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Renal vascular responsiveness to arachidonic acid in experimental diabetes

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1 Isolated perfused kidneys from diabetic rats (duration 4–6 and 20–24 weeks) were more sensitive to the vasoconstrictor effects of arachidonic acid than kidneys from age-matched control rats. Sensitivity diminised with age in both control and diabetic groups.

2 The enhanced vasoconstrictor effect of arachidonic acid in diabetic rat kidneys was associated with increased conversion to prostaglandins.

3 The renal vasoconstrictor response to arachidonic acid in both groups was reduced by thromboxane A_2/prostaglandin H_2 receptor antagonism but not by inhibition of thromboxane synthase.

4 Diabetic rat kidneys were also more sensitive to the vasoconstrictor effects of the endoperoxide analogue, U46619, while vasoconstrictor responses to phenylephrine were not markedly different from those of control rat kidneys.

5 In conclusion, prostaglandin endoperoxides appear to mediate arachidonic acid-induced vasoconstriction in diabetic and control rat kidneys. The enhanced renal vasoconstrictor response to arachidonic acid in diabetic rats results from increased sensitivity to endoperoxides and increased formation of endoperoxides from arachidonic acid.

Introduction

Diabetes mellitus results in abnormalities of vascular and renal eicosanoid production. Thus, Halushka *et al.* (1981) found increased platelet thromboxane A_2 (TxA_2) formation in diabetic patients, while several investigators have shown reduced vascular prostacyclin production in human and experimental diabetes (Harrison *et al.*, 1978; Johnson *et al.*, 1979; Valentovic & Lubawy, 1983). Moreover, we demonstrated alterations in the profile of urinary prostanoids in rats made diabetic with streptozotocin (Quilley & McGiff, 1985), while others have shown abnormalities in the generation of glomerular prostanoids (Schambelan *et al.*, 1985). Consequently, we predicted the renal vascular responses to exogenous arachidonic acid would be altered in diabetes depending upon the activity of cyclo-oxygenase, the profile of eicosanoids produced and the sensitivity of the vasculature to these eicosanoids. Indeed, in a preliminary study we have shown enhanced conversion of arachidonic acid by the kidneys of diabetic rats associated with increased vasoconstrictor responses (Sarubbi *et al.*, 1989). However, this study was complicated by the development of tachyphylaxis to repeated administration of arachidonic acid. In a recent study we provided evidence that endoperoxides, intermediates in the metabolism of arachidonic acid by cyclo-oxygenase, are responsible for the vasoconstrictor effects of arachidonic acid in the isolated perfused kidney of the rat (Quilley *et al.*, 1989). If the same pertains for the diabetic rat kidney, then the response to arachidonic acid should depend upon cyclo-oxygenase activity and the responsiveness of the vasculature to endoperoxides. The present study was designed, therefore, to determine renal vascular responsiveness to arachidonic acid in kidneys of rats with short-term (4–6 weeks) and long-term (20–24 weeks) diabetes. The role of endoperoxides in the response was examined by use of a thromboxane synthase inhibitor and a TxA_2/prostaglandin H_2 (PGH_2) receptor antagonist, while sensitivity of the vasculature to endoperoxides was assessed by testing the vascular responses to an endoperoxide analogue. Cyclo-oxygenase activity was assessed by measuring the release of PGE_2 and 6-keto-$PGF_{1\alpha}$ following arachidonic acid administration.

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Methods

Diabetes was induced in male Wistar rats (175–200 g) with streptozotocin, 70 mg kg^{-1}, i.v. Control rats were given an equivalent volume of citrate buffer, pH 4.2. Rats were used either 4–6 weeks or 20–24 weeks later. At the time of experimentation blood glucose levels were measured by a glucometer (Ames). Rats were considered diabetic if blood glucose levels were greater than 300 mg dl^{-1}. Mean blood glucose levels in citrate-treated rats were less than 100 mg dl^{-1}.

Isolated perfused kidney in situ

Following pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.) the right kidney was exposed by midline laparotomy and the mesenteric and right renal arteries cleared of surrounding tissue. Ties were loosely placed around these vessels and the vena cava just above and below the junction with the right renal vein. The right renal artery was then cannulated with a 19 gauge needle via the mesenteric artery to avoid interruption of blood flow. The vena cava was then occluded and cut to provide an exit for the perfusate. The right ureter was also cut and the animal killed by an intracardiac injection of 10 mg pentobarbitone. In some preparations the vena cava was cannulated for the collection of renal venous effluent for the subsequent measurement of immunoreactive 6-keto-$PGF_{1\alpha}$ and PGE_2.

