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# Attenuated 5-$HT_2$ receptor-mediated responses in hindquarters of diabetic rats

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**Abstract**

Vasoconstrictor responses to 5-hydroxytryptamine (5-HT), α-methyl-5-HT, endothelin-1, arachidonic acid and the thromboxane $A_2$-mimetic U46619 ((15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid) were obtained in blood-perfused hindquarters of 6-week streptozotocin-diabetic rats. When compared to responses obtained in hindquarters of control rats, responses to 5-HT, α-methyl-5-HT, and arachidonic acid were attenuated in hindquarters of diabetic rats. However, responses to endothelin-1 or U46619 were not significantly different between controls and diabetics. These results suggest that 5-$HT_2$, but not endothelin $ET_A$ receptor-mediated responses are reduced in hindquarters of diabetic rats. The results utilising arachidonic acid and U46619 suggest that there may also be a defect in the cyclo-oxygenase cascade during diabetes.

*Keywords:* Diabetes; Hindquarters; Endothelin; Arachidonic acid; 5-HT (5-hydroxytryptamine, serotonin); Thromboxane

## 1. Introduction

It has been reported that levels of endothelin-1 (Takeda et al., 1991; Miller et al., 1993; Morabito et al., 1994), thromboxane $A_2$ (for a review see Hodgson et al., 1992) and 5-hydroxytryptamine (5-HT; Pietraszek et al., 1992) are altered during clinical and experimental diabetes. Diabetes is associated with hyperaggregability of platelets (for a review see Hodgson et al., 1992) and the concomitant release of intraplatelet factors, including thromboxane $A_2$ and 5-HT, further stimulates the platelet aggregation cascade. We have previously shown that contractile responses to endothelin-1 (Fulton et al., 1991; Hodgson and King, 1992) and 5-HT (Sikorski et al., 1993; James et al., 1994) are attenuated in aortae from streptozotocin-diabetic rats. We have also identified an important interaction between endothelin-1 and 5-HT which appears to involve endothelial-derived thromboxane $A_2$ (James and Hodgson, 1995). However, these studies have utilised isolated vessels from diabetic rats. Many previous workers who have used isolated intact vascular beds have perfused their preparations with a physiological salt solution (Friedman, 1989; Sarubbi et al., 1989; Taylor et al., 1994). This presents problems due to the absence of platelets, which are important in the onset of diabetes-related cardiovascular complications, and the potential for the development of oedema. In addition, peripheral vascular disease is prevalent in diabetics with gangrene of the lower extremities being 8–150 times as frequent in diabetics than in non-diabetics (Bierman, 1987). Therefore, the present study was designed to examine vascular reactivity changes in the isolated intact blood-perfused hindquarters preparation.

## 2. Materials and methods

### 2.1. Induction of diabetes

Male Wistar rats (284–384 g) were injected with streptozotocin (60 mg/kg i.v.) or vehicle (50 mM citrate buffer) under 4% halothane anaesthesia ($O_2/N_2O$ 2:1) as previously described (Hodgson and King, 1992). The animals were then housed in treatment pairs, being allowed free access to food and water at all times. Only rats displaying elevated blood glucose levels (> 17 mM Ames Mininlab 1) after 6 weeks were

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considered to be diabetic. Control and insulin-treated rats had normal (2.6–9.3 mM) blood glucose levels over the same period.

### 2.2. Insulin treatment

Where indicated, streptozotocin-treated rats received a single daily dose of Lente MC insulin zinc suspension (5 units/day s.c.) commencing on the second day after streptozotocin administration (Hodgson and King, 1992).

