phenylephrine (1 μM) and at the peak of the contraction, relaxation responses to cumulative increasing concentrations of ACh (10^{-14}–10^{-4} M) or Sodium nitroprusside (SNP) (10^{-14}–10^{-4} M) were recorded at 3 min intervals.

3.3. Assays of serum and tissue content of nitric oxide (as nitrite)

The effect of short-term diabetes on nitrite ion concentrations (in both genders) was assessed in serum and endothelium-intact aortic tissue homogenate samples using commercially procured enzyme immunoassay (EIA) kits (Assay Design, Stressgen). At sacrifice, blood samples from normoglycaemic or diabetic animals were collected by cardiac puncture, and on coagulation, serum was obtained by centrifugation (10,000 rpm for 6 min at 4 °C) and stored in −80 °C until analyzed. Segments of normoglycaemic or diabetic aorta which were preserved in liquid nitrogen following aortic ring preparation were thawed and homogenized in a Krebs-HEPES buffer. The assay for nitrite ion was measured in supernatant fractions according to the manufacturer’s assay procedure. The level of nitrite ion was expressed as pg/mL of serum or pg/mL mg tissue wet weight.

3.4. Assays of tissue content of prostaglandins

The effect of diabetes on productions of thromboxane TXB2 (a stable metabolite of TXA2) and PGE2 and 6-keto-PGF1α (a stable metabolite of PGI2) was measured in endothelium-intact aortic tissues (homogenized in phosphate buffer solution) using commercial EIA kits (Cusabio Biotech, Co. LTD). All assays were performed in supernatant fractions in accordance with the manufacturer’s instructions. The amounts of TXB2, PGE2 and 6-keto-PGF1α in the tissue were expressed as pg/mL mg tissue per wet weight.

4. Data analysis

Responses were recorded as mean ± standard error of the mean (S.E.M.) for the number of rats. The concentration-response curve
for each experimental condition was plotted and from it were deduced the values of maximal agonist-induced response ($R_{\text{max}}$) and the concentration of the agonist (expressed as negative log molar concentration) producing 50% of $R_{\text{max}}$ ($pEC_{50}$) (Prism version 6.0, Graph Pad Software, USA). Statistical evaluation of the data was performed using the Student’s $t$-test for unpaired observations and by two-way analysis of variance (ANOVA) for multiple group comparison followed by the Bonferroni post-hoc test for selected pairs. A value of $p<0.05$ was considered statistically significant.

5. Drugs

The following drugs were used: Acetylcholine chloride (ACh), Sodium nitroprusside (SNP), Indomethacin, $N^\alpha$-nitro-$\text{L}$-arginine methyl ester (L-NAME), Methylene blue (MB), Phenylephrine-HCl, $\beta$-oestradiol, all purchased from Sigma-Aldrich (Co., St. Louis, Mo., USA). Krebs salts were purchased from BDH Limited (Poole, England). All the drugs were dissolved in distilled water with the exception of 17$\beta$-oestradiol and Indomethacin. Indomethacin was dissolved in 0.5% w/v Sodium carbonate. 17$\beta$-oestradiol stock solution was prepared in 5% (v/v) Dimethyl sulfoxide (DMSO). The final concentration was prepared by serial dilutions with distilled water which adjusted the final concentration of DMSO to less than 0.05% (v/v). All drug concentrations were expressed in the final base molar concentration present in the organ bath.

6. Results

6.1. Weight and glycaemia profiles of male and female rats

The normoglycaemic female rats weighed significantly ($p<0.001$) less than the normoglycaemic male rats. Eight weeks after treatment with STZ, male and female diabetic animals attained body weight significantly less than the respective normoglycaemic controls (Table 1). Diabetic males showed a greater loss ($p<0.001$) in body weight than the females ($p<0.05$). Diabetic male and female rats showed similar plasma glucose levels which were higher ($p<0.001$) compared to the levels in age-matched normoglycaemic controls.