The kidney was perfused by means of a Watson-Marlow pump (Model 5025) with Krebs-Henseleit buffer gassed with 95% O_2:5% CO_2 at 37°C and flow adjusted to obtain a basal perfusion pressure of 70–80 mmHg. The perfusion pressure was measured with a Harvard pressure transducer and recorded on a Soltec 1246 recorder.

Renal vascular responses to arachidonic acid

Arachidonic acid was administered as random bolus doses (0.3–10 μg) given at 10 min intervals into the perfusate line proximal to the kidney. Each kidney was used for 1 or 2 doses of arachidonic acid only, depending on whether a high or low dose was given first because of the appearance of tachyphylaxis to repeated administrations. Responses in kidneys from rats with diabetes of 4–6 weeks duration (n = 10) were

compared to those in age-matched controls ($n = 8$). Similarly, kidneys from 20–24 week diabetic rats ($n = 9$) were compared to age-matched controls ($n = 10$).

Renal vascular responses to U46619, an endoperoxide analogue

In kidneys from rats with diabetes of 4–6 weeks duration ($n = 5$) and age-matched controls ($n = 5$), U46619 was administered as random bolus doses (10–1000 ng), given at 5–7 min intervals when pressure had returned to baseline levels following the preceding dose.

In kidneys from rats with diabetes of 20–24 weeks duration ($n = 3$) and their corresponding controls ($n = 4$), only one dose of U46619 was compared, 100 ng. These same preparations were also used to test responses to 3 μg arachidonic acid and acted as the vehicle-treated group in those experiments where responses to U46619 and arachidonic acid were tested following inhibition of thromboxane synthase (see below).

Renal vascular responses to phenylephrine

The kidneys of rats with diabetes of 4–6 weeks duration ($n = 5$) and their age-matched controls ($n = 5$) were used to determine perfusion pressure responses to phenylephrine (100–3000 ng), given as random bolus doses at intervals of 5–7 min.

Influence of thromboxane synthase inhibition on the renal vascular responses to U46619 and arachidonic acid

Perfusion pressure increases in response to arachidonic acid (3 μg) and U46619 (100 ng) in vehicle-treated kidneys from 20–24 week diabetic rats ($n = 3$) and age-matched control rats ($n = 4$) were compared to those obtained in diabetic rat kidneys ($n = 3$) and control rat kidneys ($n = 4$) perfused with Krebs-Henseleit buffer containing 5 μM CGS-13080, a thromboxane synthase inhibitor. In addition, the rats were given CGS-13080, 2 mg kg^{-1} i.p., 2 h before the experiment. We have previously shown that this concentration of CGS-13080 is effective in reducing the renal efflux of immunoreactive TxB_2 following arachidonic acid administration by more than 70% (Quilley *et al.*, 1989). The vehicle-treated groups in these experiments correspond to those that were used to compare diabetic and control kidney responses to 100 ng U46619 (see above).

Influence of TxA_2/PGH_2 receptor antagonism on the renal vascular responses to arachidonic acid and U46619

In 6 diabetic (4–6 weeks) and 5 age-matched control rat kidney preparations, perfusion pressure responses to 1 μg and 3 μg arachidonic acid, respectively, were determined after the addition of 1 μM SQ 29548, a TxA_2/PGH_2 receptor antagonist, to the perfusate. These responses were compared to those obtained in the construction of the dose-response curves to arachidonic acid. In some of the preparations used to determine the effects of TxA_2/PGH_2 receptor blockade on responses to arachidonic acid, we verified that SQ 29548 was effective in reducing the responses to U46619 (100 ng), which was administered before and 10 min after addition of the antagonist to the perfusate. Thus, 6 control and 3 diabetic rat kidneys were tested in this way. The responses obtained to U46619 before the addition of SQ 29548 to the perfusate did not differ from those used in the construction of dose-response cuves to U46619 and the data were, therefore, pooled.

