Relative roles of nitric oxide and cyclo-oxygenase and lipoxygenase products of arachidonic acid in the contractile responses of rat renal arcuate arteries

X.C. Wu,^{1,*}N.T. Richards, *J. Michael & E. Johns

Department of Physiology, University of Birmingham, Edgbaston, Birmingham B15 2TT and *Department of Nephrology, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH

- 1 We have examined the effects of inhibition of nitric oxide synthase, cyclo-oxygenase and lipoxygenase on the responses of renal arcuate arteries of Wistar rats, with and without endothelium, to noradrenaline, potassium chloride, endothelin-1, acetylcholine and sodium nitroprusside.
- 2 Noradrenaline, potassium chloride and endothelin-1 caused concentration-dependent contraction of the vessels. Indomethacin (14 μ M) attenuated the contractile response to noradrenaline and to potassium chloride. The inhibitory effect of indomethacin persisted following endothelial removal.
- 3 Acetylcholine produced concentration-dependent relaxation of the vessels which was potentiated by indomethacin (14 μ M).
- 4 N^G-nitro-L-arginine methyl ester (L-NAME, 100 μ M) did not affect the contractile response to either noradrenaline or potassium chloride but abolished relaxation to acetylcholine. In addition, L-NAME abolished the effects of indomethacin on acetylcholine-induced relaxation and noradrenaline- and potassium chloride-induced contraction.
- 5 BW755C attenuated noradrenaline and potassium chloride-induced contraction. This effect persisted in the presence of indomethacin.
- 6 In vessels pretreated with CHAPS, BW755C inhibited both noradrenaline and potassium chloride-induced contraction. In these vessels BW755C had no additional inhibitory effect to indomethacin on noradrenaline- and potassium-induced contraction.
- 7 Inhibition of nitric oxide synthase with L-NAME (100 μ M) attenuated the effect of BW755C on noradrenaline- and potassium-induced contraction.
- 8 BW755C alone did not affect endothelium-dependent relaxation as assessed by the response to acetylcholine. However, in the presence of indomethacin, BW755C inhibited acetylcholine-induced relaxation.
- 9 BW755C did not affect endothelium-independent relaxation as assessed by the response to sodium nitroprusside in vessels with or without endothelium.
- 10 These data support the existence of two vasoconstrictor products of arachidonic acid released during contraction of renal arcuate arteries with noradrenaline and potassium chloride. A cyclo-oxygenase product which appears to be endothelium-independent and the other an endothelium-dependent lipoxygenase product.

Keywords: Renal arcuate arteries; cyclo-oxygenase; lipoxygenase; nitric oxide; Wistar rats

Introduction

Endothelium-derived relaxing factors play a central role in the control of vascular smooth muscle tone and therefore vascular resistance (Baylis *et al.*, 1990; Moncada *et al.*, 1991). Nitric oxide is known to be tonically released and has major influences on regional haemodynamics in particular within the kidney, although the exact site of action remains contentious (Tolins *et al.*, 1990; Rademacher *et al.*, 1990). Endothelium-dependent contracting factors have also been described in a number of different vascular beds including the aorta (Lüscher & Vanhoutte, 1986; Diederich *et al.*, 1990; Dohi *et al.*, 1990; Kato *et al.*, 1990) and renal arcuate arteries of spontaneously hypertensive rats (SHR) (Fu-Xiang *et al.*, 1992). Similar factors have also been described in Wistar-Kyoto (WKY) rats, (Diederich *et al.*, 1989; Koga *et al.*, 1989; Kato *et al.*, 1990) although not consistently. Koga *et al.* (1989) have shown that in WKY aorta, endothelium-dependent vasoconstrictor prostanoids develop with age. Noradrenaline is known to release both endothelium-dependent relaxing and contracting factors from rat arteries (Nigro *et al.*, 1990). The contracting factors described are cyclo-

oxygenase-dependent (Diederich *et al.*, 1990; Lüscher *et al.*, 1990) but the compounds responsible vary depending upon the species and vascular bed studied. In the aorta and intrarenal arteries of both WKY and SHR the contracting factor appears to be either prostaglandin H₂ (Kato *et al.*, 1990; Fu-Xiang *et al.*, 1992) or thromboxane A₂ (Lin & Nasjletti, 1991).

