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Effects of N^G-nitro-L-arginine methyl ester on regional haemodynamic responses to $MgSO_4$ in conscious rats

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1 We assessed regional haemodynamic responses to the vasodilator, $MgSO_4$, in the absence and presence of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), in conscious chronically instrumented Long Evans rats ($n = 9$).

2 $MgSO_4$ (loading dose 220 μmol kg^{-1} min^{-1} for 7 min, maintenance dose 56 μmol kg^{-1} min^{-1} for 7 min), alone, caused slight bradycardia and hypotension accompanied by reductions in renal and mesenteric flows, but a marked hyperaemic vasodilatation in the hindquarters (flow, Δ 54 ± 6%, vascular conductance, Δ 77 ± 5%).

3 L-NAME (183 nmol kg^{-1} min^{-1}) caused hypertension (29 ± 2 mmHg) accompanied by bradycardia (− 51 ± 6 beats min^{-1}) and reductions in flow and vascular conductance in the renal (− 18 ± 4% and − 35 ± 3%, respectively), mesenteric (− 35 ± 3% and − 49 ± 3%, respectively), and hindquarters (− 26 ± 3% and − 42 ± 3%, respectively) vascular beds. In the presence of L-NAME, the hypotensive and bradycardic effects of $MgSO_4$ were still apparent, but its hindquarters hyperaemic vasodilator effect was significantly attenuated.

4 In order to determine if the inhibitory action of L-NAME on the hindquarters hyperaemic vasodilator action of $MgSO_4$ was a non-specific effect, due to the change in baseline conditions caused by L-NAME, we also examined responses to $MgSO_4$ in the presence of endothelin-1 (12.5 pmol kg^{-1} min^{-1}) or angiotensin II (50 pmol kg^{-1} min^{-1}). In the presence of either peptide, the overall effects of $MgSO_4$ on hindquarters flow and vascular conductance were unchanged.

5 In a separate experiment ($n = 8$) we determined that the inhibitory effect of L-NAME on the hyperaemic vasodilator response to $MgSO_4$ was prevented by L-arginine, and also demonstrated that the β_2-adrenoceptor antagonist, ICI 118551, caused significant inhibition of the hindquarters haemodynamic effects of $MgSO_4$.

6 We conclude that the hindquarters haemodynamic effects of $MgSO_4$ in conscious rats involve a substantial L-NAME-sensitive component which depends on activation of β_2-adrenoceptors, probably as a consequence of adrenal medullary adrenaline release.

Keywords: $MgSO_4$; vasodilatation; nitric oxide; N^G-nitro-L-arginine methyl ester; β_2-adrenoceptors

Introduction

Magnesium (Mg^{2+}) can cause relaxation of vascular smooth muscle *in vitro* and vasodilatation *in vivo* (see Altura & Altura, 1985, for review). However, in normotensive rats, infusion of Mg^{2+} has been claimed to cause no change in heart rate, blood pressure or regional blood flow, other than in the heart (DiPette *et al.*, 1987). This latter observation is of interest in the light of recent reports of the beneficial effects of Mg^{2+} in myocardial infarction (Teo *et al.*, 1991; Woods *et al.*, 1992; Horner, 1992).

Some *in vitro* evidence indicates that Mg^{2+} influences endothelium-derived-relaxing-factor (EDRF) release but the effect seems to be one whereby elevation of plasma Mg^{2+} would cause inhibition of EDRF release (Ku & Ann, 1991; Zhang *et al.*, 1992); hence, the latter would not be expected to be involved in the vasodilator effects of Mg^{2+}. However, any possible involvement of EDRF in the visceral haemodynamic action of Mg^{2+} has not been investigated *in vivo*.

There is convincing support for the assertion that a major EDRF is nitric oxide (NO), which is produced through the action of the enzyme, NO synthase (see Moncada *et al.*, 1991; Gardiner & Bennett, 1993, for review). Various analogues of L-arginine, including N^G-nitro-L-arginine methyl ester (L-NAME), inhibit the production of NO, and thus any influence of L-NAME on responses to Mg^{2+} *in vivo* might indicate an involvement of NO. Therefore, in the present work we determined the regional (renal, mesenteric and hindquarters) haemodynamic responses to Mg^{2+} in conscious rats, in the absence and presence of L-NAME. However, the latter has marked cardiovascular effects itself, causing hypertension and widespread regional vasoconstriction (Gardiner *et al.*, 1990c). Since it is feasible that these changes in baseline status would affect responses to Mg^{2+}, we also compared the effects of Mg^{2+} in the absence and presence of two other substances that cause hypertension and vasoconstriction, namely endothelin-1 (ET-1) and angiotensin II (AII).

