

# Effect of bacterial lipopolysaccharide on gastric emptying of liquids in rats

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## Abstract

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The objectives of the present investigation were 1) to study the effect of bacterial lipopolysaccharide (LPS) on rat gastric emptying (GE) and 2) to investigate a possible involvement of the vagus nerve in the gastric action of LPS. Endotoxin from *E. coli* (strain 055:B5) was administered *sc*, *ip* or *iv* to male Wistar rats (220-280 g body weight) at a maximum dose of 50 µg/kg animal weight. Control animals received an equivalent volume of sterile saline solution. At a given time period after LPS administration, GE was evaluated by measuring gastric retention 10 min after the orogastric infusion of a test meal (2 ml/100 g animal weight), which consisted of 0.9% NaCl plus the marker phenol red (6 mg/dl). One group of animals was subjected to bilateral subdiaphragmatic vagotomy or sham operation 15 days before the test. A significant delay in GE of the test meal was observed 5 h after *iv* administration of the endotoxin at the dose of 50 µg/kg animal weight. The LPS-induced delay of GE was detected as early as 30 min and up to 8 h after endotoxin administration. The use of different doses of LPS ranging from 5 to 50 µg/kg animal weight showed that the alteration of GE was dose dependent. In addition, vagotomized animals receiving LPS displayed a GE that was not significantly different from that of the sham control group. However, a participation of the vagus nerve in LPS-induced delay in GE could not be clearly demonstrated by these experiments since vagotomy itself induced changes in this gastric parameter. The present study provides a suitable model for identifying the mechanisms underlying the effects of LPS on gastric emptying.

### Key words

- Gastric emptying
- Lipopolysaccharide
- Vagus nerve

Natural infection or parenterally administered lipopolysaccharide (LPS) of bacterial origin can result in various clinical manifestations in several animal species (1-3). In the gastrointestinal tract, bacterial endotoxins induce severe intestinal mucosal injury frequently accompanied by diarrhea and enteric bacterial translocation (4-6). It has been suggested that vascular damage may play a role in the pathogenesis of LPS-induced acute

diarrhea (4). In addition, LPS at sublethal doses suppresses gastric motility as suggested by the LPS-induced inhibition of gastric emptying of liquids and solids observed in mice and rats (7,8). The mechanisms underlying the toxic actions of endotoxins on gastric emptying remain unexplored.

The aim of the present study was to examine the effect of LPS from *E. coli* on gastric emptying of a saline solution in rats

evaluating the optimum route of administration, dosage and time following administration of the endotoxin. After this preliminary phase of the study, the second part was set up to determine vagal involvement in the LPS-induced alteration of gastric emptying.

Male Wistar rats (SPF, Biotério Central, Universidade Estadual de Campinas, Brazil) aged 8-10 weeks and weighing 220-280 g were employed. The rats were allowed to habituate to the laboratory environment for at least 15 days before any experimental manipulation. The animals were collectively housed and kept under conditions of controlled temperature (22-26°C) and artificial dark-light cycle of 12 h. They were fed on a standard rat chow (Labina, Purina) and received water *ad libitum*.

Before the test day, the rats were transferred to individual cages and subjected to a 20-h fasting period during which only water was available. LPS from *E. coli* (strain 055:B5, Sigma) was dissolved in 0.9% nonpyrogenic sterile NaCl and administered at a maximum dose of 50 µg/kg animal weight. Three different administration routes were employed for LPS injection: intravenous (*iv*) using the tail vein, subcutaneous (*sc*) at the external region of the posterior limbs and intraperitoneal (*ip*). Control injections consisted of an equivalent volume of the appropriate vehicle (1 ml/kg animal weight).

Gastric emptying was evaluated 5 h after parenteral administration of the endotoxin, except in the experiment testing the time dependence of the gastric response to LPS when the measurement of gastric emptying was performed at different times (up to 24 h) after *iv* injection of endotoxin. Gastric emptying was indirectly evaluated by determining the percentage of gastric retention (%GR) of a phenol red-containing test meal recovered within the gastric content as compared to that orally administered (9,10). Gastric retention was measured 10 min after orogastric infusion of the test meal in a vol-

ume of 2 ml/100 g animal weight. The test meal consisted of an aqueous solution of 0.9% NaCl containing 6 mg/dl phenol red as a marker. Saline solution has been considered a suitable test meal to evaluate gastric emptying without intestinal interference since there is no activation of intestinal receptors in contrast to that obtained with nutritive test meals (11).