Arachidonic acid may be metabolized into its vasoactive products by lipoxygenases (5-, 12- and 15-) a cytochrome P450-dependent mono-oxygenase and by cyclo-oxygenase. Lipoxygenase activity (5-, 12-, 15-) has been demonstrated in human, bovine and rabbit vascular endothelium and vascular smooth muscle, by functional studies, by the ability to convert arachidonic acid to monohydroxyeicosatetraenoic acids and by immunochemical techniques (Larrue *et al.*, 1983; Farber *et al.*, 1985; Revtyak *et al.*, 1988; Shannon *et al.*, 1991). Interest has focused on the role of lipoxygenase and cytochrome P450-dependent mono-oxygenase products of arachidonic acid in the control of vascular tone and the development of hypertension. However, the role of lipoxygenase products in the control of vascular tone is controversial.

The main vasoactive products of the 5-lipoxygenase en-

¹ Author for correspondence.

zyme are leukotriene C₄ and leukotriene D₄ which are potent vasoconstrictors of intrarenal arteries of rats (Badr *et al.*, 1987). 12-Hydroxyeicosatetraenoic acid (HETE), a product of the 12-lipoxygenase pathway, likewise appears to have vasoconstrictor properties and has been found to contribute to the effector mechanisms of angiotensin II-induced vasoconstriction (Saito *et al.*, 1992). 12-HETE also modulates the intracellular calcium response to arginine vasopressin, angiotensin II and endothelin. Other actions of 12-HETE include inhibition of prostacyclin-induced renin secretion by rat renal cortical slices (Antonipillai, 1990), and direct vasoconstrictor actions in dog renal arcuate arteries (Ma *et al.*, 1991). A product of the 15-lipoxygenase pathway, 15-HETE, is a directly acting vasoconstrictor in a number of vascular beds of the dog (Van Diest *et al.*, 1990). In addition, 15-HETE may act as an endothelium-independent vasodilator in vessels pre-contracted with prostaglandin F_{2α} (Van Diest *et al.*, 1990). This may have physiological relevance as Larrue *et al.* (1983) suggest that 15-HETE is the major lipoxygenase product of cultured endothelial cells and 15-HETE is also the major HETE of atherosclerotic vessel wall (Henriksson *et al.*, 1985).

There are at least two possible mechanisms by which the nitric oxide pathway may interact with the lipoxygenase pathway. Firstly lipoxygenase-dependent metabolism of arachidonic acid can lead to superoxide anion production (Hänsch, 1990), which may accelerate the breakdown of nitric oxide. Secondly there is evidence that nitric oxide may inhibit lipid oxidation by both lipoxygenase and cyclooxygenase (Kanner *et al.*, 1992).

It is clear that the lipoxygenase products of arachidonic acid are potent vasoactive compounds. There are few data on their effects on renal arcuate arteries. In this study we have used indomethacin, the combined lipoxygenase/cyclooxygenase blocker, 3-amino-1-[*m*-(trifluoromethyl)phenyl]-2-pyrazoline (BW755C, Higgs *et al.*, 1979) and the nitric oxide synthase blocker, N^G-nitro-L-arginine-methyl ester (L-NAME), to examine the relative roles of cyclooxygenase and lipoxygenase products of arachidonic acid and their relationship to nitric oxide in the contractile properties of rat renal arcuate arteries.

Methods

Preparation of blood vessels

Renal arcuate arteries (mean internal diameter of 168 ± 10 μm , $n = 114$) were isolated from male Wistar rats (age 3–4 months, weight range 250–400 g). The rats were either killed by CO₂ asphyxia or pentobarbitone injection. Pentobarbitone was used, in a series of animals, to allow cannulation of the main renal artery and perfusion of the kidney with 0.1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate (CHAPS) for 4 min, prior to nephrectomy, to effect endothelium removal. The method of killing does not affect the vascular responses obtained. The kidneys were removed into iced physiological salt solution (PSS) containing (mM): NaCl 119, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.17, NaHCO₃ 25, KH₂PO₄ 1.18, EDTA 0.026 and glucose 5.5, pH 7.4; the arcuate arteries were identified and dissected free of surrounding connective tissue. The vessels were mounted on 40 μm tungsten wires in a small vessel myograph (Cambustion, Cambridge), capable of measuring isometric tension. The arteries were bathed in PSS at 37°C, bubbled with 5% CO₂ in O₂. Maximal potassium activation was achieved by KPSS (PSS with equimolar substitution of KCl for NaCl resulting in final K concentration of 125 mM).

