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Role of endoperoxides in arachidonic acid-induced vasoconstriction in the isolated perfused kidney of the rat

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1 Administration of arachidonic acid caused dose-dependent vasoconstriction in the isolated rat kidney perfused *in situ* with Krebs-Henseleit solution.

2 Inhibition of cyclo-oxygenase with indomethacin or meclofenamate reduced the renal vasoconstrictor effect of arachidonic acid.

3 The renal vasoconstrictor effect of arachidonic acid was unaffected by CGS-13080 at concentrations that effectively reduced thromboxane A_2 (TxA_2) synthesis by platelets and the kidney.

4 The endoperoxide/TxA_2 receptor antagonist, SQ 29,548, abolished the renal vasoconstrictor effect of arachidonic acid and of U46619, an endoperoxide analogue. In contrast, SQ 29,548 did not affect the renal vasoconstrictor response to angiotensin II, prostaglandin E_2 or $F_{2\alpha}$.

5 These data suggest that the vasoconstrictor effect of arachidonic acid in the isolated kidney of the rat is mediated by its metabolites, including the prostaglandin endoperoxides.

Introduction

Arachidonic acid (AA), the precursor of prostaglandins, thromboxane and leukotrienes, elicits renal vasodilatation or renal vasoconstriction depending on the species. For example, the administration of AA produces vasodilatation in the dog (Chang *et al.*, 1975) and rabbit (Larson & Angaard, 1974) kidney and vasoconstriction in the rat kidney (Gerber & Nies, 1979). The renal vasodilator effect of AA in dogs and rabbits requires metabolism of the fatty acid by cyclo-oxygenase to vasodilator mediators, presumably prostaglandin E_2 (PGE_2) and PGI_2 (Larsson & Angaard, 1974; Chang *et al.*, 1975). The renal vasoconstrictor effect of AA in the rat also requires metabolism of the fatty acid by cyclo-oxygenase to a vasoconstrictor mediator(s) (Sakr & Dunham, 1982).

In the rat kidney autoperfused with blood, the renal vasoconstrictor effect of AA is incompletely inhibited by OKY-1581, an inhibitor of thromboxane synthetase, suggesting that thromboxane A_2 (TxA_2) of platelet or renal origin mediates, in part, the vascular effect of arachidonic acid in the rat kidney (Sakr & Dunham, 1982). The mediator(s) of AA-induced renal vasoconstriction which is not subject to attenuation by inhibitors of thromboxane synthetase is yet to be identified.

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The prostaglandin endoperoxides, PGG_2 and PGH_2, common precursors of prostaglandins and TxA_2, stimulate contraction of isolated vascular smooth muscle via interaction with TxA_2 receptors (Coleman *et al.*, 1981; Saussy *et al.*, 1985). Hence the possibility arises that the prostaglandin endoperoxides contribute to the renal vasoconstrictor effect of AA in the rat. To test this hypothesis we examined the vasoconstrictor effect of AA in the rat kidney perfused *in situ* with Krebs-Henseleit buffer, in the presence and the absence of cyclo-oxygenase inhibitors, thromboxane synthesis inhibitors or antagonists of TxA_2/PGH_2 receptors. If prostaglandin endoperoxides interacting with the TxA_2 receptor contribute to AA-induced vasoconstriction, treatment with blockers of TxA_2/PGH_2 receptors should be more effective than inhibitors of thromboxane synthetase in attenuating the vasoconstrictor effect of arachidonic acid.

Methods

Male Wistar rats (Charles River Labs, Wilmington, MA), 8–10 weeks of age (body weight 260–340 g) were used for these studies.

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 for exit of the perfusate. The right ureter was also cut. The animal was then killed by an intracardiac injection of 10 mg pentobarbitone. 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 the flow adjusted to approximately 13 ml min^{-1} to obtain a basal perfusion pressure of approximately 100–110 mmHg. The perfusion pressure was measured with a Harvard pressure transducer and recorded on a Soltec 1246 recorder.

In those preparations used to establish the effective concentration of CGS-13080 for inhibition of renal thromboxane synthesis, the vena cava was cannulated for the collection of renal venous effluent for the subsequent measurement of immunoreactive TxB_2.

