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# Inhibitors of nitric oxide synthetase prevent castor-oil-induced diarrhoea in the rat

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**1** Castor oil (2 ml orally) produced copious diarrhoea in rats 3 h after its administration.

**2** Pretreatment (intraperitoneal, i.p.) of rats with the NO synthesis inhibitors $N^G$-nitro-L-arginine methyl ester (L-NAME, 1–25 mg $kg^{-1}$) and $N^G$-monomethyl-L-arginine (L-NMMA, 2.5–100 mg $kg^{-1}$) inhibited or prevented castor-oil-induced diarrhoea. L-Arginine (150–600 mg $kg^{-1}$, i.p.) administered to rats pretreated with L-NAME 10 mg $kg^{-1}$, drastically reduced the antidiarrhoeal activity of L-NAME in a dose-related manner. D-Arginine (900 mg $kg^{-1}$) did not modify the protection by L-NAME.

**3** Pretreatment (i.p.) of rats with L-NAME (2.5–25 mg $kg^{-1}$) decreased the intestinal fluid accumulation and $Na^+$ secretion induced by castor oil. L-Arginine (600 mg $kg^{-1}$) but not D-arginine (900 mg $kg^{-1}$) counteracted the inhibitory effect of L-NAME (10 mg $kg^{-1}$).

**4** L-NAME (10 and 25 mg $kg^{-1}$) had no significant effect on the intestinal transit in normal rats or those given castor oil.

**5** These results provide evidence that nitric oxide (NO) could play an important role in castor-oil-induced diarrhoea.

**Keywords:** Castor oil; diarrhoea; NO; $N^G$-nitro-L-arginine methyl ester (L-NAME); $N^G$-monomethyl-L-arginine (L-NMMA); L-arginine

## Introduction

Castor oil is a very effective laxative and, like bile salts and other laxatives, decreases absorption and increases secretion in the small intestine by multiple undetected mechanisms (for ref. see Donowitz & Welsh, 1987).

Castor oil and its active principle, ricinoleic acid, reduce active $Na^+$ and $K^+$ absorption and decrease $Na^+$, $K^+$-ATPase in the small intestine and colon (Gaginella & Phillips, 1975) but have not been clearly shown to increase adenosine 3':5'-cyclic monophosphate (cyclic AMP) content in the small intestine and even in the colon this effect is disputed (Simon *et al.*, 1978; Racusen & Binder, 1979). As with other laxatives, castor oil changes the intestinal permeability and the histology but it has not been established whether this contributes to the laxative effect (Gaginella & Phillips, 1976). Castor oil also increases platelet activating factor and some other autacoids throughout the rat intestinal tract (Capasso *et al.*, 1986; Pinto *et al.*, 1989; 1992) particularly in the small intestine. However, it remains to be determined rigorously whether this contributes to the laxative action. Castor oil also alters intestinal contractility (Gaginella & Bass, 1978) but the mechanism of this action, the type of response and its involvement in diarrhoea is still debated. Nitric oxide (NO), generated from L-arginine, but not from D-arginine, may modify gastrointestinal motility when released from non-adrenergic, non-cholinergic (NANC) neurones (Boeckxtaens *et al.*, 1990; Gustafsson *et al.*, 1990; Desai *et al.*, 1991).

In order to investigate whether NO plays a role in the diarrhoea caused by castor oil we have studied the effects of $N^G$-nitro-L-arginine methyl ester (L-NAME) and $N^G$-monomethyl-L-arginine (L-NMMA), which inhibit the NO synthetase enzyme (Rees *et al.*, 1990; Gardiner *et al.*, 1990), and L-arginine, a natural substrate of NO synthetase (Moncada *et al.*, 1991).

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## Methods

### *Animals*

Male Wistar (Morini) rats, weighing 150–170 g, were used after acclimatization for a week in their housing conditions (23 ± 2°C; 60% humidity). Standard food (Morini) was withheld 14 h before experiments but there was free access to drinking water.