There is some evidence that L-NAME can act as a muscarinic receptor antagonist (Buxton *et al.*, 1993), so in a secondary experiment we assessed the ability of L-arginine to prevent the inhibitory effects of L-NAME on haemodynamic responses to $MgSO_4$. Finally, because $MgSO_4$ caused prominent hyperaemic vasodilatation in the hindquarters (see Results), and because activation of β_2-adrenoceptors has similar effects in this vascular bed (Gardiner *et al.*, 1991b,c; 1992), we investigated the influence of the β_2-adrenoceptor antagonist, ICI 118551 (Bilski *et al.*, 1983) on haemodynamic responses to $MgSO_4$.

Methods

A group of nine male, Long Evans rats (350–450 g) were used in the primary study. Animals were anaesthetized (sodium methohexitone, 60 mg kg^{-1}, i.p., supplemented as required) and had pulsed Doppler probes (Haywood *et al.*, 1981)

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implanted to allow monitoring of renal, mesenteric and hindquarters blood flows (Gardiner *et al.*, 1990c,d). At least 7 days after probe implantation, animals were briefly anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.) for the placement of intravascular catheters in the abdominal aorta (via the caudal artery) to monitor systemic arterial blood pressure and in the right jugular vein to administer $MgSO_4$ and vasoconstrictor substances. Animals were then left to recover for at least 24 h before experiments were begun, and the protocols were run over the following 4 days. The experiments were as follows:-

Effect of $MgSO_4$ alone

Animals were given an intravenous infusion of $MgSO_4$ as a loading dose (220 μmol kg^{-1} min^{-1} for 7 min) followed by a maintenance dose of 56 μmol kg^{-1} min^{-1} for 7 min. This dose schedule for $MgSO_4$ was based on a previous study in which plasma Mg^{2+} levels rose from 0.58 ± 0.01 mmol l^{-1} to 3.8 ± 0.13 mmol l^{-1} at the end of infusion, accompanied by a reduction of -10 ± 2 mmHg in mean arterial blood pressure (Kemp *et al.*, 1993). However, it is not likely that a steady state was reached for the effects of $MgSO_4$ (see Results).

Effect of $MgSO_4$ in the presence of L-NAME

L-NAME (183 mmol kg^{-1} min^{-1}) (Gardiner & Bennett, 1992) was infused continuously and after 60 min, when haemodynamic changes had stabilised, $MgSO_4$ was infused for 14 min (as above).

Effect of $MgSO_4$ in the presence of ET-1

ET-1 (12.5 pmol kg^{-1} min^{-1}) was infused continuously and after 20 min, when haemodynamic changes had stabilized, $MgSO_4$ was infused for 14 min (as above). The dose of ET-1 was chosen to have similar haemodynamic effects to L-NAME, although it was not possible to match the hindquarters vasoconstrictor responses (see Results).

Effect of $MgSO_4$ in the presence of AII

AII (50 pmol kg^{-1} min^{-1}) was infused and after 20 min, when haemodynamic changes had stabilized, $MgSO_4$ was infused for 14 min (as above). The dose of AII was chosen to have mesenteric and hindquarters vasoconstrictor effects similar to those of L-NAME, but it was not possible to match the pressor and the renal vasoconstrictor actions of AII to those of L-NAME under these circumstances. Only one experiment per day was performed on each animal and, over the first 3 days, animals received $MgSO_4$ alone, or $MgSO_4$ in the presence of ET-1 or AII. The order in which these experiments were run was randomized; however, L-NAME was always given on the fourth day because of its long-lasting effects.

One blood sample was taken during the last minute of the $MgSO_4$ infusion for determination of the plasma Mg^{2+} concentration. Plasma Mg^{2+} levels were measured on a Kodak Ektachem 700X (Department of Clinical Chemistry, University Hospital, Nottingham), for which the lowest limit of detection was 0.08 mmol l^{-1} and co-efficients of variation on standards of 0.86 and 1.97 mmol l^{-1} Mg^{2+} were less than 2.5%.

