Br. J. Pharmacol. (1994), **113**, 228–232

Nitric oxide as a mediator of the laxative action of magnesium sulphate

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1 Magnesium sulphate was studied for its effects on diarrhoea, fluid secretion, gastrointestinal transit and nitric oxide (NO) synthase activity in rats.

2 At a dose of 2 g kg^{-1} orally magnesium sulphate produced diarrhoea that was delayed in onset and intensity in a dose-related manner by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). This was prevented by the NO precursor, L-arginine and the NO donating compound, isosorbide-5-mononitrate (IMN).

3 Nitric oxide synthase activity was stimulated in gut tissue from rats given magnesium sulphate and this was inhibited by L-NAME. Dexamethasone (1 mg kg^{-1}, i.p.), an inhibitor of inducible NO synthase, had no effect on magnesium sulphate-induced diarrhoea.

4 Magnesium sulphate stimulated fluid and electrolyte accumulation in the intestinal lumen; these effects were prevented by L-NAME but not D-NAME.

5 Gastrointestinal transit of a non-absorbable marker (charcoal suspension) was increased by oral magnesium sulphate from a mean value of 54.1% to 72.9% ($P<0.01$), and this was prevented by pretreatment with L-NAME.

6 The results demonstrate that oral magnesium sulphate produces diarrhoea in rats by increasing the accumulation of fluid in the intestinal lumen and enhancing flow from the proximal to distal intestine. The mechanism involves release of NO, probably through stimulation of the constitutive form of NO synthase. Whether or not the effects of magnesium sulphate are due to an osmotic action or an intrinsic effect of the magnesium or sulphate ions cannot be determined from these experiments.

Keywords: Magnesium sulphate; laxatives; nitric oxide; intestinal secretion; gastrointestinal transit

Introduction

Pharmacological studies on the laxative action of magnesium sulphate are reported (Wood, 1908) to have been done over 100 years ago. The early studies attributed the action of this saline laxative to the osmotic activity of the constituent ions in the intestinal lumen. However, measurement of water absorption from *in situ* intestinal loops prepared in cats was impaired by an isotonic solution of magnesium sulphate (Lium & Florey, 1939), suggesting that the magnesium or sulphate ions have an action on the bowel that is independent of an osmotic effect. Harvey & Read (1973) presented arguments to support the idea that the laxative effect of magnesium sulphate is not due simply to osmotic effects, but involves the release of cholecystokinin (CCK).

Whilst the proposal that the laxative effect of magnesium sulphate could be due to the release of a hormone such as CCK is not universally accepted, perfusion of magnesium ions as part of an isosmotic solution into the duodenum of man evoked a moderate stimulatory effect on CCK release (Malagelada *et al.*, 1978). CCK is an intestinal secretagogue in rats (Hubel, 1972), guinea-pigs (Kachur *et al.*, 1991), dogs (Bussjaeger & Johnson, 1973) and man (Moritz *et al.*, 1973). In the human jejunum, isotonic magnesium sulphate induced net fluid secretion and increased transit through the small intestine (Wanitschke & Ammon, 1976). In an *in vivo* animal model, others confirmed that an isotonic magnesium sulphate solution produces secretion, possibly through changes in intracellular calcium (Reichelderfer *et al.*, 1979).

Because the laxative action of magnesium sulphate (whether isotonic or hypertonic) might be due to the release of other mediators, we considered the involvement of nitric oxide (NO). NO is an apparent mediator of the action of other laxatives (Mascolo *et al.*, 1993; 1994; Gaginella, personal communication). NO is an intestinal secretagogue (Tamai & Gaginella, 1994) and it relaxes intestinal smooth muscle (Boeckxstaens *et al.*, 1993; Grider, 1993; Stark *et al.*, 1993). This muscle relaxant effect is likely to enhance intestinal transit and contribute to a laxative/diarrhoeal effect (Gullikson & Bass, 1984).

Methods

Animals

Male Wistar (Morini) rats (150–170 g) were used after a week of acclimatization to their housing conditions (temperature 23 ± 2°C; humidity 60%). Food was withheld 18 h before experiments but there was free access to drinking water. Each rat was placed in a separate cage at the beginning of the experiment.

Laxative (diarrhoeal) test

Rats were injected intraperitoneally with N^G-nitro-L-arginine methyl ester (L-NAME, 2.5–25 mg kg^{-1}), or D-NAME (25 mg kg^{-1}) 15 min before and 3 h after oral dosing with magnesium sulphate or mannitol. L-Arginine (600 and 1500 mg kg^{-1}, i.p.) was given 15 min before, the NO donor isosorbide-5-mononitrate (IMN, 30–120 mg kg^{-1}, orally) 30 min before plus 3 h after, and the glucocorticoid, dexamethasone (1 mg kg^{-1}, i.p.) 2 h before the laxatives were given.