### 2.3. Autoperfused rat hindquarters

After 6 weeks, rats were anaesthetised with pentobarbitone sodium (60–100 mg/kg i.p.). The hindquarters were then perfused at a constant flow using the method of Brody et al. (1963) as previously described by us (Boura et al., 1986,1987). A midline incision was made in the cervical region, and the trachea, right jugular vein and the left carotid artery cannulated. Heparin (500 units/kg i.v.) was administered, an abdominal midline incision made and a segment of the abdominal aorta between the left renal artery and the posterior aortic bifurcation exposed and dissected free from the vena cava. A central ligature was placed around the aorta and cannulae inserted proximal and distal to this ligature. Blood was withdrawn through the proximal cannula and perfused at a constant flow rate through the distal cannula using a Masterflex pump (model 7013, Cole-Palmer, Chicago, USA). The flow rate was set so that hindquarter perfusion pressure approximated systemic arterial pressure and was not changed during an experiment. Perfusion and systemic arterial blood pressures were monitored using Gould Statham pressure transducers (P23). Heart rate was recorded via a triggered cardiotachometer. All variables were displayed on a Grass Polygraph (model 79E). Body temperature was maintained at approximately 37°C with a heated rat table and monitored with a rectal thermometer. Where required animals were ventilated with an Ugo Basile rodent respiratory pump (50 strokes/min; 1.0 ml/100 g body weight). Drugs were injected directly into the perfusion circuit using a microsyringe. Discrete dose-response curves were obtained to agonists with at least a 4 min interval between each addition. Antagonists were allowed to equilibrate for at least 20 min before agonists were added. Only one dose-reponse curve was performed in each animal.

### 2.4. Drugs

The following drugs were used: arachidonic acid (Sigma), α-methyl-5-hydroxytryptamine maleate (Glaxo), BQ123 (American Peptide Co.), endothelin-1 (Auspep), GR32191B [1*R*-[1α(*Z*),2β,3β,5α]]-(±)-7-[5-[[(1,1'-biphenyl)-4-yl] methoxy]-3-hydroxy-2-(1-piperidinyl) cyclopentyl]-4-heptenoic acid (Glaxo), 5-hydroxytryptamine creatine sulphate (Sigma), ketanserin tartrate (Janssen), Lente MC bovine insulin (CSL-Novo), Tween 20 (polyoxyethylene-sorbitan-monolaurate) (Sigma), U46619 ((15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid) (Upjohn). All doses were expressed in terms of their base with the exception of α-methyl-5-HT which was expressed as its salt.

Arachidonic acid stock solution was prepared in *n*-hexane, which was evaporated under nitrogen gas immediately prior to use, redissolved and further diluted in 1% $Na_2CO_3$. Indomethacin was dissolved in 1% $Na_2CO_3$ and diluted in 0.9% saline. Endothelin-1 stock was prepared in distilled water, aliquoted and frozen. On the day of use it was thawed and further diluted in 0.9% saline. U46619 stock solution was prepared in ethanol, which was evaporated under nitrogen gas immediately prior to use, and redissolved in 0.9% saline. GR32191B was dissolved in 1.0% Tween 20. All other drugs were dissolved in 0.9% saline.

### 2.5. Statistics

Hindquarter vascular responses were measured as peak change from baseline and expressed in absolute units (mm Hg) as standard error of the mean (S.E.M.). Results were statistically analysed by analysis of vari-

Table 1
Body weights and blood glucose levels of control, diabetic and insulin-treated diabetic rats

| | (*n*) | Body weights (g) | | Blood glucose levels (mM) | |
|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final |
| Control | 42 | 327 ± 4 | 452 ± 5 [b] | 5.0 ± 0.2 | 5.0 ± 0.2 |
| Diabetic | 42 | 327 ± 4 | 291 ± 5 [a,b] | 6.9 ± 0.3 | 24.0 ± 0.8 [a,b] |
| Diabetic/insulin | 5 | 327 ± 8 | 394 ± 8 [a,b,c] | 5.0 ± 0.4 | 6.0 ± 0.8 [c] |

Initial measurements were made at the time of streptozotocin or vehicle injection, and final measurements made 6-weeks later. [a] Significantly different from corresponding value in control group, $P < 0.05$, ANOVA. [b] Significantly different from initial value in same treatment group, $P < 0.05$, ANOVA. [c] Significantly different from corresponding value in diabetic group, $P < 0.05$, ANOVA.