Influence of L-arginine on the effects of $MgSO_4$ in the presence of L-NAME

In a secondary experiment, animals ($n = 8$) were given $MgSO_4$ alone (as above). At least 3 h later a primed infusion of L-arginine was begun (1.42 mmol kg^{-1} bolus, 1.42 mmol kg^{-1} h^{-1} infusion) 20 min before L-NAME which was administered for 60 min prior to $MgSO_4$ (as above).

Effect of $MgSO_4$ in the presence of ICI 118551

Prior to the experiments above, and on a separate experimental day, the same animals were given $MgSO_4$ alone or $MgSO_4$ 60 min after primed infusion of ICI 118551 (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion).

Throughout the experiments, continuous recordings were made of heart rate (HR) mean arterial blood pressure (MAP) and renal, mesenteric and hindquarters Doppler shift signals, both phasic and mean (using a modified Crystal Biotech VF-1 system) (Gardiner *et al.*, 1990b). Measurements (averaged over 20 s) were made immediately before infusion of L-NAME, ET-1 or AII, and in each animal these values were the baseline to which all the subsequent changes were referred. Sixty minutes after the start of L-NAME infusion, or 20 min after the start of ET-1, or AII infusion, cardiovascular variables were measured again to give steady-state responses to L-NAME, ET-1 or AII, respectively. Thereafter, the loading dose of $MgSO_4$ was given over 7 min and measurements were made at the end of this period, and again at the end of the subsequent 7 min period during which the maintenance dose of $MgSO_4$ was given.

In the experiment in which $MgSO_4$ was given alone, measurements were made 20 min before (i.e., baseline), and immediately before the start of $MgSO_4$ infusion (i.e., control value), and at the end of the infusion of the loading dose and of the maintenance dose of $MgSO_4$ (as above).

Percentage changes in mean Doppler shift signals were taken as indices of flow changes (Haywood *et al.*, 1981), and mean arterial blood pressure and mean Doppler shift signals were used to calculate percentage changes in renal, mesenteric and hindquarters vascular conductances (Gardiner *et al.*, 1990c,d).

Data analysis

Changes relative to baseline and changes relative to pre-$MgSO_4$ were analysed by Friedman's test (Theodorsson-Norheim, 1987). A P value < 0.05 was taken as significant.

Drugs and peptides

$MgSO_4$ was dissolved in distilled water. L-Arginine hydrochloride and L-NAME hydrochloride (Sigma) were dissolved in isotonic saline (154 mmol l^{-1} NaCl). ET-1 (Peptide Institute) and AII (Bachem, UK) were dissolved in isotonic saline containing 1% bovine serum albumin (Sigma). ICI 118551 (erythro-(±)-1[7-methylindan-4-yloxy]-3-isopropyl-aminobutan-2-ol) hydrochloride (a gift from ICI Pharmaceuticals plc) was dissolved in sterile water by gentle warming.

Infusions were given at a rate of 0.3 ml h^{-1} for all substances except $MgSO_4$ which was infused at a rate of 0.15 ml min^{-1}.

Results

Resting values for cardiovascular variables on the different experimental days are shown in Table 1.

Effects of $MgSO_4$ alone

During the 20 min control period, prior to administration of $MgSO_4$, there were no significant changes in cardiovascular variables (Figures 1 and 2). At the end of the loading dose of $MgSO_4$ there was a slight hypotension accompanied by reductions in renal and mesenteric flow, but a marked increase in hindquarters flow (Figure 1). The latter was associated with a substantial increase in vascular conductance, but there were no changes in renal or mesenteric vascular conductance (Figure 2). By the end of the maintenance dose of $MgSO_4$ there was still a slight hypotension, accompanied by a modest bradycardia. There was no longer any significant reduction in

Table 1 Resting cardiovascular variables in the same conscious Long Evans rats ($n = 9$) prior to infusion of $MgSO_4$ N^G-nitro-L-arginine methyl ester (L-NAME), endothelin-1 (ET-1) or angiotensin II (AII) on different experimental days

	pre-$MgSO_4$	*pre-L-NAME*	*pre-ET-1*	*pre-AII*
Heart rate (beats min^{-1})	309 ± 6	299 ± 6	305 ± 7	293 ± 3
Mean arterial blood pressure (mmHg)	105 ± 2	103 ± 2	104 ± 3	100 ± 2
Renal Doppler shift (kHz)	7.8 ± 0.9	7.6 ± 0.9	7.3 ± 1.0	7.6 ± 1.0
Mesenteric Doppler shift (kHz)	5.0 ± 0.4	5.5 ± 0.6	5.1 ± 0.5	5.5 ± 0.5
Hindquarters Doppler shift (kHz)	4.6 ± 0.5	4.1 ± 0.4	4.3 ± 0.5	4.4 ± 0.5
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	74 ± 7	73 ± 8	69 ± 7	75 ± 8
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	48 ± 4	54 ± 6	49 ± 5	56 ± 5
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	44 ± 5	40 ± 4	42 ± 5	44 ± 5

Values are means ± s.e.mean.

