Assessment of the effects of endothelin-1 and magnesium sulphate on regional blood flows in conscious rats, by the coloured microsphere reference technique

¹P.A. Kemp, ^{*}¹S.M. Gardiner, ¹J.E. March, ²P.C. Rubin & ¹T. Bennett

¹School of Biomedical Sciences, University of Nottingham Medical School, Queens Medical Centre, Nottingham NG7 2UH, England; and ²School of Medical and Surgical Sciences, University of Nottingham Medical School, Queens Medical Centre, Nottingham NG7 2UH, England

1 There is evidence to suggest that magnesium (Mg^{2+}) is beneficial in the treatment of a number of conditions, including pre-eclampsia and acute myocardial infarction. The mode of action of Mg^{2+} in these conditions is not clear, although the vasodilator properties of Mg^{2+} are well documented both *in vitro* and *in vivo*.

2 Previously, we demonstrated that i.v. infusion of magnesium sulphate ($MgSO_4$) alone, or in the presence of vasoconstrictors, caused increases in flow and conductance in the common carotid, internal carotid and hindquarters vascular beds, in conscious rats. Therefore, the objective of the present study was to investigate the regional and subregional changes in haemodynamics in response to the vasoconstrictor peptide endothelin-1 (ET-1) and $MgSO_4$ in more detail, using the coloured microsphere reference technique.

3 Infusion of ET-1 and $MgSO_4$ had similar effects on heart rate and mean arterial pressure as in our previous study. Infusion of ET-1 caused a rise in mean arterial pressure and a fall in heart rate, and infusion of $MgSO_4$ returned mean arterial pressure to control levels with no effect on heart rate.

4 The responses to $MgSO_4$ in the presence of ET-1 showed considerable regional heterogeneity with blood flow increasing (e.g. skeletal muscle), decreasing (e.g. stomach) or not changing (e.g. kidney). Of particular interest was the finding that $MgSO_4$ caused increases in flow in the cerebral and coronary vascular beds.

5 This, and our previous studies, have shown that $MgSO_4$ can reverse vasoconstriction in a number of vascular beds, and indicate that this compound may have therapeutic benefit in conditions associated with vasospasm.

Keywords: Endothelin-1; magnesium sulphate; blood flow; coloured microspheres

Abbreviations: $MgSO_4$, magnesium sulphate; Mg^{2+} , magnesium; ET-1, endothelin-1

Introduction

There is evidence to suggest that magnesium (Mg^{2+}) is beneficial in the treatment of a number of conditions, including pre-eclampsia (Lucas *et al.*, 1995; Eclampsia Trial Collaborative Group, 1995) and acute myocardial infarction (Woods *et al.*, 1992). The mode of action of Mg^{2+} in these conditions is not clear, although vasodilator properties of Mg^{2+} are well documented both *in vitro* (Altura & Altura, 1980, 1982; Altura & Turlapaty, 1981; Kimura *et al.*, 1989; Skajaa *et al.*, 1990; Szabo *et al.*, 1991, 1992; Noguera & D'Ocon, 1993), and *in vivo* (Seelig *et al.*, 1983; Dipette *et al.*, 1987; Nishio *et al.*, 1989; Chi *et al.*, 1990; Ram *et al.*, 1991; Sipes *et al.*, 1991; Perales *et al.*, 1991; Kemp *et al.*, 1993, 1994).

Woods (1991) suggested that Mg^{2+} is a coronary vasodilator, and that some of the beneficial effect of Mg^{2+} in the treatment of acute myocardial infarction may be due to the prevention and relief of coronary vasospasm associated with the condition. Also, infusion of Mg^{2+} has been shown to be useful in coronary artery bypass surgery (Marichal *et al.*, 1992), and to suppress exercise-induced angina in patients with variant angina (Kugiyama *et al.*, 1988). The ability of Mg^{2+} to prevent seizures in pre-eclampsia could also be due to its vasodilator action (Perales *et al.*, 1991; Belfort & Moise, 1992;

Kemp *et al.*, 1993). It is believed that the seizures may be caused by cerebral ischaemia, brought about by cerebral vasospasm, and that Mg^{2+} is effective in preventing or relieving the vasospasm and, therefore, the cerebral ischaemia (Perales *et al.*, 1991; Belfort & Moise, 1992; Kemp *et al.*, 1993).

