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N^G-Nitro-L-arginine methyl ester reduces senna- and cascara-induced diarrhoea and fluid secretion in the rat

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Abstract

Senna (60 mg/kg orally) and cascara (800 mg/kg orally)-induced diarrhoea and net fluid secretion were studied in rats for a time period of 1–8 h. N^G-Nitro-L-arginine methyl ester (L-NAME) (2.5–25 mg/kg i.p. twice, 15 min before and 4 h after laxative administration), an inhibitor of nitric oxide synthase, reduced the diarrhoeal response. This effect was counteracted by L-arginine (600 and 1500 mg/kg i.p. 15 min before laxative administration), the precursor of nitric oxide (NO). The senna- and cascara-stimulated fluid secretion was reduced by N^G-nitro-L-arginine methyl ester 25 mg/kg i.p. (twice, 15 min before and 4 h after laxative administration), while the stereoisomer N^G-nitro-D-arginine metyl ester (D-NAME) 25 mg/kg i.p. was without effect. These results suggest a possible involvement of NO in senna- and cascara-induced diarrhoea and fluid secretion.

Keywords: Nitric oxide (NO); Senna; Cascara; Anthraquinone; Laxative; Intestinal secretion

1. Introduction

Anthraquinones, including sennosides and cascara, are widely used by the public as all-purpose laxatives and also as recommended by physicians for bowel evacuation prior to diagnostic radiographs (Gaginella, 1994). The active ingredients of the anthraquinones are glycosides which are activated by intestinal flora that cleave off a sugar to produce the aglycone form (Lemli and Lemmens, 1980). The mechanism by which these drugs alter fluid transport across the intestinal mucosa is believed to be through inhibition of Na^+ absorption (Van Os, 1976), through a nonspecific metabolic effect on the epithelial cells (Verhaeren, 1980) or inhibition of Na^+-K^+ ATPase (see Gaginella, 1994). In addition an effect on the release of neurotransmitters from the myenteric plexus, which in turn may alter fluid absorption by the gut, cannot be ruled out (Gaginella, 1994). Anthraquinones also increase prostaglandin synthesis in intestinal mucosa (Beubler and Kollar, 1988), but this is not clearly involved in the effects on fluid transport since indomethacin failed to affect the anthraquinone-induced colonic transport changes (Donowitz et al., 1984).

However, despite the fact that several mechanism have been proposed for the effect of anthraquinones, the precise mechanism of action of these drugs remains elusive, partly because of their multiple effects on the gut.

Nitric oxide (NO) is a colourless gas synthesized by the enzyme NO synthase via the five-electron oxidation of one of the chemically equivalent guanidino nitrogens associated with L-arginine (Marletta, 1993). This enzyme exists as a constitutive (Ca^{2+}/calmodulin-dependent, unaffected by glucocorticoids) and an inducible (Ca^{2+}/calmodulin-independent, induction inhibited by glucocorticoids) form in many tissues (Moncada and Higgs, 1993). The muscle of the rat and canine intestine is innervated by NO synthase-containing neurons (Nichols et al., 1993; Kostka et al., 1993), supporting a role for NO in the regulation of intestinal functions. Intestinal NO can be furthermore produced by neutrophils, macrophages and mast cells, implicating it as a potential pathophysiological mediator of the secretory diarrhoea associated with mucosal inflammation and injury.

