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Augmented Potentiation of Renal Vasoconstrictor Responses by Thromboxane A_2 Receptor Stimulation in the Alloxan-diabetic Rat

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Abstract—Dose-response curves were obtained to bolus injections of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in blood and Krebs-perfused kidneys of male Wistar rats. Vasoconstrictor responses to both NA and 5-HT were significantly attenuated in blood-perfused kidneys of alloxan-treated 14 day diabetic rats compared with non-diabetic animals. Responses to low doses of NA were also significantly attenuated in Krebs-perfused kidneys from diabetic rats but responses to 5-HT were augmented. Dose-dependent potentiation of vasoconstrictor responses to NA and 5-HT in Krebs-perfused kidneys of both non-diabetic and diabetic rats occurred during infusion of the thromboxane A_2 (TxA_2)-mimetic U46619 ((15S)-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13E-dienoic acid). The potentiation by U46619 (11ng mL^{-1}) was inhibited in both groups during infusion of the thromboxane receptor antagonist AH23848 ([1α(Z), 2β, 5α]−(±)−7-[5[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid). Infusion of 5-HT in Krebs-perfused kidneys of non-diabetic rats, causing a rise in perfusion pressure of similar magnitude to that produced by infusion of 111ng mL^{-1} U46619, did not significantly affect responses to bolus injections of NA. Potentiation of vasoconstrictor responses to low concentrations of 5-HT by U46619 was significantly greater in Krebs-perfused kidneys of diabetic rats than kidneys from non-diabetic animals. Activation of vascular TxA_2 receptors augments the vasoconstrictor effects of 5-HT in Krebs-perfused diabetic rat kidneys to a greater extent than in non-diabetic kidneys.

Changes in eicosanoid metabolism during diabetes are well documented (Rosen & Hohl 1984; Halushka et al 1985). In particular, platelets from diabetic subjects synthesize and release larger quantities of thromboxane A_2 (TxA_2) than those from controls (Ziboh et al 1979; Halushka et al 1981; Kaapa et al 1986; Katayama et al 1987) whereas prostacyclin (PGI_2) production from endothelial cells is reduced (Johnson et al 1979; Subbiah & Deitemeyer 1980). It is also known that activation of vascular TxA_2 receptors (sometimes referred to as TxA_2/prostaglandin H_2 (PGH_2) receptors) potentiates vasoconstrictor responses to 5-hydroxytryptamine (5-HT) in isolated strips of human digital arteries (Young et al 1986). It is therefore possible that the increased endogenous TxA_2 production during diabetes could cause augmentation of vascular responses to other vasoconstrictor agents, particularly platelet derived 5-HT, thus contributing to the occlusive vascular disorders associated with the disease. Additionally the diabetic renal vasculature develops changes in sensitivity to constrictor and dilator stimuli (Bhardwaj & Moore 1988), and increased permeability and production of oedema (Colwell et al 1979). We now report on the interactions between TxA_2/PGH_2 and 5-HT receptor-mediated vasoconstriction in the renal vasculature, comparing responses of controls with those of alloxan-diabetic rats.

Materials and Methods

Male Wistar rats, 300–375 g, were made diabetic by subcutaneous administration of alloxan monohydrate (175 mg kg^{-1}). A solution of the diabetogen in saline (0·9% NaCl) was prepared immediately before injection, the animals being allowed free access to food and water. Two, seven and 14 days after injection, levels of glucose in the urine (Ames multiple reagent strips) and venous blood (Ames Glucometer II, Indiana, USA) were determined. Only rats with elevated blood (> 16 mmol L^{-1}) and urine glucose levels (> 60 mmol L^{-1}) at all time points were considered diabetic. Control rats were injected with saline by the same route and had normal glucose levels over the 14 day period (blood glucose 5–8 mmol L^{-1}, urine glucose undetectable).