### *Castor oil diarrhoea*

Rats were treated intraperitoneally with L-NAME 1–25 mg $kg^{-1}$, L-NMMA 2.5–100 mg $kg^{-1}$, L-arginine 150–600 mg $kg^{-1}$, D-arginine 600 and 900 mg $kg^{-1}$; 15 min later 2 ml castor oil were administered by gavage. Control rats received the same volume (2 ml) of olive oil orally. Rats were scored (blind) for copious (++), mild (+) or lack (0) of diarrhoea 3 h after oil challenge. The activity score was calculated by taking the sum of the number of '+' rats and twice the number of '++' rats. Thus, for groups of 12 rats, the maximum score indicating severe diarrhoea was 24 and 0 was no diarrhoea.

### *Intestinal fluid volume and electrolyte secretion*

Intraluminal fluid accumulation was determined by enteropooling (Robert *et al.*, 1976). L-NAME (2.5–25 mg $kg^{-1}$, i.p.) was injected 30 min before giving castor oil orally. Control rats received olive oil orally. After a further 30 min the animals were killed, the small intestine removed, its content collect in a test tube and its volume measured. Samples of the fluid were analysed for $Na^+$ concentrations by an electrochemical method after high performance liquid chromatography (h.p.l.c.) (Poole & Schuette, 1984).

### *Small intestinal transit*

The method of Vischer & Casals-Stenzel (1983) was generally followed. Rats were given orally a 10% charcoal suspension in 5% gum arabic 30 min after they had received L-NAME

10 or 25 mg kg$^{-1}$ i.p. or the vehicle. After 30 min the rats were killed and gastrointestinal tract was removed and 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. L-NAME 10–25 mg kg$^{-1}$ i.p. was also studied on intestinal transit in rats each giving 2 ml castor oil 30 min later and killed after a further 30 min.

### *Chemicals*

N$^G$-nitro-L-arginine methyl ester (L-NAME) hydrochloride, N$^G$-monomethyl-L-arginine (L-NMMA) acetate, L-arginine hydrochloride, D-arginine hydrochloride, castor oil (all Sigma) and olive oil (Carapelli) were used.

### *Statistics*

Diarrhoea was expressed as total score (Piercey & Ruwart, 1979) and a chi-squared test was used to determine the significance between groups. Fluid volume, $Na^+$ secretion and small intestinal transit were expressed as mean ± s.e; Student's unpaired or paired $t$ test was used to determine the significance of differences between means and $P<0.05$ was taken as statistically significant.

**Table 2** Diarrhoea induced by castor oil (CO) in rats treated with L- or D-arginine: effect of N$^G$-nitro-L-arginine methyl ester (L-NAME) 10 mg kg$^{-1}$

| *Treatment* (mg kg$^{-1}$, i.p.) | *Diarrhoea score* ++ | + | 0 | *Total score* (max = 24) |
|---|---|---|---|---|
| Control | 0 | 0 | 12 | 0 |
| CO (2 ml per rat) | 12 | 0 | 0 | 24 |
| CO + L-NAME | 0 | 2 | 10 | 2 |
| CO + L-NAME + L-arginine 150 | 0 | 3 | 9 | 3 |
| CO + L-NAME + L-arginine 300 | 3 | 5 | 4 | 11 |
| CO + L-NAME + L-arginine 600 | 10 | 1 | 1 | 21[a] |
| CO + L-NAME + D-arginine 900 | 0 | 2 | 10 | 2 |
| CO + L-arginine 600 | 12 | 0 | 0 | 24 |
| CO + D-arginine 900 | 12 | 0 | 0 | 24 |
| L-Arginine 600 | 0 | 0 | 12 | 0 |
| D-Arginine 900 | 0 | 0 | 12 | 0 |

Twelve animals were used for each group. N$^G$-nitro-L-arginine methyl ester (L-NAME), L-arginine or D-arginine were given i.p. 15 min before oral administration of castor oil. Results were analyzed by chi-squared test.
[a]$P<0.01$ vs castor oil (CO) + L-NAME.