The experiments with SQ29548 were only conducted in rats with diabetes of 4–6 weeks duration, while those with CGS-13080 utilized rats with diabetes of 20–24 weeks duration. This design was adopted because of the similar qualitative responses to arachidonic acid and U46619 in both diabetic and control rats irrespective of the duration of diabetes (see Results).

Renal venous efflux of 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 in response to arachidonic acid

In additional experiments, kidneys from 3 rats with diabetes of 4–6 weeks duration and 4 age-matched control rats were used to compare the venous release of immunoreactive 6-keto-$PGF_{1\alpha}$ and PGE_2 following the administration of 3 μg arachidonic acid. Thus, the renal venous effluent was collected for 1 min before and for 2 min after the administration of arachidonic acid, beginning at the onset of a change in perfusion pressure. Perfusates were stored at −20°C prior to radioimmunoassay as previously described (Quilley & McGiff, 1985). Stimulated release of 6-keto-$PGF_{1\alpha}$ and PGE_2 was obtained by subtracting basal release from that measured following arachidonic acid administration.

Statistical analyses

Dose-response curves were compared by ANOVA and individual points on the curve by Student's t test for unpaired data. Similarly, responses to individual doses of arachidonic acid and U46619 between diabetic and control and following treatment with a thromboxane synthase inhibitor or a PGH_2/TxA_2 receptor antagonist were compared by an unpaired t test. Results are expressed as mean ± standard error of the mean (s.e.mean). A P value < 0.05 was considered statistically significant.

Materials

Sodium arachidonate was obtained from NuChek, Elysian, Mn, U.S.A. and dissolved in distilled water to give a concentration of 1 mg ml^{-1} which was stored under nitrogen at −70°C. A fresh solution was thawed and used for each kidney preparation. CGS-13080 (imidazo [1,5-α] pyridine-5-hexanoic acid), a gift from Ciba-Geigy, Summit, NJ, U.S.A, was dissolved in a minimal volume of Na_2CO_3 (4%) and diluted with saline.

Solutions were freshly made each day. SQ 29,548 ([1S[1α, 2β-(5Z),3β,4α]]-7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]-methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) was a gift from E.R. Squibb and Sons, Inc., Princeton, NJ, U.S.A., and was dissolved in 95% ethanol to give a 10 mg ml^{-1} solution which was diluted with 9 volumes of 2 mM Na_2CO_3. The resulting 1 mg ml^{-1} stock solution was divided into aliquots, sealed under nitrogen and stored at −70°C until used. U46619 (11,9 epoxy methano prostaglandin H_2), a gift from the UpJohn Co., Kalamazoo, MI, U.S.A was initially dissolved in ethanol, 1 mg ml^{-1}, diluted in distilled water to 50 μg ml^{-1} and stored frozen in aliquots at −70°C. The hydrochloride salt of phenylephrine was obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A and was dissolved in distilled water, 100 μg ml^{-1}, and stored in aliquots at −70°C. Streptozotocin was also obtained from the Sigma Chemical Co. and dissolved in citrate buffer, pH 4.2, immediately before use.

Results

Renal vascular responses to arachidonic acid

In rats with diabetes of 4–6 weeks duration and age-matched control rats the renal perfusate flows were 14.6 ± 1.1 ($n = 10$) and 12.3 ± 1.1 ml min^{-1} ($n = 8$), respectively, which yielded basal perfusion pressures of 76 ± 4 mmHg and 75 ± 3 mmHg, respectively. The dose-response curve for arachidonic acid in the diabetic group was shifted to the left (Figure 1). Thus, at comparable doses, arachidonic acid elicited greater increases in renal perfusion pressure in the diabetic rat. At 1.0 μg arachidonic acid the increases in renal perfusion pressure were 137 ± 34 mmHg and 11 ± 1 mmHg for diabetic and control groups, respectively ($P < 0.01$). Similarly, 3 μg arachidonic acid increased perfusion pressure by 189 ± 3 mmHg in the

