The involvement of endothelium-derived relaxing factor in the regulation of renal cortical blood flow in the rat

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1 In the present study the role of endogenous nitric oxide (NO) was investigated, in the regulation of renal cortical blood flow (RCBF) *in vivo* in anaesthetized rats under conditions in which prostacyclin involvement had been eliminated.

2 Infusions of the NO synthesis inhibitor N^G-monomethyl-L-arginine (MeArg) at 1 or 3 mg kg⁻¹ min⁻¹, i.v., produced significant decreases in RCBF of 29 ± 7% and 35 ± 5%, respectively. These effects were reversed by co-infusion of a 3 fold excess of L-arginine (L-Arg).

3 Similarly, intravenous infusion of N_ω-nitro-L-arginine methyl ester (NO₂Arg) at 30 or 300 µg kg⁻¹ min⁻¹ attenuated RCBF by 21 ± 4% or 53 ± 4%, respectively, and these effects were reversed by L-Arg (3 or 10 mg kg⁻¹ min⁻¹, i.v.). Most importantly, a low dose of NO₂Arg (30 µg kg⁻¹ min⁻¹, i.v.), while having no pressor effect, considerably reduced RCBF, indicating that basal release of NO is important for the maintenance of renal cortical blood flow.

4 MeArg (3 mg kg⁻¹ min⁻¹, i.v.) or NO₂Arg (300 µg kg⁻¹ min⁻¹, i.v.) inhibited endothelium-dependent acetylcholine (ACh, 10 µg kg⁻¹ min⁻¹, i.v. for 3 min) increases in RCBF in an L-Arg reversible manner, but did not affect endothelium-independent (dopamine 10 µg kg⁻¹ min⁻¹, i.v., for 3 min) increases. Endothelin-1 (1 nmol kg⁻¹, i.v.), when given as a control for the vasoconstrictor effects of MeArg and NO₂Arg, produced a slight inhibition of the ACh-induced increase in RCBF, but this effect was significantly weaker than that produced by MeArg or NO₂Arg.

5 Our findings suggest that MeArg and NO₂Arg inhibit basal and ACh-stimulated release of NO in the renal cortical vasculature. Thus, endogenous NO formation may play an important role in the local regulation of renal cortical blood flow.

Keywords: Laser Doppler flowmetry; N^G-monomethyl-L-arginine; N_ω-nitro-L-arginine; L-arginine

Introduction

The endothelium releases several vasoactive agents which interact to regulate the underlying vascular smooth muscle tone, thus contributing to the control of blood pressure. One such agent is the labile humoral factor, endothelium-derived relaxing factor (EDRF), first described by Furchgott & Zawadzki (1980). In many vascular beds *in vitro*, including the rabbit isolated ear (Griffith *et al.*, 1988), rat lung (Cherry & Gillis, 1987), rat mesentery (Byfield *et al.*, 1986) and rat kidney (Bhardwaj & Moore 1988; Burton *et al.*, 1988), a role for EDRF in the control of arteriolar resistance has been proposed. However, relatively little is known about the role of EDRF in the control of renal vascular resistance *in vivo*.

EDRF, which was recently identified as nitric oxide (NO) (Palmer *et al.*, 1987) is formed from the terminal guanidino nitrogen atom(s) of L-arginine (L-Arg) (Palmer *et al.*, 1988a,b). N^G-monomethyl-L-arginine (MeArg), an inhibitor of arginine metabolism to nitrogen oxides (Hibbs *et al.*, 1987) prevents the release of NO from vascular endothelial cells (Palmer *et al.*, 1988a). In the anaesthetized rabbit (Rees *et al.*, 1989), rat (Whittle *et al.*, 1989) and guinea-pig (Aisaka *et al.*, 1989a), and in the conscious rat (Gardiner *et al.*, 1990a), MeArg causes a sustained rise in blood pressure suggesting that resting blood pressure is modulated by a continuous basal release of NO. Moreover, MeArg infusion into the forearm of human volunteers reduces blood flow by 40% and attenuates the dilator responses to acetylcholine but not to glyceryl trinitrate (Vallance *et al.*, 1989). In the rabbit aorta and rat mesentery *in vitro*, N_ω-nitro-L-arginine methyl ester (NO₂Arg) is a more potent inhibitor of endothelium-dependent vasodilatation than MeArg (Moore *et al.*, 1990). Although there is still debate as to whether EDRF is NO or a closely related substance

such as a nitrosothiol (Myers *et al.*, 1990), the inhibition of EDRF formation by L-arginine analogues, such as MeArg or NO₂Arg, lends support to the identity of EDRF as NO. We shall refer to EDRF as NO in this paper.

NO causes vasodilatation by stimulating the formation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) and a role of NO in the regulation of renal haemodynamics *in vivo* has recently been demonstrated, using excretion of urinary cyclic GMP as a biological marker of NO activity (Tolins *et al.*, 1990).

In the present study we have employed MeArg and NO₂Arg to elucidate the role of basal and stimulated release of NO in the haemodynamics of the renal cortex, using laser Doppler flowmetry for the continuous measurement of renal cortical blood flow. All animals were treated with indomethacin to eliminate any possible involvement of prostacyclin or other prostaglandins.

Methods

Surgical procedure

Male Wistar rats (195–462 g; Glaxo Laboratories, Greenford, Middx.), were anaesthetized with Trapanal (120 mg kg⁻¹, i.p.). The trachea was cannulated to facilitate respiration and body temperature was maintained by 37°C by means of a rectal probe connected to a homeothermic blanket (BioScience, Sheerness, Kent). The right carotid artery was cannulated and connected to a Transamerica type 4-422-0001 pressure transducer for the measurement of systemic blood pressure and heart rate on a Grass 7D polygraph (Grass Instruments, Quincy, Mass., U.S.A.). The left jugular vein and right femoral vein were cannulated for the administration of drugs and the left femoral vein for the administration of saline (3 ml h⁻¹) to compensate for any fluid loss. All drugs were infused with a syringe pump (Perfuser VI, Braun, Melsunge, F.R.G.). The left

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kidney was exposed via a mid-line laparotomy to facilitate renal cortical blood flow measurements.