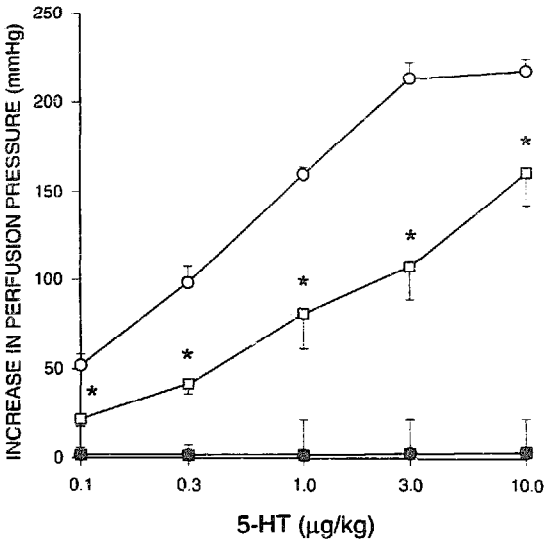


Fig. 1. Discrete dose-response curves to 5-hydroxytryptamine (5-HT), in the absence or presence of ketanserin (1 mg/kg i.v., $n = 3–4$) in blood-perfused hindquarters of control (open circles without ketanserin; closed circles with ketanserin) and diabetic (open squares without ketanserin; closed squares with ketanserin) rats. * $P < 0.05$, significantly different from corresponding control group, ANOVA. Values indicate mean ± S.E.M.

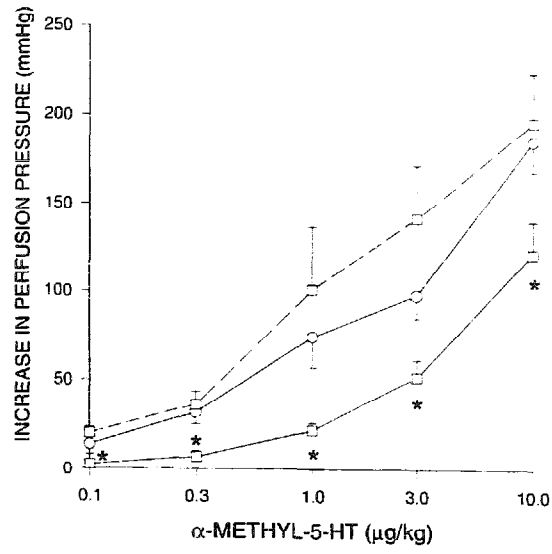


Fig. 2. Discrete dose-response curves to α-methyl-5-HT in blood-perfused hindquarters of control (open circles), diabetic (open squares with solid line) and insulin-treated diabetic (open squares with dashed line) rats. * $P < 0.05$, significantly different from corresponding control group, ANOVA. Values indicate mean ± S.E.M.

ance (ANOVA) and Tukey test on the CLR ANOVA package (Apple Macintosh). In all cases statistical significance is indicated by $P < 0.05$.

## 3. Results

As shown in Table 1, control rats displayed significantly increased body weights compared to their pre-injection weights. Diabetic rats displayed significantly reduced body weights over the same period ($P < 0.05$, ANOVA). Blood glucose levels of diabetic rats were increased significantly after the 6-week period (Table 1, $P < 0.05$, ANOVA), while blood glucose levels of control and insulin-treated diabetic animals remained within normal limits. In comparison to control rats, diabetic rats had significantly reduced heart rates and mean arterial blood pressures. There was no significant difference in basal hindquarter perfusion pressures among the three groups (i.e. control, diabetic and insulin-treated diabetic rats). However, there was a significant difference in the flow rates of insulin-treated diabetic rats compared to controls (Table 2, $P < 0.05$, ANOVA).