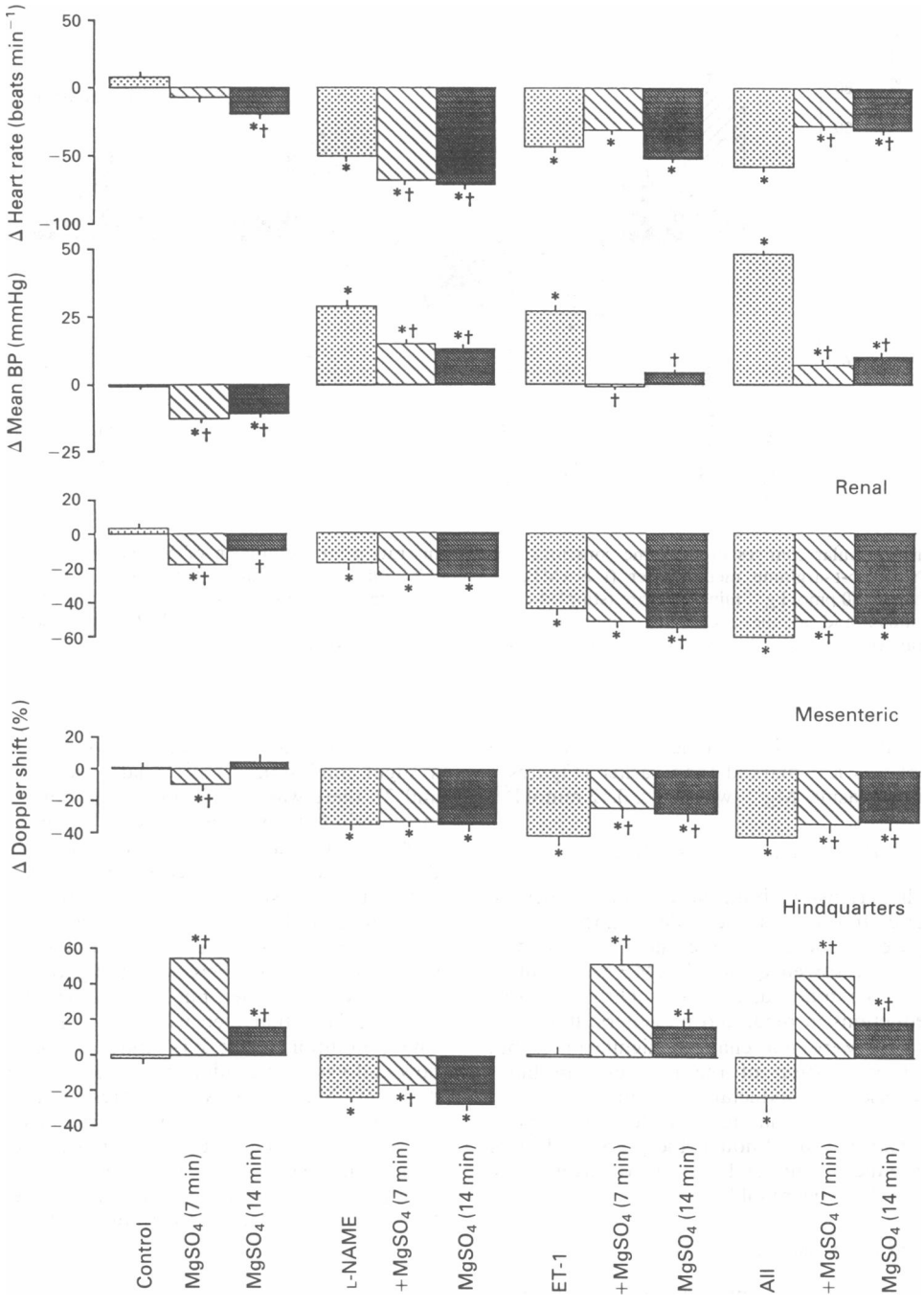

Figure 1 Cardiovascular changes in the same conscious Long Evans rats prior to infusion of $MgSO_4$ alone (control) or in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 183 nmol kg^{-1} min^{-1}), endothelin-1 (ET-1, 12.5 pmol kg^{-1} min^{-1}) or angiotensin II (AII, 50 pmol kg^{-1} min^{-1}) before (stippled columns) at the end of the loading dose of $MgSO_4$ (220 μmol kg^{-1} min^{-1}) (hatched columns), and at the end of the maintenance dose of $MgSO_4$ (56 μmol kg^{-1} min^{-1}) (solid columns). Values are mean ± s.e.mean ($n = 9$). *$P < 0.05$ versus baseline; †$P <$ versus pre-$MgSO_4$ value.

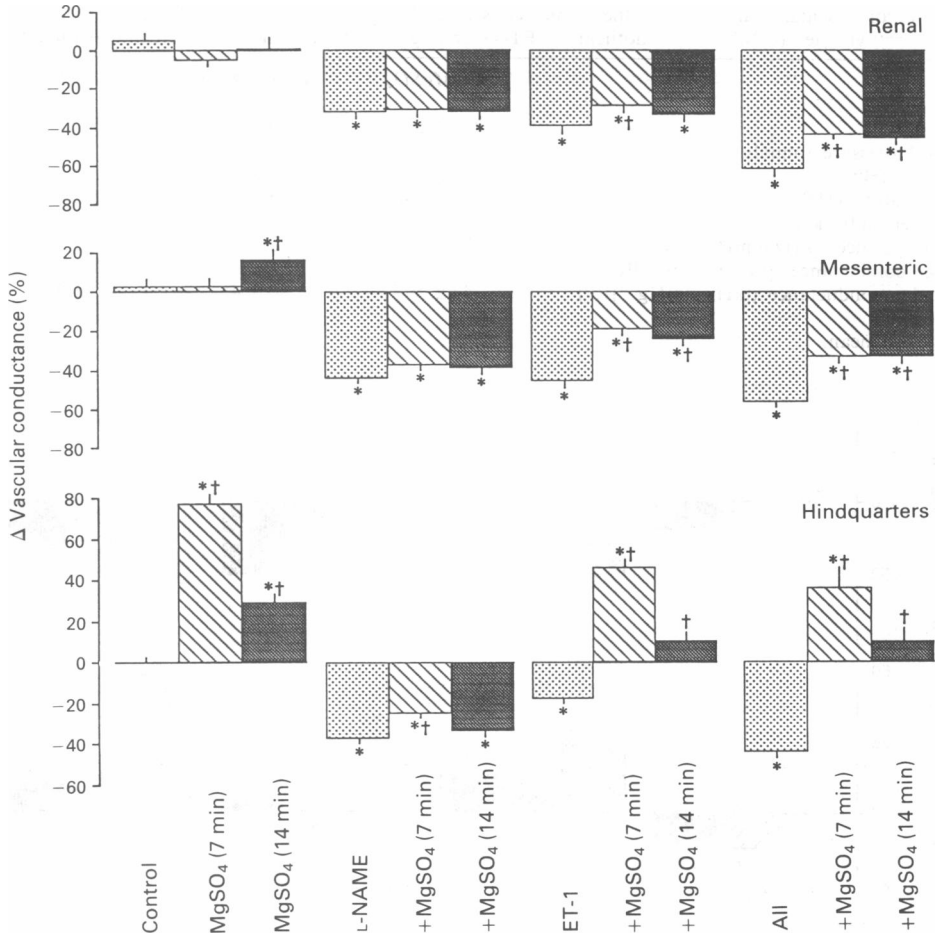

Figure 2 Cardiovascular changes in the same conscious Long Evans rats prior to infusion of $MgSO_4$ alone (control) or in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 183 nmol kg^{-1} min^{-1}), endothelin-1 (ET-1, 12.5 pmol kg^{-1} min^{-1}) or angiotensin II (AII, 50 pmol kg^{-1} min^{-1}) before (stippled columns) at the end of the loading dose of $MgSO_4$ (220 μmol kg^{-1} min^{-1}) (hatched columns), and at the end of the maintenance dose of $MgSO_4$ (56 μmol kg^{-1} min^{-1}) (solid columns). Values are mean ± s.e.mean ($n = 9$). *$P < 0.05$ versus baseline; †$P <$ versus pre-$MgSO_4$ value.

renal or mesenteric flow, and the hyperaemic vasodilatation in the hindquarters was less marked than earlier (Figures 1 and 2). At this time plasma Mg^{2+} was 3.9 ± 0.12 mmol l^{-1}.