Laser Doppler flowmetry

Renal cortical blood flow was measured with a standard laser Doppler flow probe (Periflux PF3, Perimed, Stockholm, Sweden), the principle of which has been previously described (Walder *et al.*, 1990). The probe, which was gently placed on the ventral surface of the capsule of the left kidney, was fixed to a mobile arm pivoted about a fixed axis so that it moved as the kidney moved, thus reducing respiratory artefacts. Blood flow was continuously recorded on a microcomputer and expressed in arbitrary perfusion units. Drug-induced changes in blood flow were measured as percent change in perfusion units (PU) or as the area under the curve (AUC) (calculated from recorded data with the computer software package PeriSoft, Perimed, Sweden) and expressed as units. The use of the laser Doppler technique for measurement of blood flow was first described by Riva *et al.* (1972) and has been validated as a method for study of renal blood flow (Stern *et al.*, 1977; Smits *et al.*, 1986). In particular relative changes in RCBF measured by laser Doppler flowmetry are closely correlated to absolute changes in total renal blood flow (as measured by electromagnetic flowprobes) or absolute changes in RCBF measured with radioactive microspheres (Smits *et al.*, 1986). In addition, removal of the capsule of the kidney is not a prerequisite to obtain reliable measurements of RCBF by laser Doppler flowmetry (Stern *et al.*, 1977; Smits *et al.*, 1986).

Experimental design

Effects of NO synthesis inhibition After surgery, all animals were allowed to stabilize for 10 min before receiving indomethacin (5 mg kg^{-1} , i.v.). Twenty min later infusions were started of either N^G -monomethyl-L-arginine acetate (MeArg; $1 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v., $n = 6$; or $3 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v., $n = 5$) or N^G -nitro-L-arginine methyl ester hydrochloride (NO_2Arg ; $30 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 4$; or $300 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v., $n = 5$) at 0.04 ml min^{-1} and continued throughout the experiment. These doses significantly elevate blood pressure in the anaesthetized rat *in vivo*, presumably via inhibition of NO formation (Walder *et al.*, 1990; Whittle *et al.*, 1990; Hecker *et al.*, 1990). After 20 min, the infusion of the NO synthesis inhibitors was continued but with a co-infusion of an excess of L-Arg (3 fold excess of L-Arg at either 3 or $10 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v. for MeArg and a 100 fold excess of L-Arg at 3 or $30 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v. for NO_2Arg). In some control experiments, L-Arg or D-Arg (3 , 10 or $30 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 3-9$) were infused for 10 min.

Effects of NO synthesis inhibition on stimulated release of NO Twenty min after indomethacin treatment, a sub-maximal dose of acetylcholine (ACh; $10 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) was infused at a rate of 0.1 ml min^{-1} for 3 min. This dose of ACh was repeated at 30 min and 60 min after the first dose. Ten min prior to the second ACh infusion, animals received either MeArg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 5$) or NO_2Arg ($300 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 5$), with the infusion continued throughout the experiment at a rate of 0.04 ml min^{-1} . Twenty min before the last ACh infusion, L-Arg was co-infused with either MeArg or NO_2Arg at a dose of $10 \text{ mg kg}^{-1} \text{ min}^{-1}$ or $30 \text{ mg kg}^{-1} \text{ min}^{-1}$, respectively. In some experiments, the endothelium-independent renal vasodilator, dopamine ($10 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 3$), was infused at a rate of 0.1 ml min^{-1} , instead of ACh. In these experiments, the effect of only one of the inhibitors, namely NO_2Arg , was studied. In order to control for the general vasoconstriction and rise in blood pressure induced by MeArg or NO_2Arg , one group of animals received a bolus dose of endothelin-1 (ET-1; 1 nmol kg^{-1} , i.v.; $n = 4$) which preceded the second dose of ACh by 10 min. In this group, the animals received neither L-Arg nor a third infusion of ACh. In one group of experi-

ments, the second infusion of ACh or dopamine was preceded by a 10 min infusion of L-Arg ($30 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 4-6$).

Materials

Indomethacin was prepared as a 5 mg kg^{-1} solution in 5% w/v sodium bicarbonate. Sodium-thiopentone (Trapanal) was obtained from Byk Gulden (Konstanz, F.R.G.). ACh, L-Arg, NO_2Arg , dopamine and indomethacin were obtained from Sigma (Poole, Dorset), MeArg was from Calbiochem (La Jolla, C.A., U.S.A.) and endothelin was from the Peptide Research Institute (Osaka, Japan).

Statistical comparisons

All values in the figures and text are expressed as mean $\pm$ s.e.mean of n observations. Statistical comparisons of differences within the same animal (i.e. before and after treatment) were made by Student's t test for paired determinations; comparisons of differences between groups of animals were made by Student's t test for unpaired determinations. A P value of <0.05 was considered significant.

Results

Mean resting values for mean arterial blood pressure (MAP) were $117 \pm 3 \text{ mmHg}$ ($n = 27$), for heart rate were $401 \pm 6 \text{ beats min}^{-1}$ ($n = 27$) and for renal cortical blood flow (RCBF) were $212 \pm 15 \text{ PU}$ ($n = 27$). These values were unaffected by pretreatment with indomethacin ($122 \pm 3 \text{ mmHg}$, $n = 27$, $P > 0.05$; $391 \pm 7 \text{ beats min}^{-1}$, $n = 27$, $P > 0.05$; and $199 \pm 16 \text{ PU}$, $n = 27$, $P > 0.05$).