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Blood-perfused kidneys

Fourteen days after injection of either saline or alloxan, rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1} i.p.). Heparinized saline (200 units mL^{-1}, 500 units kg^{-1}) was injected via a cannula in the right jugular vein. A midline incision was made and the abdominal aorta ligated cephalad to the right and caudal to the left renal arteries. The aorta was then cannulated, between the caudal ligature and the left renal artery, and both kidneys perfused with blood (2·0 mL min^{-1}) from the left carotid artery. Arterial blood pressure was measured via a cannula in the right carotid artery and both arterial and perfusion pressures were monitored using Gould-Statham pressure transducers (P23). Heart rate was recorded using a cardiotachometer and all variables were displayed on a Grass Polygraph (Model 7D). The animals were ventilated with a tracheal cannula using a rodent respiratory pump (50 strokes min^{-1}, 1·4 mL/stroke, C. F. Palmer, London). Dose response curves to NA and 5-HT were constructed by giving bolus injections into the aortic tubing using a 25 μL microsyringe (Scientific Glass Engineering, Ringwood, Australia).

Krebs-perfused kidneys

The technique was similar to that described above except that the animal was not artificially ventilated and Krebs solution at 37°C saturated with oxygen and 5% carbon dioxide ((mM):NaCl, 118·4; KCl, 4·7; $MgSO_4 \cdot 7H_2O$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25·0; glucose, 11·1; $CaCl_2 \cdot 2H_2O$, 2·5) was pumped retrogradely into the aorta and then through both kidneys (2·0 mL min^{-1}). Perfusion pressure was recorded as above and the venous return allowed to escape from the severed inferior vena cava. Dose response curves to noradrenaline (NA) and 5-HT were constructed as above. The TxA_2-mimetic U46619 and its antagonist AH23848 were administered by continuous infusion (0·25 mL min^{-1}) into the aortic perfusate. Dose response curves were obtained to an agonist before and after U46619 (or U46619 and AH23848). At the completion of the experiment, which lasted 2·5–2·75 h, both kidneys were removed from the rats and weighed separately.

For both blood and Krebs kidney perfusion, rats were used in pairs (in random order) with a non-diabetic and a diabetic rat being used on the same day, with the same drug dilutions and under the same experimental conditions. Experiments were fully randomized between days.

Weights of kidneys which had not been perfused were obtained from rats injected with either saline or alloxan as described above. After the 14 day period each rat was anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and the kidneys removed and weighed separately. Both the blood and Krebs-perfused kidney preparations were modified from techniques described by Hepburn & Bentley (1982).

The drugs used were: AH23848 ([1α (Z), 2β, 5α]−(±)-7-[5[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid) (Glaxo); alloxan monohydrate (Sigma); heparin sodium (David Bull); 5-hydroxytryptamine creatine sulphate (Sigma); (−)-noradrenaline bitartrate (Sigma); U46619 ((15S)-hydroxy-11α,9α-(epoxymethano) prosta-5Z, 13E-dienoic acid) (Upjohn). All doses are expressed in terms of the base used except for AH23848 which is expressed as its calcium salt.

Statistical analysis of results was performed by means of Student's *t*-test and 2-way ANOVA. Values shown are means ± s.e.mean. A 2-way ANOVA was used to analyse the effect of diabetes on response to an agonist, the effect of U46619 on response to an agonist and the interaction between diabetes and U46619 on response to an agonist. Linear regression analysis was performed where stated (Diem & Leutner 1970).

Results

Body weights and arterial blood pressures of diabetic rats were significantly less than those of non-diabetics (Table 1). Kidneys from diabetic animals that had not been perfused were significantly heavier than kidneys from non-diabetic rats. This change was still evident after Krebs or blood perfusion at 2 mL min^{-1}. However, after 2·5–2·75 h of Krebs perfusion the kidneys of both groups showed significant weight increases compared with unperfused kidneys ($P < 0.05$, Student's *t*-test). These increases, expressed in percentage terms, were not significantly different for either group (% increases for non-diabetics; left 40 ± 9%, right 40 ± 9%: diabetics; left 61 ± 9%, right 58 ± 9%). The perfusion pressure of blood-perfused (but not Krebs-perfused) kidneys of diabetic rats was less than that of non-diabetic animals.

Responses of Krebs-perfused kidneys

Injection of NA into the perfusion fluid was followed by dose-dependent increases in perfusion pressure in kidneys of non-diabetic rats (Fig. 1). The responses to the higher doses were augmented, in a concentration dependent manner, during infusion of the TxA_2-mimetic U46619 (11–555 ng mL^{-1}). Linear regression analysis showed that the log dose response curves to NA were shifted to the left in a non-

Table 1. Body and kidney weights, blood pressures and kidney perfusion pressures of non-diabetic and diabetic rats.