Several data support the involvement of NO in intestinal secretion: the NO-donating compound sodium nitroprusside (Wilson et al., 1993) and NO itself (Tamai and

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Gaginella, 1993) stimulate anion secretion by the rat colon in vitro; in addition, in vivo studies have shown that the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) reduced the fluid accumulation due to castor oil (Mascolo et al., 1993; Mascolo et al., 1994), diphenylmethane laxatives (Gaginella et al., 1994), magnesium

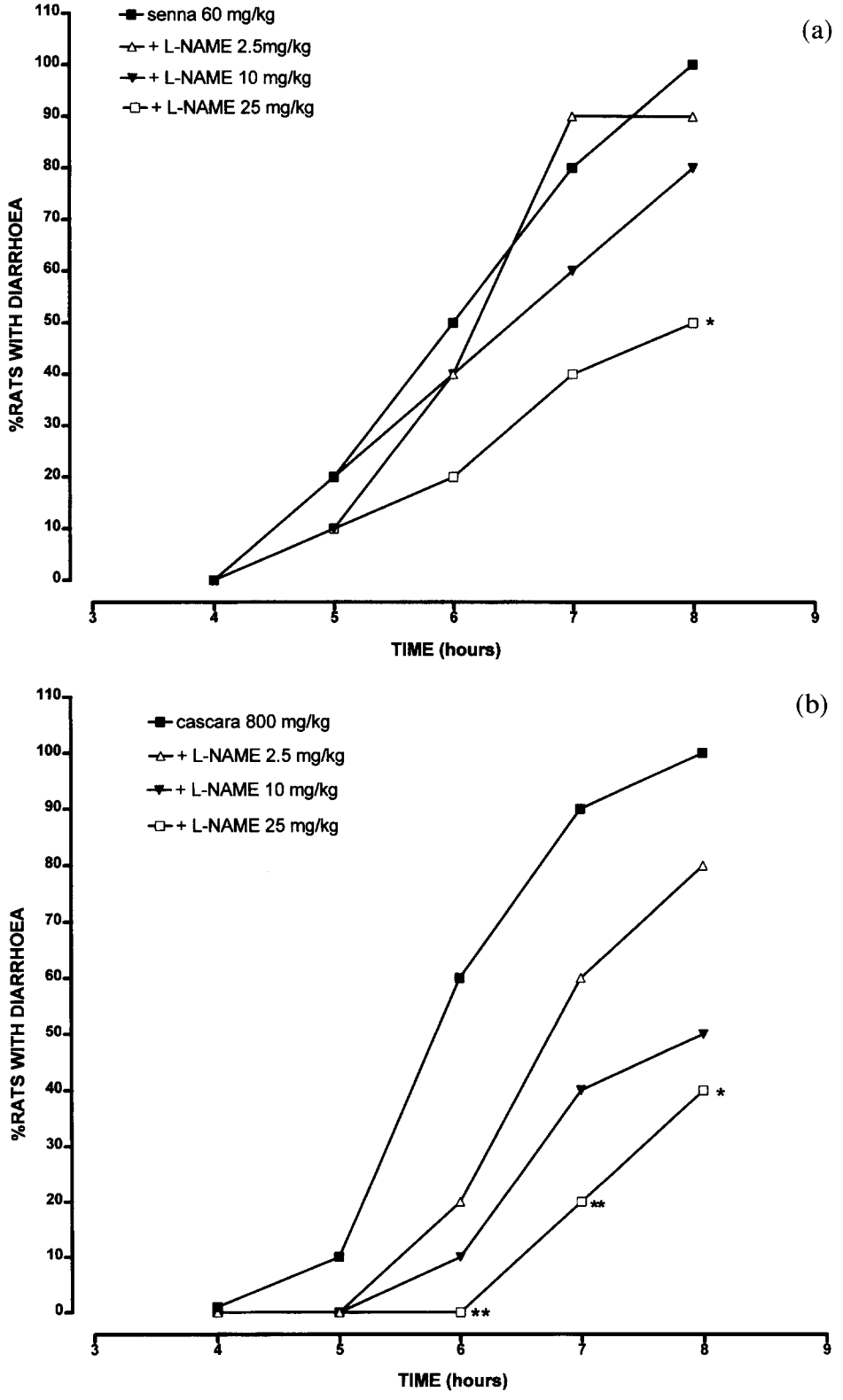

Fig. 1. Inhibitory effect of L-NAME (2.5–25 mg/kg) on the percentage of rats (out of 10) with diarrhoea at various times after oral senna 60 mg/kg (Fig. 1a) and cascara 800 mg/kg (Fig. 1b). L-NAME was given i.p. 15 min and 4 h after laxative administration. * $P < 0.05$ and ** $P < 0.01$ (Chi-square test).