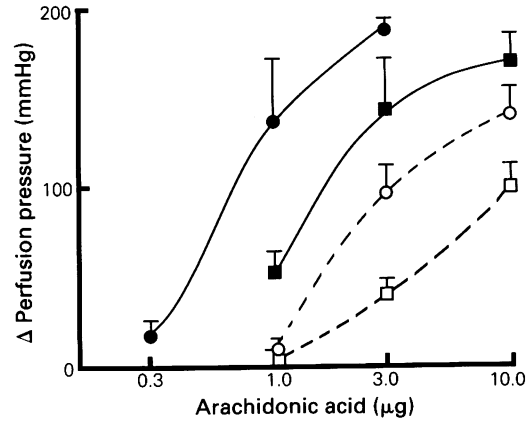

Figure 1 Increases in perfusion pressure in response to arachidonic acid in kidneys from rats with diabetes of duration 4–6 weeks ($n = 10$, ●——●) and 20–24 weeks ($n = 9$, ■——■) and their respective age-matched controls ($n = 8$, ○---○ and $n = 10$, □---□). Data are presented as the mean with s.e.mean shown by vertical bars.

diabetic group compared to 99 ± 13 mmHg in the control group ($P < 0.01$).

In kidneys from rats with diabetes of 20–24 weeks duration ($n = 9$) and age-matched control rats ($n = 10$) the perfusate flows were 23.6 ± 2.5 ml min^{-1} and 17.5 ± 1.7 ml min^{-1} ($P < 0.05$), respectively, which resulted in basal perfusion pressures of 77 ± 6 mmHg and 85 ± 4 mmHg, respectively. As before, the dose-response curve to arachidonic acid in the diabetic group was shifted to the left (Figure 1). Thus, at all doses tested (1–10 μg) the diabetic group responded with greater increases in perfusion pressure. However, the older rats, both diabetic and control, were less responsive than younger rats.

Renal vascular responses to U46619

In kidneys from 4–6 week diabetic rats ($n = 5$) and age-matched controls ($n = 5$) perfusate flow rates were 14.0 ± 0.6 ml min^{-1} and 12.9 ± 0.8 ml min^{-1}, respectively, which resulted in basal perfusion pressures of 71 ± 3 mmHg and 68 ± 2 mmHg, respectively. U46619 elicited significantly greater responses in diabetic rat kidneys compared to control (Figure 2a), such that the dose-response curve was shifted to the left.

In the older diabetic ($n = 6$) and control ($n = 8$) groups perfusate flows were 25.7 ± 1.7 ml min^{-1} and 14.2 ± 1.4 ml min^{-1}, respectively ($P < 0.01$) and basal perfusion pressures were 75 ± 5 mmHg and 77 ± 3 mmHg, respectively. The much greater flow rate in the diabetic rat kidneys could be accounted for by the greater renal mass. Thus, left kidney weight in diabetic rats was 3.95 ± 0.30 g compared to 2.35 ± 0.13 g for control ($P < 0.01$). When this weight difference was taken into account renal perfusate flows were not different, i.e., 6.0 ml min^{-1} g^{-1} for control versus 6.5 ml min^{-1} g^{-1} for diabetic rats. These groups of rats were those used to test the effects of thromboxane synthase inhibition on the vasoconstrictor responses to U46619 (100 ng) and arachidonic acid (3 μg). As thromboxane synthase inhibition did not affect the responses to U46619 the data were pooled. Thus, in the diabetic rat kidneys treated with CGS 13080, 100 ng U46619 increased perfusion pressure by 131 ± 22 mmHg compared to an increase of 47 ± 10 mmHg in control rat kidneys ($P < 0.01$). These perfusion pressure responses are comparable to those seen in the 4–6 week diabetic and control rats when 100 ng U46619 increased perfusion pressure by 146 ± 7 mmHg and 75 ± 17 mmHg, respectively.

Renal vascular responses to phenylephrine

In 4–6 week diabetic ($n = 5$) and age-matched control ($n = 5$) rats, renal perfusate flow rates were 17.2 ± 0.7 and 10.6 ± 0.7 ml min^{-1}, respectively, which resulted in similar basal perfusion pressures of 73 ± 2 and 74 ± 1 mmHg, respectively. In the diabetic rat kidneys, phenylephrine (100–3000 ng) resulted in dose-dependent increases in perfusion pressure which were slightly greater than those obtained in control rat kidneys, the difference being significant at the lower doses (Figure 2b). Thus, the diabetic rat kidney has a lower threshold for the vasoconstrictor response to phenylephrine, although the maximal response was not different from control.

