The effect of arginine and nitric oxide on resistance blood vessels of the perfused rat kidney

R. Bhardwaj & P.K. Moore

Pharmacology Group, Biomedical Sciences Division, King's College, University of London, Chelsea Campus, Manresa Road, London SW3 6LX

1 The vasodilator effects of arginine, nitric oxide (NO), acetylcholine (ACh) and sodium nitroprusside (NP) in the noradrenaline-precontracted ('high tone') perfused rat kidney have been examined.

2 L-Arginine (0.6–23 μmol) caused a biphasic change in renal perfusion pressure. D-Arginine (0.6–23 μmol) was without effect. The second vasodilator component was abolished and the first vasoconstrictor effect augmented following CHAPS-induced removal of the vascular endothelium suggesting that vasodilatation was endothelium-dependent.

3 L-Arginine salts produced transient and dose-related vasodilatation. L-Arginine methylester was the most potent with an ED_{50} of $2.2 \pm 0.4 \mu\text{mol}$ ($n = 6$). The rank order of potency of the salts tested was: methylester > hydroxamate > chloride. L-Homoarginine chloride was also vasodilator (ED_{50} , $12.0 \pm 1.3 \mu\text{mol}$, $n = 5$). D-Arginine chloride was without effect at doses up to 170 μmol . Responses to L-arginine chloride were endothelium-derived relaxing factor (EDRF)-dependent being abolished by CHAPS (4.7 mg ml^{-1} , 30 s) and significantly inhibited (>70%) by gossypol (3 μM) and nordihydroguaiaretic acid (NDGA, 10 μM).

4 Vasodilatation due to NO was unaffected by CHAPS and gossypol treatment but inhibited by NDGA. NO was approximately 3 times less potent than ACh but 3000 times more potent than L-arginine methylester.

5 Kidneys perfused for 1 h with Krebs solution containing L-arginine chloride (100 μM) or L-canavanine (50 μM) showed no change in sensitivity towards ACh or NP. Higher concentrations of L-arginine chloride (500 μM) or L-canavanine (150 μM) significantly reduced the response to both vasodilators.

6 L-Arginine salts dilate resistance blood vessels of the perfused rat kidney by a mechanism which may involve the release of EDRF from vascular endothelial cells of the perfused rat kidney.

Introduction

Endothelium-derived relaxing factor (EDRF) is a chemically unstable, locally acting vasodilator released from the vascular endothelium of intact blood vessels (Furchgott, 1984). EDRF mediates the relaxation of precontracted rings or bands of isolated arteries and veins in response to acetylcholine (ACh) and is responsible for the vasodilator effect of this substance on resistance blood vessels of several perfused organs including rat lung (Cherry & Gillis, 1987), mesentery (Furchgott *et al.*, 1987) and kidney (Bhardwaj & Moore, 1988). Recently evidence has been presented that EDRF is identical with nitric oxide (NO) (Ignarro *et al.*, 1987; Palmer *et al.*, 1987) although the existence of an additional EDRF which

relaxes blood vessels by hyperpolarizing membranes of vascular smooth muscle cells has also been proposed (Taylor & Weston, 1988).

Despite progress in the identification of EDRF the biochemical pathway for its formation remains obscure. Recent work has implicated L-arginine as the physiological precursor of NO (Palmer *et al.*, 1988; Schmidt *et al.*, 1988a,b). We have now investigated the vasodilator effect of L-arginine and a selection of its salts as well as its putative metabolite, NO, in the perfused rat kidney in an attempt to gain further insights into the biochemical mechanism of EDRF formation.

Methods

The experimental procedures employed in this study have been detailed elsewhere (Bhardwaj & Moore, 1988). Briefly, rats (male Sprague-Dawley, 250–350 g) were stunned by a blow to the head and exsanguinated. The left kidney was removed and perfused with warmed (37°C), oxygenated (95% O₂: 5% CO₂) Krebs solution containing indomethacin (8 µM) to inhibit prostanoid formation. Perfusion pressure was constantly monitored by means of a Bell & Howell pressure transducer connected to a Devices pen recorder. Drugs were injected in volumes less than 20 µl to prevent vascular effects due to an injection artefact. Vasodilator responses to ACh, L-arginine base and salts, NO and NP were assessed in so-called 'high tone' preparations which were partially precontracted by addition to the perfusing Krebs solution of a concentration of noradrenaline (0.1–0.5 µM) that increased basal perfusion pressure by 130–160 mmHg. This increase represents approximately 60–80% of the maximal response. No significant loss of vasoconstrictor tone was observed in such preparations for periods of up to 4 h. A dose cycle time of 5 min was employed for all vasodilator drugs.