Previously, we investigated the effect of intravenous infusion of magnesium sulphate ($MgSO_4$) alone, or in the presence of vasoconstrictors, on regional haemodynamics in conscious rats, using chronically implanted pulsed Doppler flow probes. The effects of $MgSO_4$ were studied in the common and internal carotid vascular beds (Kemp *et al.*, 1993), and in the renal, mesenteric and hindquarters vascular beds (Kemp *et al.*, 1994) in separate experiments. $MgSO_4$ alone, or in the presence of vasoconstrictor peptides, caused increases in flow and conductance in the common carotid, internal carotid and hindquarters vascular beds; the vasodilator effects of $MgSO_4$ were particularly marked in the hindquarters vascular bed in the presence of endothelin (Kemp *et al.*, 1994). However, in those studies, we were unable to measure coronary flow. Furthermore, although internal carotid flow and conductance increased during infusion of $MgSO_4$, we could not be certain that the changes we measured were a reflection of changes only in the cerebral vascular bed. In addition, any change in brain blood flow due to variation in vertebral arterial haemodynamics would not have been detected, although Wellers *et al.* (1976) demonstrated that, in anaesthetized rats, blood flow

* Author for correspondence.

through the right vertebral artery was mainly distributed extracranially. For these various reasons we were interested in investigating the subregional changes in haemodynamics in response to MgSO_4 in more detail. Therefore, in the present study we used the coloured microsphere technique (Kowallik *et al.*, 1991; Hakkinen *et al.*, 1995) to determine, in conscious rats, the influence of MgSO_4 on regional and subregional blood flows in the presence of exogenous endothelin-1 (ET-1).

Methods

Left atrial catheter implantation

Male, Long Evans rats ($n = 16$ in total) had left atrial catheters implanted for injection of microspheres. The method used for left atrial catheter implantation was a modified version of that described by Wicker & Tarazi (1982).

Rats were anaesthetized (sodium methohexitone, 60 mg kg^{-1} , i.p., supplemented as required), intubated, and put on to a ventilator. A thoracotomy was performed through the fourth left intercostal space, the pericardium was opened, and a tie was placed around the tip of the left atrium. The base of the atrium was clamped between a pair of forceps to prevent bleeding, and then a small cut was made in the wall of the atrial appendage. A saline-filled (heparinized saline, 15 u ml^{-1}) catheter (Portex polythene tubing, I.D. 0.28 mm), with a flared end, was inserted into the atrium and tied in place. The forceps holding the base of the atrium were then released and blood was drawn back into the catheter to check that it was positioned correctly. The catheter was flushed, and the chest wall was closed around the catheter. A drain was inserted through the chest wall and negative pressure was applied to reinflate the lungs. The drain was removed, the hole closed, and the rat was removed from the ventilator. The catheter was tunnelled under the skin to exit at the back of the neck, protruding 1–2 cm, and the end was heat sealed. The animals were allowed to recover from the anaesthetic and left for 3–4 days, before arterial and venous catheters were inserted.

Arterial and venous catheterization

Under anaesthesia (as above) an arterial catheter was inserted into the distal abdominal aorta (*via* the ventral caudal artery) and two venous catheters were inserted into the jugular vein for the infusion of drugs. All catheters were tunnelled under the skin to exit at the same site as the atrial catheter.

At this stage, an extension was attached to the atrial catheter and all the catheters were fed through a flexible spring, which was attached to a harness worn by the rat. The animals were allowed to recover for 24 h before experiments began. The arterial catheter was connected, overnight, to an infusion pump (heparinized saline 7.5 u ml^{-1} , 0.4 ml h^{-1}), *via* a fluid-filled swivel (Brown *et al.*, 1976). When experiments began, the catheters were connected to pumps for drug infusion, reference sample withdrawal, and microsphere injection. The arterial catheter was connected to a pressure transducer and arterial pressure was measured, when it was not required for reference sample withdrawal.

Coloured microsphere technique

Yellow, red and blue Dye-Trak polystyrene microspheres (Triton Technology Inc., San Diego, U.S.A.), 15 μm in diameter, were used. The microspheres were sonicated and vortexed immediately prior to injection. Approximately