In addition, a group of animals were subjected to bilateral subdiaphragmatic vagotomy 15 days before the test day (12). Sham surgery was also performed in some animals by executing laparotomy without the section of the vagus (12). Data for animals displaying severe gastric stasis after vagotomy were excluded from data analysis.

Data are reported as means  $\pm$  SEM (number of animals). The Student *t*-test ( $k = 2$ ) or analysis of variance ( $k \geq 3$ ) followed by the Tukey test were used for statistical analysis of the data, with the level of significance set at  $P < 0.05$ . Correlation coefficients were calculated for the dose-response data.

The results of the present study showed that 5 h after *iv* administration of LPS (50 µg/kg animal weight), the treated animals presented a significantly higher percentage of GR of the test meal than the control group (%GR after 10 min, control =  $34.8 \pm 3.2\%$  ( $N = 8$ ), treated =  $63.6 \pm 2.3\%$  ( $N = 8$ );  $P < 0.05$ , *t*-test). In contrast, when the endotoxin was administered *sc* or *ip* using the same experimental protocol as employed above, no statistical difference in GR was observed between the control and treated groups (%GR after 10 min, control *sc* =  $33.2 \pm 2.1\%$  ( $N = 8$ ) vs treated *sc* =  $33.7 \pm 2.2\%$  ( $N = 8$ ); control *ip* =  $33.7 \pm 2.2\%$  ( $N = 8$ ) vs treated *ip* =  $41.1 \pm 2.2\%$  ( $N = 8$ )).

To determine the dose dependence of this LPS-induced alteration of gastric emptying, different doses of LPS (ranging from 5 to 50 µg/kg animal weight) injected only *iv* were tested. As shown in Figure 1A, there was a close correlation between increasing doses of LPS and the intensity of the in-

crease of gastric retention induced by the endotoxin (correlation coefficient,  $r = 0.97$ ). Among the doses tested, 25 and 50  $\mu\text{g}/\text{kg}$  of LPS were effective in significantly increasing GR of a saline solution in rats ( $P < 0.05$  compared to the control group, Tukey test).

Figure 1B shows the time-response effect of the endotoxin. For this set of experiments, GR was determined 0.5, 2, 5, 8 and 24 h after *iv* administration of 50  $\mu\text{g}/\text{kg}$  LPS. The endotoxin induced a rapid and sustained increase in GR of the saline solution since significant differences between the treated group and its respective controls ( $P < 0.05$ , Tukey test) were observed as early as 30 min and up to 8 h after endotoxin administration. However, LPS was not effective in inducing this gastric effect 24 h after its administration (Figure 1B).

In the last part of the present study, we determined whether vagal innervation of the stomach was involved in the LPS-induced delay of gastric emptying. Figure 2 shows the results obtained. When the two subgroups treated with the endotoxin for 5 h were compared, the vagotomized animals showed significantly lower %GR values than the sham-operated animals ( $P < 0.05$ , Tukey test). These data suggest that the vagus might mediate, at least partially, the action of LPS upon gastric emptying. However, vagotomy itself had an effect on GR as indicated by the fact that vagotomized control animals displayed significantly lower %GR values than the sham control group ( $P < 0.05$ , Tukey test).

In conclusion, the present data clearly demonstrate that LPS from *E. coli* induces a significant delay of gastric emptying of an aqueous solution in rats. This alteration of gastric function was closely dependent on the administration route, dosage and time after administration of the endotoxin. This gastric disturbance was observed with considerably lower doses of LPS as compared to those employed in previous studies (7,8). Concerning the vagal involvement in the phenomenon, bilateral vagotomy seems to