Effect of $MgSO_4$ in the presence of L-NAME

L-NAME caused hypertension, bradycardia, and reductions in renal, mesenteric and hindquarters flows and vascular conductances (Figures 1 and 2). At the end of the loading dose of $MgSO_4$, the pressor effect of L-NAME was reduced by about 50%, but the bradycardia was slightly increased (Figure 1). The reductions in renal and mesenteric flows and vascular conductances were not changed significantly, but there was a slight attenuation of the reduction in hindquarters flow and vascular conductance (Figures 1 and 2). However, by the end of the maintenance dose of $MgSO_4$, these latter effects had waned, although the pressor effect of L-NAME was still reduced (Figures 1 and 2). At this juncture plasma Mg^{2+} was 3.9 ± 0.09 mmol l^{-1}.

Effect of $MgSO_4$ in the presence of ET-1

ET-1 caused hypertension and bradycardia, accompanied by constriction in the renal, mesenteric and hindquarters vascular beds, although only renal and mesenteric flows were reduced (Figures 1 and 2). The loading dose of $MgSO_4$ abolished the pressor effect of ET-1, but a bradycardia persisted (Figure 1). The ET-1-induced reduction in renal flow was not affected significantly by $MgSO_4$, although there was a slight inhibition of the renal vasoconstriction (Figures 1 and 2); there was significant attenuation of the reductions in mesenteric flow and vascular conductance (Figures 1 and 2). $MgSO_4$ caused a marked increase in hindquarters flow, and the ET-1-induced constriction in this vascular bed was reversed to a substantial dilatation (Figure 2).

At the end of the maintenance dose of $MgSO_4$, the pressor effect of ET-1 was still abolished, but a bradycardia was still present (Figure 1). There was a slightly greater reduction in renal flow than earlier, but the renal vasoconstriction was not different from that seen with ET-1 alone (Figures 1 and 2). The diminution in the ET-1-induced reduction in mesenteric flow and vascular conductance, seen at the end of the loading dose of $MgSO_4$, was still present at the end of the maintenance dose but the hindquarters hyperaemia had waned and the hindquarters vascular conductance was not significantly increased above baseline, although it was higher than in the presence of ET-1 alone (Figures 1 and 2). Plasma Mg^{2+} level was 4.4 ± 0.14 mmol l^{-1} at this time.

Effect of $MgSO_4$ in the presence of AII

AII caused hypertension, bradycardia, and reductions in renal, mesenteric and hindquarters flows and vascular conductances (Figures 1 and 2). Although the pressor effect of AII was greater than that of L-NAME or ET-1, the mesen-

teric and hindquarters vasoconstrictor effects of AII were not significantly different from those of L-NAME (Figure 2).

By the end of the loading dose of $MgSO_4$, the pressor and bradycardic effects of AII were significantly reduced (Figure 1), and there were slight inhibitions of the reductions in renal and mesenteric flows and vascular conductances (Figures 1 and 2). The AII-induced reductions in hindquarters flow and vascular conductance were reversed to significant increases (Figures 1 and 2).

At the end of the maintenance dose of $MgSO_4$ its inhibitory effects on the pressor, bradycardic, and renal and mesenteric vasoconstrictor actions of AII were still present (Figures 1 and 2). However, the hindquarters hyperaemia was less marked, and vascular conductance, although significantly higher than in the presence of AII alone, was not significantly above baseline (Figures 1 and 2). At this point plasma Mg^{2+} was 4.1 ± 0.11 mmol l^{-1}.