6.2. Effect of gender on Phenylephrine-evoked aortic contraction

Both normoglycaemic and diabetic male and female tissues (with or without endothelium) produced concentration-dependent contractions to Phenylephrine (Fig. 1/Table 2). Diabetes significantly attenuated ($p<0.001$) Phenylephrine contraction of endothelium-intact male tissues when compared to their normoglycaemic control, but had no significant effect in the female tissues. Endothelium-intact diabetic tissues from female rats contracted more than the equivalent male tissues. Removal of the endothelium abolished the observed contractile differences between the following categories: normoglycaemic and diabetic male tissues, the normoglycaemic male and female tissues, and between the diabetic male and diabetic female tissues. Denudation resulted in higher contraction of diabetic male aorta compared to the female (Fig. 1/Table 2).

6.3. Phenylephrine-evoked contraction of oestradiol-treated tissues

Exogenous oestradiol (17$\beta$-oestradiol; 0.1 μM) attenuated ($p<0.001$) Phenylephrine concentration–response curve in endothelium-intact normoglycaemic male aorta, but enhanced ($p<0.05$) it in diabetic tissues (Fig. 2/Table 2). In the female, oestradiol had no effect on Phenylephrine-induced contraction of endothelium-intact normoglycaemic or diabetic female aorta (Fig. 2/Table 2). In the absence of endothelium, oestradiol attenuated ($p<0.001$) Phenylephrine contraction in both male and female normoglycaemic and diabetic tissues. DMSO, which served as vehicle for oestradiol treatment did not alter Phenylephrine contractions in both normoglycaemic and
diabetic tissues from both genders, hence responses to Phenylephrine served as controls for studies on these tissues.

6.4. Effect of L-NAME or MB on Phenylephrine-evoked contraction

L-NAME or MB enhanced (p < 0.001) Phenylephrine concentration–response curve more in endothelium-intact normoglycaemic female tissues compared to the male. Phenylephrine contraction was enhanced more (p < 0.001) in L-NAME or MB treated diabetic tissues of male compared to the female (Fig. 2/Table 2). Treatment with either L-NAME or MB abolished differences in Phenylephrine contraction between normoglycaemic male versus the female or the diabetic male, and between diabetic male versus the diabetic female tissues.

**Table 2**

<table>
<thead>
<tr>
<th>R_{max} ± SEM</th>
<th>pEC50 ± SEM</th>
</tr>
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<tr>
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<td>Female</td>
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<table>
<thead>
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<th>Rats normoglycaemic</th>
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<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SEM</td>
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<tr>
<td>Endothelium-intact</td>
<td>Control</td>
<td>179.70±7.08</td>
<td>7.44±0.10</td>
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<td>17ß-oestradiol</td>
<td>134.90±6.77*</td>
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<td>L-NAME</td>
<td>187.70±5.49</td>
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<td>Methylene blue</td>
<td>164.10±4.85</td>
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<td>Indomethacin</td>
<td>120.10±8.04*</td>
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<td>L-NAME + Indomethacin</td>
<td>173.00±4.02*</td>
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<td>Endothelium-denuded</td>
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<td>217.70±7.44*</td>
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<td>L-NAME</td>
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<td>Methylene blue</td>
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<td>7.53±0.07</td>
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<td></td>
<td>Indomethacin</td>
<td>159.40±6.29*</td>
<td>7.51±0.12</td>
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<tr>
<td></td>
<td>L-NAME + Indomethacin</td>
<td>149.20±3.81*</td>
<td>7.58±0.08</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>238.40±5.59*</td>
<td>7.69±0.07*</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM (n=7–11). Statistics: *p<0.001, female compared to male tissues; †p<0.01, compared with corresponding male/female endothelium-intact; p<0.001, compared with corresponding male/female endothelium-denuded control tissues. p<0.001, endothelium-intact diabetic tissues compared to normoglycaemic tissues.

