Old Herborn University Seminar Monograph 9:
Gastro-intestinal motility.
Editors: Peter J. Heidt, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 35-40 (1997).

EFFECTS OF E. COLI LIPOPOLYSACCHARIDE ON GUT MOTILITY MEDIATED VIA NITRIC OXIDE PATHWAY

PER M. HELLSTRÖM

Department of Medicine, Karolinska Hospital, S-171 76 Stockholm, Sweden.

SUMMARY

The interference of endotoxin (lipopolysaccharide, LPS, *E. coli* 055:B5) with motility of the small intestine, as registered with chronically implanted bipolar electrodes, was studied in rats using a radioactive Cr-51 polyethylene glycol marker for evaluation of effects on transit in the gut. Experiments were carried out with increasing doses of LPS administered luminally, intraperitoneally and intravenously, as well as after pretreatment with L-arginine analogues and steroids that inhibit the elaboration of nitric oxide (NO) by the constitutive and inducible nitric oxide synthases (NOS), respectively. Administration of LPS at doses from 20 to 160 $\mu g\ kg^{-1}$ intravenously, but not luminally or intraperitoneally, resulted in disruption of the migrating motor complex (MMC) concomitantly with a hastened small intestinal transit and diarrhoea. The effect on MMC was abolished by pretreatment of the rats with N^G-nitro-L-arginine (L-NNA), N^G-L-arginine methyl ester (L-NAME) or dexamethasone. Simultaneously, transit was normalized with L-NNA, L-NAME and dexamethasone. Provided a changed mucosal barrier in disease states promotes absorption of LPS from the intestinal lumen this may disrupt interdigestive myoelectric activity concomitant with a hastened transit and diarrhoea.

INTRODUCTION

Gram-negative bacteria are often pathogenic organisms in infections originating in the gastro-intestinal tract. In common diarrhoeal diseases different strains of *E. coli* are frequently isolated organisms. The pathophysiological effects of these infections are to a large extent due to the action of endotoxin.

Endotoxins can be isolated from the cell wall of Gram-negative bacteria. Chemically it is described as a lipopolysaccharide (LPS) with a molecular weight between 100 and 900 kD (*Gyles*, 1992). LPS produces extensive effects in man and in animals most likely through stimulation of Ca^{2+}-independent inducible nitric oxide synthase (NOS) in different cell types such as macrophages (*Rees* et al., 1990, 1990; *Leone* et al., 1991), thereby activating the nitric oxide (NO) pathway with elaboration and release of huge amounts of NO locally and systemically. The general signs of such an endotoxemia include fever, hypotension, tachycardia and reduced responses to pressor agents as well as inhibited gastric motility. Recently it has been found that after challenge with LPS, NO is produced by a Ca^{2+}-independent enzyme in macro-

phages, with profound generalized effects. Therefore, NO has been suggested to play a role in host defence mechanisms against bacteria and other invading organisms (*Moncada* et al., 1990).

Less is known about effects of LPS on small intestinal motility. Such effects could be of interest in the explanation of intestinal paralysis in connection with severe abdominal infections and after surgical procedures involving endotoxin containing organs as the large intestine.

The interdigestive migrating myoelectric complex (MMC) is a general motor pattern that can be predicted to recur at certain time intervals and therefore the MMC has been used to study effects of different agents on motility. The function of the MMC has been considered a transport mechanism, cleansing the bowel lumen of secretion, cell debris and undigested food particles (*Al-Saffar* et al., 1984, 1985).

The aim of the present study was to investigate the effects of LPS on the MMC and transit of contents in the small intestine of fasted rats. Further, the involvement of NO in the LPS-induced changes of motor activity was evaluated using L-NNA and L-NAME as inhibitors of the NO pathway, and dexamethasone as an inhibitor of inducible NOS.