	Non-diabetic	(n)	Diabetic	(n)
Body weight (g)	351 ± 4	51	315 ± 5**	50
Kidney weight (g) (without perfusion)				
Left	1·36 ± 0·05	8	1·58 ± 0·06**	7
Right	1·35 ± 0·06		1·63 ± 0·06**	
Kidney weight (g) (after blood perfusion)				
Left	1·36 ± 0·04	10	1·64 ± 0·09*	10
Right	1·29 ± 0·04		1·62 ± 0·08**	
Kidney weight (g) (after Krebs perfusion)				
Left	1·91 ± 0·05†	41	2·54 ± 0·06**,†	40
Right	1·89 ± 0·05†		2·58 ± 0·06**,†	
Blood pressure (mmHg)				
Diastolic	91 ± 4	10	54 ± 8*	10
Systolic	122 ± 6		91 ± 7*	
Heart rate (beats min^{-1})	332 ± 18	10	290 ± 17	10
Perfusion pressure of blood-perfused kidneys (mmHg)	48 ± 4	10	33 ± 3*	10
Perfusion pressure of Krebs-perfused kidneys (mmHg)	25 ± 2	41	22 ± 1	40

Figures shown are means ± s.e. means.
* $P < 0.05$, ** $P < 0.01$ significantly different from non-diabetic, Student's *t*-test.
† $P < 0.05$ significantly different from unperfused kidneys, Student's *t*-test.

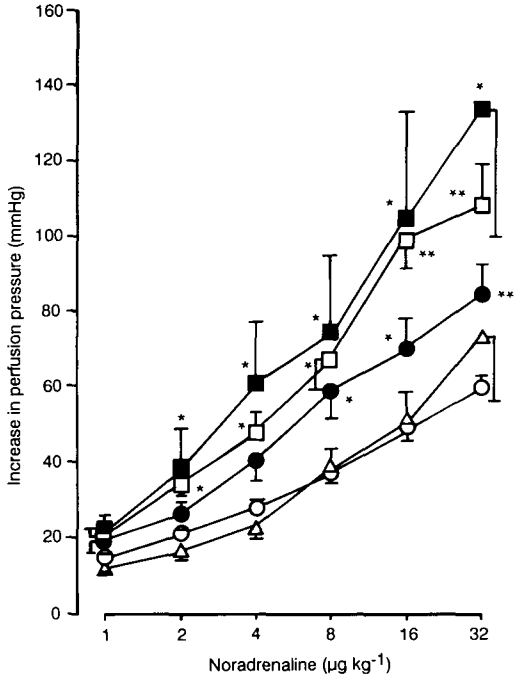

FIG. 1. Effect of U46619 infusion on constrictor responses to noradrenaline in Krebs-perfused kidneys from non-diabetic rats. Without U46619 (○——○, n = 25), U46619 11 ng mL^{-1} (●——●, n = 5); 111 ng mL^{-1} (□——□, n = 5); 555 ng mL^{-1} (■——■, n = 5); U46619 11 ng mL^{-1} with AH23848 111 ng mL^{-1} (△——△, n = 5). Vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.01$, significantly different from responses in the absence of U46619, 2-way ANOVA.