Influence of L-arginine on the effects of $MgSO_4$ in the presence of L-NAME

In the presence of L-arginine, L-NAME had no significant effect on renal vascular conductance ($-3 \pm 5\%$) or mesenteric vascular conductance ($3 \pm 22\%$), but there was still a significant reduction in hindquarters vascular conductance ($-31 \pm 6\%$). (Such a differential effect of L-arginine on the regional vasoconstrictor responses to L-NAME has been reported previously (Gardiner *et al.*, 1990c)). The hindquarters vasoconstrictor effect of L-NAME, in the presence of L-arginine, was abolished at the end of the loading dose of $MgSO_4$ (Δ vascular conductance $= 1 \pm 6\%$ (from $-31 \pm 6\%$)) and was still significantly reduced at the end of the maintenance dose ($-16 \pm 6\%$). Thus, in the presence of L-arginine and L-NAME, there was an underlying hindquarters vasodilator effect of $MgSO_4$ which was significantly greater than in the absence of L-arginine.

Effect of $MgSO_4$ in the presence of ICI 118551

In the presence of ICI 118551, the hindquarters vasodilator response to $MgSO_4$ (Δ vascular conductance at 7 min $= 15 \pm 6\%$, and at 14 min $= 5 \pm 5\%$) was significantly less than in the absence of ICI 118551 (at 7 min $= 67 \pm 9\%$ and at 14 min $= 29 \pm 5\%$).

Discussion

In the present work we measured renal, mesenteric and hindquarters responses to $MgSO_4$ infusion and found that the slight hypotensive effect of this intervention was accompanied by a selective hindquarters hyperaemia and vasodilatation. It is feasible that the apparently differential vasodilator influence of $MgSO_4$ was due to baroreflex-mediated sympathoadrenal stimulation, and activation of renin-angiotensin and vasopressin-mediated mechanisms, opposing any $MgSO_4$-induced vasodilatation in the renal and mesenteric vascular beds. If so, this situation might be analogous to that in which calcitonin gene-related peptide (CGRP), when infused *in vivo*, causes hindquarters vasodilatation and mesenteric vasoconstriction (Gardiner *et al.*, 1989a,b), in spite of the fact that, *in vitro*, CGRP is a potent mesenteric vasodilator (Marshall *et al.*, 1986; Kawasaki *et al.*, 1988). However, in that circumstance, the hypotensive response to CGRP was much greater than observed here with $MgSO_4$, and thus activation of vasoconstrictor mechanisms was probably more marked with CGRP. Moreover, since sympathoadrenal activity and vasopressin can exert hindquarters vasoconstrictor effects (Gardiner & Bennett, 1988; Gardiner *et al.*, 1988), it is probable (see later) that the early, sizeable increase in hindquarters flow and vascular conductance induced by $MgSO_4$ was due, at least partly, to more effective activation of vasodilator mechanism(s) in that vascular bed. We cannot dismiss the possibility that the waning vasodilator effect of $MgSO_4$ was due to the plasma Mg^{2+} level falling during infusion of the maintenance dose.

If the vasodilator effect of $MgSO_4$ *in vivo* was due to diminution in intracellular Ca^{2+} levels (Altura & Altura, 1985; D'Angelo *et al.*, 1992), it is not obvious why this influence should be confined to the hindquarters vascular bed. The finding that the hindquarters hyperaemic vasodilator action of $MgSO_4$ was clearly attenuated in the presence of L-NAME, and that this effect was largely prevented by pretreatment with L-arginine, indicates that a large component of the effect of $MgSO_4$ under normal conditions was due to activation of NO-mediated processes. However, as with a direct vasodilator action of $MgSO_4$ (above), it is, at first sight, puzzling that such an effect should be so prominent in the hindquarters vascular bed, particularly since, in other protocols, we have found the mesenteric vascular bed shows more marked vasodilatation than the hindquarters vascular bed in response to NO donors (Gardiner *et al.*, 1990d; 1991b; Phillips *et al.*, 1991). Thus, it would have to be argued that $MgSO_4$ more effectively activated NO-mediated processes in the hindquarters, than in the mesenteric, vascular bed. This is not without precedent, since CGRP has such an action (Gardiner *et al.*, 1991a). However, the influence of CGRP is a receptor-mediated event (Gardiner *et al.*, 1990e), whereas an effect of Mg^{2+} on NO release, for example, would be likely to be due to an influence on the disposition of Ca^{2+} (Altura & Altura, 1985). In this connection, *in vitro* findings indicate that elevated Mg^{2+} should inhibit NO release (Ku & Ann, 1991; Zhang *et al.*, 1992), thus our results are the opposite of what would be predicted from such *in vitro* studies. However, it is feasible that elevated Mg^{2+} levels suppress Na^+/Ca^{2+} exchange, thereby increasing intracellular Ca^{2+} levels and promoting NO release (Cocks *et al.*, 1988). In addition, there is evidence that Mg^{2+} is required for agonist-induced, endothelium-dependent vasorelaxation (Altura & Altura, 1987; Ku & Ann, 1991), and clearly our results could have been due to factors other than a direct stimulatory influence of Mg^{2+} on endothelial NO release, e.g., change in shear force on endothelial cells, or Mg^{2+}-induced release of other mediators (see below).