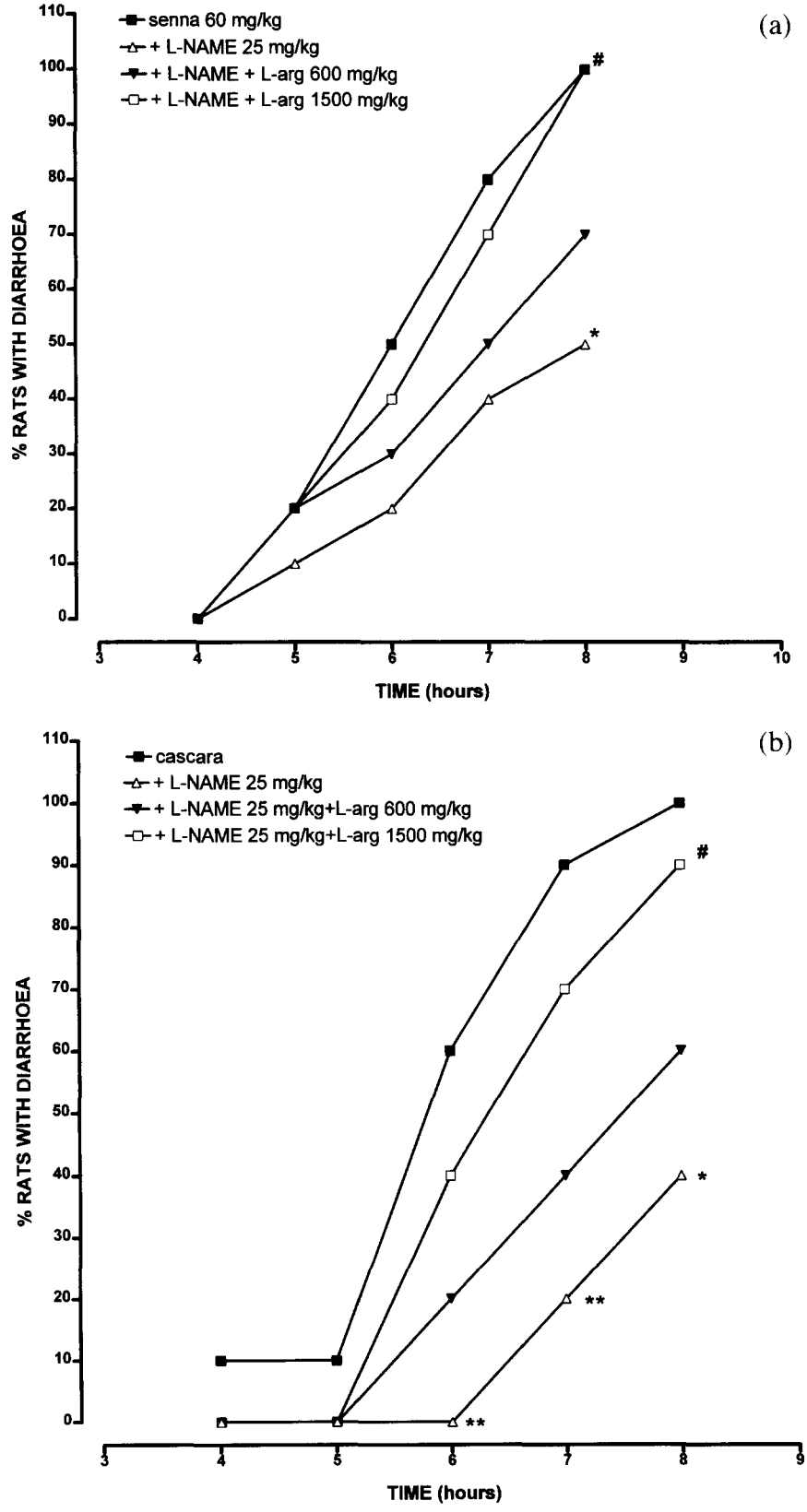

Fig. 2. Reversal by L-arginine (L-arg 600 and 1500 mg/kg i.p.) of the inhibitory effect of L-NAME (25 mg/kg i.p.) on diarrhoea in rats ($n = 10$) given senna (Fig. 2a) and cascara (Fig. 2b) orally. L-NAME was given 15 min before and 4 h after laxative administration, while L-arginine was given (i.p.) 15 min before the laxatives. * $P < 0.05$ and $P < 0.01$ vs. control and $^{\#}$ $P < 0.05$ vs. laxative + L-NAME.

sulphate (Izzo et al., 1994), sodium choleate (Mascolo et al., 1994) or trinitrobenzene sulphonic acid-induced colitis (Miller et al., 1993a). Furthermore, L-arginine, the precursor of NO, also induces fluid secretion in the rat jejunum (Mourad et al., 1993). In contrast with these findings, Rao et al. (1994) have shown that NO produced a net proabsorptive effect in isolated sheets of mouse ileum in vitro. Consistent with these results, prostaglandin E_2- or *Escherichia coli* heat-stable enterotoxin-induced fluid secretion is enhanced by infusion of L-NAME in the rat jejunum in vivo (Schirgi-Degen and Beubler, 1995). Therefore the role of NO in mediating intestinal secretion seems to be uncertain, but probably depends upon whether the conditions under study are physiological or pathophysiological.

In order to determine whether or not the mechanism of laxative action of senna and cascara involves NO, we studied their ability to produce diarrhoea and stimulate fluid secretion in rat. Comparisons were made in the absence and presence of L-NAME, its inactive isomer N^G-nitro-D-arginine methyl ester (D-NAME) and L-arginine, the substrate for NO synthase.