Fig. 2. Cumulative Phenylephrine (10^{-11}–10^{-5} M) concentration–response curves of 17ß-oestradiol-treated or untreated endothelium-intact (upper panel) or -denuded (lower panel) aortic rings from male and female normoglycaemic/diabetic WKY rats. n=4–9 in each group. (*p<0.001, female compared with male; †p<0.001, 17ß-oestradiol-treated compared with the corresponding male or female normoglycaemic/diabetic untreated controls).
Fig. 3. Cumulative Phenylephrine (10$^{-11}$–10$^{-5}$ M) concentration–response curves of N$\omega$-nitro-l-arginine methyl ester (l-NAME) (upper panel) or Methylene blue (MB) (lower panel)-treated or untreated endothelium-intact aortic rings from male and female normoglycaemic/diabetic WKY rats. n=6–13 in each group. (*p<0.01, female compared with male; #p<0.001, diabetic (treated/untreated) compared with the corresponding male/female normoglycaemic group; ‡p<0.01, l-NAME-treated compared with the corresponding male or female normoglycaemic/diabetic untreated controls).

Fig. 4. Cumulative Phenylephrine (10$^{-11}$–10$^{-5}$ M) concentration–response curves of N$\omega$-nitro-l-arginine methyl ester (l-NAME), Indomethacin or l-NAME + Indomethacin-treated (or their untreated controls) endothelium-intact aortic rings from male and female normoglycaemic (upper panel)/diabetic (lower panel) WKY rats. n=6–13 in each group. (*p<0.01, female compared with male; ‡p<0.001; l-NAME and/or Indomethacin-treated compared with the corresponding male or female normoglycaemic/diabetic untreated controls).
Values represent mean±SEM (n=6 normoglycaemic and diabetic WKY aorta pre-contracted with Phenylephrine (10⁻⁶ M)).

6.5. Effect of Indomethacin or l-NAME plus Indomethacin on Phenylephrine-evoked contraction

In the male rat, Indomethacin attenuated (p<0.001) Phenylephrine-evoked contraction of endothelium-intact normoglycaemic aorta, but enhanced (p<0.001) it in the diabetic aorta. In the female, it did not alter responses in normoglycaemic tissues but attenuated (p<0.001) contraction in diabetic tissues (Fig. 4/Table 2). l-NAME reversed the relaxant action of Indomethacin in normoglycaemic male and diabetic female tissues, but did not alter its contractile effect in diabetic male tissues (Fig. 4/Table 2).

6.6. The effect of ACh and SNP on Phenylephrine-evoked contraction

Both male and female endothelium-intact normoglycaemic and diabetic aorta pre-contracted with Phenylephrine showed dose-dependent relaxation to ACh or SNP. Normoglycaemic and diabetic female tissues were more responsive to ACh compared to the respective male tissues (Fig. 5/Table 3). In the diabetic group, responses to the exogenous NO releasing compound, SNP, was similar in both genders, but diabetes reduced the sensitivity of the male tissues to SNP (Fig. 5/Table 3).

6.7. Nitric oxide (nitrite ion) levels in normoglycaemic and diabetic tissues

Serum nitrite levels were higher (p<0.001) in normoglycaemic and diabetic female tissues compared to the respective male tissues. Diabetes significantly elevated (p<0.001) serum nitrite content in both genders when compared with the corresponding normoglycaemic controls, but aortic tissue nitrite level was enhanced (p<0.001) only in the female diabetic aorta (see Fig. 6).

6.8. Prostanoid levels in normoglycaemic and diabetic tissues

TXA₂ (TXB₂) and PGE₂ contents were higher (p<0.001) in normoglycaemic male tissues compared to the corresponding female tissues. In the male, diabetes significantly reduced (p<0.001) tissue concentrations of TXA₂ and PGE₂ in contrast to the female where the content of TXA₂ but not PGE₂ was elevated (p<0.001). The levels of both prostanoids, however, are significantly elevated in the diabetic female compared to the male tissues (Fig. 7). Normoglycaemic female tissues exhibited higher (p<0.01) PGL₂ (6-keto-PGF₁α) content compared to the male, and diabetes elevated (p<0.001) its levels in aortic tissues.