MATERIALS AND METHODS

Electromyography and transit

Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden), 300-350 g, were anaesthetized with pentobarbital (50 mg.kg-1; Apoteksbolaget, Umeå, Sweden). Through a midline incision three bipolar stainless steel electrodes (SS-5T, Clark Electromedical Instruments, Reading , UK) were implanted into the muscular wall of the small intestine at 5, 20 and 35 cm distal to the pylorus. The animals were provided with a catheter implanted 6 cm from the pylorus for instillation of LPS or a transit marker. All animals also had a jugular vein catheter or an intraperitoneal catheter for administration of LPS and drugs. The electrodes and catheters were tunnelled subcutaneously to exit at the back of the animals' neck. After surgery the animals were allowed to recover for at least 7 days before experiments were undertaken. During recovery the rats were trained to accept experimental conditions. Experiments were then carried out in conscious animals after a 24-h fasting period in wire-bottomed cages with free access to water. During the experiments the rats were placed in Bollman cages. The electrodes were connected to an EEG preamplifier (7P5B) operating a Grass Polygraph 7B (Grass Instruments, Quincy, MA, USA). The time constant was set at 0.015 s and the low and high cut-off frequencies were at 10 Hz and 35 Hz, respectively.

To study effects of LPS on the MMC, all experiments started with a recording of basal myoelectric activity with four propagated activity fronts over all three registration sites. After five activity fronts had passed the first electrode site, the substance to be studied was administered and an effect on the MMC pattern was analysed visually over a period of 90 min. For detailed analysis of the characteristics of activity fronts a computerized method for calculations was employed (*Hellström* et al., 1993). The main characteristic feature of myoelectric activity of the small intestine in the fasted state, the activity front, or phase 3 of the MMC, was identified as a period of at least 1 min with clearly distinguishable intense spiking activity and an amplitude at least twice that of

the preceding baseline, propagating aborally through the recording segment and followed by a period of quiescence, phase 1 of MMC. Phase 2 of MMC was characterized by irregular spiking preceding the activity front. Periods of more than 30 min with spike potentials, but no discernible cyclic activity, were considered as periods of irregular spiking activity.

To study effects on transit, the effect of LPS on the MMC was first established over a period of one hour. Then, 0.4 ml of a transit marker consisting of polyethylene glycol 4000 with 1.48 MBq $Na_2{}^{51}CrO_4$ per ml of marker solution (pH 7.2, 300 mOsm.kg-1) was instilled through the duodenal catheter over a period of 30 s.

Thirty min later the rats were killed with an overdose of pentobarbital. The abdomen was opened and after ligation of the gastroduodenal and ileo-caecal junctions, the small intestine was carefully removed in its entire length. The small intestine was divided into 10 equal segments and analysed for distribution of the radioactive marker in a gamma counter (Beckman, Fullerton, CA, USA). Intestinal transit was quantified by calculating the leading edge of the distribution of the marker in the gut.

Values are given as mean ± standard error of the mean.

Design of the study

In a first session, dose-response relationships for LPS on MMC and transit were investigated. The importance of different administration routes of LPS were studied. LPS was given at doses of 5-160 $\mu g.kg^{-1}$, each as a 0.2 ml bolus intraluminally, intraperitoneally or intravenously. As a control, saline solution (NaCl 154 $mmol.l^{-1}$) of an equal volume given the same routes of administration was used.

In a second session, the effect of LPS at a dose of 20 $\mu g.kg^{-1}$ i.v. on the MMC and transit were investigated in conjunction with different agents that to inhibit NOS pathways. The action of the NOS inhibitors L-NNA or L-NAME on the effect of LPS was studied, as was the effect of dexamethasone which inhibits the inducible form of NOS. L-NNA or L-NAME were both given at a dose of 1 $mg.kg^{-1}$ i.v. bolus plus 0.1 $mg.kg^{-1}.min^{-1}$ i.v. infusion, while dexamethasone was given at a dose of 0.25 $mg.kg^{-1}$ i.v. and 6 h later, LPS was given and the effects on MMC and transit evaluated.

Drugs and chemicals

Lipopolysaccharide, LPS, 055:B5), NG-nitro-L-arginine (L-NNA) and NG-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Dexamethasone was a kind gift from Merck, Sharp and Dohme (MSD, Rahway, NJ, USA). All compounds were diluted in saline before use *in vivo*, except dexamethasone which was supplied as solution.

RESULTS

Effects of lipopolysaccharide

LPS at doses of 5-160 $\mu g.kg^{-1}$ i.v. caused a dose-dependent disruption of the MMC and irregular spiking. At a dose of 20 $\mu g.kg^{-1}$ i.v. LPS inhibited MMC at all registration levels ($p<0.01$). The LPS-induced myoelectric pattern was characterized by the occurrence of successive bursts of spike potentials separated by short quiescent periods. The effect occurred 30 min after LPS administration and remained during the infusion period.