Effects of NO synthesis inhibition

Infusion of MeArg ($1 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v., $n = 6$; or $3 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v., $n = 5$) produced dose-dependent increases in MAP of $33 \pm 8 \text{ mmHg}$ and $60 \pm 4 \text{ mmHg}$, respectively. These effects were reversed by a 3 fold excess of L-Arg (3 or $10 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.) (Figure 1a). The increases in MAP brought about by 1 or 3 mg kg^{-1} of MeArg were accompanied by concomitant decreases in RCBF of $29 \pm 7\%$ ($45 \pm 11 \text{ PU}$) and $35 \pm 5\%$ ($56 \pm 8 \text{ PU}$) which were not significantly different from each other. When calculated as change in conductance, MeArg (1 or $3 \text{ mg kg}^{-1} \text{ min}^{-1}$) decreased renal vascular conductance by $43 \pm 7\%$ and $56 \pm 4\%$, respectively. These values were also not significantly different from each other, suggesting that the low dose of MeArg ($1 \text{ mg kg}^{-1} \text{ min}^{-1}$) was sufficient to achieve a maximal effect on RCBF. These reductions in RCBF were reversed by a 3 fold excess of L-Arg. An infusion of $10 \text{ mg kg}^{-1} \text{ min}^{-1}$ of L-Arg not only reversed the effects of MeArg, but resulted in a $49 \pm 10\%$ increase in RCBF (Figure 2a). However, L-Arg had some vasodilator role of its own. Infusions of L-Arg (3 , 10 and $30 \text{ mg kg}^{-1} \text{ min}^{-1}$; $n = 3-5$) for 10 min significantly increased RCBF by $28 \pm 3 \text{ PU}$, $47 \pm 7 \text{ PU}$, $59 \pm 3 \text{ PU}$, respectively ($P < 0.05$). The same doses of D-Arg did not significantly increase RCBF ($18 \pm 1 \text{ PU}$, $14 \pm 2 \text{ PU}$, $29 \pm 8 \text{ PU}$, respectively) when compared to saline ($9 \pm 5 \text{ PU}$) ($n = 3-9$).

When compared to MeArg ($1 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.), NO_2Arg ($300 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 5$) produced a similar increase in MAP of $34 \pm 7 \text{ mmHg}$ indicating that NO_2Arg is approximately 3 fold more potent than MeArg (Figure 1b). An infusion of a 100 fold excess of L-Arg ($30 \text{ mg kg}^{-1} \text{ min}^{-1}$), however, failed to reverse the pressor effect of NO_2Arg . This dose of NO_2Arg had a much greater effect on RCBF than MeArg ($1 \text{ mg kg}^{-1} \text{ min}^{-1}$), significantly reducing it by $53 \pm 4\%$ ($95 \pm 16 \text{ PU}$) (Figure 2b). This higher potency of NO_2Arg on RCBF was also reflected by a significantly greater fall in renal vascular conductance ($64 \pm 2\%$). More importantly, a lower dose of NO_2Arg ($30 \mu\text{g kg}^{-1} \text{ min}^{-1}$; $n = 4$), without having any effect on MAP, significantly reduced RCBF by $21 \pm 4\%$ ($56 \pm 9 \text{ PU}$), (Figure 1b, 2b and Figure 3) and renal vascular

Figure 1 N^G -monomethyl-L-arginine (MeArg) or N^G -nitro-L-arginine methyl ester (NO_2 Arg) increase mean arterial blood pressure (MAP). (a) MeArg ($1 \text{ mg kg}^{-1} \text{ min}^{-1}$; solid column; or $3 \text{ mg kg}^{-1} \text{ min}^{-1}$; hatched column) increased MAP, whilst a 3 fold excess of L-arginine (L-Arg, $3 \text{ mg kg}^{-1} \text{ min}^{-1}$; solid column or $10 \text{ mg kg}^{-1} \text{ min}^{-1}$; hatched column, respectively) reversed this effect. (b) NO_2 Arg ($30 \text{ µg kg}^{-1} \text{ min}^{-1}$; open column) had no significant effect on MAP, whilst NO_2 Arg ($300 \text{ µg kg}^{-1} \text{ min}^{-1}$; stippled column) significantly increased MAP. A 100 fold excess of L-Arg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$; open column or $30 \text{ mg kg}^{-1} \text{ min}^{-1}$; stippled column, respectively) produced no significant reversal. Columns are means with s.e.mean shown by vertical bars. * $P < 0.05$ when compared to basal; † $P < 0.05$ when compared to MeArg.

conductance by $28 \pm 8\%$. The decreases in RCBF achieved with NO_2 Arg were significantly reversed by a 100 fold excess of L-Arg (Figure 2b).

Effect of NO synthesis inhibition on stimulated release of NO

Figure 4 illustrates the fall in blood pressure in response to 3 min infusions of ACh in the presence and absence of MeArg. Infusion of ACh ($10 \text{ µg kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 14$) for 3 min resulted in a fall of MAP, which at maximum was $46 \pm 2 \text{ mmHg}$ and was $35 \pm 3 \text{ mmHg}$ after 3 min of infusion. This fall in MAP was not associated with any significant change in heart rate ($P > 0.05$; data not shown). Although MeArg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.) had no effect on the maximum fall in MAP induced by ACh, it did, however, reduce the duration of the response so that a significant inhibition was seen after 3 min of infusion (Figures 4 and 5a). This inhibition was partially, but significantly, reversed by a 20 min infusion of L-Arg. Similarly, NO_2 Arg ($300 \text{ µg kg}^{-1} \text{ min}^{-1}$, i.v.) did not affect the maximum fall in MAP produced by ACh, but significantly reduced the duration of the response. This effect was significantly reversed by a co-infusion of a 100 fold excess of L-Arg (Figure 5b).