Vasoconstrictor responses to 5-HT (0.1–10 μg/kg i.a.) were significantly reduced in hindquarters of diabetic rats when compared to those from control rats (Fig. 1, $P < 0.05$, ANOVA). Similarly, vasoconstrictor responses to α-methyl-5-HT (0.1–10 μg/kg i.a.) were significantly reduced in diabetic rats compared to controls (Fig. 2, $P < 0.05$, ANOVA). Responses to 5-HT (Fig. 1) and α-methyl-5-HT (data not shown) in hindquarters of control and diabetic rats were abolished by ketanserin (1 mg/kg i.v.) ($P < 0.05$, ANOVA). In hindquarters of insulin-treated diabetic rats, vasoconstrictor responses to α-methyl-5-HT were not significantly different to those from control rats but significantly augmented compared to responses from diabetic rats (Fig. 2, $P < 0.05$, ANOVA).

Vasoconstrictor responses to endothelin-1 (0.01–3 μg/kg i.a.) obtained in hindquarters of diabetic rats were not significantly different to those obtained in control rats (Fig. 3). In the presence of the endothelin

Table 2
Blood pressures, flow rates and basal perfusion pressures of control, diabetic and insulin-treated diabetic rats

| | Heart rate (bpm) | Mean arterial blood pressure (mm Hg) | Flow rate (ml/min) | Basal perfusion pressure (mm Hg) |
|---|---|---|---|---|
| Control | 369 ± 8 | 129 ± 3 | 1.7 ± 0.1 | 65 ± 3 |
| Diabetic | 267 ± 9 [a] | 89 ± 5 [a] | 1.5 ± 0.1 | 57 ± 4 |
| Diabetic/insulin | 384 ± 20 [b] | 133 ± 14 [b] | 1.3 ± 0.1 [a] | 70 ± 7 |

[a] Significantly different from corresponding value in control group, $P < 0.05$, ANOVA. [b] Significantly different from corresponding value in diabetic group, $P < 0.05$, ANOVA.

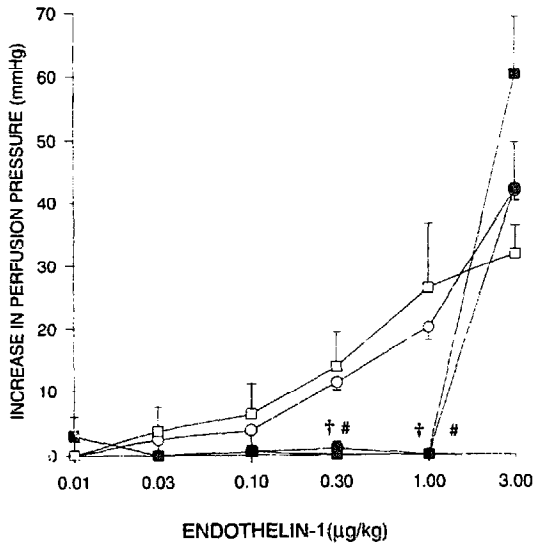


Fig. 3. Discrete dose-response curves to endothelin-1, in the absence or presence of BQ123 (0.1 mg/kg i.v., $n = 3$–6) in blood-perfused hindquarters of control (open circles without BQ123; closed circles with BQ123) and diabetic (open squares without BQ123; closed squares with BQ123) rats. $^{\dagger} P < 0.05$, significantly different from corresponding control group without BQ123, ANOVA. $^{\#} P < 0.05$, significantly different from corresponding diabetic group without BQ123, ANOVA. Values indicate mean ± S.E.M.