Table 3

<table>
<thead>
<tr>
<th>Rmax</th>
<th>pEC50</th>
<th>Rmax</th>
<th>pEC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>SEM</td>
<td>Male</td>
<td>SEM</td>
</tr>
<tr>
<td>Normoglycaemic</td>
<td>ACh</td>
<td>57.03 ± 3.88</td>
<td>7.63 ± 0.28</td>
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<tr>
<td>SN</td>
<td>100.40 ± 2.82</td>
<td>10.31 ± 0.15</td>
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</tr>
<tr>
<td>Diabetic</td>
<td>ACh</td>
<td>75.57 ± 3.39</td>
<td>7.05 ± 0.22*</td>
</tr>
<tr>
<td>SNP</td>
<td>102.50 ± 2.90</td>
<td>10.01 ± 0.15*</td>
<td></td>
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</tbody>
</table>

Values represent mean±SEM (n=6-9). Statistics: *p<0.05, female compared to male tissues; **p<0.01, ACh or SNP compared with corresponding control tissue.
from both genders (significantly \( p < 0.001 \)) more so in diabetic female tissues compared to the male (Fig. 7).

7. Discussion

This study investigated the effect of gender and STZ-induced diabetes (8 weeks) on the responses of WKY rat aorta to the \( \alpha_1 \)-adrenoceptor agonist, Phenylephrine. The role of the nitric oxide and cyclooxygenase pathways in regulating the contraction and the gender differences therein were also studied. The results show that Phenylephrine-induced contraction of normoglycaemic and diabetic tissues is influenced by endothelium-mediated and gender-dependent factors.

7.1. The role of the endothelium

Eight weeks following induction of diabetes, endothelium-intact normoglycaemic male tissues contracted more than the normoglycaemic female or the diabetic male which in turn contracted lesser than the diabetic female (Fig. 1). Removal of the endothelium or pre-treatment with L-NAME or MB (Figs. 1 and 3) abolished or reversed the contractile differences between the normoglycaemic male and the diabetic male or the normoglycaemic female, and between the diabetic male versus the diabetic female aorta. These observations suggest that endothelium-dependent factors modulate these differences in contraction. This result is consistent with earlier studies (Sanchez et al., 1996; Stallone et al., 1991; Robert et al., 2005) which showed that normoglycaemic male respond significantly more to agonist-induced vasoconstriction compared to the female. It also supports previous observations (Head et al., 1987; Myers and Messina, 1996; Misurski et al., 2001) in the male which showed that vascular hyporeactivity to endogenous vasoconstrictors existed in short-term diabetes, perhaps an early pathophysiological tissue response to counter the subsequent increased contractility seen in this condition (Alosachie and Godfraind, 1988; Myers and Messina, 1996; Misurski et al., 2001). Furthermore, while no gender difference was observed in the contraction of endothelium-denuded normoglycaemic controls, contraction in endothelium-denuded diabetic tissues were differentiated, suggesting that the factors modulating the gender differences are endothelium-dependent and -independent, respectively, in normoglycaemic and diabetic tissues. The result also suggests that diabetes induced desensitization of the female smooth muscle cells to Phenylephrine stimulation, as inferred from a comparison of the pEC\(_{50}\) values of endothelium-denuded normoglycaemic versus diabetic tissues. The data in Fig. 3 suggest that NO modulates Phenylephrine contraction in male and female normoglycaemic and diabetic tissues, since blockade of NO (with L-NAME) enhanced contraction in all these conditions. In addition, the data suggest that the NO signal transduction in all these tissues is via cGMP as blockade of this enzyme with Methylene blue enhanced contraction in all the conditions, except in the diabetic female tissues, indicating an attenuation of the relaxant effect of cGMP in diabetic female. This may be part of the mechanism for the observed worse outcome in the female gender when disease ensues (Barrett-Conner et al., 1991; Bolego et al., 1999).