100,000 yellow, 100,000 red, and 200,000 blue spheres, in 0.2 ml of 0.02% Tween in saline, were injected (0.8 ml min^{-1} , Sage pump, model 351) into the left atrium, followed by 0.3 ml of saline. Starting 10 s before, and continuing for 55 s following injection of the spheres, a reference sample was withdrawn (Sage pump, model 351) from the distal abdominal aorta at a rate of 0.85 ml min^{-1} . At the end of the experiment the animals were killed (Euthatal, sodium pentobarbitone solution, 200 mg ml^{-1} , 0.3–0.5 ml) and the tissues of interest removed and weighed (see below). The coloured microspheres were extracted from the tissue and blood by overnight digestion with 4 M potassium hydroxide, followed by vacuum filtration through 10 μm pore filters (Triton Technology Inc.). The filters were carefully folded, to prevent sphere loss, and placed in a 1-ml Eppendorf tube. Dyes were recovered in 200 μl of dimethylformamide. The tubes were vortexed and centrifuged, and 50 μl of the supernatant was taken for spectrophotometry. The dye mixtures were scanned, between 340 and 800 nm, in a Beckman DU 650 spectrophotometer, and the scans saved to disk. On computer, the scans were copied to the Dye-Trak matrix inversion program (Triton Technology Inc.), which corrects for overlap of the absorption spectra of the colours; sample weights were added, and blood flows calculated (Kowallik *et al.*, 1991).

One group of animals ($n = 8$) was given an i.v. infusion of MgSO_4 (220 $\mu\text{mol min}^{-1}$ at 0.15 ml min^{-1}) (Kemp *et al.*, 1993) for 7 min, beginning 20 min after the onset of ET-1 infusion (12.5 pmol min^{-1} at 0.3 ml h^{-1}) (Kemp *et al.*, 1993), and the other group ($n = 8$) was infused with isotonic saline in place of ET-1 and MgSO_4 . In both groups of animals, three measurements of flow were made in the way described above. Yellow spheres were injected immediately before onset of saline (control group) or ET-1 (experimental group). Red spheres were injected 20 min after onset of saline (control group) or ET-1 (experimental group), and blue spheres were injected during the last min of saline (control group) or MgSO_4 (experimental group) infusion. Blood pressure and heart rate were monitored throughout the experiment, except for during the three flow measurement periods when the arterial catheter was required for reference sample withdrawal. Measurements of mean arterial pressure and heart rate were made just before and immediately after each flow measurement.

At the end of the experiment animals were killed, and the kidneys, adrenal glands, spleen, stomach, testicles, heart (ventricles only), tongue, eyes, and brain were removed whole. Samples of small intestine, hindquarters and head skin, and hindquarters muscle were taken. The tissues were then weighed, and tissue and reference samples were processed, as described above, for removal of spheres from the tissues, and for estimation of amount of dye of each colour present in the samples. Calculations of blood flow, corrected for weight, were made as described above.

For reasons unknown, some samples of skin and hindquarters muscle could not be filtered and it was not possible, therefore, to estimate flow in those samples. Therefore, the numbers of measurements made for those tissues were: Head skin: Control group ($n = 7$), Experimental group ($n = 6$); Hindquarters skin: Control group ($n = 4$), Experimental group ($n = 4$); Hindquarters muscle: Control group ($n = 8$), Experimental group ($n = 7$). For all other tissues $n = 8$ in both groups.

Data analysis

All data are given as means $\pm$ s.e.mean. Flow data were compared by analysis of variance followed by *post hoc*

Bonferroni/Dunn test. Heart rate and pressure changes relative to baseline, changes relative to pre-saline or MgSO_4 and changes relative to pre-measurement values, were analysed by Friedman's test (Theodorsson-Norheim, 1987). A P value <0.05 was taken as significant.

Drugs

MgSO_4 was dissolved in distilled water. ET-1 (Peptide Institute, Osaka, Japan) was dissolved in isotonic saline containing 1% bovine serum albumin (Sigma).

Results

Heart rate and mean arterial pressure

There were no significant changes in heart rate in the control group during the experiment. However, mean arterial pressure showed a small, but significant, increase after the first measurement (Table 1). In the experimental group, the changes seen in heart rate and mean arterial pressure were similar to those seen in the previous studies during infusion of ET-1 and MgSO_4 (Kemp *et al.*, 1994). Infusion of ET-1 caused a significant rise in mean arterial pressure and a reduction in heart rate. Subsequent co-infusion with MgSO_4 caused mean arterial pressure to return to baseline levels, with no significant effect on heart rate (Table 1).

Effects of ET-1 and MgSO_4 on regional and subregional blood flows

Effects on cranial flow (brain, eye, tongue, cranial skin) There were no significant differences in baseline flows between groups, for any of the tissues measured, except the head skin in which the baseline flow was slightly, but significantly, higher in the experimental group compared to the control group. During infusion of saline (control group) there were no significant changes in tissue blood flows (Table 2). In the experimental group infusion of ET-1 had no effect on brain or eye blood flow, but reduced flow through the cranial skin. MgSO_4 had no effect on blood flow through the eyes or cranial skin, but increased brain and tongue blood flows to above baseline levels (Table 2).