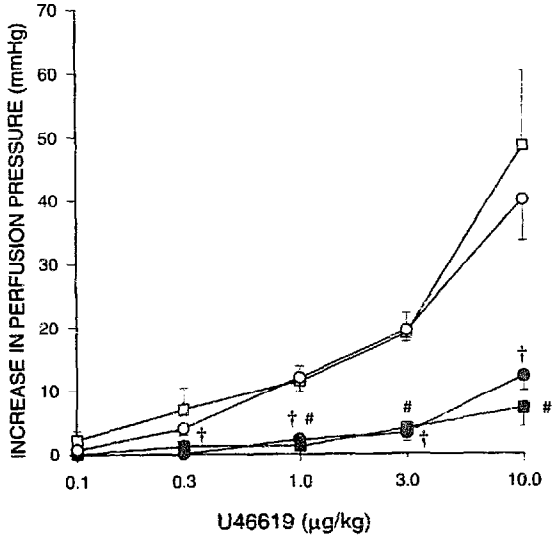


Fig. 5. Discrete dose-response curves to U46619, in the absence or presence of GR32191B (1 mg/kg i.v., $n = 4$–5), in blood-perfused hindquarters of control [illegible] circles without GR32191B; closed circles with GR32191B) and diabetic rats (open squares without GR32191B; closed squares with GR32191B). $^{\dagger} P < 0.05$, significantly different from corresponding control group without GR32191B, ANOVA. $^{\#} P < 0.05$, significantly different from corresponding diabetic group without GR32191B. Values indicate mean ± S.E.M.

$ET_A$ receptor antagonist BQ123 (0.1 mg/kg i.v.), constrictor responses to endothelin-1 (0.3–1 μg/kg i.a.) were significantly inhibited in hindquarters of control and diabetic rats ($P < 0.05$, ANOVA). However, vasoconstrictor responses at the highest dose of endothelin used, 3 μg/kg (i.a.), were not significantly inhibited by BQ123 (0.1 mg/kg i.v.) in hindquarters of control and diabetic rats.

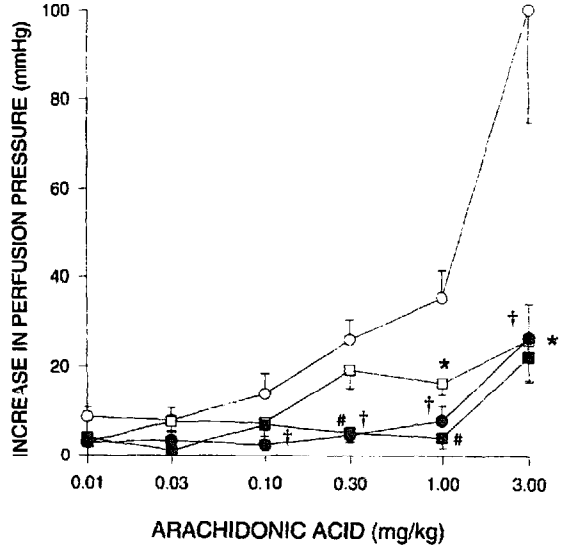


Fig. 4. Discrete dose-response curves to arachidonic acid, in the absence or presence of GR32191B (1 mg/kg i.v., $n = 4$–6) in blood-perfused hindquarters of control (open circles without GR32191B; closed circles with GR32191B) and diabetic (open squares without GR32191B; closed squares with GR32191B) rats. $^{*} P < 0.05$, significantly different from corresponding control group, ANOVA. $^{\dagger} P < 0.05$, significantly different from corresponding control group without GR32191B. $^{\#} P < 0.05$, significantly different from corresponding diabetic group without GR32191B. Values indicate mean ± S.E.M.

Vasoconstrictor responses to arachidonic acid (0.01–3 mg/kg i.a.) were significantly reduced in hindquarters of diabetic rats compared to responses in control rats (Fig. 4, $P < 0.05$, ANOVA). The thromboxane $A_2$/prostaglandin $H_2$ receptor antagonist, GR32191B (1 mg/kg i.v.) significantly reduced responses to arachidonic acid in hindquarters of control (0.1–3 mg/kg i.a.) and diabetic (0.3–1 mg/kg i.a.) rats (Fig. 4, $P < 0.05$, ANOVA).

Responses to the thromboxane $A_2$-mimetic U46619 (0.1–10 μg/kg i.a.) obtained in hindquarters of diabetic rats were not significantly different to those obtained in control rats (Fig. 5). GR32191B (1 mg/kg i.v.) significantly reduced vasoconstrictor responses to U46619 in both groups (Fig. 5, $P < 0.05$, ANOVA).