One hour after dosing with the laxatives and each hour for 8 h, the individual rat cages were inspected (by an observer unaware of the particular treatment) for the presence of

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unformed water faecal pellets; their absence was recorded as a positive result, indicating protection from diarrhoea at that time.

Water and electrolyte secretion

Four and one-half hours after dosing with magnesium sulphate or mannitol, the rats were anaesthetized with urethane (1.3 g kg^{-1}, i.p.). The colon was rinsed carefully with sterile 0.9% (w/v) NaCl solution (37°C) to remove the bowel contents. After 30 min, the colon was ligated after filling with 2.5 ml sterile Tyrode solution consisting of (in g l^{-1}): NaCl 8.00, KCl 0.20, $NaH_2PO_4 \cdot 7H_2O$ 0.05, $MgSO_4 \cdot 7H_2O$ 0.05, D-glucose $\cdot H_2O$ 1.0, $NaHCO_3$ 1.00 and $CaCl_2 \cdot H_2O$ 0.26. One hour later, the animals were killed and the colon was quickly removed. Net water transport was calculated from the volume of the fluid content of the colon minus the 2.5 ml of the solution used to fill the colon.

Electrolyte content was analysed in the supernatant (after centrifugation) by high performance liquid chromatography (h.p.l.c.) utilizing a conductivity detector (Poole & Shuette, 1984). Net transport was calculated by difference (Van Hoestenberghe *et al.*, 1992). L-NAME (25 mg kg^{-1}, i.p.) or D-NAME (at the same dose, i.p.) was given 15 min before and 3 h after laxative challenge.

Gastrointestinal transit

Magnesium sulphate or mannitol, at the same doses used in the diarrhoea and fluid transport protocols, was administered orally 2 h before the oral administration of 1 ml of a transit marker (10% charcoal suspension in 5% gum arabic). After 30 min, the rats were killed and the gastrointestinal tract was removed. The distance travelled by the marker was measured and expressed as a percentage of the total length of the intestine from the pylorus to caecum. A dose of 25 mg kg^{-1} of L-NAME or D-NAME was administered i.p. 30 min before oral administration of laxatives. Control rats received water.

NO synthase assay

The activity of NO synthase in colonic tissue from control animals and those treated with magnesium sulphate and mannitol, some of which were treated with L-NAME or dexamethasone, was assessed. Five hours after giving the laxatives the animals were anaesthetized and killed. Full thickness segments of the colon (0.5 g) were homogenized for 20 s on ice in 2.5 ml of a buffer containing sucrose (0.32 M), dithiothreitol (1 mM), soybean trypsin inhibitor (10 $\mu g\ ml^{-1}$). The homogenates were centrifuged at 10,000 *g* for 5 min (5°C) and the supernatant processed for colorimetric determination of citrulline as described by Boyde & Rahmatullah (1980). In brief, 0.1 ml of supernatant was added to 3 ml of chromogenic solution, vortexed for 30 s and boiled at 100°C for 5 min. Colorimetric readings were made at room temperature, measuring the absorbance at 530 nm. Citrulline standard was determined simultaneously with the samples. The NO synthase activity was expressed as nmol g^{-1} tissue.

Chemicals

L-NAME hydrochloride, magnesium sulphate, mannitol, citrulline, dithiothreitol, soybean trypsin inhibitor and dexamethasone were purchased from Sigma Chemical Co. (Milan, Italy); Isosorbide-5-mononitrate, and D-NAME came from Astra and RBI respectively (Milan, Italy). These compounds were dissolved in saline before being used except for IMN, which was suspended in 1% carboxymethylcellulose. All chemicals used for the Tyrode and other solutions were of the highest purity available, from commercial sources.

Statistics

The Chi-Squared test was used to determine the significance between groups with or without diarrhoea. Intestinal fluid volume, electrolyte secretion and small intestinal transit were expressed as mean ± s.e. and compared by One-way Analysis of Variance (ANOVA) followed by Duncan's New Multiple-Range Test and Student's *t* test respectively. A *P* value less than 0.05 was considered significant.