The depressor response to ACh was associated with a hyperaemia of the renal cortex of $44 \pm 3\%$ amounting to an AUC of 19616 ± 2280 units ($n = 14$). Infusion of MeArg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.) significantly attenuated the ACh-induced hyperaemia by 86% (25711 ± 4300 to 4559 ± 3000 units; $n = 5$), whilst co-infusion of L-Arg ($10 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.) significantly reversed this inhibitory effect (25147 ± 2489 units) (Figure 6a). A representative trace of the effects of MeArg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.) on basal and ACh-stimulated release of NO is shown in Figure 7. A 3 fold lower concentra-

Figure 2 N^G -monomethyl-L-arginine (MeArg) or N^G -nitro-L-arginine methyl ester (NO_2 Arg) decrease renal cortical blood flow (RCBF) in an L-arginine (L-Arg) reversible manner. (a) MeArg ($1 \text{ mg kg}^{-1} \text{ min}^{-1}$; solid column; or $3 \text{ mg kg}^{-1} \text{ min}^{-1}$; hatched column) decreased RCBF, whilst a co-infusion of a 3 fold excess of L-Arg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$; solid column) abolished this effect or ($10 \text{ mg kg}^{-1} \text{ min}^{-1}$; hatched column) resulted in an increase in RCBF. (b) NO_2 Arg ($30 \text{ µg kg}^{-1} \text{ min}^{-1}$; open column or $300 \text{ µg kg}^{-1} \text{ min}^{-1}$; stippled column) produced a more striking decrease in RCBF which was reversed by a 100 fold excess of L-Arg ($3 \text{ mg kg}^{-1} \text{ min}^{-1}$; open column or $30 \text{ mg kg}^{-1} \text{ min}^{-1}$; stippled, respectively). Columns are means with s.e.mean shown by vertical bars. * $P < 0.05$ when compared to basal; † $P < 0.05$ when compared to MeArg or NO_2 Arg.

tion of NO_2 Arg ($300 \text{ µg kg}^{-1} \text{ min}^{-1}$, i.v.; $n = 5$) produced a similar 96% inhibition of the ACh-induced increase in renal blood flow (15667 ± 3064 to 837 ± 513 units), whilst a 100 fold excess of L-Arg ($30 \text{ mg kg}^{-1} \text{ min}^{-1}$, i.v.) resulted in a partial, but significant reversal (8591 ± 2515 units) (Figure 6b).

Ten min after injection of ET-1 (1 nmol kg^{-1} , i.v.) the MAP was increased by $32 \pm 5 \text{ mmHg}$. The associated fall in RCBF ($43 \pm 7 \text{ PU}$) was not significantly different from that produced

Figure 3 N^G -nitro-L-arginine methyl ester (NO_2 Arg) decreases renal cortical blood flow (RCBF), without having any effect on mean arterial blood pressure (MAP). NO_2 Arg ($30 \text{ µg kg}^{-1} \text{ min}^{-1}$; hatched bar) reduced RCBF, by approximately 20%, without any significant effect on MAP. A co-infusion of L-arginine (L-Arg, $3 \text{ mg kg}^{-1} \text{ min}^{-1}$; open bar) reversed this effect.

Figure 4 The figure shows two original traces illustrating the reductions in phasic arterial blood pressure in the anaesthetized rat in response to 3 min infusions of acetylcholine (ACh) (a) in the absence and (b) in the presence of N^G -monomethyl-L-arginine (MeArg). Infusion of ACh ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.v. for 3 min) produced a maximum fall in mean arterial blood pressure (MAP) of 48%, which after 3 min remained at -44% (a). Although MeArg ($3 \text{ mg kg}^{-1} \text{min}^{-1}$, i.v.) did not affect the maximum fall in MAP induced by ACh (-48%), MeArg caused a partial inhibition of the ACh-induced reduction in blood pressure at 3 min (-26%; b).

by MeArg. Likewise, the increase in MAP brought about by ET-1 was not significantly different from that induced by NO_2Arg , even though the fall in RCBF was greater. Thus, the effects of ET-1 on either MAP or RCBF can be considered as a reasonable control for the changes brought about by both MeArg and NO_2Arg . This injection of ET-1 (1 nmol kg^{-1}) did not significantly affect the depressor response to ACh at 3 min (Figure 5c). The renal cortical hyperaemia induced by ACh was inhibited to some extent by a bolus dose of ET-1 (1 nmol kg^{-1} ; $n = 4$) (16933 ± 2828 to 12019 ± 2560 units). However, this was significantly different from the inhibition produced by MeArg and NO_2Arg ($P < 0.05$; Figure 6c). A 3 min infusion of dopamine ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$) had no signifi-

Figure 5 N^G -monomethyl-L-arginine (MeArg) or N^G -nitro-L-arginine methyl ester (NO_2Arg) inhibit the duration of acetylcholine (ACh)-induced decreases in mean arterial blood pressure (MAP). (a) At the 3 min time point after commencing infusion, ACh ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$) produced a decrease in MAP which was attenuated by MeArg ($3 \text{ mg kg}^{-1} \text{min}^{-1}$; open column) and reversed by L-arginine (L-Arg, $10 \text{ mg kg}^{-1} \text{min}^{-1}$; hatched column). (b) The ACh-induced decrease in MAP is similarly attenuated by NO_2Arg ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$; open column) and reversed by L-Arg ($30 \text{ mg kg}^{-1} \text{min}^{-1}$; hatched column). (c) The fall in MAP produced by ACh was not significantly affected by endothelin-1 (ET-1; 1 nmol kg^{-1}). (d) Dopamine ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$; for 3 min) had no significant effect on MAP either in the presence (open column) or absence (stippled column) of NO_2Arg ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$). Data are expressed as means $\pm$ s.e.mean. * $P < 0.05$ when compared to control; † $P < 0.05$ when compared to MeArg or NO_2Arg .