Effects on coronary flow Baseline values for flow through the heart were not significantly different between the two groups. In the control group there was a significant increase in flow to the heart after the first measurement of flow, which was still

apparent in the second measurement (Table 2). Infusion of ET-1 had no significant effect on blood flow to the heart, but MgSO_4 caused a large increase in flow to above the baseline value (Table 2).

Effects on flow in the kidneys, adrenal glands, spleen, stomach and small intestine Baseline flows for all tissues, except the stomach, were not significantly different between groups (Table 2). Resting flow through the stomach was significantly higher in the control group than in the experimental group (Table 2). In all tissues measured, except for the adrenal glands, there were no significant changes in flow during infusion of saline. The adrenal glands were combined for flow measurement because of their small size, and, like the heart, showed a significant increase in flow in the control group after the first flow measurement, with no further change after that (Table 2). Infusion of ET-1 caused significant reductions in flow through the kidneys and adrenal glands, and infusion of MgSO_4 had no significant effect on these reductions in flow. In the spleen, infusion of ET-1 and MgSO_4 had no significant effects. Infusion of ET-1 caused a significant rise in blood flow to the stomach, which returned towards baseline levels during infusion of MgSO_4 (Table 2).

Effects on flow in the hindquarters region (testicles, hindlimb skin, hindlimb muscle) In all tissues measured, baseline flows were not different between groups (Table 2) and flow was unaffected by infusion of saline. Infusion of ET-1 caused a significant reduction in flow to the testicles, with no significant change in flow taking place during infusion of MgSO_4 . In the hindquarters skin, infusion of ET-1 and MgSO_4 caused little change in blood flow. In hindquarters muscle, infusion of ET-1 did not cause a significant reduction in blood flow; however, infusion of MgSO_4 caused a significant increase in flow (Table 2).

Discussion

The results from this study, using the coloured microsphere technique to measure flow, produced values for resting flows in all tissues that compared well to measurements made by other workers using either radio-labelled, or coloured, microspheres to measure flow in rats (Malik *et al.*, 1976; Ishise *et al.*, 1980; Wicker & Tarazi, 1982; Hakkinen *et al.*, 1995). Our observations substantiate the finding of Hakkinen *et al.* (1995) that coloured microspheres are a suitable alternative to radio-labelled spheres for measuring blood flow in rats.

In our previous study (Kemp *et al.*, 1993) we found that ET-1 caused a fall in common and internal carotid blood flow,

Table 1 Heart rate and mean arterial pressure measured just before, and immediately after, each determination of flow

	Control group			Experimental group		
	Control (beats min ⁻¹)	Saline (beats min ⁻¹)	Saline (beats min ⁻¹)	Control (beats min ⁻¹)	ET-1 (beats min ⁻¹)	ET-1 + Mg^{2+} (beats min ⁻¹)
Heart rate						
Before	385 ± 8	403 ± 14	398 ± 10	368 ± 10	298 ± 25†	260 ± 12†
After	389 ± 9	396 ± 7	373 ± 14	365 ± 14	264 ± 18*	265 ± 12
Mean arterial pressure	Control (mmHg)	Saline (mmHg)	Saline (mmHg)	Control (mmHg)	ET-1 (mmHg)	ET-1 + Mg^{2+} (mmHg)
Before	106 ± 3	111 ± 3†	114 ± 3†	108 ± 4	146 ± 4†	111 ± 3‡
After	110 ± 3	114 ± 2	116 ± 2	112 ± 4	140 ± 4	111 ± 3

Values are means ± s.e.mean; *denotes a significant change in the post-measurement value compared to the pre-measurement value; †denotes significantly different from the first measurement (control); ‡denotes significantly different from the second measurement (ET-1).

Table 2 Subregional blood flows ($\text{ml min}^{-1} 100 \text{ g}^{-1}$) in conscious Long Evans rats in control ($n=8$) and experimental groups ($n=8$); Values are means $\pm$ s.e.mean

