J. Pharm. Pharmacol. 1997, 49: 988–990
Received March 6, 1997
Accepted June 5, 1997

Nitric Oxide Modulates the Acute Increase of Gastrointestinal Transit Induced by Endotoxin in Rats: a Possible Role for Tachykinins

M. A. MARTÍNEZ-CUESTA, M. D. BARRACHINA, B. BELTRÁN, S. CALATAYUD AND J. ESPLUGUES

Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain

Abstract

Because of the evidence that endogenous nitric oxide (NO) plays an essential role in the physiological regulation of gastrointestinal motility we have investigated, by use of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), the role of endogenous NO in the acute endotoxin-induced changes of gastrointestinal transit.

Pre-treatment with *E. coli* endotoxin (100 μg kg^{-1}, i.v.) induced a significant increase in the gastrointestinal transit of a charcoal suspension in anaesthetized rats. Previous administration of the NO synthase inhibitor, L-NAME (10 mg kg^{-1}, i.v.) significantly prevented the effects of endotoxin. L-arginine (200 mg kg^{-1}, i.v.) and the substance P antagonist [D-Pro^2, D-Trp^{7,9}]-substance P (SPA), significantly reversed the effects of L-NAME on gastrointestinal transit in rats treated with endotoxin. Pre-treatment with dexamethasone (5 mg kg^{-1}, s.c., twice), an inhibitor of the expression of inducible NO synthase, did not affect the increase in the gastrointestinal transit through constitutive NO synthesis.

The results suggest that constitutive nitric oxide is involved in the increase of gastrointestinal transit induced by endotoxin and that the reduction in transit induced by L-NAME in endotoxin-treated rats is mediated by endogenous tachykinins.

There is substantial evidence that endogenous nitric oxide (NO) plays an essential role in the physiological regulation of gastrointestinal motility. Thus, NO directly relaxes gastrointestinal muscle and modulates spontaneous motility and peristalsis by mediating non-adrenergic-non-cholinergic relaxations or regulating the release of contractile mediators from local neuronal or intestinal sources (Sanders & Ward 1992). Furthermore, it has been shown that the synthesis of NO mediates gastrointestinal motility and secretion changes induced by various drugs (Calignano et al 1992; Barry et al 1994; Izzo et al 1994a, b; Kadowaki et al 1996). Likewise, pathological changes of intestinal motility such as paralytic ileus (Salzman 1995) and diarrhoea (Gaginella et al 1994; Izzo et al 1994a, b; Mascolo et al 1994) have been attributed to formation of excess NO.

Early clinical symptoms of bacteraemia and septic shock are associated with abnormal gut motility. It is well established that endotoxin can stimulate the expression of a Ca^{2+}-independent NO-synthase in various cell types (Radomski et al 1990) and, furthermore, recent evidence suggests that some of the early gastrointestinal changes induced by endotoxin are mediated by NO and free radicals (Pons et al 1991).

In this study we have investigated, by the use of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), the role of endogenous NO in the acute endotoxin-induced changes of gastrointestinal transit. We have also evaluated the possible involvement of endogenous tachykinins in such events because it has been suggested that interactions between endogenous nitric oxide and tachykinins affect the modulation of the spontaneous motility of rat duodenum (Martínez-Cuesta et al 1996).

Correspondence: M. A. Martínez-Cuesta, Departamento de Farmacología, Facultad de Medicina Avda. Blasco Ibañez 15, 46010-Valencia, Spain.

Material and Methods

Charcoal, *E. coli* endotoxin (serotype 0111:B4), [D-Pro^2, D-Trp^{7,9}]-substance P, hydroxypropylmethylcellulose, L-NAME and L-arginine were purchased from Sigma. Dexamethasone (fortecortin, Merck) and ketamine (ketolar, Parke-Davis) were used as clinically available preparations. All drugs were dissolved in saline immediately before use and administered in a volume of 1 mL kg^{-1}.

Male Wistar rats, 200–250 g, were fasted for 24 h but allowed drinking water. Under ketamine anaesthesia (150 mg kg^{-1}, i.m.) the trachea was intubated and the jugular vein was cannulated. Gastrointestinal propulsion was assessed by the gastrointestinal transit of a charcoal test meal comprising an aqueous suspension of 1% (w/w) hydroxypropylmethylcellulose and 10% (w/w) carbon black (Howd et al 1978). The suspension was continuously stirred, pre-warmed to body temperature and given intragastrically by means of an oesophageal catheter. Fifteen minutes later the rats were killed by cervical dislocation, and after laparotomy the front of the charcoal suspension in the small intestine was detected visually and marked with a ligation. Gastrointestinal transit was expressed as the percentage of the length of the small intestine traversed by the charcoal marker.