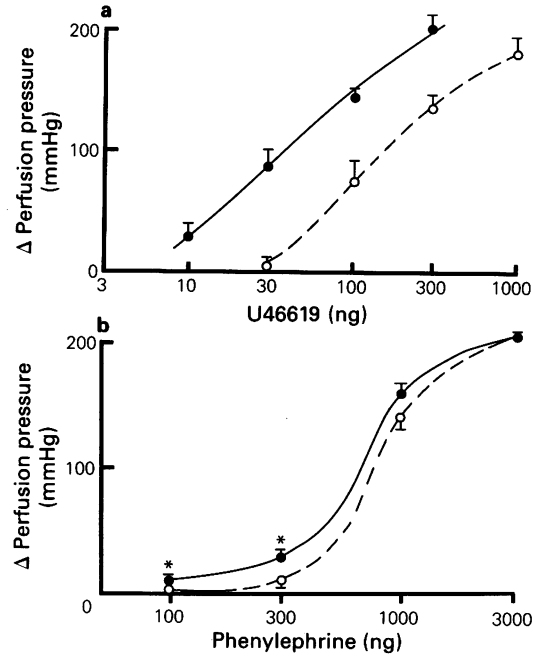

Figure 2 Increases in perfusion pressure in response to (a) the thromboxane A_2/prostaglandin H_2 agonist, U46619, and (b) phenylephrine in kidneys from rats with diabetes of 4–6 weeks duration ($n = 5$ for each agonist) and from age-matched control rats ($n = 5$ for each agonist). (●——●) Diabetic; (○---○) control. Data are presented as the mean with s.e.mean shown by vertical bars.

Effects of inhibition of thromboxane synthase on renal vascular responses to U46619 and arachidonic acid

In both control rats and those with diabetes of 20–24 weeks duration, inhibition of thromboxane formation by CGS-13080 did not alter responses to U46619 (100 ng) or arachidonic acid (3 μg). Thus, in vehicle-treated diabetic rat kidneys perfusion pressure increases to U46619 and arachidonic acid were 140 ± 13 mmHg and 182 ± 13 mmHg, respectively, compared to increases of 121 ± 47 mmHg and 186 ± 5 mmHg, respectively, in kidneys treated with CGS-13080 (Figure 3). Similarly, in control rat kidneys the perfusion pressure responses to U46619 and arachidonic acid were 54 ± 18 mmHg and 78 ± 15 mmHg, respectively, in the absence of CGS-13080 compared to 40 ± 11 mmHg and 66 ± 19 mmHg, respectively, in the presence of CGS-13080.

Effects of TxA_2/PGH_2 receptor antagonism on renal vascular responses to U46619 and arachidonic acid

In rats with diabetes of 4–6 weeks duration and age-matched control rats, the renal perfusate flow rates were 17.7 ± 1.5 ml min^{-1} and 11.8 ± 1.6 ml min^{-1}, respectively, resulting in basal perfusion pressures of 76 ± 3 mmHg and 77 ± 2 mmHg, respectively. In diabetic rat kidneys, the administration of 1 μg arachidonic acid, following SQ 29548 treatment, increased perfusion pressure by 60 ± 22 mmHg compared to 137 ± 34 mmHg in untreated preparations, a reduction of almost 60% (Figure 4). Similarly, in control preparations after SQ 29548, 3 μg arachidonic acid increased pressure by 12 ± 10 mmHg compared to 99 ± 13 mmHg in

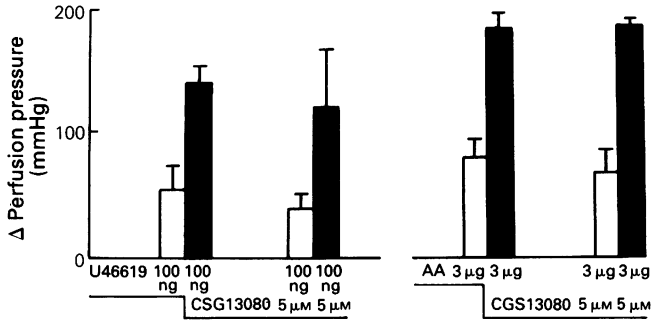

Figure 3 Effects of thromboxane synthesis inhibition with CGS 13080 (5 μM) on perfusion pressure responses to the endoperoxide analogue, U46619, and arachidonic acid (AA) in kidneys from rats with diabetes of 20–24 weeks duration ($n = 3$; solid columns) and from age-matched controls ($n = 4$; open columns). Data are presented as the means with s.e.mean shown by vertical bars.