Results

Diarrhoea

Diarrhoea occurred in the magnesium sulphate-treated group of rats from 3–8 h and from 2–8 h in the mannitol-treated group; dexamethasone (an inhibitor of inducible nitric oxide synthase) had no effect on either of these laxatives (Table 1). Four hours after administration of the laxatives and for the next 4 h, diarrhoea was evident in all the animals. L-NAME dose-dependently delayed the onset of diarrhoea to both drugs and reduced the total number of animals with diarrhoea over the time frame studied (Figure 1). The 25 mg kg^{-1} dose significantly ($P<0.05$) reduced the incidence of diarrhoea from 4–8 h after magnesium sulphate. L-Arginine (1500 mg kg^{-1}) reversed the effect of 25 mg kg^{-1} L-NAME

Table 1 Effect of dexamethasone (1 mg kg^{-1}, i.p., 2 h before laxative challenge) on the diarrhoea induced by magnesium sulphate (2 g kg^{-1}) and mannitol (10 g kg^{-1})

Laxative (oral)	*Number of rats (12) with diarrhoea at different times*							
	1	2	3	4	5	6	7	8 (h)
Magnesium sulphate	0	0	2	8	12	12	12	12
+ Dexamethasone	0	0	1	9	12	12	12	12
Mannitol	0	2	6	10	12	12	12	12
+ Dexamethasone	0	3	5	9	11	12	12	12

Results were analysed by the Chi-squared test. There were no significant effects of dexamethasone on any of the laxatives.

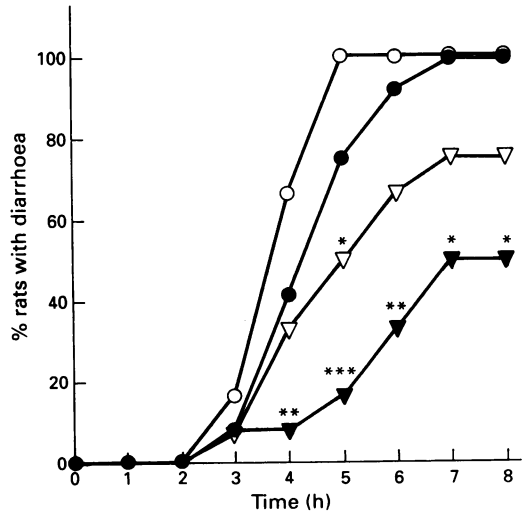

Figure 1 Inhibitory effect of N^G-nitro-L-arginine methyl ester (L-NAME) (2.5–25 mg kg^{-1}) on the percentage of rats (out of 12) with diarrhoea at various times after oral magnesium sulphate (2 mg kg^{-1}, ○). The L-NAME was given (i.p.) 15 min before and 3 h after the laxative: L-NAME, 2.5 mg kg^{-1} (●); 10 mg kg^{-1} (▽) and 25 mg kg^{-1} (▼). Asterisks indicate significance compared to control (laxative only) at $*P<0.05$, $**P<0.01$ and $***P<0.001$ by the Chi squared test.

on the magnesium sulphate-induced diarrhoea (Figure 2). L-Arginine (1500 mg kg^{-1}) itself did not modify the diarrhoeal effect nor did D-NAME (25 mg kg^{-1}), inhibit the response (data not shown). The NO donating compound IMN prevented the inhibitory effect of L-NAME in rats with magnesium sulphate-induced diarrhoea (Table 2).

Secretion

Magnesium sulphate produced fluid secretion into the intestinal loops. This was reversed by L-NAME but unaffected by D-NAME (Table 3); L-NAME and D-NAME had no effect on the mannitol response. Likewise, L-NAME inhibited the magnesium sulphate but not the mannitol-induced electrolyte secretion (Table 4).

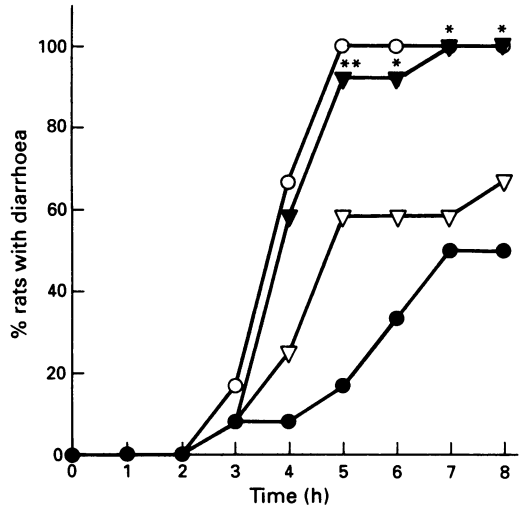

Figure 2 Reversal by L-arginine (600, ▽, and 1500 mg kg^{-1}, ▼, i.p.) of the inhibitory effect of N^{G}-nitro-L-arginine methyl ester (L-NAME, 25 mg kg^{-1}, i.p., ●) on diarrhoea in animals ($n = 12$) dosed orally with magnesium sulphate (2 mg kg^{-1}, ○). The L-NAME alone was given 15 min before and 3 h after the laxative, while L-arginine was given (i.p.) only 15 min before the laxative. *$P<0.05$ and **$P<0.01$ compared to control (laxative only) by the Chi Squared test.