In some experiments, endothelial cells lining resistance blood vessels in the non-constricted (i.e. 'low tone') rat kidney were removed by addition to the perfusing Krebs solution of the detergent, 3,3 cholamidopropyl dimethylammonio 1-propanesulphonate (CHAPS, 4.7 mg ml⁻¹, 30 s). In other experiments, L-arginine chloride (100–700 µM), L-canavanine (50–250 µM), gossypol (3 µM) or nordihydroguaiaretic acid (NDGA, 10 µM) were added to the Krebs solution and allowed to perfuse the pre-constricted (i.e. 'high tone') kidney for a minimum of 1 h before further challenge with vasodilator drugs. Kidneys were weighed before and at the end of the experiment to determine the extent of oedema formation.

Solutions of NO (BDH, 99.9%) were prepared essentially as described by Shikano *et al.* (1988). Briefly, NO (10 ml) was transferred without contact with air into rubber-sealed, Wheatman flasks containing 25 ml nitrogen-degassed distilled water and kept on ice throughout the experiment. Assuming complete saturation with gas the concentration of NO in solution is approximately 3.3 mM. This concentration was used to calculate the doses of NO administered. Indomethacin was dissolved in 0.5% Na₂CO₃. Other drugs were dissolved in saline. All drugs were purchased from Sigma Ltd and kept on ice throughout the experiment. NDGA was dissolved in Krebs solution. Solutions of NDGA and NO were prepared fresh each day. NDGA was used within 1 h.

Data are presented as mean ± s.e.mean. Statistical analysis was performed by use of Student's *t* test.

Results

Effects of acetylcholine, nitric oxide and sodium nitroprusside on renal perfusion pressure

The basal perfusion pressure of rat kidney preparations used in this study was 148.5 ± 7.2 mmHg (*n* = 18). No significant change in kidney weight was observed following perfusion for up to 4 h (2.9 ± 0.6 g, *n* = 18, at the start compared with 3.3 ± 0.2 g, *n* = 18, at the end of the experiment) suggesting the absence of significant oedema formation over this period.

ACh, NO and NP produces dose-related vasodilatation of the noradrenaline-precontracted, perfused rat kidney. Responses to ACh and NO were transient whilst responses to similarly effective doses of NP were characteristically more prolonged. Representative traces are shown in Figure 1. The doses of each drug required to produce 50% of the maximal response (ED₅₀) were 0.18 ± 0.05 nmol, *n* = 6, 0.66 ± 0.11 nmol, *n* = 5 and 1.3 ± 0.3 nmol, *n* = 6, while the maximal falls in perfusion pressure which could be achieved were 61.5 ± 4.5 mmHg, *n* = 6, (1.1 nmol), 53.2 ± 4.5 mmHg, *n* = 6, (3.3 nmol) and 70.6 ± 4.1 mmHg, *n* = 6, (7.6 nmol) respectively.

Removal of endothelial cells lining resistance blood vessels within the kidney abolished the vasodilator effect of ACh without influencing responses to NO or NP. Similarly, responses to approximate ED₇₀ doses of ACh (0.22 nmol, 5.5 ± 3.4 mmHg, cf. 45.7 ± 4.4 mmHg, *n* = 5, *P* < 0.05) but not NO (1.65 nmol, 38.9 ± 6.7 mmHg, cf. 41.4 ± 4.2 mmHg, *n* = 6, *P* > 0.05) or NP (2.2 nmol, 51.2 ± 4.5 mmHg, cf. 45.6 ± 6.7 mmHg, *n* = 6, *P* > 0.05) were reduced by gossypol (3 µM). In contrast, in separate preparations NDGA (10 µM) reduced the response to ACh (0.22 nmol, 4.4 ± 2.3 mmHg, cf. 48.7 ± 3.2 mmHg, *n* = 5, *P* < 0.05) and NO (1.65 nmol, 6.7 ± 3.3 mmHg, cf. 45.4 ± 4.5 mmHg, *n* = 6, *P* < 0.05) and partially blocked vasodilatation due to NP (2.2 nmol, 34.4 ± 2.2 mmHg, cf. 43.3 ± 1.9 mmHg, *n* = 6, *P* < 0.05).