untreated kidneys ($P < 0.01$), a reduction of 88%. We verified that this concentration of SQ 29548 was effective in blocking the responses to U46619. Thus, in control rat kidneys the perfusion pressure response to 100 ng U46619 was reduced from 85 ± 12 mmHg to zero after SQ 29548 treatment (Figure 4). Similarly, in diabetic rat preparations the response to U46619 was markedly reduced from 181 ± 11 mmHg to 8 ± 7 mmHg after SQ 29548 ($P < 0.01$).

Release of 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin E_2

In diabetic (4–6 weeks) and control rat kidneys the basal release of 6-keto-$PGF_{1\alpha}$, an index of prostacyclin, into the venous effluent was 0.5 ± 0.1 ng min^{-1} and 0.3 ± 0.1 ng min^{-1}, respectively (NS). In contrast, basal release of PGE_2 was lower in diabetic rats (0.7 ± 0.1 ng min^{-1}) than control (2.3 ± 0.7 ng min^{-1}) rats ($P < 0.05$). However, administration of 3 μg arachidonic acid resulted in greater increases in the release of 6-keto-$PGF_{1\alpha}$ and PGE_2 from diabetic rat kidneys than control rat kidneys. Thus, 6-keto-$PGF_{1\alpha}$ and PGE_2 release increased by 6.0 ± 0.6 ng min^{-1} and 7.1 ± 1.2 ng min^{-1}, respectively, from diabetic rat kidneys and 2.7 ± 0.4 ng min^{-1} ($P < 0.05$) and 3.9 ± 0.5 ng min^{-1} ($P < 0.05$), respectively, from control rat kidneys. The increased release of prostanoids from diabetic rat kidneys was associated with greater perfusion pressure increases to arachidonic acid, i.e., 165 ± 22 mmHg vs 110 ± 22 mmHg in control rat kidneys ($P < 0.05$).

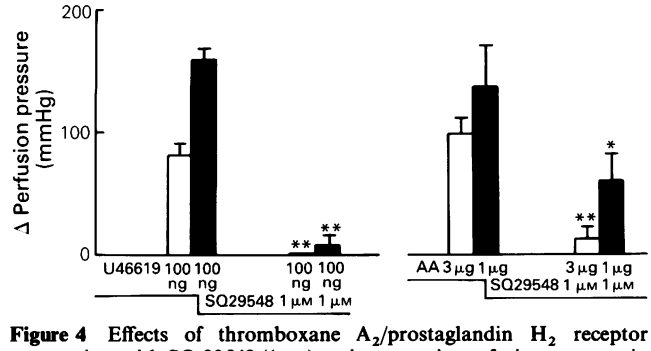

Figure 4 Effects of thromboxane A_2/prostaglandin H_2 receptor antagonism with SQ 29548 (1 μM) on increases in perfusion pressure in kidneys from rats with diabetes of 4–6 weeks duration (solid columns) and from age-matched control rats (open columns) in response to the endoperoxide analogue, U46619 ($n = 6$ for control and 3 for diabetic), and arachidonic acid ($n = 5$ for control and 6 for diabetic). The pretreatment values for U46619 also include data from the dose-response curves to U46619 (Figure 2) so that $n = 11$ for control and 8 for diabetic. The values for the arachidonic acid (AA) responses were obtained from Figure 1. Data are presented as the means with s.e.mean shown by vertical bars. *$P < 0.05$, **$P < 0.01$, compared to responses with SQ 29548.

Discussion

This study demonstrates enhanced renal vasoconstrictor responses to arachidonic acid in rats with diabetes induced by streptozotocin and confirms our earlier preliminary study (Sarubbi *et al.*, 1989). However, the previous study was complicated by the development of tachyphylaxis to the vasoconstrictor effects of arachidonic acid. This vascular tachyphylaxis to arachidonic acid was avoided by administering only one or two doses of arachidonic acid to each kidney depending on whether a high or low dose was given first. Our earlier work has now been extended to characterize more fully the renal vascular response in diabetic rats.