	Control group			Experimental group		
	Control (ml min^{-1} 100 g^{-1})	Saline (ml min^{-1} 100 g^{-1})	Saline (ml min^{-1} 100 g^{-1})	Control (ml min^{-1} 100 g^{-1})	ET-1 (ml min^{-1} 100 g^{-1})	ET-1 + Mg^{2+} (ml min^{-1} 100 g^{-1})
Head skin	21 $\pm$ 3	20 $\pm$ 2	15 $\pm$ 2	27 $\pm$ 5*	12 $\pm$ 2†	11 $\pm$ 2†
Tongue	95 $\pm$ 25	79 $\pm$ 16	85 $\pm$ 14	60 $\pm$ 8	40 $\pm$ 7	116 $\pm$ 22‡
Eyes	165 $\pm$ 20	169 $\pm$ 14	172 $\pm$ 22	170 $\pm$ 14	140 $\pm$ 10	178 $\pm$ 6
Brain	125 $\pm$ 10	139 $\pm$ 10	132 $\pm$ 16	123 $\pm$ 9	124 $\pm$ 9	170 $\pm$ 13‡
Heart	403 $\pm$ 24	523 $\pm$ 31†	581 $\pm$ 26†	393 $\pm$ 24	265 $\pm$ 17	614 $\pm$ 57‡
Left kidney	505 $\pm$ 37	575 $\pm$ 70	503 $\pm$ 57	464 $\pm$ 44	186 $\pm$ 11†	178 $\pm$ 22†
Right kidney	389 $\pm$ 79	470 $\pm$ 81	370 $\pm$ 86	431 $\pm$ 44	145 $\pm$ 23†	204 $\pm$ 14†
Adrenal glands	565 $\pm$ 46	837 $\pm$ 87†	848 $\pm$ 72†	583 $\pm$ 46	244 $\pm$ 24†	388 $\pm$ 26†
Spleen	241 $\pm$ 16	338 $\pm$ 60	371 $\pm$ 107	355 $\pm$ 27	256 $\pm$ 23	287 $\pm$ 45
Stomach intestine	269 $\pm$ 22	285 $\pm$ 19	261 $\pm$ 17	217 $\pm$ 30	162 $\pm$ 25	166 $\pm$ 24
Stomach	233 $\pm$ 14	193 $\pm$ 15	194 $\pm$ 17	108 $\pm$ 13*	212 $\pm$ 13†	129 $\pm$ 15‡
Testicles	41 $\pm$ 3	47 $\pm$ 4	51 $\pm$ 3	40 $\pm$ 3	29 $\pm$ 2†	25 $\pm$ 2†
Hindquarters skin	19 $\pm$ 3	21 $\pm$ 6	19 $\pm$ 4	18 $\pm$ 4	7 $\pm$ 1	5 $\pm$ 1†
Hindquarters muscle	14 $\pm$ 2	16 $\pm$ 2	14 $\pm$ 2	12 $\pm$ 2	8 $\pm$ 1	17 $\pm$ 4‡

The first flow measurement was made immediately before infusion of saline (control) or ET-1 (experimental). The second flow measurement was made 20 min after onset of saline or ET-1 infusion, and immediately before onset of MgSO_4 infusion. The third flow measurement was made in the last minute of a 7 min infusion of MgSO_4 . Values are means $\pm$ s.e.mean; *denotes a significant difference between the control values for the two groups; †denotes significantly different from the first flow measurement (control); ‡denotes significantly different from the second flow measurement (ET-1).

and infusion of MgSO_4 caused flow to increase in both vessels, in the presence of ET-1. Torregrosa *et al.*, (1994) showed that ET-1 caused a reduction in cerebral blood flow in the conscious goat, but found that MgSO_4 caused an increase in flow, in the presence of ET-1, only when given directly into the cerebroarterial supply, and not when given as an intravenous infusion. In the present study, ET-1 and MgSO_4 had heterogeneous effects on blood flow through the cranial tissues supplied by the internal carotid artery. Infusion of ET-1 reduced flow only in the skin, and MgSO_4 caused increases in flow only in the tongue and brain. Although ET-1 did not cause a reduction in flow to the brain, infusion of MgSO_4 markedly increased cerebral blood flow, in the presence ET-1. This shows that MgSO_4 is able to increase cerebral blood flow, and provides further evidence that MgSO_4 may have the ability to relieve cerebral vasospasm (Perales *et al.*, 1991; Belfort & Moise, 1992; Kemp *et al.*, 1993). Heterogeneous haemodynamic effects of ET-1 and MgSO_4 were not confined to the cranial tissues and possible explanations are considered below.

At first sight our findings of a lack of effect of ET-1 on cerebral blood flow appears at odds with the report from Macrae *et al.* (1993) showing severe reductions in cerebral blood flow in response to ET-1. However, those workers applied ET-1 directly to the adventitial surface of the exposed middle cerebral artery, rather than giving the peptide intravenously. Using the latter route of administration, Takahashi *et al.* (1992) found, as we did, that ET-1 did not affect cerebral blood flow. Since ET-1 caused a significant increase in systemic arterial blood pressure with no change in cerebral blood flow, there would have been a decrease in calculated cerebral vascular conductance. Under these circumstances this cerebral vasoconstriction is likely to have been an autoregulatory response.