7.2. The role of exogenous oestradiol (17\( \beta \)-oestradiol)

The effect of exogenous oestradiol on vascular responses to contractile agents is controversial. Both attenuation (Thomas et al., 1995) and enhancement (Miller and Vanhoutte, 1990) of contraction have been reported. In this study oestradiol attenuated Phenylephrine contraction of normoglycaemic male aorta, but had no effect on the female...
tissues (Fig. 2) in line with earlier observations (Nadarali et al., 2001; Tep-areenan et al., 2003). It is suggested that the lower contraction of normoglycaemic female tissues was partly promoted by its oestriadiol-rich content. In the diabetic tissues, oestriadiol contracted rather than relax diabetic male tissues while eliciting no apparent effect on the female, suggesting that oestriadiol relaxant action is reversed in diabetes mellitus (at least in the male) in line with previous findings (Maggi et al., 2003) and supportive of the loss of oestriadiol protective (vasorelaxant) function in cardiovascular diseases (Barrett-Conner et al., 1991; Bolego et al., 1999). The apparent absence of oestriadiol effect in both normoglycaemic and diabetic female tissues may reflect a number of reasons: 1) the female tissues were already fully primed with oestriadiol and hence, were insensitive to the physiological concentration of oestriadiol tested; 2) the vasorelaxant action of oestriadiol may have been masked by the diabetic state promotion of vasoconstrictor prosta-glandins (TXA2 and PGE2) or vasodilators (EDNO and PGI2) or both (Figs. 5–7). This latter view is strongly supported by the observation that the vasodilator effect of oestriadiol was promoted by endothelial denudation (and thus removal of EDNO and PGI2) of both diabetic male and female tissues; and 3) It is possible that the relaxant effects of oestriadiol will become more observable in the later stages of diabetes in the female tissues when endothelial function deteriorates even more. These are interesting speculations requiring further studies.

Oestriadiol is known to exert vascular effects via genomic and non genomic mechanisms (Orshal and Khalil, 2004). Thus, the classic genomic pathway (producing delayed vascular effects of oestriadiol) requires receptor-specific transcription and protein synthesis, while in the non genomic (rapid onset) pathway, oestriadiol promotes vasodilation by directly regulating Ca2+ entry mechanisms. Since the duration of vascular tissue exposure to oestriadiol (1 h or more for genomic (Binko and Majweski, 1998); 5–25 min for non genomic events (Teoh et al., 2000)), determines which of the two mechanisms is triggered, the 20–25 min exposure employed in the current study probably supports a non genomic mechanism for the vasodilator effect of this hormone in male normoglycaemic tissues, and probably the impairment of this mechanism in the male diabetic tissues.

7.3. The role of NO-cGMP pathway

Compared to the male, the endothelium of healthy (normoglycaemic) female is in a higher NO-cGMP-mediated vasorelaxant state as inferred by the greater percentage increase in contraction following endothelial denudation, L-NAME or MB treatment (Figs. 1 and 3/Table 2) and as demonstrated by others (Hayashi et al., 1992; Kauser and Rubanyi, 1994). Since increase in aortic contraction in response to L-NAME indirectly reflects on the levels of basal NO release (Hayashi et al., 1992), our current data also suggest that normoglycaemic female tissues exhibited higher basal release of EDNO compared to the male (Hayashi et al., 1992; Kauser and Rubanyi, 1994). This is consistent with the data in Fig. 5, where normoglycaemic female aorta exhibited a higher percentage relaxation to the endothelium-dependent vasodilator, Acetylcholine, but which was not the case with the responses to the endothelium-independent NO donor, Sodium nitroprusside, where male and female tissue responses were similar. Equally, in Fig. 6, normoglycaemic female tissues exhibited higher nitrite ion content (a biomarker of NO function) than the male. Taken together, these results suggest that a higher activation of NO-cGMP pathway in normoglycaemic female tissues was responsible for its lesser contraction to Phenylephrine compared to the male. In Figs. 1–3, this female ‘advantage’ appears to have been considerably diminished by the diabetic state (Bolego et al., 1999; Pinna et al., 2001).