## Results

### *Castor oil diarrhoea*

Three hours after castor oil administration all rats produced copious diarrhoea and a maximum score of 24 (Table 1). L-NAME 1 mg kg$^{-1}$ had little effect while 2.5, 5 and 10 mg kg$^{-1}$ reduced the diarrhoea score to 14, 8 and 2 respectively (Table 1). L-NAME 25 mg kg$^{-1}$ gave complete protection. Lower doses of L-NMMA 2.5 and 25 mg kg$^{-1}$ had little or no effect but 100 mg kg$^{-1}$ caused a reduction. The highest dose of either L-NAME and L-NMMA or L-arginine did not alter the consistency or the number of faecal pellets of rats not given castor oil (Table 2). L-Arginine did not modify the castor-oil-induced diarrhoea, but it counteracted the effect of L-NAME 10 mg kg$^{-1}$ in a dose-related manner (Table 2). In contrast, D-arginine 900 mg kg$^{-1}$ did not modify the antidiarrhoeal effect of L-NAME (Table 2); it was also inactive in control rats, and did not modify diarrhoea in rats given castor oil.

### *Intestinal fluid accumulation*

In preliminary experiments castor oil increased the volume of intestinal fluid, the effect being maximal at 30 min but absent after 60 min. In the following experiments the influence of L-NAME was therefore assessed at 30 min. Pretreatment with L-NAME dose-dependently reduced castor oil-induced intestinal fluid accumulation; L-NAME 25 mg kg$^{-1}$ gave complete protection but did not alter intestinal fluid volume in control rats (Table 3). L-Arginine (600 mg kg$^{-1}$) but not D-arginine (900 mg kg$^{-1}$) counteracted the inhibitory effect of L-NAME (10 mg kg$^{-1}$). Neither L- nor D-arginine altered intestinal fluid volume in untreated (olive oil) rats or rats given castor oil.

### *Electrolyte secretion*

$Na^+$ secretion induced by castor oil 30 min after its administration was inhibited by L-NAME (2.5–25 mg kg$^{-1}$) in a dose-related manner (Table 3). In rats given both 25 mg kg$^{-1}$ of L-NAME and castor oil the values were similar to controls. L-NAME had a similar inhibitory effect on both $Na^+$ levels and fluid volume. L-Arginine (600 mg kg$^{-1}$) but not D-arginine (900 mg kg$^{-1}$) counteracted the L-NAME (10 mg kg$^{-1}$) effect.

**Table 1** Effect of N$^G$-nitro-L-arginine methyl ester (L-NAME) and N$^G$-monomethyl-L-arginine (L-NMMA) on diarrhoea induced by castor oil (CO)

| *Treatment* (mg kg$^{-1}$, i.p.) | *Diarrhoea score* ++ | + | 0 | *Total score* (max = 24) | *Protection* (%) |
|---|---|---|---|---|---|
| Control | 0 | 0 | 12 | 0 | – |
| CO (2 ml per rat) | 12 | 0 | 0 | 24 | – |
| CO + L-NAME 1.0 | 9 | 2 | 1 | 20 | 16.7 |
| CO + L-NAME 2.5 | 6 | 2 | 4 | 14 | 41.6 |
| CO + L-NAME 5.0 | 2 | 4 | 6 | 8[a] | 66.7 |
| CO + L-NAME 10.0 | 0 | 2 | 10 | 2[c] | 91.7 |
| CO + L-NAME 25.0 | 0 | 0 | 12 | 0[c] | 100.0 |
| CO + L-NMMA 2.5 | 11 | 1 | 0 | 23 | 4.2 |
| CO + L-NMMA 25.0 | 8 | 2 | 2 | 18 | 25.0 |
| CO + L-NMMA 100.0 | 6 | 0 | 6 | 12[b] | 50.0 |
| L-NAME 25.0 | 0 | 0 | 12 | 0 | – |
| L-NMMA 100.0 | 0 | 0 | 12 | 0 | – |

Twelve animals were used for each group. N$^G$-nitro-L-arginine methyl ester and N$^G$-monomethyl-L-arginine were given i.p. 15 min before oral administration of 2 ml castor oil. Results were analyzed by chi-squared test.
[a]$P<0.05$; [b]$P<0.01$ and [c]$P<0.001$ vs castor oil (CO).