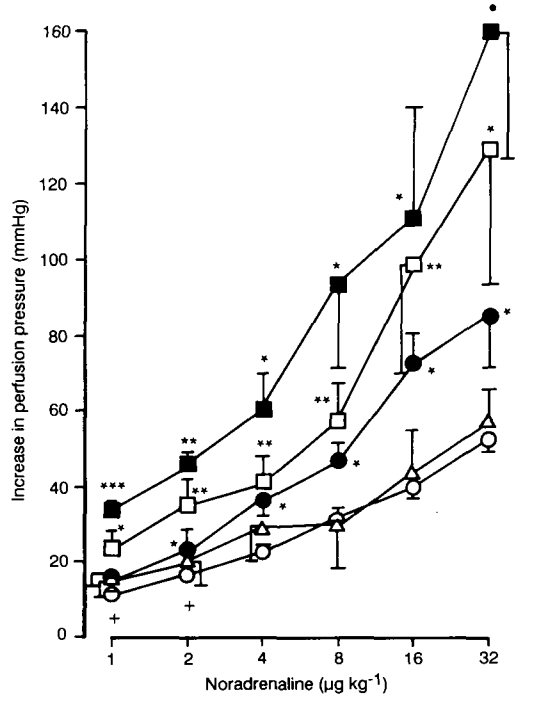

FIG. 2. Effect of U46619 infusion on constrictor responses to noradrenaline in Krebs-perfused kidneys from diabetic rats. Without U46619 (○——○, n = 25), U46619 11 ng mL^{-1} (●——●, n = 5); 111 ng mL^{-1} (□——□, n = 5); 555 ng mL^{-1} (■——■, n = 5), and U46619 11 ng mL^{-1} with AH23848 111 ng mL^{-1} (△——△, n = 5). Vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from responses in the absence of U46619, 2-way ANOVA. † $P < 0.05$, significantly different from non-diabetic rats without U46619 (see Fig. 1), 2-way ANOVA.

parallel fashion by U46619. U46619 also augmented responses to NA in the kidneys of diabetic rats (Fig. 2). However, analysis by 2-way ANOVA showed that although U46619 significantly potentiated responses in both diabetics and non-diabetics the degree of potentiation of responses to NA (at any of the doses used) by U46619 was not significantly greater in diabetics than non-diabetics. The increases in perfusion pressure produced by continuous infusion of U46619 (11, 111 and 555 ng mL^{-1}) were 0 (undetectable), 15·5 ± 5·0, and 14·0 ± 1·0 mmHg (n = 15, non-diabetics); and 0 (undetectable), 13·0 ± 4·6, and 14·5 ± 1·7 mmHg (n = 15, diabetics), respectively.

The log dose-response curve to NA (1–32 μg kg^{-1}) obtained in Krebs-perfused kidneys of either non-diabetic or diabetic rats was not significantly changed by combined continuous infusion of U46619 (11ng mL^{-1}) plus the TxA_2 receptor antagonist AH23848 (111ng mL^{-1}, Figs 1, 2, n = 5). Comparison of responses to NA in the absence of U46619 (Figs 1, 2) showed a reduced sensitivity of diabetic compared with non-diabetic kidneys at low doses of NA (1, 2 μg kg^{-1}, ANOVA, $P < 0.05$).

Infusion of 5-HT (111 ng mL^{-1}) causing an increase in perfusion pressure of 15·6 ± 6·0 mmHg (n = 5, non-diabetics) and 13·0 ± 2·0 mmHg (n = 5, diabetics), i.e. of similar magnitude to that caused by 111 and 555 ng mL^{-1} U46619, did not significantly affect the log dose-response curve obtained for bolus injections of NA in kidneys of non-diabetic rats, but significantly augmented responses in kidneys from diabetic rats (4, 8, 32 μg kg^{-1}, Fig. 3).

As shown in Fig. 4, 5-HT (1–64 μg kg^{-1}) caused vasoconstrictor responses in Krebs-perfused kidneys from non-diabetic rats. These were increased during continuous infusion of U46619 (1·1 and 11 ng mL^{-1}). Potentiation of the effects of higher doses of 5-HT was significant. During simultaneous infusion of AH23848 (111 ng mL^{-1}) in the

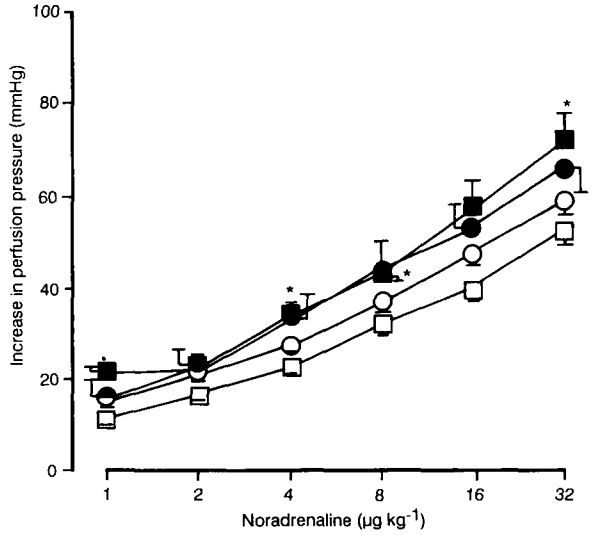

FIG. 3. Effect of 5-hydroxytryptamine (5-HT) infusion on constrictor responses to noradrenaline in Krebs-perfused kidneys. Without 5-HT (○——○, non-diabetics, n = 5; □——□, diabetics, n = 5); 5-HT 111 ng mL^{-1} (●——●, non-diabetics, n = 5; ■——■, diabetics, n = 5). Vertical lines show s.e.mean. * $P < 0.05$, significantly different from responses in the absence of 5-HT, 2-way ANOVA.