After an equilibration period of 1 h in PSS, the passive tension/internal circumference characteristics were determined (Mulvany & Halpern, 1977). The vessels were then set to an internal circumference equivalent to 90% of that they would have had when relaxed *in situ* under a transmural

pressure of 100 mmHg. The maximum active tension is developed at approximately this circumference (Mulvany & Halpern, 1977). The arteries were then maximally contracted for 2 min every 10 min on four occasions. The first two contractions being with KPSS and 5 μM noradrenaline followed by 5 μM noradrenaline and finally KPSS. Any vessel which failed to develop a maximum tension equivalent to a pressure of 100 mmHg was discarded.

Experiment protocol

Cumulative concentration-response curves were constructed sequentially, to acetylcholine, 0.01–100 μM , (arteries precontracted with 5 μM noradrenaline), noradrenaline 0.01–10 μM and potassium chloride 12–125 mM. Each concentration of agonist was left in contact with the vessels for 2 min, the tension being recorded at the end of the period. Following each concentration-response curve the vessels were washed with PSS and rested in PSS for 10 min. Following the three concentration-response curves the vessels were washed again and allowed to rest for a further 10 min. Groups of vessels (6–8 vessels in each group) were then incubated in a number of different agents (one agent or pair of agents per group): indomethacin 14 μM ; L-NAME 100 μM ; indomethacin 14 μM and L-NAME 100 μM ; BW755C 50 μM or 180 μM ; BW755C 50 μM and indomethacin 14 μM ; BW755C 50 μM and L-NAME 100 μM . Concentration-response curves were then repeated to acetylcholine, noradrenaline and potassium chloride in the presence of the appropriate agent or combination of agents. The above protocol was repeated in groups of vessels pretreated with CHAPS.

The experimental protocol involves repeating concentration-response curves to acetylcholine, noradrenaline and potassium chloride on two occasions. In order to exclude changes in sensitivity of the vessels with repeated exposure, timed controls were performed. Concentration-response curves were obtained to acetylcholine, noradrenaline and potassium chloride as above; the vessels were washed in PSS and allowed to rest for 10 min. The concentration-response curves were then repeated.

In two groups of vessels concentration-response curves were constructed to endothelin-1 0.1 nM–1 μM , alone and to endothelin-1 in the presence of BW755C 50 μM . Due to the irreversible nature of endothelin-1-induced contraction it was not possible to perform sequential concentration-response curves to endothelin-1 alone and then with BW755C. Concentration-response curves were also constructed to sodium nitroprusside 0.1 nM–10 μM , in vessels precontracted with 5 μM noradrenaline, with or without pretreatment with CHAPS, alone and in the presence of BW755C 50 μM .

Drugs

Acetylcholine, indomethacin, L-NAME, CHAPS, endothelin-1 (Sigma, Poole, Dorset), sodium nitroprusside (Central Laboratories, Dublin, Ireland), noradrenaline (Winthrop Laboratories, Guildford, Surrey) and BW755C (Wellcome Laboratories, Beckenham, Kent) were used. All other reagents were obtained from BDH (Poole, Dorset). All reagents were of Analar grade and solutions were prepared fresh daily.

Statistical analysis

A Hill plot was constructed for each concentration-response relationship. The $-\log$ concentration of the drug required to produce 50% of the maximum response (pEC_{50}) was calculated with a computerised curve fitting software package (Graphpad Inplot version 3.14). Where curve fitting was not appropriate, the maximum responses were compared. In the figures, concentrations are given as negative logarithms in molar units. Tension was expressed as mN mm^{-1} artery length. Relaxation to acetylcholine and sodium nitroprusside

was expressed as a percentage of the initial precontraction to noradrenaline. All values are given as mean $\pm$ s.e.mean. Differences between means were assessed by Student's *t* test for paired or unpaired data as appropriate. $P < 0.05$ was taken as statistically significant.