**Table 3** Inhibition of castor oil (CO)-stimulated fluid and $Na^+$ secretion in rat small intestine by $N^G$-nitro-L-arginine methyl ester (L-NAME)

| *Treatment* (mg kg$^{-1}$, i.p.) | *Volume* (ml) | *$Na^+$* (mM) | *$Na^+$ amount* (μmol) |
|---|---|---|---|
| Control | 0.24 ± 0.05 | 152.3 ± 12.8 | 36.2 ± 3.5 |
| CO | 1.45 ± 0.15[a] | 152.9 ± 13.0 | 220.4 ± 18.3[a] |
| CO + L-NAME 2.5 | 1.35 ± 0.32 | 160.7 ± 14.0 | 216.9 ± 13.6 |
| CO + L-NAME 10.0 | 0.37 ± 0.10[b] | 139.5 ± 9.4 | 51.6 ± 5.4[b] |
| CO + L-NAME 25.0 | 0.24 ± 0.07[b] | 139.6 ± 10.0 | 33.5 ± 4.2[b] |
| CO + L-NAME 10.0 + L-arginine 600.0 | 1.40 ± 0.16[c] | 150.6 ± 12.7 | 210.8 ± 11.4[c] |
| CO + L-NAME 10.0 + D-arginine 900.0 | 0.37 ± 0.11 | 140.7 ± 7.8 | 52.1 ± 4.7 |
| CO + L-arginine 600.0 | 1.43 ± 0.13 | 152.5 ± 10.5 | 218.1 ± 16.4 |
| CO + D-arginine 900.0 | 1.46 ± 0.15 | 151.7 ± 13.0 | 221.5 ± 15.0 |
| L-NAME 25.0 | 0.26 ± 0.04 | 176.8 ± 15.5 | 46.0 ± 4.8 |
| L-Arginine 600.0 | 0.27 ± 0.06 | 153.6 ± 11.5 | 41.5 ± 6.6 |
| D-Arginine 900.0 | 0.25 ± 0.04 | 152.4 ± 10.7 | 38.1 ± 4.5 |

Results are expressed as means ± s.e. of 7–9 experiments.
[a]$P<0.001$ vs control; [b]$P<0.001$ vs castor oil (CO); [c]$P<0.001$ vs castor oil (CO) + $N^G$-nitro-L-arginine (L-NAME) 10.0 mg kg$^{-1}$.

### *Intestinal transit*

In normal rats the marker usually travelled more than half of the total length of the small intestine in 30 min: the mean ± s.e. was 58.0 ± 3.4% ($n = 10$). In rats given castor oil the value was 47.2 ± 3.0% ($n = 10$) (18.6% less, $P > 0.05$). L-NAME had no significant effect on the intestinal transit in normal (L-NAME 10 mg kg$^{-1}$: 56.4 ± 2.7% transit $n = 10$; L-NAME 25 mg kg$^{-1}$: 55.5 ± 3.0%, $n = 10$) or castor-oil-treated rats (L-NAME 10 mg kg$^{-1}$: 48.3 ± 3.0%, $n = 10$; L-NAME 25 mg kg$^{-1}$: 51.7 ± 3.4%, $n = 8$).

## Discussion

It has been reported that castor oil causes diarrhoea 1–2 h after administration in rats (Niemegeers *et al.*, 1972; Vischer & Casals Stenzel, 1983). We obtained a highly reproducible response 3 h after castor oil administration (Pinto *et al.*, 1989; and the present results) and we used this 3 h time-period because at this time all rats exhibited scored diarrhoea.