2. Materials and methods

2.1. Animals

Male Wistar (Morini) rats (150–170 g) were used after 1 week of acclimation (temperature 23 + 2°C humidity 60%). Rats were fasted for 18 h, but received water ad libitum. Each rat was placed in a separate cage at the beginning of the experiment.

2.2. Laxative (diarrhoeal) test

Rats were injected (i.p.) with L-NAME 2.5–25 mg/kg (or D-NAME 25 mg/kg i.p.) 15 min before and 4 h after p.o. dosing with 60 mg/kg of senna or 800 mg/kg of cascara. L-Arginine (600–1500 mg/kg i.p.) or D-arginine (1500 mg/kg) was given 15 min before laxative administration.

One hour after dosing with the laxatives, and each hour for 8 h, the individual cages were inspected (by an observer unaware of the particular treatment) for the presence of characteristic wet 'diarrhoeal' droppings; their absence was recorded as a positive result, indicating protection from diarrhoea at that point in time.

2.3. Fluid secretion

Five hours after senna (60 mg/kg) or cascara (800 mg/kg) treatment, rats were anaesthetized with urethane (1.3 g/kg i.p.) and the entire colon was rinsed with warm saline solution to remove the contents. Thirty minutes later the colon was filled with 2.5 ml of Tyrode solution and ligated. After 60 min the rats were killed and the colon was removed. Net water transport was calculated from the volume of the fluid content minus the 2.5 ml of the solution used to fill the colon (Gaginella et al., 1994; Izzo et al., 1994; Mascolo et al., 1994). L-NAME 25 mg/kg i.p. (or D-NAME 25 mg/kg i.p.) was given 15 min before and 4 h after laxative administration.