In the heart, there was a significant increase in flow after the first injection of microspheres in the control group. In contrast, there was a fall in flow during infusion of ET-1 in the experimental group, although this was not significant. However, infusion of MgSO_4 , in the presence of ET-1, caused a large increase in flow to levels significantly higher than the baseline value. These findings demonstrate that MgSO_4 is

capable of increasing flow to the heart in rats, and supports the view that MgSO_4 may be beneficial in the treatment of acute myocardial infarction because of its ability to improve coronary flow (Woods, 1991).

In our previous study, using Doppler flow probes (Kemp *et al.*, 1994), renal and mesenteric flows were significantly reduced by ET-1. Renal flow was not affected significantly by infusion of MgSO_4 , but the reduction in flow in the mesenteric vascular bed was attenuated by a small, but significant, amount by MgSO_4 . In the present study, the kidneys showed the same pattern of changes as in the previous study. Interestingly, flow through the stomach rose significantly during infusion of ET-1 and returned to control level in the presence of MgSO_4 . Flow in the spleen was unaffected by ET-1 and MgSO_4 . Thus, it appears there might be substantial variation in the changes in inter-organ flow, not apparent from monitoring with Doppler probes. A vasodilator effect of ET-1 in the stomach has also been reported by Allcock *et al.*, (1995) in the anaesthetized, ganglion-blocked rat. In that study the vasodilator effect of ET-1 was shown to be converted to a vasoconstriction in the presence ET_A -, and/or ET_B -receptor antagonists. Allcock *et al.* (1995) suggested these observations were consistent with the ability of ET-1 to stimulate local prostanoid production. If this was the case, our finding that MgSO_4 suppressed the ET-1 induced gastric hyperaemia may indicate an inhibitory influence of MgSO_4 on prostanoid synthesis, although this is not consistent with the ability of MgSO_4 to stimulate prostacyclin production (Laurant *et al.*, 1992).

In our earlier study (Kemp *et al.*, 1994), flow to the hindquarters region was unaffected by infusion of ET-1. However, MgSO_4 alone, and in the presence of ET-1, caused a marked increase in hindquarters flow. In the present study, the testicles showed a significant reduction in blood flow during infusion of ET-1, which was unaffected by MgSO_4 . Although hindquarters muscle blood flow was not altered by infusion of ET-1, infusion of MgSO_4 caused an increase in flow which was significantly different to basal flow and flow in the presence of ET-1 alone. The increase in flow caused by MgSO_4 (42%) was comparable to that seen in the hindquarters region, in the previous study, in the presence of ET-1 (46%).

The striking heterogeneity in the regional and subregional haemodynamic effects of ET-1 and MgSO_4 is likely to be due to the wide range of interacting factors which influence microvascular behaviour. In addition to those considered above, we (Kemp *et al.*, 1994) have suggested there might be contributions from baroreflex-mediated sympathoadrenal and neurohumoral activation. As pointed out by Malmström *et al.* (1997) a further factor to be considered is the variation in endothelial permeability between different vascular beds, since

this will influence tissue access of exogenous substances administered intravenously.

In conclusion this, and our previous studies, have shown that MgSO_4 can reverse vasoconstriction in a number of vascular beds, and indicate that this compound may have therapeutic benefit in conditions associated with vasospasm.

This work was supported by a grant (BHF 91/10) from the British Heart foundation.