Examination of renal vascular responses to arachidonic acid

AA was administered as random bolus doses (1–100 μg) given into the perfusate line just proximal to the kidney. Each kidney was used for 1 or 2 doses of AA only, depending on whether a high or low dose was given first. For example, if the first injection of AA was at a dosage of 10 μg or greater, no subsequent injections of the fatty acid were made. This procedure was adopted because of the appearance of a tachyphylactic-type effect upon repeated administration of AA.

Effect of cyclo-oxygenase and thromboxane synthase inhibition on the renal vascular response to arachidonic acid

The renovascular response to injections of AA was studied in rat kidneys perfused with Krebs-Henseleit buffer containing indomethacin (1 μM) or meclofenamate (3 μM) to inhibit cyclo-oxygenase, $n = 5$ (Flower, 1974), CGS-13080, $n = 7$ (5 and 25 μM) or OKY-046, $n = 3$ (5 μM) to inhibit thromboxane synthase (McNab *et al.*, 1984; Garcia-Szabo *et al.*, 1984), or with an appropriate vehicle, $n = 10$. When studying the effects of CGS-13080, in addition to adding the drug to the renal perfusate, the rats were treated 2 h prior to perfusion of the kidney with CGS-13080 at 2 mg kg^{-1}, i.p.

Effect of thromboxane A_2/endoperoxide receptor antagonism on the arachidonic acid renovascular response

Rat kidneys were perfused with Krebs buffer containing either SQ 29,548 at a final concentration of 1 μM ($n = 6$) or vehicle ($n = 7$) and dose-response curves to AA (1–30 μg) were constructed as described previously. In the isolated perfused kidneys the effectiveness of receptor antagonism by SQ 29,548 was tested by use of 100 ng U46619, an endoperoxide analogue. This dose of U46619 corresponded to an ED_{40} obtained from dose-response curves constructed from preliminary experiments. In addition, the response to 10 ng angiotensin II was compared in kidneys treated with SQ 29,548 or vehicle. In six other experiments the renal vasoconstrictor responses to PGE_2 or $PGF_{2\alpha}$ were compared before and after SQ29548 was added to the perfusate.

In 5 other preparations another TxA_2/PGH_2 receptor antagonist was used (Stegmeier *et al.*, 1984). Thus, BM13.177, at a concentration (20 μM) that inhibited pressor responses to U46619 (100 ng), was included in the perfusate and the responses to 10 μg AA and 10 ng angiotensin II recorded and compared to responses obtained in vehicle-treated kidneys.

Statistical analyses

Individual points on the curves were compared by Student's t test for unpaired data. Comparison of responses to U46619 and angiotensin II as well as basal perfusion pressure and flow in different groups was also made using an unpaired t test. Comparison of responses to PGE_2 and $PGF_{2\alpha}$ in the same preparations before and after SQ 29,548 were compared by a paired 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. Indomethacin was a gift from Merck, Sharpe and Dohme and was dissolved in 4% Na_2CO_3 and diluted in distilled water; sodium

meclofenamate was obtained from Parke-Davis and Co., Holland, MI, U.S.A. and dissolved immediately before use in distilled water. 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 use. U46619 (11,9 epoxy methano-prostaglandin H_2), a gift from the UpJohn Co., 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. OKY-046 (sodium-(E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoate) was obtained from Ono Pharmaceuticals Ltd., Japan, dissolved in distilled water to yield a stock solution of 2 mg ml^{-1} which was stored in aliquots at −70°C. BM 13,177 (4-[2-benzenesulphonamido)-ethyl]-phenoxyacetic acid) was obtained from Boehringer Mannheim, F.R.G.

Results

Effect of cyclo-oxygenase and thromboxane synthetase inhibitors on arachidonic acid responses in rat kidneys

The flow rates in the control group, CGS-13080 group and indomethacin or meclofenamate group were 13.1 ± 0.8 ml min^{-1}, 13.5 ± 1.5 ml min^{-1} and 13.8 ± 1.3 ml min^{-1}, respectively, and the basal perfusion pressures were 117 ± 5 mmHg, 110 ± 7 mmHg and 101 ± 3 mmHg ($P < 0.05$ vs control), respectively.

