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Changes in cardiovascular sensitivity of alloxan-treated diabetic rats to arachidonic acid

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1 Arachidonic acid (AA, 0.125–2.0 mg kg^{-1}) administered intravenously to male Wistar rats produced a dose-dependent fall in diastolic blood pressure. However AA (0.125–1.0 mg kg^{-1}) injected into the autoperfused hindquarters via the aorta produced a dose-dependent increase in perfusion pressure. Both these responses to AA were inhibited by indomethacin (5 mg kg^{-1}).

2 The thromboxane A_2 receptor antagonist AH23848 (5 mg kg^{-1}, i.v.) inhibited pressor responses to AA in the autoperfused hindquarters, but potentiated depressor responses to AA (0.125–0.5 mg kg^{-1}) in the whole animal.

3 Alloxan-treated diabetic rats (14 days after a single s.c. injection of alloxan, 175 mg kg^{-1}) displayed reduced sensitivity to the depressor effects of AA (1–2 mg kg^{-1}) in the whole animal, increased sensitivity to the pressor effects of AA (0.5–1.0 mg kg^{-1}) in the perfused hindquarters, and reduced sensitivity to the pressor effects of the thromboxane A_2 mimetic U46619 (0.5–8.0 μg kg^{-1}, i.a.) in the perfused hindquarters.

4 These results suggest that AA can be predominantly converted to either pressor or depressor metabolites depending on the vasculature. In the diabetic state the ratio of the metabolites formed appears to change favouring a major pressor metabolite, which is probably thromboxane A_2.

Introduction

It has been postulated that changes in eicosanoid metabolism and sensitivity in the cardiovascular system may play a role in the onset of the cardiovascular complications of diabetes mellitus (Harrison *et al.*, 1978; 1980; Johnson *et al.*, 1979; Silberbauer *et al.*, 1979; Rosen & Hohl, 1984).

The present study describes sensitivity changes in the alloxan diabetic autoperfused hindquarters and the systemic blood pressure of rats to the eicosanoid precursor arachidonic acid and to the thromboxane A_2 (TXA_2)-mimetic, U46619 (Coleman *et al.*, 1980). The effect of the TXA_2 receptor antagonist AH23848 (Brittain *et al.*, 1985) on these responses was also examined. Some of the data have already been communicated in a brief form (Hodgson & Boura, 1986).

Methods

Male Wistar rats, weighing between 300–375 g were made diabetic by subcutaneous administration of alloxan monohydrate (175 mg kg^{-1}). A solution of the diabetogen in saline was prepared immediately before injection, the animals being allowed free access to food and water at all times. Two, seven and fourteen days after injection, urine glucose levels (Ames multiple reagent strips) and blood glucose levels (Ames dextrostix) from a tail-vein sample were tested. Only rats displaying at all these times elevated blood glucose (> 13.9 mmol l^{-1}) and urine glucose levels (> 60 mmol l^{-1}) were considered to be diabetic. Control rats were injected with saline by the same route and showed normal glucose levels over the 14 day period (blood glucose 5.0–7.2 mmol l^{-1}, urine glucose undetectable).

Autoperfused hindquarters

After 2 or 14 days rats from both the control and diabetic groups were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). Heparinized saline (200 u ml^{-1}, 500 u kg^{-1}) was injected via a cannula in the jugular vein. The hindquarters were then perfused at a constant flow using the method described by Brody *et al.* (1963). A midline incision was made and the abdominal aorta tied off. Cannulae were inserted above and below the tie. The cephalad aortic blood was pumped, using a Masterflex pump (Model 7013,

Cole-Palmer, Chicago), into the caudal aorta. Central aortic arterial blood pressure and perfusion pressure were monitored using Gould Statham pressure transducers (P23) and heart rate recorded using a cardiotachometer. All variables were displayed on a Grass Polygraph (Model 7D). The perfusion flow rate (1.5–1.8 ml min^{-1}) was set so blood pressure and perfusion pressure were approximately equal after switching on the pump. The animals were ventilated using a rodent respiratory pump (50 strokes min^{-1}, 1.4 ml per stroke, C.F. Palmer, London). Drugs were injected directly into the aortic tubing using a 25 µl microsyringe (Scientific glass engineering, Ringwood, Australia).