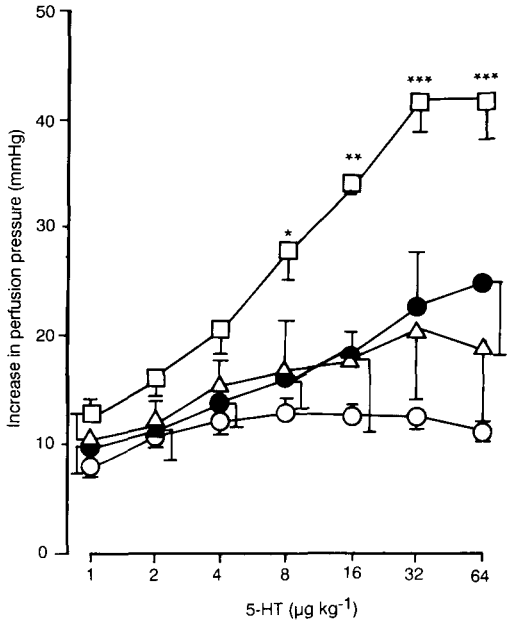

FIG. 4. Effect of U46619 infusion on constrictor responses to 5-hydroxytryptamine in Krebs-perfused kidneys from non-diabetic rats. Without U46619 (○——○, n = 16), U46619 1·1 ng mL^{-1} (●——●, n = 5), 11 ng mL^{-1} (□——□, n = 6), and U46619 11 ng mL^{-1} with AH23848 111 ng mL^{-1} (△——△, n = 5). Vertical lines show s.e.mean. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, significantly different from responses in the absence of U46619, 2-way ANOVA.

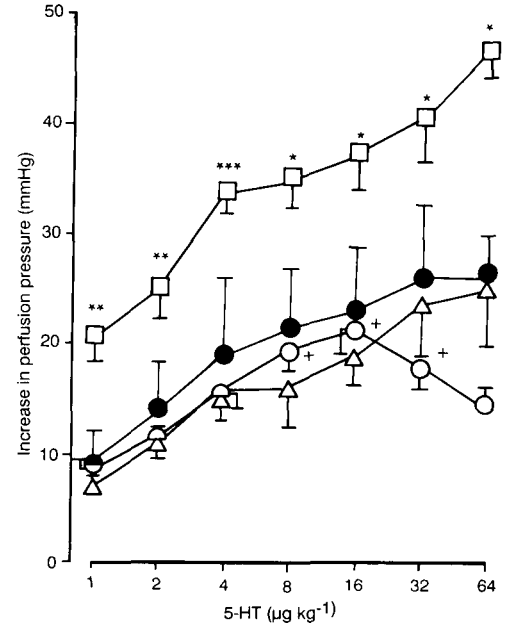

FIG. 5. Effect of U46619 infusion on constrictor responses to 5-hydroxytryptamine in Krebs-perfused kidneys from diabetic rats. Without U46619 (○——○, n = 15), U46619 1·1 ng mL^{-1} (●——●, n = 5); 11 ng mL^{-1} (□——□, n = 5), and U46619 11 ng mL^{-1} with AH23848 111 ng mL^{-1} (△——△, n = 5). Vertical lines show s.e.mean. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, significantly different from responses in the absence of U46619, 2-way ANOVA. † $P < 0{\cdot}05$, significantly different from non-diabetic without U46619 (see Fig. 4), 2-way ANOVA.

presence of the higher concentration of U46619, no potentiation was seen. In Krebs-perfused kidneys from diabetic rats (Fig. 5), continuous infusion of 11 ng mL^{-1} U46619 potentiated responses to all doses. Comparison of responses to 5-HT in the absence of U46619 (Figs 4, 5) showed an increased sensitivity of kidneys from diabetic compared with those from non-diabetic rats (4–16 μg kg^{-1}, ANOVA, $P < 0{\cdot}05$). 2-way ANOVA of data from Figs 4 and 5 showed that U46619 (11 ng mL^{-1}) significantly potentiated responses to 5-HT (4-64 μg kg^{-1}), and the degree of potentiation at 1–4 μg kg^{-1} 5-HT was significantly greater in diabetics than non-diabetics.