Figure 1 shows the effect of AA on renal perfusion pressure in rat kidneys perfused with Krebs-Henseleit buffer only or buffer containing 5 μM CGS-13080. In kidneys perfused without the enzyme inhibitors, injections of arachidonic acid resulted in dose-dependent increases in perfusion pressure which is indicative of vasoconstriction. This response is specific for arachidonic acid as linolenic acid has no effect on renal perfusion pressure when injected in doses of up to 30 μg ($n = 2$). The renal vasoconstrictor effect of arachidonic acid at all dose levels was greatly inhibited by indomethacin (1 μM) or meclofenamate (3 μM). Thus, after cyclo-oxygenase inhibition, 10 μg and 30 μg AA increased pressure by 7 ± 5 mmHg and 34 ± 15 mmHg, respectively, whereas in untreated kidneys 1 μg and 3 μg AA increased perfusion pressure by 38 ± 12 mmHg ($P < 0.01$) and 121 ± 22 mmHg ($P < 0.01$), respectively. In contrast, the thromboxane synthase inhibitor, CGS-13080 at 5 μM, had no effect on the dose-response curve to arachidonic acid. The effectiveness of CGS-13080 in inhibiting thromboxane synthase was ascertained in several experiments. First, we showed that CGS-13080 at 5–25 μM inhibits by more than 97% the synthesis of platelet TxB_2 during clotting of rat blood *in vitro* ($n = 2$). Thus, 1, 5 and 25 μM CGS-13080 reduced TxB_2 from 70.8 ng ml^{-1} to 3.5, 2.1 and 1.7 ng ml^{-1}, respectively. Second, we found that the pretreatment of rats with CGS-13080 at 2 mg kg^{-1}, i.p., reduces the levels of serum TxB_2 measured by radioimmunoassay from 87.6 ± 7.4 ng ml^{-1} ($n = 7$) to 15.6 ± 4.6 ng ml^{-1} ($n = 4$), an inhibition of more than 80% ($P < 0.01$). Third, in two control and two treated preparations the increase in renal venous efflux of immunoreactive TxB_2 following a renal arterial injection of 10 μg arachidonic acid fell from 817 and 449 pg min^{-1} in control kidneys to 72 and 237 pg min^{-1} in kidneys perfused with Krebs-Henseleit buffer containing 5 μM CGS-13080. Moreover, CGS-13080 at 25 μM did not affect the increase in perfusion pressure caused by 10 μg of AA, perfusion pressure being increased by 150 ± 38 mmHg and 152 ± 29 mmHg in kidneys perfused with and without the inhibitor of thromboxane synthesis, respectively. Similarly, OKY-046 at a concentration of 5 μM, was without effect on the perfusion pressure response obtained to 10 μg AA (176 ± 40 mmHg vs 183 ± 26 mmHg in control, $P > 0.05$).

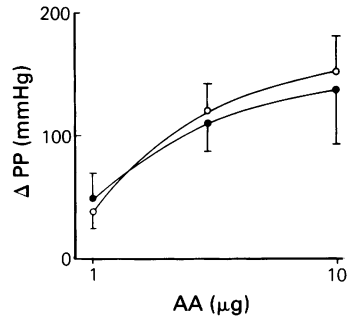

Figure 1 Effect of CGS-13080 (5 μM, ●) on the perfusion pressure responses to arachidonic acid (AA, 1–10 μg) in rat kidneys perfused with Krebs solution; (○) control. Data are presented as the mean with s.e.mean shown by vertical bars.

Effects of thromboxane A_2/prostaglandin H_2 receptor blockade on aracidonic acid responses in control rat kidneys

The flow rates in control and SQ 29,548-treated kidneys were 13.4 ± 0.7 ml min^{-1} and 12.4 ± 1.0 ml min^{-1}, respectively, which resulted in perfusion pressures of 102 ± 3 mmHg in both groups. Figure 2 shows the dose-response curves for

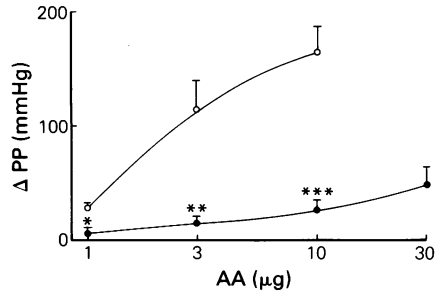

Figure 2 Effects of thromboxane A_2/prostaglandin H_2 receptor antagonism with SQ 29,548 (1 μM) on perfusion pressure responses to arachidonic acid (AA, 1–30 μg) in rat kidneys perfused with Krebs solution. Data are presented as the mean with s.e.mean shown by vertical bars; (○) Control; (●) SQ 29,548 1 μM. * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ when compared to corresponding value in control.