Results

Noradrenaline, potassium chloride and endothelin-1 produced concentration-dependent contraction and acetylcholine and sodium nitroprusside concentration-dependent relaxation of the vessels. Repetition of the concentration-response curves, as timed controls did not significantly affect either the pEC_{50} or maximum response obtained to noradrenaline (pEC_{50} 6.22 ± 0.02 curve 1, versus 6.22 ± 0.02 curve 2; maximum response 2.03 ± 0.34 mN mm $^{-1}$ curve 1, versus 2.02 ± 0.37 mN mm $^{-1}$ curve 2), potassium chloride (pEC_{50} 1.35 ± 0.02 curve 1, versus 1.40 ± 0.02 curve 2); maximum response

1.09 ± 0.18 mN mm $^{-1}$ curve 1, versus 0.98 ± 0.18 mN mm $^{-1}$ curve 2) and acetylcholine (pEC_{50} 5.95 ± 0.30 curve 1 versus 6.26 ± 0.11 curve 2; maximum response $23.8 \pm 4.7\%$ curve 1 versus $23.1 \pm 2.4\%$ curve 2).

Indomethacin

Indomethacin attenuated the contractile response to noradrenaline (pEC_{50} 6.28 ± 0.04 versus 6.13 ± 0.06 with indomethacin, $P < 0.05$; maximum response 1.84 ± 0.27 mN mm $^{-1}$ versus 1.48 ± 0.21 mN mm $^{-1}$ with indomethacin, $P < 0.01$) and potassium (maximum response 0.77 ± 0.20 mN mm $^{-1}$ versus 0.55 ± 0.11 mN mm $^{-1}$ with indomethacin, $P < 0.01$) (Figure 1). Indomethacin potentiated acetylcholine-induced relaxation (pEC_{50} 5.96 ± 0.06 versus 6.80 ± 0.13 with indomethacin, $P < 0.001$; maximum response $17.2 \pm 1.7\%$ versus $36.5 \pm 3.3\%$ with indomethacin, $P < 0.001$) (Figure 1).

Figure 1 The effect of indomethacin, $14 \mu\text{M}$, on the concentration-response relationship of renal arcuate arteries to (a) noradrenaline, (b) potassium chloride and (c) acetylcholine. In (a) and (b): ($\circ$, ∇) control; ($\bullet$, $\blacktriangledown$) indomethacin; $n = 8$; diameter = $180 \pm 7 \mu\text{m}$. In (c): ($\square$) control, ($\blacksquare$) indomethacin; $n = 8$; diameter = $160 \pm 7 \mu\text{m}$.

Figure 2 The effect of indomethacin and N^G -nitro-L-arginine methyl ester (L-NAME) on the concentration-response relationship of rat renal arcuate arteries to (a) noradrenaline, (b) potassium chloride and (c) acetylcholine. In (a) and (b): ($\circ$, ∇) control; ($\bullet$, $\blacktriangledown$) indomethacin plus L-NAME; $n = 8$; diameter = $184 \pm 10 \mu\text{m}$. In (c): ($\square$) control; ($\blacksquare$) indomethacin plus L-NAME; $n = 8$; diameter = $156 \pm 11 \mu\text{m}$.

L-NAME

L-NAME had no significant effect on the concentration-response curves to either noradrenaline (pEC_{50} 6.38 ± 0.05 versus 6.47 ± 0.05 with L-NAME; maximum response 2.03 ± 0.28 $mN\ mm^{-1}$ versus 2.20 ± 0.34 $mN\ mm^{-1}$ with L-NAME) or to potassium chloride (pEC_{50} 1.43 ± 0.04 versus 1.43 ± 0.03 with L-NAME). Acetylcholine-induced relaxation was virtually abolished by L-NAME (maximum relaxation $19.1 \pm 2.2\%$ versus $-3.2 \pm 1.2\%$ with L-NAME, $P < 0.001$. Negative relaxation implies contraction).