7.4. The role of cyclooxygenase pathway

Inhibition of the cyclooxygenase pathway with Indomethacin decreased aortic contraction in the normoglycaemic male, but not significantly so in the normoglycaemic female tissues. (Fig. 4) This suggests that contractile COX product(s) was induced more in the normoglycaemic male than the female tissues in response to Phenylephrine and its inhibition led to vasodilatation of the male aorta. A similar pattern of responses were observed in the diabetic female tissues (Fig. 4) with Indomethacin treatment causing a relaxant effect which, like in the male normoglycaemic tissues, was reversed by L-NAME. Taken together, we postulate that in a state of mild oxidative stress, such as exists in the normoglycaemic male and diabetic female (but not the oestriadiol-tempered normoglycaemic female) tissues, there is a compensatory up-regulation of eNOS (NO) with a COX-eNOS cross-talk that tempers the eNOS effect through the release of contractile COX product, TXA2, the main vasoconstrictor released from the endothelium in response to COX-1 activity (Muscaria et al., 2000). Inhibition of the contractile COX product unravels the up-regulated eNOS leading to a reduced contraction. This hypothesis calls for more studies, but is strongly supported by the increased TXB2 levels recorded in both normoglycaemic male and diabetic female tissues (Fig. 7), and by the fact that treatment with L-NAME reversed the Indomethacin-induced relaxation in both tissues (Fig. 4). Increased contractile PGE2 was also observed in the normoglycaemic male tissues and it perhaps exerted similar effects as did TXB2. PGE2 and PGI2 (6-keto-PGF1α) are major disease-induced products of COX-II
activation (Wallace et al., 1999) and while TXA2 and PGE2 promote platelet aggregation/atherosclerosis and may lead to vasocostriction (Rolland et al., 1984; Yamamoto et al., 1993), PGI2, which inhibits platelet aggregation, may lead to vasodilatation (Lam et al., 1993). Not surprising, therefore, the higher TXB2 and PGE2 levels in the normoglycaemic male is perhaps the basis for its greater contractile response to Phenylephrine in comparison to the hypoglycaemic male is perhaps the basis for its greater contractile response to Phenylephrine in comparison to the hypoglycaemic male. We postulate that the enhancement of contractile prostaglandins (TXA2 and PGE2). We postulate that the enhancement of contractile prostaglandins in diabetic female compared to male tissues is perhaps the less-than-anticipated increase in contraction of the contractile-prostaglandin-rich diabetic female tissues. On the other hand, the diabetes-induced reductions in TXB2 and PGE2 coupled with increases in vasodilator PGI2 (and EDNO) levels in diabetic tissues from both genders is perhaps, part of an early pathophysiological mechanism induced in diabetic tissues to counter or compensate for increased tissue contractility seen in this condition (Myers and Messina, 1996; Misurski et al., 2001).

7.5 Future areas of investigations

Although it has been suggested that EDHF may not contribute significantly to vasodilatation of diabetic male (Browne et al., 2007) or female aorta (Csanyi et al., 2007), we do not rule out its possible involvement in the observed diabetes-mediated increase in vasoconstriction. Further experiments are required to address this issue. The hyperglycaemic state is a major contributor to the vascular pathophysiology in diabetes. The levels of hyperglycaemia achieved in this study, although similar to levels in other studies (Pinna et al., 2001; Sanz et al., 2003) were uncontrolled. The possibility that various controlled (e.g. with insulin) levels of hyperglycaemia could yield different patterns of response, is worth exploring for its clinical relevance. Similarly, there is a need to repeat the current investigation in resistance vessels for correlation with the microvascular angiopathy of clinical diabetes.

In conclusion, our data provide evidence of gender differences in the effect of short-term (8 weeks) diabetes on the reactivity of WKY rat aortic tissue. These differences are endothelium-dependent and are attributable to an increase (male) or decrease (female) in the synthesis of contractile PGs (TXA2 and PGE2) in normoglycaemic tissues, in addition to greater increased EDNO function in the female. In diabetic tissues the difference is attributable to a reduction (male) or an enhancement (female) in the levels of contractile prostaglandins (TXA2 and PGE2). We postulate that the enhancement of contractile prostaglandin in the female is probably a factor in the well-documented reversal of the female cardiovascular protective effect once cardiovascular disease (e.g. diabetes) develops. There is also an enhanced release of EDFRs (nitric oxide and PGI2) in aorta from both genders, which probably is a pathophysiologic compensatory mechanism for the increased diabetic tissue contractility.

Statement of conflicts of interest

None

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