After irregular spiking was estab-

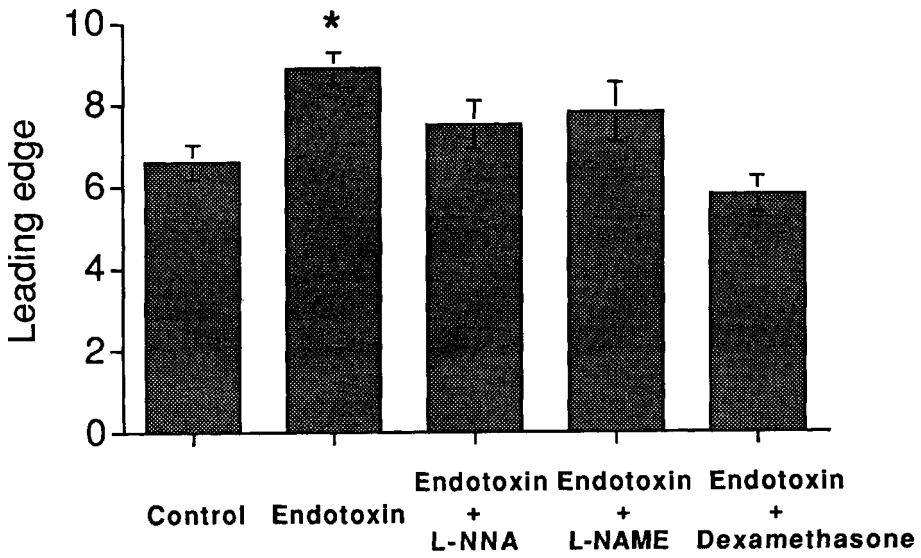

Figure 1: Stimulatory effect of lipopolysaccharide of E. coli (20 mg.kg^{-1} i.v.) on transit of the leading edge of a luminal marker, and its inhibition by the nitric oxide synthase inhibitors L-NNA or L-NAME (1 mg.kg^{-1} + 0.1 mg kg^{-1}.min^{-1}), and by dexamethasone (0.25 mg.kg^{-1}) which inhibits inducible nitric oxide synthase.
* $p<0.05$.

lished with LPS at a dose of 20 mg.kg^{-1} ($p<0.01$) the transit was hastened, as reflected by an increased propagation of the marker in the gut ($p<0.05$) (Figure 1). In all rats diarrhoea was observed ($p<0.01$).

Employing the same dose range for intraduodenal or intraperitoneal administration did not affect the MMC pattern and no diarrhoea was observed.

Inhibition of nitric oxide pathway

Inhibition of NOS with L-NNA or L-NAME inhibited the disruption of the MMC and irregular spiking induced by LPS, and normalized the distribution of the leading edge of the transit marker (Figure 1). No diarrhoea was seen in these two groups of rats.

Inhibition of inducible NOS with dexamethasone efficiently inhibited the effect of LPS on myoelectric activity and normalized the transit of the leading edge of the luminal marker (Figure 1).

Small bowel weight

The dry weight of the small intestine was determined after freeze-drying of the small bowel specimen. The relation dry/wet weight was 0.2±0.04 in control animals and 0.3±0.05 in animals treated with LPS 20 mg.kg^{-1} i.v., indicating an increase of fluid contents after LPS of about 10%.

DISCUSSION

The main finding in the present investigation was that LPS given intravenously at a dose that did not affect the general condition of the animals, induced a complete disorganisation of normal fasting motility, as measured

both directly by electromyographic recordings from chronically implanted electrodes, and indirectly by measurements of the leading edge of a radioactive luminal marker. Thus, LPS besides its great variety of other biological effects, such as inflammation, appears to be capable of affecting small intestinal motility.

A question of major concern was whether the recorded motility alteration could be mediated via an unspecific effect through inflammation, swelling and water accumulation in the tissues. This is unlikely due to the fact that the increase in dry/wet weight ratio of the tissues was only about 10%. Whether this is due to increased intraluminal accumulation of fluid, or intramural oedema could not be determined. However, preliminary data from experiments of the small intestine with extravasation of Evans blue after challenge with LPS do not show any major effect of LPS at this dose on the permeability of the small intestine . Because the increase was only 10% it does not seem likely that this accumulation of fluid could disturb the results of the transit study. Also, the method used for transit studies has been shown to be insensitive to changes in the volume of luminal contents. The distribution of radioactivity is not disturbed by cholera toxin when administered at a dose that causes a prominent increase of intestinal secretion. Finally, the hastened transit registered after LPS is in line with our results in myoelectric recordings.