Whole animals

Rats from both groups were used after 14 days and anaesthetized in the same way as the hindquarters preparation. The jugular vein was cannulated for the administration of drugs (which were washed in with 0.2 ml saline) and the femoral artery cannulated for recording of blood pressure. Heart rate was recorded as before.

The following drugs were used: AH23848 ([1 α (Z), 2 β, 5 α]-(±)-7-[5[[(1, 1'-biphenyl)-4-yl] methoxy] - 2 - (4-morpholinyl) - 3 - oxocyclopentyl] - 4 - heptenoic acid) (Glaxo); alloxan monohydrate (Sigma); arachidonic acid (Sigma); heparin sodium (David Bull); indomethacin (Merck, Sharp & Dohme); mepacrine HCl (May & Baker) and U46619 ((15S)-hydroxy-11 α, 9 α-(epoxymethano) prosta-5Z, 13E-dienoic acid) (Upjohn).

Statistical analysis of results was performed by means of student's *t* test. Unpaired *t* tests were used to compare results from control and diabetic rats. Paired *t* tests were used when effects in the same animal were compared. Values shown are means ± s.e.mean.

Results

Table 1 shows body weights, heart rates, blood pressures, and perfusion pressures and flow rates in perfused hindquarters, of control and diabetic rats. In comparison to control rats, diabetic rats were found to have significantly reduced body weights, heart rates and diastolic and systolic blood pressures.

Effects of arachidonic acid on systemic blood pressure

As shown in Figure 1a, arachidonic acid (AA, 0.5–2.0 mg kg^{-1}) injected into the jugular vein of control rats caused a fall in both diastolic and systolic blood pressures. Figure 2a shows that the mean fall in diastolic blood pressure was dose-dependent over the range of doses of AA used (0.125–2.0 mg kg^{-1}). After injection of the thromboxane A_2 receptor antagonist (AH23848, 5 mg kg^{-1}), the fall in diastolic blood pressure following injection of low doses of AA (0.125–0.5 mg kg^{-1}) was significantly increased (Figure 2a).

In day 14 diabetic rats, AA (0.125–2.0 mg kg^{-1}) also caused falls in diastolic blood pressure (Figure 2b). However, after doses of 1–2 mg kg^{-1} depressor responses were significantly less than those which occurred in control animals ($P < 0.05$). After injection of AH23848 (5 mg kg^{-1}) into diabetic rats, the fall in diastolic blood pressure following low doses of AA (0.125–0.25 mg kg^{-1}) was significantly increased (Figure 2b). The cyclo-oxygenase inhibitor indomethacin inhibited the depressor effects of AA (1.0 and 2.0 mg kg^{-1}) in non-diabetic whole animals; the decreases in diastolic blood pressure to 0.125, 0.25, 0.5, 1.0 and 2.0 mg kg^{-1} AA following indomethacin (5 mg kg^{-1}) were 11.3 ± 3.2, 12.5 ± 1.4, 17.5 ± 1.4, 37.0 ± 7.2, 38.0 ± 7.0 mmHg, respectively (mean ± s.e.mean, $n = 5$, significantly different from controls in Figure 2a, $P < 0.05$ for 1.0 and 2.0 mg kg^{-1} AA).

Effects of arachidonic acid on perfusion pressure of autoperfused hindquarters

As shown in Figure 1b, AA (0.25–1.0 mg kg^{-1}) caused increases in perfusion pressure of autoperfused hindquarters in control rats. This vasoconstrictor effect of

Table 1 Body weights, heart rates, blood pressures, flow rates and perfusion pressures of perfused hindquarters of control and 14 day diabetic rats

	Body weight (g)	*Heart rate* (beats min^{-1})	*Blood pressure* (mmHg) *Diastolic*	*Systolic*	*Flow rate* (ml min^{-1})	*Perfusion pressure* (mmHg)
Control	347.9	326.4	71.6	97.7	1.7	48.9
($n = 28$)	±7.4	±11.1	±4.9	±4.6	±0.07	±2.6
Diabetic	326.6	242.6	47.4	73.4	1.6	39.2
($n = 19$)	±7.0*	±11.7*	±5.9*	±5.4*	±0.07	±5.1

Results show mean ± s.e.mean. * $P < 0.05$, significantly different from control.