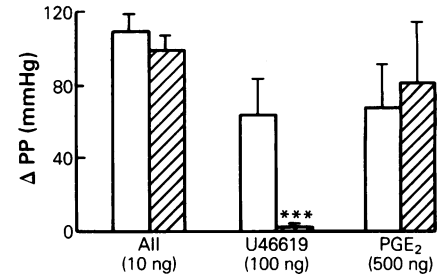

Figure 3 Changes in perfusion pressure to angiotensin II (AII, 10 ng), U46619 (100 ng) and prostaglandin E_2 (PGE_2, 500 ng) in isolated kidneys from rats. Kidneys were perfused with Krebs buffer containing vehicle (open columns) or 1 μM SQ 29,548 (hatched columns). Data are presented as means with s.e.mean shown by vertical bars. *** $P < 0.01$ compared to control.

arachidonic acid (1–30 μg) in kidneys perfused with Krebs buffer alone or buffer containing 1 μM SQ 29,548. This concentration of SQ 29,548 has been demonstrated to be effective in antagonizing responses to 9,11-azo PGH_2 in guinea-pig tracheal spirals and rat aortic strips (Ogletree *et al.*, 1985). SQ 29,548 significantly reduced the perfusion pressure responses to arachidonic acid at all doses tested, shifting the dose-response curve to the right. Thus in control rat kidneys, 10 μg AA increased pressure by 165 ± 21 mmHg compared to only 25 ± 9 mmHg in treated preparations. The responses obtained to U46619 (100 ng) in untreated and treated kidneys (Figure 3) show that the concentration of SQ 29,548 (1 μM) was sufficient to block TxA_2/PGH_2 receptors in the kidney. In contrast, SQ 29,548 was without effect on the perfusion pressure increases produced by 10 ng angiotensin II or 500 ng PGE_2 (Figure 3). Microgram quantities of $PGF_{2\alpha}$ were required to elicit increases in perfusion pressure which were unaffected by SQ 29,548.

Like SQ 29,548, 20 μM BM 13.177 significantly reduced the response to 10 μg AA, $P < 0.05$ (Figure 4). The concentration of BM 13.177 used, effectively blocked TxA_2/PGH_2 receptors shown by the response to U46619 (100 ng), $P < 0.02$ (Figure 4). However, BM 13.177 was without effect on the pressor response obtained to 10 ng AII, ($P > 0.05$).

Discussion

This study demonstrates that the vasoconstrictor effect of AA in the rat kidney involves interaction of one or more of its metabolites by cyclo-oxygenase with TxA_2/PGH_2 vascular receptors. The conclusion that products of AA metabolism by cyclo-oxygenase mediate the renal vasoconstrictor effect of the fatty acid is based on the finding that inhibitors of cyclo-oxygenase, indomethacin and meclofenamate, greatly inhibited the rise in perfusion pressure elicited by AA in the rat kidney perfused *in situ* with Krebs-Henseleit buffer. This finding confirms reports that inhibition of cyclo-oxygenase results in attenuation of AA-induced renal vasoconstriction in the rat (Sakr & Dunham, 1982). The conclusion that the eicosanoid(s) mediating the renal vasoconstrictor effect of AA does so via interaction with TxA_2/PGH_2 receptors is inferred from our finding that two dissimilar antagonists of TxA_2/PGH_2 receptors, SQ 29,548 and BM 13.177, greatly inhibited the rise in perfusion pressure caused by arachidonic acid in the rat kidney.