Indomethacin and L-NAME

In these vessels, the inhibitory effect of indomethacin on the contractile response to noradrenaline and potassium was abolished by the presence of L-NAME (noradrenaline pEC_{50} 6.22 ± 0.04 versus 6.41 ± 0.05 with indomethacin and L-NAME, $P < 0.05$; maximum response 3.57 ± 0.46 $mN\ mm^{-1}$ versus 3.65 ± 0.47 $mN\ mm^{-1}$ with indomethacin and L-NAME, NS; potassium pEC_{50} 1.40 ± 0.06 versus 1.44 ± 0.05 with indomethacin and L-NAME, NS; maximum response 1.98 ± 0.03 $mN\ mm^{-1}$ versus 2.08 ± 0.28 $mN\ mm^{-1}$ with indomethacin and L-NAME, NS) (Figure 2). The enhanced acetylcholine-induced relaxation seen in the presence of indomethacin, was abolished by L-NAME (maximum relaxation $11.5 \pm 2.7\%$ versus $-0.5 \pm 1.7\%$ with indomethacin and L-NAME, $P < 0.05$) (Figure 2).

BW755C

BW755C attenuated the contractile response of the vessels, in a concentration-dependent manner, to noradrenaline (pEC_{50} 6.47 ± 0.03 versus 6.02 ± 0.01 with BW755C $50\ \mu M$, $P < 0.001$, 6.48 ± 0.03 versus 5.56 ± 0.04 with BW755C $180\ \mu M$, $P < 0.01$; maximum response 1.94 ± 0.53 $mN\ mm^{-1}$ versus 1.27 ± 0.15 $mN\ mm^{-1}$ with BW755C $50\ \mu M$, NS, 2.32 ± 0.59 $mN\ mm^{-1}$ versus 0.72 ± 0.22 $mN\ mm^{-1}$ with BW755C $180\ \mu M$, $P < 0.05$) and potassium (pEC_{50} 1.45 ± 0.04 versus 1.40 ± 0.03 with BW755C $50\ \mu M$, NS, 1.40 ± 0.07 versus 1.13 ± 0.22 with BW755C $180\ \mu M$, NS; maximum response 0.77 ± 0.20 $mN\ mm^{-1}$ versus 0.20 ± 0.08 $mN\ mm^{-1}$ with BW755C $50\ \mu M$, $P < 0.05$, 0.92 ± 0.31 $mN\ mm^{-1}$ versus 0.10 ± 0.06 $mN\ mm^{-1}$ with BW755C $180\ \mu M$, $P < 0.05$) (Figure 3). BW755C did not alter the response to either acetylcholine (pEC_{50} 5.49 ± 0.12 versus 5.67 ± 0.35 with BW755C, NS; maximum response $11.5 \pm 2.3\%$ versus $12.1 \pm$

5.1% with BW755C, NS) or endothelin-1 (pEC_{50} 7.13 ± 0.13 versus 7.44 ± 0.18 with BW755C, NS; maximum response 2.24 ± 0.71 $mN\ mm^{-1}$ versus 1.83 ± 0.16 $mN\ mm^{-1}$ with BW755C, NS).

BW755C and indomethacin

In the presence of indomethacin, BW755C still attenuated the contractile response to potassium (maximum response 1.02 ± 0.22 $mN\ mm^{-1}$ versus 0.38 ± 0.10 $mN\ mm^{-1}$ with BW755C and indomethacin, $P < 0.01$) and caused a shift to the right of the concentration-response curve to noradrenaline (pEC_{50} 6.05 ± 0.04 versus 5.88 ± 0.03 with BW755C and indomethacin, $P < 0.01$) although the magnitude of the effect was small compared to that seen with BW755C alone (Figure 4). The combination of BW755C and indomethacin caused a shift to the right of the acetylcholine concentration-response curve (pEC_{50} 6.58 ± 0.09 versus 5.97 ± 0.15 with BW755C and indomethacin, $P < 0.001$) and reduced the maximum relaxation seen ($23.2 \pm 1.15\%$ versus $9.4 \pm 2.24\%$ with BW755C and indomethacin, $P < 0.05$). This effect was directly opposite to that seen with indomethacin alone (Figure 1).