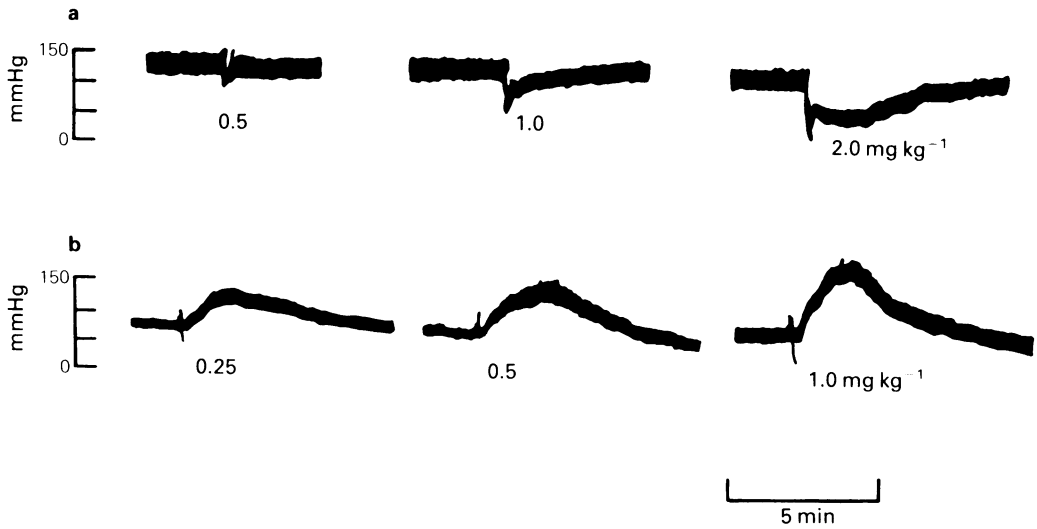

Figure 1 Responses of (a) whole animal femoral artery blood pressure and (b) hindquarters vasculature perfusion pressure to arachidonic acid in non-diabetic control rats.

AA in hindquarters contrasted with its depressor effect in the whole animal. Figure 3 shows that the responses to AA (0.125–1.0 mg kg^{-1}) in the hindquarters of control rats were significantly reduced after injection of AH23848 (5 mg kg^{-1}). The increases in perfusion pressure induced by AA (0.5–1.0 mg kg^{-1}) in hindquarters of 14 day diabetic rats were significantly greater than in controls (Figure 3). As shown in Table 2, in hindquarters of control rats the phospholipase A_2 inhibitor mepacrine (Vigo *et al.*, 1980) 5 mg kg^{-1}, caused no significant change in the pressor response to AA (0.125–1.0 mg kg^{-1}), whereas indomethacin (5 mg kg^{-1}) significantly inhibited responses.

Rats treated with a single dose of alloxan (175 mg kg^{-1}) 2 days previously had elevated blood and urine glucose levels that were similar to those of 14 day diabetic rats. However, as shown in Table 2 the hindquarters of the 2 day diabetics showed no significant change from non-diabetic controls in their sensitivity to the pressor effects of AA (0.125–1.0 mg kg^{-1}). Table 2 also shows that control rats which were weight-matched with 14 day diabetic rats showed no significant differences from normal age-matched controls in their responses to AA (0.125–1.0 mg kg^{-1}).

Effects of U46619 on perfusion pressure of autoperfused hindquarters

As shown in Figure 4, the thromboxane A_2-mimetic compound U46619 (0.5–8.0 μg kg^{-1}) produced a

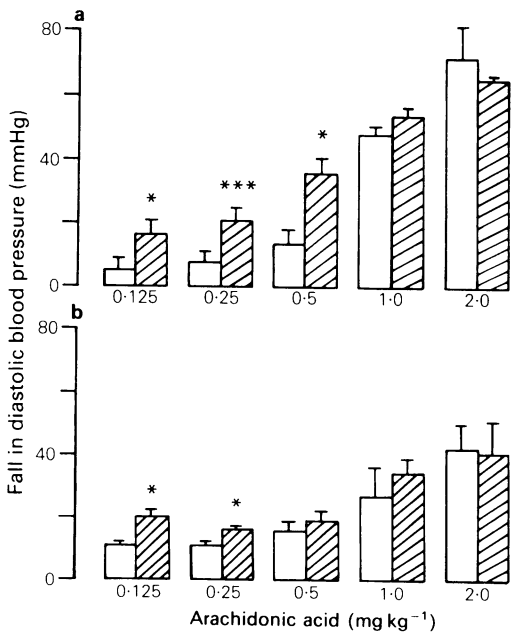

Figure 2 The effect of AH23848, administered 30 min before, on depressor responses to arachidonic acid in (a) non-diabetic ($n = 5$), and (b) 14 day diabetic whole animals ($n = 5$). Open columns before, and hatched columns after 5 mg kg^{-1} AH23848. Vertical lines represent s.e.mean. $^*P < 0.05$, $^{***}P < 0.001$, significantly different from corresponding value in absence of AH23848.