Effect of arginine base and salts on renal perfusion pressure

In preliminary experiments, L- and D-arginine base (0.6–23 µmol) were assessed for renal vasodilator activity. In 'high tone' perfused rat kidney preparations, L-arginine produced a biphasic effect viz. vasoconstriction followed by vasodilatation (Figure 2). For example, administration of 11.5 µmol L-arginine caused an increase in perfusion pressure of 53.9 ± 7.5 mmHg followed immediately thereafter by a fall of 16.9 ± 5.0 mmHg (both *n* = 8). In contrast,

Figure 1 Representative traces showing the vasodilator effect of approximate ED_{50} doses (nmol) of acetylcholine (ACh), nitric oxide (NO) and nitroprusside (NP) in noradrenaline-precontracted, perfused rat kidneys. (a) CHAPS (4.7 mg ml^{-1} , 30 s) was administered in a 'low tone' perfused rat kidney preparation and the effect on vasodilatation due to ACh, NO and NP assessed 30 min thereafter following noradrenaline (NA) addition to the perfusing Krebs solution. (b and c): Effect of gossypol ($3 \mu\text{M}$) and nordihydroguaiaretic acid (NDGA) ($10 \mu\text{M}$) administered to 'high tone' rat kidneys on vasodilatation due to ACh, NO and NP. Preparations were exposed to the inhibitor for 1 h before further injection of vasodilator drug. Vertical bar indicates perfusion pressure in mmHg. Horizontal bar shows time in min.

D-arginine base had no effect on kidney perfusion pressure over the dose-range used.

Unlike L-arginine base, bolus injection of L-arginine chloride, L-arginine hydroxamate, L-arginine methylester or L-homoarginine chloride resulted in dose-related vasodilatation of similar duration to that observed with ACh (Figure 2). The ED_{50} values (maximum response in brackets) for each of these L-arginine salts are: $14.0 \pm 1.2 \mu\text{mol}$ ($55.2 \pm 6.4 \text{ mmHg}$, $95.2 \mu\text{mol}$), $4.4 \pm 0.5 \mu\text{mol}$

Figure 2 Representative traces showing the biphasic effect of L-arginine base (μmol) on perfusion pressure in the noradrenaline-precontracted, perfused rat kidney (a) and vasodilator effect of approximate ED_{50} doses (μmol) of L-arginine chloride (C), L-arginine hydroxamate (H), L-arginine methylester (M) and L-homoarginine chloride (HC) (b). The effect of CHAPS (4.7 mg ml^{-1} , 30 s) administered to 'low tone' perfused rat kidney preparations (as in Figure 1) on responses to L-arginine base and L-arginine chloride are also shown. Vertical bar indicates perfusion pressure in mmHg. Horizontal bar shows time in min.

($67.0 \pm 9.2 \text{ mmHg}$, $70 \mu\text{mol}$), $2.2 \pm 0.4 \mu\text{mol}$ ($76.5 \pm 2.3 \text{ mmHg}$, $51.2 \mu\text{mol}$) and $12.0 \pm 1.3 \mu\text{mol}$ ($73.3 \pm 10.0 \text{ mmHg}$, $96 \mu\text{mol}$) (all $n = 4-7$). No vasoconstrictor response was observed with any of these L-arginine salts at doses up to $100 \mu\text{mol}$. Of all the arginine salts tested, only D-arginine chloride failed to affect perfusion pressure even at doses as high as $170 \mu\text{mol}$ (results from 6 experiments).