Table 2 Reversal of the antidiarrhoeal effect of N^{G}-nitro-L-arginine methyl ester (L-NAME) on magnesium sulphate (2 g kg^{-1}, p.o.) by isosorbide-5-mononitrate (IMN)

Treatment	*Number of rats with diarrhoea* ($n = 12$)	*% reversion*
Magnesium sulphate	12	–
+ L-NAME	2	–
+ L-NAME + IMN	5	30
+ L-NAME + IMN	9	70
+ L-NAME + IMN	10*	80

Results were analysed by the Chi squared test.
*$P<0.05$ vs corresponding laxative + L-NAME.
Magnesium sulphate was given orally. L-NAME and IMN were given twice i.p. 15 min before and 3 h after laxative challenge. Diarrhoea was assessed at 4 h.

NO synthase activity

Magnesium sulphate significantly stimulated nitric oxide synthase activity and this was inhibited by L-NAME (25 mg kg^{-1}) (Figure 3). Mannitol was not tested for this effect because neither fluid nor electrolyte secretion were affected by L-NAME.

Gastrointestinal transit

The gastrointestinal transit of charcoal was increased from 54.1 ± 2.1% ($n = 12$) of the total length of intestine (control group) to 72.9 ± 2.0% (magnesium sulphate group) ($P<0.01$). L-NAME (25 mg kg^{-1} twice) had no effect (48.2 ± 2.2%) on the control response and prevented ($P<0.05$) the magnesium sulphate effect (58.2 ± 2.3% transit compared to to the 72.9% for the laxative).

Discussion

Our results support previous suggestions (Harvey & Read, 1973; Stewart *et al.*, 1975; Wanitschke & Ammon, 1976) that magnesium sulphate produces a laxative effect through a mechanism that is not solely due to an osmotic gradient. We used mannitol as an osmotic control for the magnesium sulphate. Assuming an animal weight of 150 g and dilution in 10 ml of gastrointestinal fluid, magnesium sulphate and mannitol at the doses used here would yield intraluminal concentrations of approximately 570 and 825 milliosmolar, respectively. Even though mannitol represented nearly 1.5 times more osmotic equivalents than magnesium sulphate and both agents produced diarrhoea, only the effects of magnesium sulphate were influenced by modulating the generation of NO. These findings suggest that NO probably serves as an intermediate in the laxative action of magnesium sulphate. This does not exclude the possibility that other, perhaps osmotic factors, also contribute to its mechanism of action.

Dexamethasone, a glucocorticoid that inhibits the inducible form of NO synthase (see Moncada *et al.*, 1991), failed to inhibit diarrhoea after dosing with magnesium sulphate or mannitol, but L-NAME (a competitive inhibitor of constitutive and inducible NO synthases) dose-dependently antagonized the diarrhoeal effect of magnesium sulphate. Assay of NO synthase confirmed that magnesium sulphate stimulated NO synthase activity in the intestine and that L-NAME can block this effect. The NO synthesis precursor, L-arginine, and the NO donating compound, IMN, both antagonized the diarrhoeal effect of magnesium sulphate, providing additional support for the involvement of NO. Taken together, the data indicate that the NO release arises from magnesium and/or sulphate activation of the constitutive, not the inducible form of NO synthase.

Table 3 Effect of N^{G}-nitro-L-arginine methyl ester (L-NAME, 25 mg kg^{-1}, i.p.) and D-NAME (25 mg kg^{-1}, i.p.) on magnesium sulphate and mannitol-induced water flux in the ligated rat colon

Treatment	*Net fluid accumulation* (ml) *Saline*	*L-NAME*	*D-NAME*
Control[a]	– 1.08 ± 0.08	– 1.00 ± 0.07	– 1.10 ± 0.07
Magnesium sulphate	0.17 ± 0.10[b]	– 0.75 ± 0.04[c]	0.14 ± 0.07
Mannitol	0.16 ± 0.07	0.17 ± 0.10	0.14 ± 0.09

[a]Results are expressed as mean ± s.e. for 6–8 experiments. The colon was rinsed with 2 ml saline solution. A negative value represents net absorption and a positive value net secretion.
[b]$P<0.01$ vs control.
[c]$P<0.001$ vs corresponding laxative + saline group.