Figure 6 N^G -monomethyl-L-arginine (MeArg) or N^G -nitro-L-arginine methyl ester (NO_2Arg) inhibit endothelium-dependent (acetylcholine, ACh) but not endothelium-independent increases in cortical blood flow (RCBF). RCBF responses were measured as the area under the curve (AUC). (a) ACh ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$; for 3 min) results in an increase in RCBF which is attenuated by MeArg ($3 \text{ mg kg}^{-1} \text{min}^{-1}$; open column) and reversed by L-arginine (L-Arg, $10 \text{ mg kg}^{-1} \text{min}^{-1}$; hatched column). (b) The ACh-induced increase in RCBF is similarly attenuated by NO_2Arg ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$; open column) and reversed by L-Arg ($30 \text{ mg kg}^{-1} \text{min}^{-1}$; hatched column). (c) The increase in RCBF produced by ACh was inhibited by endothelin-1 (1 nmol kg^{-1}). However, this inhibition was significantly weaker than the inhibition seen with MeArg or NO_2Arg . (d) The increase in RCBF produced by dopamine ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$; for 3 min) was not significantly affected by NO_2Arg ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$). Data are expressed as means $\pm$ s.e.mean. * $P < 0.05$ when compared to control; † $P < 0.05$ when compared to MeArg or NO_2Arg .

cant effect on MAP, either alone or in the presence of NO_2Arg ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$) (Figure 5). However, an infusion of this dose of dopamine for 3 min resulted in an increase in renal cortical blood flow of $21 \pm 1\%$ amounting to an AUC of 10468 ± 880 units ($n = 3$), which was unaffected by NO_2Arg ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$) (10785 ± 815 units) (Figure 6d).

Infusions of high doses of L-Arg ($30 \text{ mg kg}^{-1} \text{min}^{-1}$, i.v.) did not affect the increase in RCBF induced by dopamine (7465 ± 1115 to 6442 ± 277 units, $n = 4$; $P > 0.05$). Although L-Arg potentiated the increase in RCBF induced by ACh in 3 out of 6 experiments, this effect failed to attain statistical significance (21951 ± 1851 to 38509 ± 7583 units, $n = 6$; $P > 0.05$).

Figure 7 N^G -monomethyl-L-arginine (MeArg) inhibits the increase in renal cortical blood flow (RCBF) produced by acetylcholine (ACh), in an L-arginine (L-Arg) reversible manner. MeArg ($3 \text{ mg kg}^{-1} \text{min}^{-1}$; hatched bar) reduces the basal, and subsequently inhibits the ACh-stimulated ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$; for 3 min) increase in RCBF whilst a co-infusion of L-Arg ($10 \text{ mg kg}^{-1} \text{min}^{-1}$; open bar) reverses these inhibitory effects of MeArg.

Discussion

After the discovery that aspirin-like drugs inhibit prostaglandin biosynthesis (Vane, 1971), many workers studied their effects on renal blood flow, in order to define any prostaglandin contribution (see Patrono & Dunn, 1987). There is now general agreement that prostaglandin formation does not contribute to normal renal blood flow but comes into play when the kidney is compromised, such as in circulatory shock or renal failure (Clive & Stoff, 1984; Carmichael & Shankel, 1985; Morrison, 1986; Patrono & Dunn, 1987; Oates *et al.*, 1988). Our results, in which indomethacin had no effect on renal cortical blood flow, fit into this general concept.

From our results, the arginine-NO system clearly participates in the physiological maintenance of renal cortical blood flow. The present study demonstrates that the NO synthesis inhibitor, MeArg, reduced renal cortical blood flow (RCBF) in an L-Arg reversible manner. Moreover, NO₂Arg produced a dose-dependent reduction in RCBF, which was partly reversed by L-Arg. In addition, both compounds inhibited endothelium-dependent (ACh), but not endothelium-independent (dopamine) increases in RCBF in an L-Arg reversible manner. Most importantly, a low dose of NO₂Arg, while having no pressor effect, considerably reduced RCBF.

Stimulated release of NO

ACh causes renal vasodilatation in isolated perfused kidneys (Burton *et al.*, 1987; Bhardwaj & Moore, 1988; Burton *et al.*, 1989) and *in vivo* (Baer *et al.*, 1970; Baylis *et al.*, 1976; Thomas *et al.*, 1988; Walder *et al.*, 1989). In the isolated perfused kidney of the rat, the vasodilator response to ACh and the concomitant increase in urinary cyclic GMP are inhibited by haemoglobin or gossypol (Burton *et al.*, 1990). More importantly, in the anaesthetized rat *in vivo*, ACh induces a dose-dependent increase in urinary cyclic GMP levels, the rates of increase of cyclic GMP being correlated with the fall in systemic blood pressure (Tolins *et al.*, 1990). The increase in urinary cyclic GMP levels, the hypotensive effect and renal haemodynamic effects of ACh are all inhibited by MeArg, suggesting that these effects of ACh are due to stimulation of NO formation, which then activates guanylate cyclase (Tolins *et al.*, 1990). This observation is supported by the work of Lahera *et al.* (1990) who also demonstrated that the ACh-induced increase in renal blood flow and diuresis in the dog are inhibited by MeArg.