References

- ALLCOCK, G.H., WARNER, T.D. & VANE, J.R. (1995). Roles of endothelin receptors in the regional and systemic vascular responses to ET-1 in the anaesthetized ganglion-blocked rat: use of selective antagonists. *Br. J. Pharmacol.*, **116**, 2482–2486.
- ALTURA, B.M. & ALTURA, B.T. (1981). Magnesium ions and contraction of vascular smooth muscle. *Fed. Proc.*, **40**, 2672–2679.
- ALTURA, B.M. & TURLAPATY, P.D.M.V. (1982). Withdrawal of magnesium enhances coronary arterial spasms produced by vasoactive agents. *Br. J. Pharmacol.*, **77**, 649–659.
- ALTURA, B.T. & ALTURA, B.M. (1980). Withdrawal of magnesium causes vasospasm while elevated magnesium produces relaxation of tone in cerebral arteries. *Neurosci. Lett.*, **20**, 323–327.
- BELFORT, M.A. & MOISE JR K.J. (1992). Effect of magnesium sulphate on maternal brain blood flow in preeclampsia: A randomized, placebo-controlled study. *Am. J. Obstet. Gynecol.*, **167**, 661–666.
- BROWN, Z.W., AMIT, Z. & WEEKS, J.R. (1976). Simple flow-thru swivel for infusions into unrestrained animals. *Pharmacol. Biochem. Behav.*, **5**, 363–365.
- CHI, O.Z., POLLACK, P. & WEISS, H.R. (1990). Effects of magnesium sulphate and nifedipine on regional cerebral blood flow during middle cerebral artery ligation in the rat. *Arch. Int. Pharmacodyn. Ther.*, **304**, 196–205.
- DIPETTE, D.J., SIMPSON, K. & GUNTUPALLI, J. (1987). Systemic and regional hemodynamic effect of acute magnesium administration in the normotensive and hypertensive state. *Magnesium*, **6**, 136–149.
- ECLAMPSIA TRIAL COLLABORATIVE GROUP. (1995). Which anticonvulsant for women with eclampsia? Evidence from the collaborative eclampsia trial. *Lancet*, **345**, 1455–1463.
- HAKKINEN, J.P., MILLER, M.W., SMITH, A.H. & KNIGHT, D. (1995). Measurement of organ blood flow with coloured microspheres in the rat. *Cardiovasc. Res.*, **29**, 74–79.
- ISHISHE, S., PEGRAM, B.L., YAMAMOTO, J., KITAMURA, Y. & FROHLICH, E.D. (1980). Reference sample microsphere method: cardiac output and blood flows in conscious rat. *Am. J. Physiol.*, **233**, H443–H449.
- KEMP, P.A., GARDINER, S.M., BENNETT, T. & RUBIN, P.C. (1993). Magnesium sulphate reverses carotid vasoconstriction caused by endothelin-1, angiotensin II and neuropeptide-Y, but not that caused by N^G -nitro-L-arginine methyl ester, in conscious rats. *Clin. Sci.*, **85**, 175–181.
- KEMP, P.A., GARDINER, S.M., MARCH, J.E., BENNETT, T. & RUBIN, P.C. (1994). Effects of N^G -nitro-L-arginine methyl ester on regional haemodynamic responses to MgSO_4 in conscious rats. *Br. J. Pharmacol.*, **111**, 325–331.
- KIMURA, T., YASUE, H., SAKAINO, N., ROKUTANDA, M., JOUGASAKI, M. & ARAKI, H. (1989). Effects of magnesium on the tone of isolated human coronary arteries. *Circulation*, **79**, 1118–1124.
- KOWALLIK, P., SCHULZ, R., GUTH, B.D. & SCHADE, A. (1991). Measurement of regional myocardial blood flow with coloured microspheres. *Circulation*, **83**, 974–982.
- KUGIYAMA, K., YASUE, H., OKUMURA, K., GOTO, K., MINODA, K., MIYAGI, H., MATSUYAMA, K., KOJIMA, A., KOGA, Y. & TAKAHASHI, M. (1988). Suppression of exercise-induced angina by magnesium sulphate in patients with variant angina. *J. Am. Coll. Cardiol.*, **12**, 1177–1183.
- LAURANT, P., MOUSSARD, C., ALBER, D., HENRY, J.C. & BERTHELOT, A. (1992). *In vivo* and *in vitro* magnesium effects on aortic prostacyclin generation in DOCA-salt hypertensive rats. *Prostaglandins Leukot. Essent. Fatty Acids*, **47**, 183–186.
- LUCAS, M.J., LEVENO, K.J. & CUNNINGHAM, F.G. (1995). A comparison of magnesium sulphate with phenytoin for the prevention of eclampsia. *New Engl. J. Med.*, **333**, 201–205.
- MACRAE, I.M., ROBINSON, M.J., GRAHAM, D.I., REID, J.L. & McCULLOCH, J. (1993). Endothelin-1-induced reductions in cerebral blood flow: Dose dependency, time course, and neuropathological consequences. *J. Cereb. Blood Flow Metab.*, **13**, 276–284.
- MALIK, A.B., KAPLAN, J.E. & SABA, T.M. (1976). Reference sample method for cardiac output and regional blood flow determination in the rat. *J. Appl. Physiol.*, **40**, 472–475.
- MALMSTRÖM, R.E., BALMER, K.C. & LUNDBERG, J.M. (1997). The neuropeptide Y (NPY) Y_1 receptor antagonist BIBP 3226: equal effects on vascular responses to exogenous and endogenous NPY in the pig *in vivo*. *Br. J. Pharmacol.*, **121**, 595–603.
- MARICHAL, A., HESS, W. & SCHIEBER, G. (1992). Haemodynamics in patients undergoing coronary artery bypass grafting after magnesium aspartate infusion. *Anaesthetist*, **41**, 752–759.
- NISHIO, A., GREBREWOLD, A., ALTURA, B.T. & ALTURA, B.M. (1989). Comparative vasodilator effects of magnesium salts on rat mesenteric arterioles and venules. *Arch. Int. Pharmacodyn. Ther.*, **298**, 139–163.
- NOGUERA, M.A. & D'OCÓN, M.P. (1993). Modulatory role of magnesium on the contractile response of rat aorta to several agonists in normal and calcium-free medium. *J. Pharm. Pharmacol.*, **45**, 697–700.
- PERALES, A.J., TORREGROSA, G., SALOM, J.B., MIRANDA, F.J., ALABADI, J.A., MONLEON, J. & ALBORCH, E. (1991). *In vivo* and *in vitro* effects of magnesium sulphate in the cerebrovascular bed of the goat. *Am. J. Obstet. Gynecol.*, **165**, 1534–1538.
- RAM, Z., SADEH, M., SHACKED, I., SAHAR, A. & HADANI, M. (1991). Magnesium sulphate reverses experimental delayed cerebral vasospasm after subarachnoid hemorrhage in rats. *Stroke*, **22**, 922–927.
- SEELIG, J.M., WEI, E.P., KONTOS, H.A., CHOI, S.C. & BECKER, D.P. (1983). Effect of changes in magnesium ion concentration on cat cerebral arterioles. *Am. J. Physiol.*, **245**, H22–H26.
- SIPES, S.L., CHESTNUT, D.H., VINCENT JR R.D., WEINER, C.P., THOMPSON, C.S. & CHATTERJEE, M.S. (1991). Does magnesium sulphate alter the cardiovascular response to vasopressor agents in gravid ewes? *Anesthesiology*, **75**, 1010–1018.
- SKAJA, K., FORMAN, A. & ANDERSSON, K.-E. (1990). Effects of magnesium on isolated human foetal and maternal uteroplacental vessels. *Acta Physiol. Scand.*, **139**, 551–559.
- SZABO, C., FARAGO, M., DORA, E., HORVATH, I. & KOVACH, A.G.B. (1991). Endothelium-dependent influence of small changes in extracellular magnesium concentration on tone of feline middle cerebral arteries. *Stroke*, **22**, 785–789.
- SZABO, C., HARDEBO, J. & SALFORD, L.G. (1992). Role of endothelium in the responses of human intracranial arteries to a slight reduction of extracellular magnesium. *Exp. Physiol.*, **77**, 209–211.
- TAKAHASHI, H., NAKANISHI, T., NISHIMURA, M., TANAKA, H. & YOSHIMURA, M. (1992). Effects of endothelin-1 and inhibition of nitric oxide production with N^G -monomethyl-L-arginine on arterial pressure and regional blood flow in anaesthetized rats. *J. Cardiovasc. Pharmacol.*, **20**, S176–S178.
- THEODORSSON-NORHEIM, E. (1987). Friedman and Quade tests: BASIC computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.*, **17**, 85–99.