The mechanism by which the LPS of *E. coli* brings about motility changes seems to involve stimulation of the nitric oxide pathway. Pretreatment of the animals with L-NNA or L-NAME, two different inhibitors of NOS, inhibited the effect of LPS on MMC and reversed the transit effect induced by LPS. Such data speak in favour of NO as a main contributor to the effect of LPS on gut motility. In fact, other studies have shown that NO is an important mediator of reflex relaxation of the stomach (*Desai* et al., 1991, 1991). Further, because also dexamethasone was able to block the effect of NO on MMC and transit, it seems that the involvement of NO in this reaction is mediated through the inducible Ca^{2+}-independent form of NOS (*Rees* et al., 1990; *Gonzalez* et al., 1992). This type of NOS is localized mainly in macrophages, and the induction of the enzyme with liberation of NO may reflect an effect of a primary host defence mechanism (*Rees* et al., 1990). The finding is interesting in view of the fact that NO has been demonstrated to exert cytotoxic actions on various organisms such as bacteria and protozoa, which are known to cause diarrhoea (*Liew* et al., 1990; *Moncada* et al., 1991; *Sherman* et al., 1991).

Since the effect of LPS was restricted to administration via the intravenous route the pathophysiological role of the endotoxin effects is less clear. We can only speculate that in disease states with a disturbed or broken mucosal barrier LPS may be absorbed from the intestinal lumen to the circulation, thereby being able to produce pathophysiological cascade effects through activation of the NO pathway.

ACKNOWLEDGEMENTS

The study was supported by the Swedish Medical Research Council (no. 7916) and by professor Nanna Svartz fond.

LITERATURE

Al-Saffar, A., Hellström, P.M., Nylander, G., and Rosell, S.: Influence of fasting and bombesin-induced myoelectric activity on the transit of small intestinal contents in the rat. Scand. J. Gastroenterol. 19, 541-546 (1984).

Al-Saffar, A., Hellström, P.M., and Nylander, G.: Correlation between peptide YY-induced myoelectric activity and transit of small intestinal contents in the rat. Scand. J. Gastroenterol. 20, 577-582 (1985).

Desai, K.M., Sessa, W.C., and Vane, J.R.: Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. Nature 351, 477-479 (1991).

Desai, K.M., Zembowicz, A., Sessa, W.C., and Vane, J.R.: Nitrergic nerves mediate vagally induced relaxation in the isolated stomach of the guinea pig. Proc. Natl. Acad. Sci. USA 88, 11490-11494 (1991).

Gonzalez, C., Fernandez, A., Martin, C., Moncada, S., and Estrada, C.: Nitric oxide from endothelium and smooth muscle modulates responses to sympathetic nerve stimulation: implications for endotoxin shock. Biochem. Biophys. Res. Comm. 186, 150-156 (1992).

Gyles, C.L.: Escherichia coli cytotoxins and enterotoxins. Can. J. Microbiol. 38, 734-746 (1992).

Hellström, P.M., Bränström, R.O., and Al-Saffar, A.: Computer program "MMC" to summarize characteristics of activity fronts of migrating myoelectric complex in rat small intestine. Surg. Res. Comm. 14, 51-63 (1993).

Leone, A.M., Palmer, R.M.J., Knowles, R.G., Francis, P.L., Ashton, D.S., and Moncada, S.: Constitutive and inducible nitric oxide synthases are NG, CG-dioxygenases. J. Biol. Chem. 226, 23790-23795 (1991).

Liew, F.Y., Millott, A., Parkinson, C., Palmer, R.M.J., and Moncada, S.: Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J. Immunol. 144, 4794-4797 (1990).

Moncada, S., Palmer, R.M.J., and Higgs, E.A.: Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109-142 (1991).

Rees, D.D., Palmer, R.M.J., Schulz, R., Hodson, H.F., and Moncada, S.: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746-752 (1990).

Rees, D.D., Cellek, S., Palmer, R.M.J., and Moncada, S.: Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: An insight into endotoxin shock. Biochem. Biophys. Res. Comm. 173, 541-547 (1990).

Sherman, M.P., Loro, M.L., Wong, V.Z., and Tashkin, D.P.: Cytokine- and Pneumocystis carinii-induced L-arginine oxidation by murine pulmonary alveolar macrophages. J. Protozool. 38, 234S-236S (1991).