Endotoxin (*E. coli* lipopolysaccharide, 100 μg kg^{-1}) was administered intravenously 30 min before the test meal. Animals were injected intravenously with the NO synthase inhibitor L-NAME (10 mg kg^{-1}) 10 min before the endotoxin, and an additional group of rats was pre-treated with L-arginine (200 mg kg^{-1}, i.v.) 5 min before L-NAME. Another group of

animals was given an intravenous dose of 0·65 μmol kg^{-1} of the substance P antagonist [D-Pro^2, D-Trp^{7,9}]-substance P 10 min before L-NAME. In a further experiment rats were treated with dexamethasone (5 mg kg^{-1}, s.c.) 16 and 4 h before endotoxin. In all experiments a control group received a similar regimen of treatment with intravenous isotonic saline.

Results are expressed as means ± s.e.m. A one-way analysis of variance then the Bonferroni t-test were used for multiple comparison; P values less than 0·05 were taken as indicative of significance.

Results

As shown in Table 1, a charcoal meal administered intragastrically to control animals under ketamine anaesthesia covered 33 ± 2% of the total length of the small intestine in 15 min. Administration of endotoxin (100 μg kg^{-1}) 30 min before the test meal induced a significant ($P < 0·001$) increase in gastrointestinal transit. Pre-treatment of the animals with the NO synthesis inhibitor L-NAME (10 mg kg^{-1}) reversed ($P < 0·05$) the increase in gastrointestinal transit induced by endotoxin. These effects of L-NAME were prevented by previous intravenous administration of the substrate of NO synthesis, L-arginine (200 mg kg^{-1}). In control animals neither L-NAME (10 mg kg^{-1}, n = 3) nor L-arginine (200 mg kg^{-1}, n = 4) significantly modified the gastrointestinal transit of the charcoal meal; values were 30·7 ± 3% and 28 ± 4%, respectively. Pre-treatment with dexamethasone (5 mg kg^{-1}, s.c.) 16 and 4 h before endotoxin failed to modify the endotoxin-induced increase in gastrointestinal transit. The dose of 0·65 μmol kg^{-1} of [D-Pro^2, D-Trp^{7,9}]-substance P significantly ($P < 0·05$) prevented the inhibitory effect of L-NAME on gastrointestinal transit in rats treated with endotoxin. This antagonist had no significant effect on gastrointestinal transit in rats treated only with endotoxin (64 ± 5%, n = 4).

Discussion

This study shows that inhibition of NO synthesis with L-NAME reversed the increase in gastrointestinal transit induced by endotoxin. The effect of L-NAME was prevented by pre-treatment with the precursor of NO, L-arginine, thus further suggesting that NO plays a role in the effects of endotoxin on gastrointestinal transit. Although there is clear evidence that NO modulates basal intestinal motility in-vivo, as shown by the increase in intraluminal pressure and phasic intestinal contractions induced by L-NAME (Calignano et al 1992), the results of this effect on physiological peristalsis and intestinal transit are not clear. Our results show that administration of L-NAME in control animals does not modify gastrointestinal transit, an observation previously reported by other authors (Izzo et al 1994a).

Previous studies have demonstrated similar mediation by NO in the increased gastrointestinal transit induced by drugs such as magnesium sulphate (Izzo et al 1994b), salts (Mascolo et al 1994) or diphenylmethane laxative (Gaginella et al 1994). The inducible form of NO-synthase has been regarded as the possible source of the NO implicated in most of these in-vivo motility responses, with the exception of the effects of saline laxatives in which the constitutive form of NO-synthase was implicated (Izzo et al 1994b). The inducible NO-synthase is responsible for the increased synthesis of NO that occurs 3–6 h after exposure to endotoxin. It has also been suggested that the in-vivo induction of NO synthesis by *E. coli* endotoxin is responsible for alterations in the spontaneous activity of the isolated duodenum (Martínez-Cuesta et al 1996). However, it is unlikely that the inducible isoenzyme could be involved in the endotoxin-induced increase of gastrointestinal transit, because in our experiments the endotoxin was administered only 45 min before the evaluation of charcoal transit. In addition, the effects of endotoxin were not modified by the glucocorticoid dexamethasone at doses previously shown to inhibit the expression of the inducible NO synthase (Radomski et al 1990). In previous studies we have demonstrated that low doses of endotoxin can acutely modify other gastrointestinal functions, such as gastric acid secretion (Martínez-Cuesta et al 1992, 1994; Barrachina et al 1995a) or gastric mucosal resistance against injury (Barrachina et al 1995b), by activation of a pathway involving the release of NO through stimulation of the constitutive form of NO synthase. On this basis it seems reasonable to assume that NO has a leading role in the acute gastrointestinal changes triggered by endotoxin although it remains to be elucidated if the nature of NO involvement is always the same.