It is possible that the selective hindquarters vasodilator response to Mg^{2+} was due to increased prostacyclin production since there is evidence that Mg^{2+} has this action (Nadler *et al.*, 1987; Laurant *et al.*, 1992) and, in conscious rats, prostacyclin causes marked hindquarters hyperaemia (Steinberg *et al.*, 1988). However, such an effect should not be susceptible to L-NAME, unless NO mediates Mg^{2+}-induced prostacyclin release, but what evidence there is indicates that NO might suppress prostacyclin release (Mitchell *et al.*, 1993); thus L-NAME should augment any haemodynamic effects of Mg^{2+} that were due to prostacyclin release. It is feasible that the apparent inhibitory effect of L-NAME on the hindquarters response to Mg^{2+} was a non-specific influence, due to the change in baseline status caused by L-NAME, but this is not likely because, when animals were pretreated with a dose of AII that caused a reduction in hindquarters flow and vascular conductance matched to the effect of L-NAME, the hyperaemic vasodilator effect of $MgSO_4$ was not inhibited as it was in the presence of L-NAME. The hindquarters vasodilator effect of $MgSO_4$ was also seen in the presence of ET-1, although the latter did not have hindquarters haemodynamic effects that matched those of L-NAME, probably because ET-1, itself, activates hindquarters vasodilator mechanisms in conscious rats (Gardiner *et al.*, 1989c,d; 1990a).

Differences between the effects of $MgSO_4$ in the presence of L-NAME, compared to those seen in the presence of ET-1 and AII, were also apparent from the changes in mean arterial blood pressure and renal and mesenteric haemodynamics. Thus, $MgSO_4$ was more effective at reversing the pressor effects of ET-1 and AII than it was that of L-NAME. While this is consistent with the more marked hindquarters

vasodilator action of $MgSO_4$ in the former conditions, there was a clear dissociation between the slight vasodilator effect of $MgSO_4$ in the presence of L-NAME and its antihypertensive action. Thus, by the end of the maintenance dose of $MgSO_4$, in the presence of L-NAME, regional haemodynamic status was not different from that seen in the presence of L-NAME alone, but the pressor effect of L-NAME remained reduced. However, in this circumstance there was a significant bradycardia and hence a reduction in cardiac output might have been contributing to the reduction in mean arterial blood pressure.

Apart from the possibilities discussed above, we considered it feasible that the hindquarters hyperaemic vasodilator response to $MgSO_4$ might involve activation of β_2-adrenoceptors (consequent upon adrenal medullary adrenaline release), since the effect of adrenaline is also sensitive to L-NAME (Gardiner *et al.*, 1991c). We confirmed an involvement of β_2-adrenoceptors in the hindquarters haemodynamic effects of $MgSO_4$, inasmuch as pretreatment with ICI 118551 caused substantial inhibition of the responses to $MgSO_4$. It is likely that any effect of $MgSO_4$ on adrenaline release was indirect, since a direct effect of increased plasma Mg^{2+} would be expected to inhibit adrenaline secretion (Nakazoto *et al.*, 1986).

Although, as discussed above, the vasodilator effect of $MgSO_4$, and its susceptibility to L-NAME, was most obvious in the hindquarters vascular bed, $MgSO_4$ did exert significant, albeit modest, renal and mesenteric vasodilator effects in the presence of ET-1 and AII that were not apparent in the presence of L-NAME. Thus, it may be that $MgSO_4$ exerts NO-mediated vasodilator effects in all these vascular beds, but for the reasons given above this effect is most obvious in the hindquarters vascular bed. From our previous studies it appears that $MgSO_4$ also has a prominent, L-NAME-sensitive, vasodilator effect in the carotid vascular bed (Kemp *et al.*, 1993). It remains to be determined in which tissues in these vascular territories the vasodilator responses occur, but it is unlikely that β_2-adrenoceptor mechanisms are involved, since salbutamol does not increase carotid blood flow (Gardiner *et al.*, 1991b).

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