We have shown that L-NAME and L-NMMA, inhibitors of NO synthesis from L-arginine (Rees *et al.*, 1990; Gardiner *et al.*, 1990) prevented or reduced the diarrhoea induced when given 15 min before the castor oil. Both compounds at the doses used in the present study can inhibit the enzyme(s) forming NO both in peripheral tissues (for ref. see Moncada & Higgs, 1991) and in the central nervous system (Knowles *et al.*, 1989). The finding that L-NAME was more potent and effective than L-NMMA in inhibiting the diarrhoea is consistent with previous reports that L-NAME is more potent than L-NMMA (Mulsch & Bussel 1990; Moore *et al.*, 1990). The dose of L-arginine, a substrate of NO synthesis, able to counteract the L-NAME effect can be 3–100 fold higher than that of NO synthesis inhibitor, depending on tissues and species (Rees *et al.*, 1990). In the present study it was 60 fold higher. In addition, as has been reported (Palmer *et al.*, 1988; Sakuma *et al.*, 1988; Amezcua *et al.*, 1989) the effect of arginine was enantiomer-specific; D-arginine was without effect. These results suggest that nitric oxide could be involved in castor-oil-induced diarrhoea.

Castor oil and its active ingredient ricinoleic acid change the transport of water and electrolytes to a net hypersecretory response. Our results show that the stimulated fluid and $Na^+$ secretion induced by castor oil were inhibited by L-NAME in a dose-related manner, whereas control rats were unaffected. Castor-oil-induced diarrhoea might also result from changes in intestinal transit (Gaginella & Phillips, 1975). Most reports suggest that motor activity is decreased during castor oil diarrhoea (Gaginella & Bass, 1978; Sarna, 1991) while others indicate the opposite (Vischer & Casals-Stenzel, 1983; Melo *et al.*, 1988). Our results show a tendency of castor oil to delay the transit to a rate below normal but this effect was not significant. In the light of the accepted hypothesis that NO may mediate the relaxation of intestinal muscle (Bult *et al.*, 1990; Christinck *et al.*, 1990; Boeckxstaens *et al.*, 1991) it was expected that transit might be modified in rats given L-NAME. In the present results, L-NAME did not modify the effect of the castor oil or influence the intestinal transit in control rats. The ability of L-NAME given i.p. to inhibit diarrhoea at doses which had no effect on the transit rate strongly suggests that the antidiarrhoeal activity of L-NAME is related to antisecretory mechanism and not to inhibition of transit. The mechanism underlying the antisecretory effect of L-NAME is however unclear. Most active secretagogues are potent vasodilators (Granger *et al.*, 1987). The antidiarrhoeal activity of L-NAME is not, however, just due to its vasoconstrictor effect because L-NMMA in doses causing the same vasoconstrictor effect as L-NAME did not block the diarrhoea. On the other hand vasopressin, a well known vasoconstrictor agent, causes water and electrolyte secretion in the small intestine (Donowitz & Welsh, 1987). In our results the failure of L-NAME to affect normal defaecation (but also secretion and transit) in rats probably implies that NO is not synthesized normally in the intestine in amounts sufficient to play a role in defaecation but in response to stimuli (i.e. castor oil) and the supply of L-arginine for NO synthesis is not rate-limiting. Hutcheson *et al.* (1990) suggest that endotoxin, an intestinal secretagogue (Tsurumi & Fujimura, 1983), induces the formation of NO from L-arginine by endothelial cells or possibly activated leucocytes. Most probably during castor oil-induced diarrhoea there is in the gut a generation of NO and this could explain the antidiarrhoeal effect by L-NAME. NO, once generated, could modulate cell activation and consequently release endogenous secretagogues or alternatively act directly to influence water and electrolyte secretion. The inhibition of diarrhoea could also be achieved if the NO synthesis inhibitors possess anti-acetylcholine, anti-histamine or anti-5-hydroxytryptamine (5-HT) activity, substances that facilitate NO synthesis (Amezcua *et al.*, 1988; Garrison, 1990) and are involved (5-HT and histamine) in diarrhoea caused by castor oil (Capasso *et al.*, 1986).

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