The mechanisms involved in the NO-mediated increase of gastrointestinal transit in endotoxin-treated rats could be related to changes in intestinal secretions (Barry et al 1994; Izzo et al 1994a; Kadowaki et al 1996) or motility (Sanders & Ward 1992), or both. This effect might be achieved via excessive relaxation of the smooth muscle by NO or, indirectly, through interaction of NO with other mediators such as tachykinins, especially as tachykinins are probably primary transmitters of enteric sensory neurones (McConalogue & Furness 1994). Accordingly, NK-2 and NK-1 receptors have been located in

Table 1. Effects of N^G-nitro-nitro-L-arginine methyl ester on the increase in gastrointestinal transit induced by endotoxin in anaesthetized rats.

Treatment	n	Transit (% length of small intestine)
Control	18	33 ± 2
Endotoxin	7	51 ± 5*
Endotoxin + N^G-nitro-L-arginine methyl ester	5	35 ± 5†
Endotoxin + N^G-nitro-L-arginine methyl ester + L-arginine	5	55 ± 5‡
Endotoxin + N^G-nitro-L-arginine methyl ester + [D-Pro^2, D-Trp^{7,9}]-substance P	4	61 ± 8§

100 μg kg^{-1} endotoxin was administered intravenously. Pre-treatment with L-arginine (200 mg kg^{-1}, i.v.) or substance P antagonist [D-Pro^2, D-Trp^{7,9}]-substance P (0·65 μmol kg^{-1}) reversed the effects of L-NAME (10 mg kg^{-1}, i.v.). Gastrointestinal transit is expressed as the percentage of the length of the small intestine traversed in 15 min by a charcoal test meal administered intragastrically. Values are the means ± s.e.m. of results from n experiments. *$P < 0·001$, significant compared with control group; †$P < 0·05$, significant compared with group treated with endotoxin only; ‡$P < 0·01$ and §$P < 0·05$, significant compared with group treated with L-NAME and endotoxin.

gastrointestinal tissues (Zagorodnyuk et al 1995). Recently, it has been shown that an interaction between endogenous NO and tachykinins affects the regulation of intestinal motility. Furthermore, it has been reported that substance P or NK-2 tachykinins might accentuate the contraction produced by NO inhibitors in the intestine of control animals (Garcia-Villar et al 1996; Martínez-Cuesta et al 1996). In the current study, which is focused on the effects of endotoxin, the substance P antagonist (SPA) blocked the effect of L-NAME on gastrointestinal transit. This action was produced at the optimum dose conferring specificity for tachykinin receptors (Jensen et al 1984; Holzer et al 1986). In contrast, SPA did not modify the NO-mediated increase of gastrointestinal transit induced by endotoxin. Thus, in endotoxaemia, the increase in gastrointestinal transit is initially a consequence of activation of NO biosynthesis, which in turn modulates the role of tachykinins on gastrointestinal transit. The mechanisms responsible for NO-tachykinin interactions remain controversial. Whereas both receptor (NK-2) (Martínez-Cuesta et al 1996) and non-receptor mechanisms (Garcia-Villar et al 1996) have been described, further studies will be required to elucidate the nature of such an interaction.

In summary, constitutive nitric oxide is involved in the increase of gastrointestinal transit induced by endotoxin and the inhibitory effect of L-NAME on the transit is mediated by endogenous tachykinins.

Acknowledgements

This study was supported by grants PM 92-0254 from Dirección General de Investigaciones Científicas y Técnicas (DGICYT and 95/1343 from Fondo de Investigaciones Sanitarias (FIS). M. A. Martínez-Cuesta is the recipient of a fellowship from Ministerio de Educación y Ciencia. M. D. Barrachina and S. Calatayud are the recipients of a fellowship sponsored by Conselleria de Educació i Ciencia (Generalitat Valenciana). B. Beltrán is the recipient of a 'Schering-Plough Fellowship for Gastrointestinal Research'.