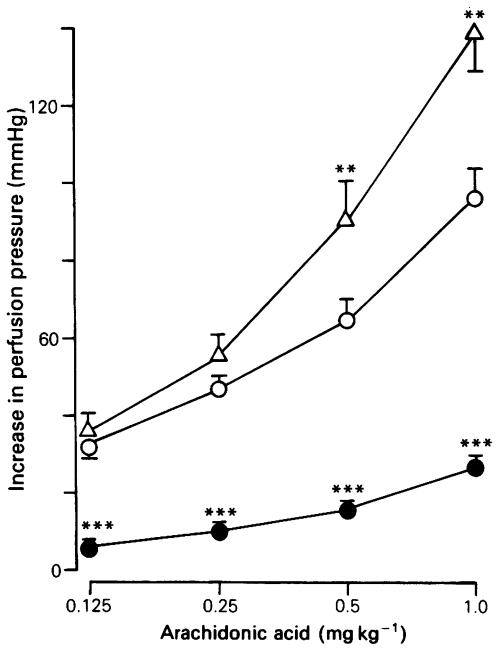

Figure 3 Increases in perfusion pressure caused by arachidonic acid in rat autoperfused hindquarters. (Δ——Δ) Fourteen day diabetics ($n = 10$); (○——○) non-diabetics ($n = 10$); (●——●) after AH23848 (5 mg kg^{-1}) in non-diabetic controls ($n = 10$). Vertical lines represent s.e.mean. **$P < 0.01$, ***$P < 0.001$, significantly different from control.

dose-dependent increase in perfusion pressure of hindquarters of control rats, which was inhibited by AH23848 (5 mg kg^{-1}). In hindquarters of 14 day diabetic rats, the mean pressor responses to U46619 were less than those in controls (Figure 4). This contrasted with the pressor effect of arachidonic acid in diabetic hindquarters which was greater than in controls (Figure 3).

Discussion

These data indicate that in the systemic circulation of the non-diabetic rat, exogenous arachidonic acid is converted to both pressor and depressor metabolites with the effects of the latter predominating. The increase in the depressor effects of low doses of arachidonic acid in the whole animal, together with the inhibition of its vasoconstrictor effects in the perfused hindquarters seen after administration of the specific TXA_2 antagonist AH23848 (Brittain *et al.*, 1985), strongly suggested that a major pressor metabolite was TXA_2. Both effects appeared to be indirect, mediated at least in part by production of products of cyclo-oxygenase, since they were reduced after administration of indomethacin. It has been found that arachidonic acid has both indirectly mediated vasoconstrictor and vasodilator properties in the coronary arteries of the rat (Belo & Talesnik, 1982) and also the dog (Sterin-Borda *et al.*, 1981).

During diabetes, apparent conversion of arachidonic acid to depressor metabolites in the whole animal continued to predominate but the contribution made by the constrictor metabolite or metabolites

Table 2 Effect of mepacrine, indomethacin and 2 days diabetes on the pressor response to arachidonic acid (AA) in hindquarters, and responses in control rats weight-matched with 14 day diabetic rats

	Body weight (g)	*Increase in perfusion pressure* (mmHg) *Dose of AA* (mg kg^{-1}) *0.125*	*0.25*	*0.5*	*1.0*
Controls ($n = 10$)	349.8 ±8.0	31.6 ±3.6	46.3 ±3.2	64.0 ±5.8	95.6 ±10.9
Controls, weight-matched with 14 day diabetics ($n = 5$)	324.8 ±8.2	29.0 ±3.3	49.5 ±8.1	66.3 ±6.7	92.0 ±6.4
Mepacrine (5 mg kg^{-1}) ($n = 5$)	340.3 ±7.7	37.8 ±6.6	51.3 ±6.8	62.7 ±5.7	92.5 ±8.3
Indomethacin (5 mg kg^{-1}) ($n = 5$)	344.0 ±6.8	13.8 ±5.2*	17.5 ±6.0*	22.5 ±6.0*	51.0 ±19.0*
2 day diabetics ($n = 5$)	351.2 ±9.1	34.9 ±7.5	44.2 ±4.3	70.3 ±9.8	99.7 ±6.6

Results are mean values ± s.e.mean. *$P < 0.05$, significantly different from control.