In the current study, the ACh-induced systemic hypotension and increase in RCBF were inhibited by MeArg or NO₂Arg in an L-Arg reversible manner. Although ET-1, given to produce a similar pressor effect and fall in RCBF to that of MeArg or NO₂Arg, produced a slight inhibition of the ACh-induced increase in RCBF, this inhibition of the endothelium-dependent vasodilatation was significantly weaker than the one produced by MeArg or NO₂Arg. This suggests that a vasoconstrictor agent, such as ET-1, can exert a weak functional antagonism of endothelium-dependent vasodilator responses. However, the main mechanism of action of the inhibition of endothelium-dependent vasodilator responses produced by MeArg or NO₂Arg is most likely via specific inhibition of NO synthesis, for it was largely attenuated by L-Arg. Our finding here that the increase in RCBF induced by the endothelium-independent renal vasodilator dopamine (which acts via direct activation of cyclic AMP) was unaffected by NO₂Arg supports this conclusion. In addition, infusion of the highest dose of L-Arg did not significantly affect the ACh- or dopamine-induced increases in RCBF. This suggests that the L-Arg reversal of the inhibitory effects of MeArg and NO₂Arg on ACh-stimulated increases in RCBF are not due to direct effect of L-Arg.

Interestingly, the maximum systemic hypotensive effect produced by a 3 min infusion of ACh was unaffected by MeArg or NO₂Arg, but the duration of the response was shortened. Similarly, MeArg does not affect the maximum hypotensive

effect produced by bolus injections of ACh in the anaesthetized guinea-pig, but significantly shortens the response to the endothelium-dependent vasodilator (Aisaka *et al.*, 1989b). In contrast, Rees *et al.* (1989) clearly demonstrated that the maximum hypotensive effects of ACh were inhibited by MeArg in the anaesthetized rabbit. In addition, Whittle *et al.* (1989) have also shown that MeArg inhibits the maximum hypotensive effects of ACh, ET-1, substance P and bradykinin in the anaesthetized rat *in vivo*. Although in our study, MeArg or NO₂Arg had only a weak inhibitor effect on the maximum systemic vasodilator responses brought about by ACh, it is clear that the ACh-induced increase in RCBF was largely attenuated by both inhibitors of NO formation. This finding indicates that the systemic hypotensive effect of ACh *in vivo* is only partly mediated by an ACh-stimulated release of NO. In support of this concept, NO₂Arg (10 mg kg⁻¹, i.v.) did not affect the fall in blood pressure produced by bolus injections of ACh in conscious rats (Gardiner *et al.*, 1990c).

When compared to MeArg, at a tenth of the dose, NO₂Arg produced a similar inhibition of the duration of the hypotensive effect and inhibition of the increase in RCBF brought about by ACh. In addition, the effects of MeArg were reversed by a 3 fold excess of L-Arg, while the effects of NO₂Arg were only partially reversed by a 100 fold excess of L-Arg. These results support the current hypothesis that NO₂Arg is a more potent inhibitor of NO synthesis (Moore *et al.*, 1990; Ishii *et al.*, 1990; Hecker *et al.*, 1990; Gardiner *et al.*, 1990b). The recent finding that bovine endothelial cells in culture metabolize MeArg, but not NO₂Arg, to L-citrulline and subsequently to L-Arg may explain the differences in potency between MeArg and NO₂Arg observed *in vitro* and *in vivo* (Hecker *et al.*, 1990).

Basal NO release

The substantial renal cortical vasoconstriction induced by the inhibition of arginine metabolism clearly indicates that the 'basal' release of NO is important for the regulation and maintenance of cortical blood flow in anaesthetized rats. Our findings here that MeArg or NO₂Arg increase blood pressure, in an L-Arg reversible manner, are consistent with the current understanding that 'basal' NO release plays a major role in the modulation of systemic blood pressure *in vivo* (Rees *et al.*, 1989; Whittle *et al.*, 1989; Aisaka *et al.*, 1989; Vallance *et al.*, 1989; Hecker *et al.*, 1990; Gardiner *et al.*, 1990a).

Using laser Doppler flowmetry, we clearly demonstrate that the increases in MAP produced by MeArg or NO₂Arg were accompanied by L-Arg reversible falls in RCBF (ranging from 21 to 53%) demonstrating a local role for NO in the control of RCBF. Similarly, MeArg (Gardiner *et al.*, 1990a) and NO₂Arg (Gardiner *et al.*, 1990b) increase blood pressure and reduce total renal blood flow in the conscious rat chronically instrumented with pulsed Doppler flow probes. In contrast, Lahera *et al.* (1990), using an electromagnetic flow probe to measure renal arterial blood flow showed that intrarenal infusion of low concentrations of MeArg (97 µg kg⁻¹ min⁻¹) had no effect on basal renal blood flow. Similarly, when renal plasma flow is measured by *p*-aminohippurate (PAH) clearance, an intravenous bolus injection of MeArg (15 mg kg⁻¹) failed to affect basal renal plasma flow (Tolins *et al.*, 1990). However, these studies (Lahera *et al.*, 1990; Tolins *et al.*, 1990) did not separate cortical from medullary flow which may account for the differences in our data on the effects of MeArg on basal RCBF.

In the present study we demonstrated that infusions of L-Arg, but not D-Arg, produced an increase in RCBF. This finding is not entirely surprising, for it is well documented that the oral intake of protein or the infusion of amino acids, in particular L-Arg, induces an increase in renal plasma flow and glomerular filtration rate in various species including man, dogs and rats (Bosch *et al.*, 1983; Palmore, 1983; Meyer & Brenner, 1983; Hostetter, 1986). Interestingly, Bhardwaj & Moore

(1989) showed that the vasodilator activity of L-Arg in the isolated perfused kidney of the rat can be abolished by removal of the endothelium. More recently, Tolins & Raji (1990) demonstrated that the increases in glomerular filtration rate and renal plasma flow, induced by amino acid infusion, were completely inhibited by intrarenal MeArg. Thus, on the one hand, NO may mediate some of the renal haemodynamic responses to L-Arg infusions. On the other hand, a direct vasodilator effect of L-Arg may contribute to some of its renal effects. At present it is not possible to differentiate between these two possibilities.