References

Barrachina, M. D., Whittle, B. J. R., Moncada, S., Esplugues, J. V. (1995a) Endotoxin inhibition of distension-stimulated gastric acid secretion in rat: mediation by NO in the central nervous system. Br. J. Pharmacol. 114: 8–12

Barrachina, M. D., Calatayud, S., Moreno, L., Martínez-Cuesta, M. A., Whittle, B. J. R., Esplugues, J. V. (1995b) Nitric oxide and sensory afferent neurones modulate the protective effects of low-dose endotoxin on rat gastric mucosal damage. Eur. J. Pharmacol. 280: 339–342

Barry, M. K., Aloisi, J. D., Pickering, S. P., Yeo, C. J. (1994) Nitric oxide modulates water and electrolyte transport in the ileum. Ann. Surg. 219: 382–388

Calignano A., Moncada S., Dio Rosa M. (1991) Endogenous nitric oxide modulates morphine-induced constipation. Biochem. Biophys. Res. Commun. 181: 889–893

Calignano, A., Whittle, B. J. R., Di Rossa, M., Moncada, S. (1992) Involvement of endogenous nitric oxide in the regulation of rat intestinal motility in vivo. Eur. J. Pharmacol. 229: 273–276

Gaginella, T. S., Mascolo, N., Izzo, A. A., Autore, G., Capasso, F. (1994) Nitric oxide as mediator of bisacodyl and phenolphthalein laxative actions: induction of nitric oxide synthase. J. Pharmacol. Exp. Ther. 270: 1239–1245

Garcia-Villar, R., Dupuis, C., Martinolle, J. P., Fioramonti, J., Bueno, L. (1996) Functional evidence for NO-synthase activation by substance P through a mechanism not involving classical tachykinin receptors in guinea-pig ileum in vitro. Br. J. Pharmacol. 118: 1253–1261

Holzer P., Holzer-Petsche, U., Leander S. (1986) A tachykinin antagonist inhibits gastric emptying and gastrointestinal transit in the rat. Br. J. Pharmacol. 89: 453–459

Howd, R. A., Adamovics, A., Palekar, A. (1978) Naloxone and intestinal motility. Experientia 34: 1310

Izzo, A. A., Mascolo, N., Di-Carlo, G., Capasso, F. (1994a) N^G-nitro-L-arginine methyl ester modulates intestinal secretion and motility produced by carbacol. Eur. J. Pharmacol. 271: 31–35

Izzo, A. A., Gaginella, T. S., Mascolo, N., Capasso, F. (1994b) Nitric oxide as a mediator of the laxative action of magnesium sulphate. Br. J. Pharmacol. 113: 228–232

Jensen, R. T., Jones, S. W., Lu, Y. A., Xu J. C., Folkers K., Gardner J. C. (1984) Interaction of substance P antagonists with substance P receptors on dispersed pancreatic acini. Biochim. Biophys. Acta. 804: 181–191

Kadowaki, M., Gershon, M. D., Kunahara, A. (1996) Is nitric oxide involved in 5-HT-induced fluid secretion in the gut? Behav. Brain Res. 73: 293–296

Martínez-Cuesta, M. A., Barrachina, M. D., Piqué, J. M., Whittle, B. J. R., Esplugues, J. V. (1992) Role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Eur. J. Pharmacol. 218: 351–354

Martínez-Cuesta, M. A., Barrachina, M. D., Whittle, B. J. R., Piqué, J. M., Esplugues, J. V. (1994) Involvement of neuronal processes and nitric oxide in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Naunyn Schmiedebergs Arch. Pharmacol. 349: 523–527

Martínez-Cuesta, M. A., Esplugues, J. V., Whittle, B. J. R. (1996) Modulation by nitric oxide of the rat isolated duodenum: role of tachykinins. Br. J. Pharmacol. 118: 1335–1340

Mascolo, N., Gaginella, T. S., Izzo, A. A., Di-Carlo, G., Capasso, F. (1994) Nitric oxide involvement in sodium choleate-induced fluid secretion and diarrhoea in rats. Eur. J. Pharmacol. 264: 21–26

McConalogue, K., Furness, J. B. (1994) Gastrointestinal neurotransmitters. Bailliers Clin. Endocrinol. Metab. 8: 851–876

Pons, L., Drov-Lefaix, M. T., Braquet, P., Bueno, L. (1991) Role of free radicals and platelet-activating factor in the genesis of intestinal motor disturbances induced by *Escherichia coli* endotoxins in rats. Gastroenterology 100: 946–953

Radomski, M. W., Palmer, R. M. J., Moncada, S. (1990) Glucocorticoids inhibited the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cell. Proc. Natl Acad. Sci. USA 87: 10043–10047

Salzman, A. L. (1995) Nitric oxide in the gut. New Horiz. 3: 33–45

Sanders, K. M., Ward, S. M. (1992) Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. Am. J. Physiol. 262: G379–G392

Zagorodnyuk, C., Santicioli, P., Maggi, C. A., Giache, A. (1995) Evidence that tachykinins NK1 and NK2 receptors mediate non-adrenergic non-cholinergic excitation and contraction in the circular muscle of guinea-pig duodenum. Br. J. Pharmacol. 115: 237–246