Removal of endothelial cells from intra-renal blood vessels by CHAPS perfusion augmented the vasoconstrictor component whilst abolishing the vasodilator effect of injected L-arginine base (Figure 2). Similarly, removal of endothelium with CHAPS abolished and exposure of perfused kidneys to either gossypol ($3 \mu\text{M}$) or NDGA ($10 \mu\text{M}$) significantly reduced renal vasodilatation due to an approximate ED_{50} dose of L-arginine chloride ($10 \mu\text{mol}$) by $84.8 \pm 7.6\%$ and $81.9 \pm 6.6\%$ ($n = 6$).

Effect of arginine 'loading' on the response to acetylcholine and sodium nitroprusside

Attempts were also made to 'load' rat kidney preparations with L-arginine chloride by addition of this amino acid to the Krebs perfusate. 'High tone' perfused rat kidneys exposed for 1 h to a high concentration of L-arginine chloride (700 μ M) responded with a slowly developing fall in perfusion pressure which resulted in complete loss of noradrenaline-induced tone within 15–25 min. Kidneys routinely perfused with Krebs containing a lower concentration (100 μ M) of L-arginine chloride exhibited no reduction in vascular tone over a 2 h period. In these circumstances no change in the vasodilator potency of ACh (ED_{50} , 0.21 ± 0.07 nmol, maximum response, 64.3 ± 3.9 mmHg, 1.2 nmol, $n = 5$, both $P > 0.1$) or NP (ED_{50} , 1.6 ± 0.6 nmol, maximal response, 66.6 ± 7.8 mmHg, 7.6 nmol, $n = 5$, both $P > 0.1$) was observed. Increasing the concentration of L-arginine even further (500 μ M) produced in the first instance a small (approx. 12 mmHg) fall in perfusion pressure which reversed within 5 to 10 min to restore full 'tone'. Thereafter, in the continued presence of this concentration of L-arginine chloride responses to both ACh (ED_{50} , 1.1 ± 0.05 nmol, maximum response 28.7 ± 3.2 mmHg, 9.6 nmol, $n = 5$, both $P < 0.05$) and NP (ED_{50} , 4.7 ± 0.4 nmol, maximum response, 36.1 ± 4.6 mmHg, 30.4 nmol, $n = 5$, both $P < 0.05$) were non-selectively reduced.

Effect of L-canavanine on the response to acetylcholine and sodium nitroprusside

Addition of L-canavanine (50 μ M) to the Krebs perfusate did not affect responses to any of the vasodilator drugs tested. Increasing the concentration of L-canavanine (150 μ M) non-selectively antagonized the renal vasodilator effect of approximate ED_{70} doses of ACh (e.g. 0.22 nmol, 26.3 ± 6.2 mmHg, cf. 48.3 ± 8.1 mmHg, $n = 5$, $P < 0.05$) and NP (e.g. 2.2 nmol, 24.5 ± 3.4 mmHg, cf. 46.5 ± 4.5 mmHg, $n = 6$, $P < 0.05$). Even higher concentrations of L-canavanine (250 μ M) added to the Krebs perfusate reduced or more often totally abolished the tone of kidney preparations and thus could not be used.

Discussion

The vasodilator effect of ACh in the perfused rat kidney is reduced by removal of the endothelium with CHAPS or exposure to gossypol or NDGA and is thus dependent upon the formation of EDRF which confirms the results of a previous study

(Bhardwaj & Moore, 1988). In addition we describe here the powerful vasodilator activity of NO on renal resistance blood vessels. The response to NO is unaffected by CHAPS or gossypol but reduced by NDGA treatment and in consequence is most likely a direct effect on vascular smooth muscle.

A major aim of the present investigation was to clarify the part played by L-arginine in the formation of EDRF within the kidney. To this end we have attempted to duplicate some of the experiments performed either in large arteries (Schmidt *et al.*, 1988a) or on cultured endothelial cells (Palmer *et al.*, 1988) which have suggested that L-arginine is the physiological precursor of EDRF.