2.4. Chemicals

L-NAME hydrochloride, L-arginine hydrochloride, D-arginine hydrocloride and D-NAME hydrocloride were

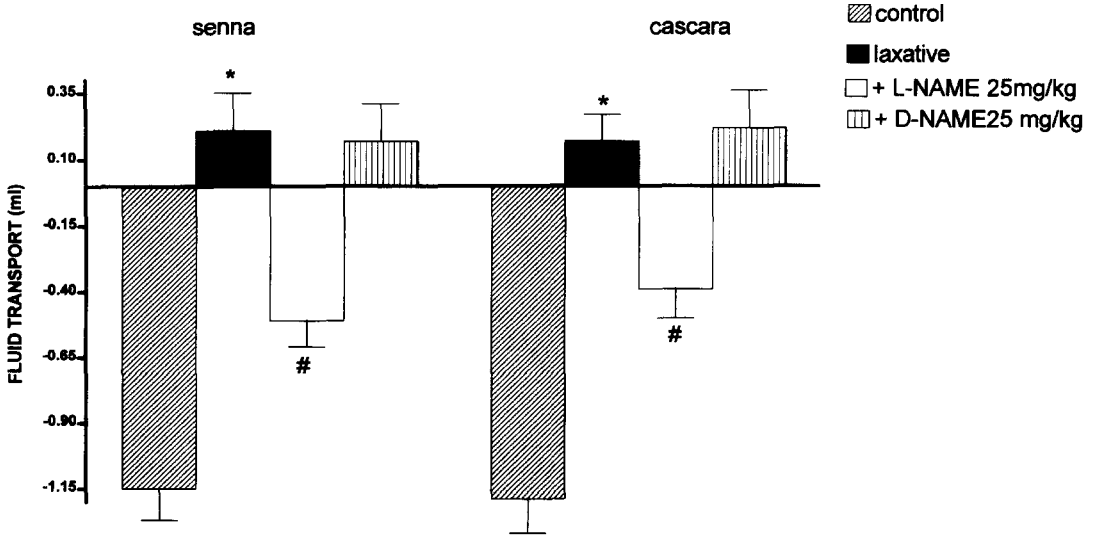

Fig. 3. Effect of L-NAME 25 mg/kg and D-NAME 25 mg/kg on water flux induced by senna (60 mg/kg) and cascara (800 mg/kg) given orally. A negative value represents net absorption and a positive value net secretion. L-NAME was administered 15 min and 3 h after the laxatives. $P < 0.01$ vs. control and $^{\#}$ $P < 0.05$ vs. laxative.

purchased from Sigma (Milan, Italy), senna pod extract (*Cassia angustifolia*) containing 45% sennoside B and cascara water extract (*Rhamnus purshiana cortex*) containing 20% cascaroside A were a gift from Indena (Settala, Italy). Drugs were dissolved in distilled water before being used.

2.5. Statistics

The Chi-square test was used to determine the significance between groups with or without diarrhoea. Intestinal fluid volume was expressed as mean ± S.E. and compared by Student's *t*-test for unpaired data. A P value less than 0.05 was considered significant.

3. Results

3.1. Diarrhoea

Eight hours after p.o. administration of the laxatives, diarrhoea was evident in all the animals. L-NAME dose dependently reduced the total number of rats with diarrhoea over the time frame studied (Fig. 1). The 25 mg/kg dose significantly ($P < 0.05$) reduced the incidence of diarrhoea 8 h after senna challenge and from hours 6 to 8 in the cascara-treated group. L-Arginine (but not D-arginine) reversed the effect of 25 mg/kg of L-NAME on senna- and cascara-induced diarrhoea (Fig. 2), but the same doses (600 and 1500 mg/kg) did not by themselves modify the diarrhoeal effect of the drugs (data not shown).