In addition, we have demonstrated that RCBF is much more sensitive than MAP to the effects of MeArg and NO₂Arg and to reversal by L-Arg. More importantly, a low dose of NO₂Arg, while having no systemic pressor effect, caused a 21% reduction in RCBF. This finding clearly indicates that the renal cortical vasculature has a relatively high basal release of NO, which substantially contributes to the regulation and maintenance of RCBF. Furthermore, our data here and our previous finding that MeArg and NO₂Arg did not influence basal blood flow in the rat gastric vasculature (Walder *et al.*, 1990), indicate that the pressor responses to MeArg and NO₂Arg in the anaesthetized rat *in vivo* are unlikely to be due to a uniform vasoconstriction in all vascular beds. Gardiner *et al.* (1990a,b), using pulsed Doppler probes, also showed differential haemodynamic effects of MeArg and NO₂Arg respectively, on renal, mesenteric and hindquarter vascular beds in the conscious rat. However, in

those studies, the dose-dependent and long-lasting vasoconstrictions of both NO-inhibitors were more pronounced in the mesenteric than in the renal or hindquarter vascular beds.

It is still not clear whether the pressor responses to inhibitors of NO formation, such as MeArg, are entirely due to inhibition of 'basal' NO formation. Here, we demonstrate that MeArg and NO₂Arg substantially reduce RCBF at very low doses. Such a reduction in RCBF may be associated with an activation of the renin/angiotensin system. Thus, another potential underlying mechanism of the pressor effects obtained with MeArg or NO₂Arg *in vivo* may be related to an enhancement of renin/angiotensin II plasma levels secondary to a reduction in renal blood flow. However, recent work suggests that the renin/angiotensin II system is not responsible for the pressor effects of MeArg since the angiotensin II receptor antagonist, Sar-Gly-angiotensin II, did not prevent the rise in blood pressure induced by MeArg (Tolins & Raji, 1990).

In conclusion, our findings suggest that MeArg and NO₂Arg inhibit basal and ACh-stimulated release of NO in the renal cortical vasculature. The discovery that NO₂Arg can substantially reduce RCBF without having an effect on systemic blood pressure indicates that the renal cortical vasculature has a relatively high basal release of NO, which substantially contributes to the regulation and maintenance of RCBF.

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References

- AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989a). N^G-methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis, is a potent pressor agent in the guinea-pig: does nitric oxide regulate blood pressure *in vivo*? *Biochem. Biophys. Res. Commun.*, **160**, 881–886.
- AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989b). L-Arginine availability determines the duration of acetylcholine-induced systemic vasodilatation *in vivo*. *Biochem. Biophys. Res. Commun.*, **163**, 710–717.
- BAER, P.G., NAVAR, L.G. & GUYTON, A.C. (1970). Renal autoregulation filtration rate and electrolyte excretion during vasodilatation. *Am. J. Physiol.*, **219**, 619–625.
- BAYLIS, C., DEEN, W.M., MYERS, B.D. & BRENNER, B.M. (1976). Effects of some transcapillary fluid exchange in renal cortex. *Am. J. Physiol.*, **230**, 1148–1158.
- BHARDWAJ, R. & MOORE, P.K. (1988). Endothelium-derived relaxing factor and the effects of acetylcholine and histamine on resistance blood vessels. *Br. J. Pharmacol.*, **95**, 835–843.
- BHARDWAJ, R. & MOORE, P.K. (1989). The effects of arginine and nitric oxide on resistance blood vessels of the perfused rat kidney. *Br. J. Pharmacol.*, **97**, 739–744.
- BOSCH, J.P., SACCAGGI, A., LAUER, A., RONCO, C., BELLEDONNE, M. & GLABMAN, S. (1983). Renal functional reserve in humans. *Am. J. Med.*, **75**, 943–949.
- BURTON, G.A., HAYLOR, J.L. & DE JONGE, A. (1987). Is the relaxation produced by acetylcholine in the isolated perfused rat kidney endothelium-dependent? *J. Endocrinol.*, **115**, suppl., 63.
- BURTON, G.A., HAYLOR, J.L. & DE JONGE, A. (1988). Effect of gossypol on acetylcholine and sodium nitroprusside induced vasodilatation in the isolated rat kidney. *Br. J. Pharmacol.*, **95**, 802P.
- BURTON, G.A., GRIFFITH, T.M. & EDWARDS, D.H. (1989). EDRF-mediated dilatation in the rat isolated perfused kidney: a microangiographic study. *Br. J. Pharmacol.*, **98**, 1207–1212.
- BURTON, G.A., MACNEIL, S., DE JONGE, A. & HAYLOR, J. (1990). Cyclic GMP release and vasodilatation induced by EDRF and atrial natriuretic factor in the isolated perfused kidney of the rat. *Br. J. Pharmacol.*, **99**, 364–368.
- BYFIELD, R.A., SWAYNE, G.T.J. & WARNER, T.J. (1986). A method for the study of endothelial derived relaxing factor (EDRF) in the perfused rat mesentery. *Br. J. Pharmacol.*, **88**, 438P.
- CARMICHAEL, J. & SHANKEL, S.W. (1985). Effects of nonsteroidal antiinflammatory drugs on prostaglandin and renal function. *Am. J. Med.*, **78**, 992–1000.
- CHERRY, P.D. & GILLIS, C.N. (1987). Evidence for the role of endothelium-derived relaxing factor in acetylcholine induced vasodilatation in the rat lung. *J. Pharmacol. Exp. Ther.*, **241**, 516–520.
- CLIVE, D.M. & STOFF, J.S. (1984). Renal syndromes associated with nonsteroidal antiinflammatory drugs. *New Engl. J. Med.*, **310**, 563–572.
- FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–375.
- GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990a). Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension*, **15**, 486–492.
- GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990b). Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.*, **101**, 625–631.
- GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990c). Regional and cardiac haemodynamic responses to glycyl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.*, **101**, 632–639.
- GRIFFITH, T.M., EDWARDS, D.H., DAVIES, R.L.I., HARRISON, T.J. & EVANS, K.T. (1988). Endothelium-dependent relaxing factor (EDRF) and resistance vessels in an intact vascular bed: a microangiographic study of the isolated rabbit ear. *Br. J. Pharmacol.*, **93**, 654–662.
- HECKER, M., HARRIS, H., MITCHELL, J.A., KATSURA, M., THIEMERMANN, C. & VANE, J.R. (1990). Endothelial cells metabolize N^G-monomethyl-L-arginine to L-citrulline and subsequently to L-arginine. *Biochem. Biophys. Res. Commun.*, **167**, 1037–1043.
- HIBBS, J.B., TAINTOR, R.R. & VAVRIN, Z. (1987). Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science*, **235**, 473–476.
- HOSTETTER, T.H. (1986). Human renal response to a meat meal. *Am. J. Physiol.*, **250**, F613–F618.
- ISHII, K., CHANG, B., KERWIN, J.F., HUANG, Z. & MURAD, F. (1990). N^G-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur. J. Pharmacol.*, **176**, 219–223.
- LAHERA, V., SALOM, M.G., FIKSEN-OLSEN, M.J., RAJI, L. & ROMERO, J.C. (1990). Effects of N^G-monomethyl-L-arginine and L-arginine on acetylcholine renal responses. *Hypertension*, **15**, 659–663.
- MEYER, T.W. & BRENNER, B.M. (1983). The renal hemodynamic response to amino acid infusions in the rat. *Clin. Res.*, **31**, 55A.
- MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.S.W., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.*, **99**, 408–412.
- MORRISON, A.R. (1986). Biochemistry and pharmacology of renal arachidonic acid metabolism. *Am. J. Med.*, (suppl. 1A) **80**, 3–11.