L-Arginine salts (chloride, hydroxamate, methylester) as well as L-homoarginine chloride were vasodilators in the perfused rat kidney, albeit some 3 orders of magnitude less potent than either NO or ACh. D-Arginine chloride was inactive. Like ACh, responses to L-arginine were reduced or abolished by removal of the endothelium with CHAPS, or exposure to gossypol or NDGA, implying that the response of the rat renal vasculature to L-arginine salts is EDRF-dependent. By similar reasoning the secondary vasodilator component of the response to L-arginine base is also EDRF-dependent. The renal vasoconstriction that occurred following injection of L-arginine base is not endothelium-dependent since it is augmented by CHAPS. Furthermore, renal vasoconstriction is unrelated to the pH of the injection medium since D-arginine base, which exhibits an identical pH to that of L-arginine base, is devoid of activity in the perfused rat kidney.

That L-arginine and its salts are vasodilator in the perfused rat kidney by a mechanism involving EDRF is in accord with recently published evidence that this amino acid is the physiological precursor of NO in vascular endothelial cells. Schmidt and colleagues (1988a) came to this conclusion when they demonstrated that L-canavanine, which prevents conversion of L-arginine to nitrite by macrophages (Iyengar *et al.*, 1987), antagonized ATP- and ACh-induced relaxation of the isolated rat aorta. In the present study, L-canavanine non-selectively blocked responses to both endothelium-dependent (ACh) and endothelium-independent (NP) vasodilators indicating a depressant effect on vascular smooth muscle reactivity, rather than or in addition to an effect on EDRF biosynthesis. That L-canavanine prevents NO release has been confirmed in the perfused rabbit aorta by a selective chemiluminescence technique (Schmidt *et al.*, 1988b). Whether L-canavanine inhibits NO release by inhibiting arginine catabolism is not clear. For example, L-canavanine exhibits potent cytotoxic activity which underlies its clinical efficacy in the treatment of certain cancers in man (Green & Ward, 1983). Similar cytotoxicity directed towards

vascular endothelial cells may also contribute to the diminished responses to ACh observed in both the rat kidney (this study) and aorta (Schmidt *et al.*, 1988a). For these reasons L-canavanine may not be a suitable tool for investigating the biosynthesis of EDRF.

Additional evidence that L-arginine is the precursor for EDRF has been obtained in cultured endothelial cells (Palmer *et al.*, 1988). Cells grown in culture containing L-arginine were shown to release larger amounts of NO following bradykinin challenge than did similar cells cultured in the absence of L-arginine. In contrast, Thomas & Ramwell (1988) have recently demonstrated that L-arginine does not potentiate the relaxant effect of ACh on isolated arteries such as the rat pulmonary artery and furthermore has no vasodilator activity in the perfused rat mesentery. These authors conclude that an arginine-containing peptide rather than L-arginine itself is the natural precursor to NO. In the present experiments we have confirmed, in the perfused rat kidney, that arginine does not potentiate renal vasodilatation due to ACh although, following bolus injection, it does dilate resistance blood vessels of the perfused rat kidney (this study) and mesentery (Al-Swaiyeh & Moore, unpublished work).

The present results do not allow us to confirm the precise mechanism of action of L-arginine and its

salts in the rat kidney. The rapidity with which L-arginine salts produce vasodilatation following injection makes it most unlikely that this amino acid is incorporated into a peptide prior to enzyme attack and NO biosynthesis as suggested by Thomas & Ramwell (1988). Additionally, if L-arginine is enzymatically converted to NO in the rat kidney then, based upon their relative potency as vasodilators, the rate of conversion is likely to be minimal (i.e. <0.01%). Furthermore, the nonselective effect of L-canavanine, the failure to increase EDRF release by ACh following arginine loading and the vasodilator effect of L-homoarginine – not a substrate for EDRF formation in cultured endothelial cells (Palmer *et al.*, 1988) – all suggest that exogenous L-arginine is not converted into NO in the kidney. Thus, we propose that L-arginine and its salts release EDRF from rat renal endothelial cells i.e. an effect similar to that of ACh. In this context it may be significant that L-arginine stimulates calcium influx into β cells of rat isolated pancreatic islets (Herchuelz *et al.*, 1984). A similar action in vascular endothelial cells would be expected to trigger EDRF efflux (see Luckhof *et al.*, 1988). Further experiments are necessary to determine whether L-arginine indeed releases EDRF or is converted into EDRF by the rat kidney. The possibility that L-arginine exhibits both such activities cannot be discounted.

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