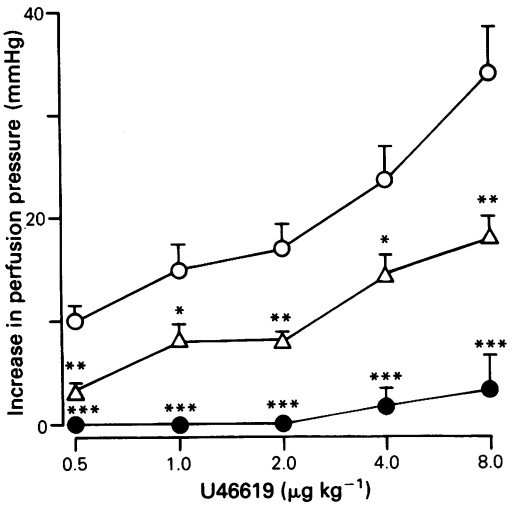

Figure 4 Increases in perfusion pressure in rat autoperfused hindquarters caused by U46619 in controls ($n = 8$) (O——O); 14 day diabetics ($n = 9$) (Δ——Δ); and controls after AH23848 (5 mg kg^{-1}, $n = 5$) (●——●). Vertical lines represent s.e.mean. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from control.

appeared to increase. In rats with established diabetes of two weeks' duration, depressor responses to the higher doses of arachidonic acid were reduced when compared with controls and its vasoconstrictor effects in the hindlimb were augmented. These findings cannot be explained by increased responsiveness to TXA_2 due to increased sensitivity of receptors or vascular smooth muscle, as responses to the TXA_2-mimetic U46619 were decreased in diabetic animals when compared with controls. Sterin-Borda *et al.* (1981) have also shown that arachidonic acid causes constriction in coronary arteries from diabetic dogs, whereas it relaxes those from control animals although, in contrast to our results, they found increased sensitivity of the coronary arteries to U46619 during diabetes.

Changes in the metabolism of arachidonic acid during diabetes have been well documented. Increased production of thromboxane A_2 has been found in both human (Ziboh *et al.*, 1979; Halushka *et al.*, 1981) and rat platelets during diabetes (Gerrard *et al.*, 1980; Subbiah & Deitemeyer, 1980; Valentovic & Lubawy, 1983). Vascular production of prostacyclin has also been found to be reduced during diabetes in man (Johnson *et al.*, 1979; Silberbauer *et al.*, 1979; Dollery *et al.*, 1979) and rats (Harrison *et al.*, 1978; 1980; Subbiah & Deitemeyer, 1980; Gerrard *et al.*, 1980; Roth *et al.*, 1983; Gilbert *et al.*, 1983). Quilley & McGiff (1985) recently demonstrated that experimentally-induced diabetes in rats was accompanied by marked increases in urinary TXB_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) excretion. They also found reduced formation of 6-keto-$PGF_{1\alpha}$ from arachidonic acid by aortic rings from diabetic rats. Canga *et al.* (1985) found that diabetic rat atria produced more TXB_2 than PGI_2 whereas in contrast the opposite was the case in normal tissue.

A temporal factor appeared to influence the changes in response to arachidonic acid seen in the present experiments. The animals studied two days after induction of diabetes showed no significant differences from controls in contrast to those examined after fourteen days of diabetes. Quilley & McGiff (1985) have already drawn attention to the fact that in the rat with experimental diabetes the proportion of urinary arachidonic metabolites varies depending on the time after induction of diabetes.

Our results therefore suggest that arachidonic acid can be converted into both pressor and depressor metabolites in the rat. During alloxan-induced diabetes, this conversion appears to be changed so that the effect of the pressor metabolite, which is probably TXA_2, achieves greater prominence.

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