BW755C and L-NAME

Inhibition of nitric oxide synthase with L-NAME significantly reduced the shift to the right of the concentration-response curves to noradrenaline and abolished that for potassium, seen in the presence of BW755C alone, (noradrenaline pEC_{50} 6.12 ± 0.04 versus 5.93 ± 0.02 with L-NAME and BW755C, $P < 0.01$; potassium pEC_{50} 1.33 ± 0.01 versus 1.34 ± 0.07 with L-NAME and BW755C, NS) (Figure 5). The maximum response to both noradrenaline and potassium in the presence of BW755C and L-NAME (2.00 ± 0.24 $mN\ mm^{-1}$ and 0.55 ± 0.14 $mN\ mm^{-1}$ respectively) were significantly greater than that seen with BW755C alone (1.27 ± 0.15 $mN\ mm^{-1}$ and 0.20 ± 0.08 $mN\ mm^{-1}$ respectively), $P < 0.02$ and $P < 0.05$ respectively. The combination of BW755C and L-NAME virtually abolished acetylcholine-induced relaxation (maximum relaxation $1.30 \pm 2.10\%$). This effect was not significantly different from that seen with L-NAME alone.

CHAPS

Endothelial removal with CHAPS increased the maximum contractile response to both noradrenaline (2.03 ± 0.34 mN

Figure 3 The effect of BW755C on the concentration-response relationship of rat renal arcuate arteries to (a) noradrenaline and (b) potassium chloride. In (a) and (b): (○) control; (●) BW755C $50\ \mu M$; $n = 7$, mean diameter = $183 \pm 10\ \mu m$; (▽) control; (▼) BW755C $180\ \mu M$; $n = 5$; mean diameter = $175 \pm 15\ \mu m$.

Figure 4 The effect of BW755C on the concentration-response relationship of rat renal arcuate arteries to (a) noradrenaline and (b) potassium chloride in the presence of indomethacin ($1.4 \mu\text{M}$). In (a): (○) indomethacin; (●) indomethacin plus BW755C $50 \mu\text{M}$. In (b): (▽) indomethacin; (▼) indomethacin plus BW755C $50 \mu\text{M}$. In (a) and (b): $n = 6$; diameter = $197 \pm 8 \mu\text{m}$.

Figure 5 The effect of BW755C on the concentration-response relationship of rat renal arcuate arteries to (a) noradrenaline and (b) potassium chloride in the presence of N^{G} -nitro-L-arginine methyl ester (L-NAME $100 \mu\text{M}$). In (a): (○) control; (●) L-NAME plus BW755C $50 \mu\text{M}$. In (b): (▽) control; (▼) L-NAME plus BW755C $50 \mu\text{M}$. In (a) and (b): $n = 8$; diameter = $138 \pm 6 \mu\text{m}$.

Figure 6 The effect of indomethacin ($1.4 \mu\text{M}$) on the concentration-response relationship of rat renal arcuate arteries, pretreated with CHAPS, to (a) noradrenaline and (b) potassium chloride. In (a): (○) control; (●) indomethacin. In (b): (▽) control; (▼) indomethacin. In (a) and (b): $n = 7$; diameter = $162 \pm 13 \mu\text{m}$.

Figure 7 The effect of BW755C ($50 \mu\text{M}$) on the concentration-response relationship of rat renal arcuate arteries, pretreated with CHAPS, to (a) noradrenaline and (b) potassium chloride. In (a): (○) control; (●) BW755C. In (b): (▽) control; (▼) BW755C. In (a) and (b), $n = 6$; diameter = $180 \pm 18 \mu\text{m}$.

mm^{-1} versus $4.52 \pm 0.48 \text{ mN mm}^{-1}$ following CHAPS, $P < 0.001$) and potassium chloride (1.09 ± 0.18 versus 2.50 ± 0.31 following CHAPS, $P < 0.006$) without causing a shift of the concentration-response curve. Acetylcholine-induced relaxation was largely abolished (maximum relaxation $23.8 \pm 4.7\%$ versus $5.10 \pm 1.4\%$ following CHAPS, $P < 0.003$).

CHAPS and indomethacin

In renal arcuate arteries pretreated with CHAPS, indomethacin reduced the maximum contractile response to noradrenaline, ($4.52 \pm 0.48 \text{ mN mm}^{-1}$ versus $3.80 \pm 0.38 \text{ mN mm}^{-1}$ with indomethacin, $P < 0.01$) and potassium ($2.50 \pm 0.31 \text{ mN mm}^{-1}$ versus $1.65 \pm 0.26 \text{ mN mm}^{-1}$ with indomethacin, $P < 0.001$). There was a tendency for a shift to the right of the concentration-response curves to both noradrenaline and potassium but this did not reach statistical significance (Figure 6).