## 4. Discussion

The present study showed that although changes in reactivity were observed in the hindquarters vascular bed during diabetes, these changes were not non-specific. While vasoconstrictor responses to 5-HT, α-

methyl-5-HT and arachidonic acid were attenuated in diabetic rats, responses to the thromboxane $A_2$ mimetic, U46619, and endothelin-1 were not altered.

5-HT (Nakaki et al., 1985), endothelin-1 (Highsmith et al., 1992) and thromboxane $A_2$ (Dorn and Becker, 1993) produce their effects in vascular smooth muscle by similar mechanisms. That is, the activation of phospholipase C and the subsequent increase in inositol-1,4,5-triphosphate and diacylglycerol levels. In turn, diacylglycerol stimulates protein kinase C. We have previously suggested a possible abnormality in this second messenger system in aortae from diabetic animals (James and Hodgson, 1995). However, it would appear that changes in the phospholipase C second messenger system are not entirely responsible for the altered responses observed to 5-HT, $\alpha$-methyl-5-HT and arachidonic acid in the hindquarters vasculature, since no differences were observed to U46619 or endothelin-1.

Vasoconstrictor responses to 5-HT and $\alpha$-methyl-5-HT were attenuated in the hindquarters of diabetic rats compared to controls confirming previous work utilising alloxan-diabetic rats (Boura et al., 1987). In many vascular smooth muscle preparations, 5-HT and $\alpha$-methyl-5-HT produce their constrictor responses via 5-$HT_{2A/2C}$ receptors. This was confirmed in the present study by the administration of ketanserin, a selective 5-$HT_{2A/2C}$ receptor antagonist, which abolished the responses to both agonists. However, ketanserin is approximately 1000-fold more potent at inhibiting 5-$HT_{2A}$ receptors than 5-$HT_{2C}$ receptors (Hoyer et al., 1994). At the dose of ketanserin used in the present study, 1 mg/kg, it is most likely that 5-$HT_{2A}$ receptors would be antagonized but not 5-$HT_{2C}$ receptors. Previous workers have shown that 0.1 mg/kg ketanserin produces a substantial block of 5-$HT_{2A}$ receptors (Kalkman et al., 1984). However, at least 10 mg/kg ketanserin is required for even a threshold 5-$HT_{2C}$ receptor blockade. Therefore, it appears that, in the hindquarters vascular bed, 5-HT and $\alpha$-methyl-5-HT produce their constrictor responses via 5-$HT_{2A}$ receptor activation. The mechanism(s) responsible for the reduction in 5-$HT_{2A}$ receptor activation during diabetes are yet to be elucidated. Down-regulation of 5-HT receptors, as a result of increased levels of platelet-derived 5-HT may contribute (Pietraszek et al., 1992). However, we have previously shown that the density and affinity of [$^3$H]ketanserin for binding sites were not altered during diabetes (James et al., 1994). Although this work was performed on membrane preparations from isolated aortae and tissue variability has been well documented in diabetes. Indeed, as previously mentioned, contractile responses to endothelin-1 (Hodgson and King, 1992) and 5-HT (Sikorski et al., 1993) are attenuated in aortae from 6-week control and diabetic rats, while in the present study vasoconstrictor responses to 5-HT were attenuated in hindquarters from diabetic rats and responses to endothelin-1 were not different between control and diabetic rats.

We have previously reported that the reactivity changes observed during streptozotocin-diabetes can be reversed by chronic insulin treatment (Hodgson and King, 1992; Booth and Hodgson, 1993; James et al., 1994). In the present study vasoconstrictor responses to $\alpha$-methyl-5-HT from insulin-treated diabetic rats were not significantly different from controls indicating that the changes observed were not due to a localized toxic effect of the diabetogen per se, but linked to the metabolic imbalance produced by insulin deficiency. Interestingly, in the present study, a slightly lower flow rate through the hindquarters vascular bed of the insulin-treated diabetic rats was required to obtain a similar basal perfusion pressure to that of the control animals. This may indicate that despite chronic insulin normalising the streptozotocin-induced hyperglycaemia there is still some alteration in resistance in the vessels of these rats. However, if this is true, there appears to be no significant effect on vascular reactivity as responses to $\alpha$-methyl-5-HT between the two groups were similar.