- TORREGROSA, G., PERALES, A.J., SALOM, J.B., MIRANDA, F.J., BARBERA, M.D. & ALBORCH, E. (1994). Different effects of Mg^{2+} on endothelin-1 and 5-hydroxytryptamine-elicited responses in goat cerebrovascular bed. *J. Cardiovasc. Pharmacol.*, **23**, 1004–1010.
- WELLERS, D., WOUTERS, L., NIJKAMP, F.P. & WYBREN DE JONG. (1976). Distribution of blood flow supplied by the vertebral artery in rats: Anatomical, functional and pharmacological aspects. *Experientia*, **32**, 85–87.
- WICKER, P. & TARAZI, R.C. (1982). Coronary blood flow measurements with left atrial injection of microspheres in conscious rats. *Cardiovasc. Res.*, **16**, 580–586.
- WOODS, K.L. (1991). Possible pharmacological actions of magnesium in acute myocardial infarction. *Br. J. Clin. Pharmacol.*, **32**, 3–10.
- WOODS, K.L., FLETCHER, F., ROFFE, C. & HAIDER, Y. (1992). Intravenous magnesium sulphate in suspected acute myocardial infarction: results of the second Leicester intravenous magnesium intervention trial (LIMIT-2). *Lancet*, **339**, 1553–1558.

(Received July 29, 1998

Revised October 21, 1998

Accepted November 6, 1998)