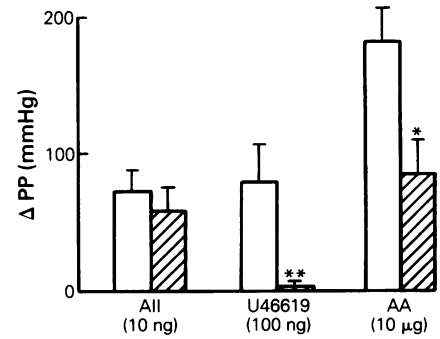

Figure 4 Changes in perfusion pressure to angiotensin II (AII, 10 ng), U46619 (100 ng) and arachidonic acid (AA, 10 μg) in rat isolated kidneys perfused with Krebs buffer containing vehicle (open columns) or 20 μM BM 13.177 (hatched columns). Data are presented as means with s.e.mean shown by vertical bars. * $P < 0.05$, ** $P < 0.02$ compared to control.

In the kidney, the metabolism of AA via the cyclo-oxygenase pathway results in the formation of various eicosanoids including PGI_2, PGE_2, $PGF_{2\alpha}$, TxA_2 and their common endoperoxide precursors, PGG_2 and PGH_2. PGE_2, $PGF_{2\alpha}$, TxA_2 and PGH_2 cause vasoconstriction in the rat isolated kidney perfused with a balanced salt solution and, consequently, are candidates for mediating the renal vasoconstrictor effect of AA (Malik & McGiff, 1975; Shibouta *et al.*, 1981). In the present study, the finding that blockade of TxA_2/PGH_2 receptors with SQ 29,548 or BM 13.177 inhibits the renal vasoconstrictor effect of AA and of U46619, a prostaglandin endoperoxide- and thromboxane-mimetic agent (Coleman *et al.*, 1981), suggests that TxA_2 and/or the prostaglandin endoperoxides mediate the effect of AA on the renal vasculature. The possibility that SQ 29,548 and BM 13.177 inhibit the renal vasoconstrictor effect of AA via a nonspecific mechanism can be excluded because neither receptor antagonist interfered with the renal vasoconstriction elicited by angiotensin II, PGE_2, and $PGF_{2\alpha}$. That the antagonists of TxA_2/PGH_2 receptors did not interfere with the renal vasoconstrictor effect of PGE_2 and $PGF_{2\alpha}$, while inhibiting that of AA, argues against any significant contribution of PGE_2 and $PGF_{2\alpha}$ to AA-induced renal vasoconstriction.

In the rat kidney autoperfused with blood, the lowering of renal blood flow caused by a renal arterial infusion of AA is partially inhibited by pretreatment with OKY-1581, an inhibitor of thromboxane synthetase, suggesting that TxA_2 contributes to the renal vasoconstrictor effect of AA (Sakr & Dunham, 1982). In the present study, however, the thromboxane synthetase inhibitor CGS-13080, at concentrations which significantly diminished the renal output of TxB_2 during AA administration, did not inhibit the renal vasoconstrictor effect of arachidonic acid. Another inhibitor of thromboxane synthesis, OKY-046, also was without effect on AA-induced renal vasoconstriction. One interpretation of these findings is that in the rat kidney perfused with Krebs-Henseleit buffer the vasoconstrictor effect of AA is mediated by a pressor eicosanoid(s) other than TxA_2, presumably a prostaglandin endoperoxide. Yet, based on our findings, one cannot exclude a role for TxA_2 as a mediator of AA-induced renal vasoconstriction. More specifically, the renal vasodilatation expected to result from the reduction in TxA_2 due to inhibition of thromboxane synthetase may be offset by the renal vasoconstriction caused by the increased levels of prostaglandin endoperoxides. If so, the renal vasoconstrictor effect of AA may be mediated by prostaglandin endoperoxides when thromboxane synthetase is inhibited, and by the prostaglandin endoperoxides and/or TxA_2 when it is not. Be that as it may, our finding that AA-induced renal vasoconstriction in the rat is inhibited by antagonists of TxA_2/PGH_2 receptors but not by inhibitors of thromboxane synthetase suggests a role for the prostaglandin endoperoxides in the mediation of the vasoconstrictor response to AA in the rat kidney.

In summary, this study demonstrates that the vasoconstrictor effect of AA in the rat kidney perfused *in situ* with Krebs-Henseleit buffer is inhibited by cyclo-oxygenase inhibitors and by antagonists of TxA_2/PGH_2 receptors, but not by inhibitors of thromboxane synthetase. We conclude that the renal vasoconstrictor response to AA is mediated by the prostaglandin endoperoxides when thromboxane synthetase is inhibited, and by the prostaglandin endoperoxides and/or TxA_2 when it is not.

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