As mentioned above, responses to exogenous arachidonic acid were significantly reduced in diabetic rats. Arachidonic acid is converted to thromboxane $A_2$, via prostaglandin $H_2$, both of which produce vasoconstriction via the activation of thromboxane $A_2$/prostaglandin $H_2$ receptors. This pathway appears to be responsible for the constrictor effect of arachidonic acid in the hindquarters preparation as we have previously shown that responses to arachidonic acid are significantly inhibited by the cyclo-oxygenase inhibitor indomethacin (Boura et al., 1987) and, in the present study, by the selective thromboxane $A_2$/prostaglandin $H_2$ receptor antagonist GR32191B. Interestingly, we were unable to obtain constrictor responses to arachidonic acid in Krebs-perfused hindquarters (Boura et al., 1987) indicating that a constituent of blood, presumably platelets, is required for this action. However, the reduction in responsiveness to arachidonic acid, observed in the present study, cannot be explained by a decrease in sensitivity of thromboxane $A_2$/prostaglandin $H_2$ receptors as responses to exogenous thromboxane (U46619) were unaltered in diabetic rats. Arachidonic acid may also be metabolised by lipoxygenase, which leads to the production of the leukotrienes, and epoxygenase, leading to the production of epoxy and dihydroxy acids. It is therefore possible that the responses to arachidonic acid were partially due to the production of other constrictor eicosanoids. However, as the change does not appear to be at a receptor level there may be alterations in the cyclo-oxygenase cascade in diabetes (e.g. alterations in enzyme activity).

Vasoconstrictor responses to endothelin-1 (0.3–1 μg/kg) were abolished by the presence of the selective endothelin $ET_A$ receptor antagonist BQ123. However, at the highest dose of endothelin-1 (3 μg/kg), responses were not attenuated by BQ123. The reason for this anomaly is unknown. At this dose, endothelin-1 may be activating endothelin $ET_{B2}$ receptors which have been recently shown to cause direct vasoconstriction (Sumner et al., 1992; Douglas et al., 1994; Wellings et al., 1994). It is also possible that this response may be due to the production of a vasoconstrictor prostanoid stimulated by endothelin-1 (Rubanyi and Polokoff, 1994).

Previous work in our laboratory has shown that constrictor responses to arachidonic acid were augmented in the hindquarters of 2-week alloxan-treated diabetic rats while responses to 5-HT and U46619 were attenuated. In contrast, in the present study responses to U46619 were unaltered while responses to arachidonic acid were significantly reduced. Previous workers have described differences in vascular sensitivity of alloxan-diabetic rats that were dependent on the duration of diabetes (Turlapaty et al., 1980). Therefore, the difference in duration of diabetes between this study and previous work (i.e. 6 weeks versus 2 weeks, respectively) may contribute to the differences in results observed. Alternatively, the differences between these two studies may be due to the different diabetogens used, streptozotocin and alloxan. Streptozotocin has replaced alloxan as the primary compound used to produce experimental diabetes in rats (and many other laboratory animals) because it has a more selective effect on the β-cells of the pancreas (Srivastava et al., 1982). It has also been reported that spontaneous remisssion is less likely to occur when streptozotocin is used (Rerup, 1970).

In conclusion, the present study demonstrates decreased reactivity to 5-HT, α-methyl-5-HT and arachidonic acid in blood-perfused hindquarters of 6-week diabetic rats. However, as there was no change in reactivity to endothelin-1 or U46619, these changes do not appear to be due to a non-specific alteration in the phospholipase C second messenger system.

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