Table 4 Effect of N^G-nitro-L-arginine methyl ester (L-NAME, 25 mg kg^{-1}, i.p.) on electrolyte movements in response to laxatives in the rat ligated colon

Treatment	*Net electrolyte transport* (μEq) *Sodium*	*Chloride*	*Potassium*
Control[a]	− 202.4 ± 14.6	− 210.0 ± 11.4	7.4 ± 1.7
Magnesium sulphate	17.5 ± 12.2[b]	12.3 ± 10.5[b]	19.2 ± 1.9[b]
+ L-NAME	− 170.2 ± 16.5[c]	− 179.1 ± 11.3[c]	10.1 ± 2.9[c]
Mannitol	− 10.2 ± 10.5[b]	− 20.1 ± 14.2[b]	12.2 ± 10.5[b]
+ L-NAME	− 5.2 ± 12.3	− 12.3 ± 16.5	9.3 ± 2.2

[a]Results are expressed as mean ± s.e. for 6–8 experiments. A negative value represents net absorption and a positive value net secretion. L-NAME was administered 15 min before and 3 h after laxatives challenge.
[b]$P<0.001$ vs control.
[c]$P<0.01$ vs magnesium sulphate.

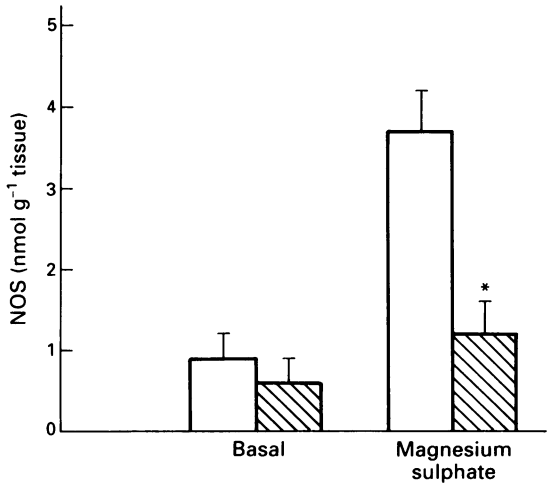

Figure 3 Effect of magnesium sulphate on nitric oxide synthase (NOS) activity under control conditions (open column) and after pretreatment (i.p.) with N^G-nitro-L-arginine methyl ester (L-NAME) (25 mg kg^{-1}, hatched column). Columns are means with s.e. (n = 6–8). *$P<0.01$ compared to control by Duncan's test.

The laxative response to magnesium sulphate results from effects on gut smooth muscle (enhancement of transit) and mucosal electrolyte transport (Gullikson & Bass, 1984; Stewart *et al.*, 1975). The inhibition of electrolyte absorption or stimulation of secretion causes the accumulation of fluid in the gut lumen, which is what we observed in the present experiments. Consistent with the results of the experiments on diarrhoea, L-NAME reversed the effect of magnesium sulphate but not mannitol on electrolyte transport and luminal fluid accumulation. Furthermore, the effect was enantiomer-specific because D-NAME was inactive.

We also found that magnesium sulphate increased the transit of a non-absorbable marker through the gut. L-NAME attenuated the enhanced transit, implicating NO in this effect. Relaxation of colonic circular smooth muscle reduces resistance to flow and promotes movement of material through the intestine (Gullikson & Bass, 1984). Such an effect would explain the reversal of the constipation due to morphine by L-arginine in the mouse (Calignano *et al.*, 1991); a reduction in the force of contraction of ileal and colonic circular muscle in dogs has been reported (Stewart *et al.*, 1975). NO also relaxes small intestinal and colonic circular smooth muscle (Boeckxstaens *et al.*, 1993; Stark *et al.*, 1993) and also seems to be involved in relaxation of the gut during the peristaltic reflex (Grider, 1993). In the latter case, vasoactive intestinal peptide (VIP) is postulated to interact with NO, whereby VIP stimulates the influx of calcium into muscle cells and activates constitutive NO synthase, liberating NO as the agonist of relaxation (Murthy *et al.*, 1993). We have no evidence for the involvement of VIP in the responses to magnesium sulphate in the present study but we cannot rule out this possibility, as VIP is not only a smooth muscle relaxant but also a potent intestinal secretatogue and diarrhoeagenic peptide (Gaginella *et al.*, 1982).

This study was supported by CNR (Rome) and Murst 60%.

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(Received March 9, 1994
Revised May 12, 1994
Accepted May 23, 1994)