3.2. Fluid secretion

In preliminary experiments, senna and cascara both reversed net water absorption to net secretion between 5.5 and 6.5 h. The influence of L-NAME (or D-NAME) was therefore assessed over this interval. Neither L-NAME nor D-NAME affected fluid transport in control rats (data not shown), but L-NAME prevented the effect of senna and cascara on fluid secretion (Fig. 3).

4. Discussion

Senna and cascara are widely used, but their precise mechanism of action is still a matter of investigation. Several mediators have been postulated to be involved in senna and cascara fluid accumulation and diarrhoea. These include prostaglandins (Beubler and Kollar, 1988), histamine (Capasso et al., 1986), serotonin (Beubler and Schirgi-Degen, 1993; Capasso et al., 1986) and Ca^{2+} (Donowitz et al., 1984). In recent years it has been demonstrated that NO is one of the mediators of castor oil (Mascolo et al., 1993)-, diphenylmethane laxative (Gaginella et al., 1994)-, magnesium sulphate (Izzo et al., 1994)-, sodium cholate (Mascolo et al., 1994)- but not mannitol (Izzo et al., 1994)-induced laxation. In this study we postulate that NO may be involved in senna- and cascara-induced diarrhoea and intraluminal fluid accumulation.

We have shown that the potent NO synthase inhibitor, L-NAME, at doses previously reported to be effective (Gaginella et al., 1994; Mascolo et al., 1993, Mascolo et al., 1994; Izzo et al., 1994), reduces the diarrhoeal effect of oral administration of senna and cascara. The doses of cascara and senna were chosen on the basis of our laboratory experience; they produced diarrhoea of the same intensity. The effect of L-NAME was prevented by L-arginine but not by its enantiomer, D-arginine, implying that the effect of senna and cascara could involve NO synthesis from L-arginine.

Senna and cascara (or their active ingredients) change intestinal mucosal fluid transport from absorption to net secretion. In our study the stimulated fluid secretion was inhibited by L-NAME, the effect being stereospecific since D-NAME was inactive. These data and the fact that NO stimulates intestinal secretion in vitro (Tamai and Gaginella, 1993) support an involvement of NO in the fluid secretion observed after cascara and senna administration.

The effect of NO on fluid transport in the intestine seems to depend upon the conditions under study. In normal anaesthetized rats L-NAME (25 mg/kg i.v.) reverses jejunal fluid absorption to secretion (Schirgi-Degen and Beubler, 1995), indicating a proabsortive tone of NO under physiological conditions. This proabsorptive tone also may downregulate fluid secretion induced by *Escherichia coli* heat-stable enterotoxin or prostaglandin E_2. However, in some pathophysiological states (such as the diarrhoea associated with laxative administration or trinitrobenzene sulphonic acid-induced colitis), NO may be produced at higher concentrations capable of evoking net secretion. Indeed, Miller et al. (1993b) have proposed that NO may be protective under basal conditions, but deleterious under chronic pathological conditions. However, the discrepancy between our results (NO as a secretagogue) and those of Schirgi-Degen and Beubler (1995) (NO as a proabsorbtive substance) could be also due to other causes such as the route of administration (i.p. vs. i.v.), different anaesthetic used (urethane vs. sodium pentobarbitone) or different regions of the gut (colon vs. jejunum).

The laxative (diarrhoeal) effect of senna and cascara could also be attributed to disordered motiliy and hence an increase in the transit of intraluminal material. The increase in intestinal transit is believed to be due to a reduced resistance to flow, because of relaxation of the circular muscle of the intestine (Gullikson and Bass, 1984). Anthraquinone derivatives are reported to relax intestinal smooth muscle (Gaginella and Bass, 1978), and NO mediates relaxation of intestinal muscle through neural mecha-

nisms under various experimental conditions (Grider, 1993; Sanders and Ward, 1992).

In conclusion, this study provides further evidence that NO could be an important mediator of intestinal secretion in pathological conditions and that NO is involved in senna- and cascara-induced diarrhoea and fluid secretion in rats.

Acknowledgements

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