- MYERS, P.R., MINOR JR., R.L., GUERRA JR., R., BATES, J.N. & HARRISON, D.G. (1990). Vasorelaxant properties of the endothelium-derived relaxing factor more closely resembles S-nitrosocysteine than nitric oxide. *Nature*, **345**, 161–163.
- OATES, J.A., FITZGERALD, G.A., BRANCH, R.A., JACKSON, E.K., KNAPP, H.R. & ROBERTS, L.J. (1988). Clinical implications of prostaglandin and thromboxane A₂ formation. *New Engl. J. Med.*, **319**, 689–98, 761–767.
- PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988a). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.
- PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium derived-relaxing factor. *Nature*, **327**, 524–526.
- PALMER, R.M.J., REES, D.D., ASHTON, D.S. & MONCADA, S. (1988b). L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem. Biophys. Res. Commun.*, **153**, 1251–1256.
- PALMORE, W.P. (1983). Glucagon and alanine-induced increases in the canine renal glomerular filtration rate. *Q. J. Exp. Physiol.*, **68**, 319–327.
- PATRONO, C. & DUNN, M.J. (1987). The clinical significance of inhibition of renal prostaglandin synthesis. *Kidney Int.*, **32**, 1–12.
- REES, D.D., PALMER, R.M.J. & MONCADA, S. (1989). Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3375–3378.
- RIVA, C., ROSS, B. & BESNEDEK, G.B. (1972). Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. *Invest. Ophthalmol.*, **11**, 936–944.
- SMITS, G.J., ROMAN, R.J. & LOMBARD, J.H. (1986). Evaluation of laser-Doppler flowmetry as a measure of tissue blood flow. *J. Appl. Physiol.*, **61**, 666–672.
- STERN, M.D., LAPPE, D.L., BOWEN, P.D., CHIMOSKY, J.E., HOLLOWAY, G.A., KEISER, H.R. & BOWMAN, R.L. (1977). Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. *Am. J. Physiol.*, **232**, H441–H448.
- TOLINS, J.P. & RAIJ, L. (1990). Effects of amino acid infusion on renal hemodynamics: role of endothelium-derived relaxing factor. *Hypertension*, **16**, P54.
- TOLINS, J.P., PALMER, R.M.J., MONCADA, S. & RAIJ, L. (1990). Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. *Am. J. Physiol.*, **258**, H655–H622.
- THOMAS, G.R., THIEMERMANN, C., WALDER, C. & VANE, J.R. (1988). The effects of endothelium-dependent vasodilators on cardiac output and their distribution in the anaesthetized rat: a comparison with sodium nitroprusside. *Br. J. Pharmacol.*, **95**, 986–992.
- VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.*, **231**, 232–235.
- VALLANCE, P., COLLIER, J. & MONCADA, S. (1989). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*, **ii**, 997–1000.
- WALDER, C.E., THOMAS, G.R. & VANE, J.R. (1989). The effects of acetylcholine, bradykinin, sodium nitroprusside and AP-II on renal cortical blood flow in the rat. *Br. J. Pharmacol.*, **98**, 620P.
- WALDER, C.E., THIEMERMANN, C. & VANE, J.R. (1990). Endothelium-derived relaxing factor participates in the increased blood flow in response to pentagastrin in the rat stomach mucosa. *Proc. R. Soc. B.*, **241**, 195–200.
- WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D.D. (1989). Modulation of the vasodepressor actions of acetylcholine bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.*, **98**, 646–652.

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