Responses of blood-perfused kidneys

Responses to both NA and 5-HT (0·5–4 μg kg^{-1}) were significantly attenuated ($P < 0{\cdot}05$, Student's *t*-test) in blood-perfused kidneys of diabetic rats when compared with age-matched non-diabetics (data not shown).

Discussion

Subsensitivity of the blood-perfused diabetic renal vasculature to noradrenaline (NA) or 5-hydroxytryptamine (5-HT) could be explained as a consequence of vasodilatation, indicated by the lower basal perfusion pressure when the diabetic rat kidneys were perfused at identical flow rates to the controls. However, subsensitivity of the diabetic renal vasculature to low doses of NA, although not to 5-HT, was additionally found in the Krebs-perfused diabetic preparations and in these, basal perfusion pressures of controls and diabetics were not significantly different.

Oedema developed in the Krebs-perfused kidneys of the controls but was not significantly more pronounced in kidneys obtained from diabetic animals. However, many previous studies have been performed without the use of an oncotic agent (Gagnon et al 1974; Bhardwaj & Moore 1988; Mohy El-Din & Malik 1988; Quilley et al 1989) and in some of these cases the degree of oedema had not been adequately assessed. The changes in renal vasculature sensitivity during diabetes found in the present study are unlikely to be a consequence of oedema, since the percentage increase in weight following Krebs perfusion was not significantly different for kidneys from diabetic rats compared with those from non-diabetic rats. Also, subsensitivity to NA was found in the kidneys from diabetic rats that were blood-perfused but which did not develop oedema. Furthermore, whereas responses to low doses of NA were depressed during diabetes, those to 5-HT were augmented in the Krebs-perfused preparations, indicating that the sensitivity changes were not non-specific as might be expected if they were a consequence of oedema or as a result of alloxan itself. However, diabetic-induced changes in vasculature permeability could possibly affect passage of some substances from the vascular lumen through the intima to the tunica media thus contributing to the changed responsiveness to the interacting effects of 5-HT and U46619 discussed below.

The magnitude of the potentiation of threshold amounts of 5-HT by U46619 was increased in kidneys from the diabetic rats, compared with controls, suggesting that in the renal vasculature of the diabetic animal, substantial vasoconstriction could develop in the presence of concentrations of 5-HT and TxA_2 (or PGH_2) which individually would be below the threshold for increasing vascular tone. During diabetes, arachidonic acid metabolism is altered, resulting in

increased TxA_2 production by platelets and decreased PGI_2 production by the vascular endothelium (Rosen & Hohl 1984). There is also indirect evidence for an increase in formation of TxA_2 in the circulation of alloxan-diabetic rats as used in this study (Boura et al 1986; 1987). As platelet aggregation liberates both 5-HT and TxA_2 (Moncada & Vane 1979) the present data indicate that the effects of these two autacoids synergize during diabetes to increase vascular tone. The mechanisms responsible for potentiation of 5-HT and NA during TxA_2/PGH_2 receptor stimulation by U46619 remain to be completely elucidated. The effect appeared to be mediated by TxA_2/PGH_2 receptor activation since it was inhibited by AH23848. We hypothesize that the increased vascular responsiveness caused by U46619 was unlikely to be a non-specific effect resulting from increased tone of the vessels. Although infusion of 5-HT (111 ng mL^{-1}) caused the same degree of renal vasoconstriction as infusion of the higher concentrations of U46619, the combined effects of 5-HT and NA were no more than additive, whereas the combined effects of U46619 and NA (or U46619 and 5-HT) were more than additive. Also, the effect of 5-HT was potentiated by U46619 in concentrations too low to cause a measurable change in renal vascular resistance. Thus, the present study supports the growing evidence that changes in arachidonic acid metabolism during diabetes and vessel changes in permeability and sensitivity could contribute to the vascular pathological conditions characteristic of the disease.

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