CHAPS and BW755C

Following endothelial removal, BW755C still produced a shift to the right of the concentration-response curve to noradrenaline (pEC_{50} 6.20 ± 0.02 versus 5.78 ± 0.01 with BW755C, $P < 0.01$) and depressed the maximum response to both noradrenaline ($3.21 \pm 0.78 \text{ mN mm}^{-1}$ versus $2.77 \pm 0.70 \text{ mN mm}^{-1}$ with BW755C, $P < 0.05$) and potassium ($1.98 \pm 0.66 \text{ mN mm}^{-1}$ versus $1.06 \pm 0.37 \text{ mN mm}^{-1}$ with BW755C, $P < 0.05$) (Figure 7).

CHAPS and BW755C with indomethacin

In the presence of indomethacin, in vessels pretreated with CHAPS, there was no additional effect of BW755C on either the maximum response or pEC_{50} for noradrenaline (pEC_{50} 5.90 ± 0.06 versus 5.81 ± 0.04 with indomethacin and BW755C; maximum response 4.88 ± 0.62 versus 4.78 ± 0.61 with indomethacin and BW755C). In the presence of indomethacin, BW755C still reduced the maximum contractile response to potassium (2.75 ± 0.40 versus 1.63 ± 0.30 with indomethacin and BW755C, $P < 0.001$).

Sodium nitroprusside

Neither endothelial removal nor BW755C affected either the maximum response or the pEC_{50} to sodium nitroprusside

(pEC_{50} 4.30 ± 0.09 versus 4.41 ± 0.28 with BW755C; 3.90 ± 0.18 versus 4.17 ± 0.32 with BW755C following CHAPS; maximum response $99.8 \pm 0.10\%$ versus $98.4 \pm 0.80\%$ with BW755C; $95.6 \pm 0.30\%$ versus $98.3 \pm 0.40\%$ with BW755C following CHAPS).

Discussion

In these vessels indomethacin enhanced endothelium-dependent relaxation, as assessed by the response to acetylcholine and attenuated the contractile responses to noradrenaline and potassium chloride. These data confirm previous findings of a cyclo-oxygenase-dependent vasoconstrictor in renal arcuate arteries (Kato *et al.*, 1990). The inhibitory effect of indomethacin persisted in the absence of a functioning endothelium suggesting that the vasoconstrictor responsible originates within the vascular smooth muscle rather than the endothelium.

Inhibition of nitric oxide synthesis with L-NAME had no effect on the contractile response to either noradrenaline or potassium, but inhibited acetylcholine-induced relaxation. This suggests that there is little stimulated nitric oxide release during contraction of these vessels with noradrenaline or potassium chloride. This may also explain the relatively poor relaxation to acetylcholine and resistance to sodium nitroprusside, but does not explain the potentiation of contraction seen following endothelial removal. These findings are compatible with those of Imig & Roman (1992) who examined the effect of nitric oxide inhibition on the pressure-diameter relationship of renal arterioles. Nitric oxide inhibition had no effect on the diameter of either arcuate or interlobular vessels whilst reducing the diameter of afferent arterioles at all perfusion pressures studied.

Kanner *et al.* (1992) have suggested that nitric oxide may inhibit arachidonic acid metabolism via both cyclo-oxygenase and lipoxygenase pathways modulating the arachidonic acid cascade and the generation of reactive oxygen species. In these arteries, indomethacin potentiated acetylcholine-induced relaxation; this relaxation was inhibited by L-NAME. Indomethacin alone attenuated the contractile response to both noradrenaline and potassium and this effect again was inhibited by L-NAME. Thus these data suggest that nitric oxide is released in these vessels by acetylcholine and on contraction with noradrenaline and potassium chloride. However the effects of nitric oxide are only apparent follow-

ing removal of the cyclo-oxygenase-derived vasoconstrictor.