In both diabetic and non-diabetic rat kidneys the vasoconstrictor effect of arachidonic acid is cyclo-oxygenase-dependent, as the response is reduced by indomethacin or meclofenamate (Sarubbi *et al.*, 1989; Quilley *et al.*, 1989). In non-diabetic rat kidneys, we have previously shown that the vasoconstrictor response to arachidonic acid is mediated by the endoperoxides (Quilley *et al.*, 1989). The results of the present study indicate that the same pertains to the diabetic rat kidney, as the vasoconstrictor response to arachidonic acid is not affected by thromboxane synthetase inhibition but is markedly reduced by TxA_2/PGH_2 receptor antagonism. However, we cannot exclude the possibility that prostanoids other than endoperoxides also contribute to the arachidonic acid response in the diabetic rat, although in normal rats we have shown that microgram quantities of PGD_2, PGE_2 and $PGF_{2\alpha}$ are required to elicit vasoconstriction while responses to PGE_2 and $PGF_{2\alpha}$ are unaffected by SQ 29548 (Quilley *et al.*, 1989).

There are several possible mechanisms whereby experimentally-induced diabetes may lead to an increase in renal vascular responsiveness to arachidonic acid. These include a generalized increase in sensitivity to vasoconstrictor agents which would probably reflect an enhancement of signal transducing mechanisms, an increase in conversion of arachidonic acid to endoperoxides or enhanced responsiveness to endoperoxides, possibly resulting from increased TxA_2/PGH_2 receptor number or affinity. A generalized increase in sensitivity to vasoconstrictor agents can be excluded, as previous studies have shown reduced renal vascular responsiveness to angiotensin II and arginine vasopressin in the diabetic rat (Reineck & Kreisberg, 1983; Sarubbi *et al.*, 1989). Moreover, in this study we found that the vasoconstrictor effects of phenylephrine were not markedly different in diabetic versus control rat kidneys. An increase in the conversion of arachidonic acid by cyclo-oxygenase contributes to the enhanced vasoconstrictor effects in diabetes. Thus, the increase in venous efflux of PGE_2 and PGI_2, measured as 6-keto-$PGF_{1\alpha}$, from the diabetic rat kidney after arachidonic acid administration was twice that of control rat kidneys. These results confirm those of a previous study and are in agreement with other studies demonstrating enhanced conversion of exogenous arachidonic acid in various tissues from diabetic rats (Rosen *et al.*, 1983; Roth *et al.*, 1983; Schambelan *et al.*, 1985). Finally, increased sensitivity of the renal vasculature of diabetic rats to endoperoxides also appears to contribute to the enhanced response to arachidonic acid. Thus, the dose-response curve to the endoperoxide analogue, U46619, in the diabetic rat kidney is shifted to the left. This is in contrast to the work of Boura *et al.* (1986) who demonstrated reduced responsiveness to U46619 in the autoperfused hindquarters of the alloxan diabetic rat. However, Boura *et al.* obtained increased vasoconstrictor responses to arachidonic acid, which were attributed to increased TxA_2 formation as blockade of the receptor reduced response. Nonetheless, the observations of Boura *et al.* can be explained by increased platelet formation of either endoperoxides or TxA_2 in diabetic rats. Thus, in human and experimental diabetes there are accounts of increased platelet cyclo-oxygenase and TxA_2 synthesis (Ziboh *et al.*, 1979; Subbiah & Deitemeyer, 1980; Halushka *et*

al., 1981). The increased sensitivity of the diabetic rat kidney to the vasoconstrictor effects of U46619 could be the result of increased receptor number or affinity, although this cannot be determined without radioligand binding studies which we have not done.

Our study indicates that increased renal vascular responsiveness to arachidonic acid in the diabetic rat results from increased cyclo-oxygenase activity, leading to the formation of more endoperoxides as well as enhanced renal responsiveness to the endoperoxides. The age-dependent diminution in vascular responsiveness to arachidonic acid in both diabetic and control rats is most probably due to reduced cyclo-oxygenase activity, as the decline in responsiveness to the endoperoxides is less suggesting that reduced formation of vasoconstrictor products, rather than reduced sensitivity, is responsible. Finally, enhanced renal vascular sensitivity to endoperoxides and increased conversion of arachidonic acid in diabetes may be of pathological significance, particularly under conditions of platelet aggregation whereupon greater vasoconstriction would be anticipated.

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