Inhibition of the lipoxigenase/cyclo-oxygenase pathway with BW755C reduced the contractile responses of rat renal arcuate arteries to noradrenaline and potassium chloride. Acetylcholine-induced relaxation (endothelium-dependent) was not affected by BW755C. The effect of BW755C on the contractile response was unlikely to have been the result of cyclo-oxygenase inhibition alone as the shift to the right of the noradrenaline concentration-response curve seen with BW755C alone was greater than that seen with indomethacin alone and in the presence of indomethacin, BW755C still caused a shift to the right of the noradrenaline concentration-response curve and reduced the maximum response to potassium chloride. Endothelial removal with CHAPS attenuated the effect of BW755C and in these vessels BW755C had little additional effect in the presence of indomethacin. Thus these data support the presence of two vasoconstrictors, one an endothelium-dependent lipoxigenase derivative and the other an endothelium-independent cyclo-oxygenase derivative. The cyclo-oxygenase derived vasoconstrictor has been previously described by ourselves (Richards *et al.*, 1990) and others (Lüscher *et al.*, 1990) and is probably prostaglandin H₂ (Kato *et al.*, 1990). BW755C predominately inhibits 5- and 12-lipoxigenase in addition to cyclo-oxygenase having little activity against 15-lipoxigenase (Higgs *et al.*, 1979; Salmon *et al.*, 1983). There has been very little investigation of the effects of lipoxigenase products in the renal vasculature but Badr *et al.* (1987) demonstrated that leukotriene D₄ constricted intra-renal and efferent arterioles in the rat and Ma *et al.* (1991) have shown that 12-HETE is a directly acting vasoconstrictor in dog renal arcuate arteries. Thus it is entirely feasible that these intermediates are active in mediating the responses seen in this study.

Whilst it is possible that BW755C is inhibiting the production of a 5- or 12-lipoxigenase-derived vasoconstrictor, another possibility is that inhibition of cyclo-oxygenase and 5- and 12-lipoxigenase leads to increased metabolism of arachidonic acid by either 15-lipoxigenase or cytochrome P450-dependent mono-oxygenase and the effects seen are related to a vasoactive product of one of these pathways such as 15-HETE or 5,6-, 8,9- and 11,12-EET which have all been shown to have vasodilator properties (Van Diest *et al.*, 1990; Carroll *et al.*, 1992).

The vasoconstrictor response to endothelin-1 was not affected by BW755C. This finding is contrary to the data of Fretschner *et al.* (1991) who examined the effect of endothelin-1 in the split hydronephrotic kidney model. They demonstrated that the response to endothelin-1 was attenuated by a competitive leukotriene antagonist. The differences

between our findings are not readily explained but may relate to differences in the model or inhibitors used.

L-NAME, greatly attenuated the inhibitory effect of BW755C suggesting that BW755C may either induce nitric oxide release or inhibit its breakdown. Moncada *et al.* (1986), using a rabbit aortic strip bioassay system, suggested that BW755C inhibited endothelium-dependent relaxation through cyclo-oxygenase-mediated production of superoxide anions. However, Semb & Vaage (1991) demonstrated that BW755C prevented oxygen free radical-induced injury in rat isolated heart and Hänsch (1990) showed that oxygen free radical generation from arachidonic acid was dependent on lipoxigenase rather than cyclo-oxygenase activity. Both these findings contradict those of Moncada *et al.* (1986) suggesting that BW755C decreases rather than increases free radical production. Reactive oxygen species accelerate nitric oxide breakdown, thus agents which either prevent free radical production or are free radical scavengers will potentiate the action of nitric oxide. If BW755C inhibits lipoxigenase-dependent free radical production it would potentiate the action of nitric oxide and thus inhibit contraction. If this were the case then the effect of BW755C should be inhibited by a nitric oxide synthase blocker, which is consistent with our findings. However, BW755C had no effect on endothelium-dependent relaxation which was unexpected as BW755C should enhance endothelium-dependent relaxation if it reduces free radical synthesis. The opposite was seen as BW755C reduced the response to acetylcholine in the presence of indomethacin and the action of BW755C was only partially endothelium-dependent which also argues against an action through nitric oxide.

In conclusion we have demonstrated that BW755C inhibits contraction of renal arcuate arteries induced by both noradrenaline and potassium chloride in the rat. This effect is additional to that seen with indomethacin, is partially endothelium-dependent and may be inhibited by blockade of nitric oxide synthase. The exact mechanism of action of BW755C in the renal vasculature has not been elucidated but it would appear that lipoxigenase derivatives could be involved in the control of renal vascular tone. Further work is required to define the roles of the individual lipoxigenase enzymes in the production of vasoactive substances, including reactive oxygen species.

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