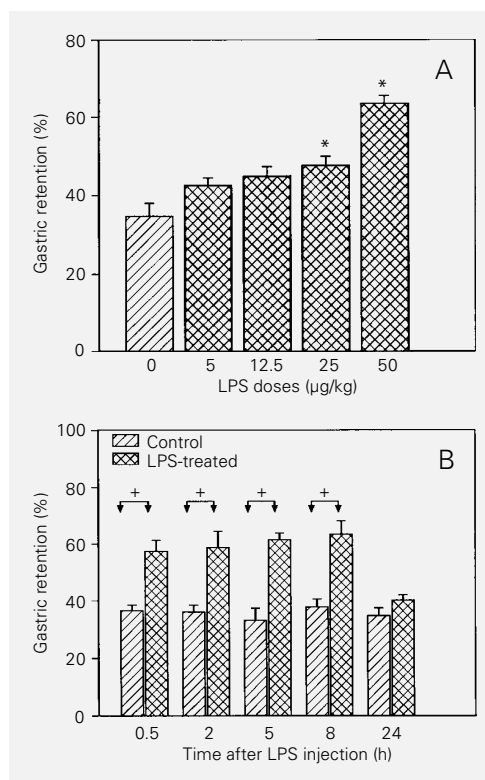


Figure 1 - Effect of *E. coli* lipopolysaccharide on gastric retention of 0.9% NaCl by rats. A, Gastric retention was evaluated 5 h after the *iv* administration of different LPS doses. B, Gastric retention was determined 0.5, 2, 5, 8 and 24 h after *iv* administration of 50  $\mu\text{g}/\text{kg}$  LPS. Control groups received vehicle instead of endotoxin. Data are reported as means  $\pm$  SEM for 8 animals (panel A) or 6 animals (panel B) in each subgroup. \* $P < 0.05$  compared to control group (Tukey test).  $r = 0.97$  for dose-response correlation. + $P < 0.05$  compared to control group (Tukey test).

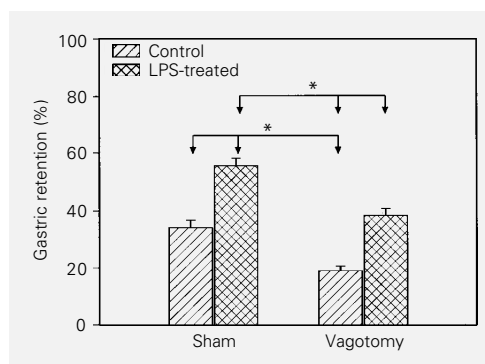


Figure 2 - Effect of vagotomy on the *E. coli* lipopolysaccharide (LPS)-induced increase in gastric retention of 0.9% NaCl solution by rats. The animals were subjected to bilateral subdiaphragmatic vagotomy 15 days before the test. Gastric retention was evaluated 5 h after the *iv* administration of LPS (50  $\mu\text{g}/\text{kg}$  animal weight). The control subgroups received vehicle instead of endotoxin. Data are reported as means  $\pm$  SEM for 8 animals in each subgroup. The %GR values between control and treated groups were significantly different. \* $P < 0.05$  compared to the indicated experimental group (Tukey test).

“normalize” the gastric emptying process in rats treated with LPS since their %GR values were partially restored to control values (Figure 2 compares sham control vs sham-treated and sham control vs vagotomized-treated groups). These data suggest that the vagus nerve may play a role in the endotoxin-induced change in gastric emptying. It is well established that afferent and efferent vagal innervation of the stomach modulates the gastric motility and function via vagal cholinergic excitatory fibers and non-adrenergic

and non-cholinergic inhibitory fibers (13). The faster gastric emptying of liquids observed in the present study after sectioning of the vagus trunk has also been described by others (13). However, the above conclusion drawn from the present data seems not to be straightforward since vagotomy itself had an effect on gastric emptying. In this respect, one may suggest that the sectioning of the vagus nerve does not block the effect of endotoxin on gastric emptying since the injection of LPS induced a two-fold increase in %GR in the vagotomized group (vagotomized control %GR = 19.1%, vagotomized LPS = 38.4%), similar to the values observed in the sham group (sham control %GR = 34.2%, sham LPS = 55.6%).

Further work is needed to identify the exact mechanisms by which the *E. coli* endotoxin affects gastric motility. An attractive possibility is that LPS induces alterations in the paracrine control of gastric emptying via locally released cytokines. For instance, endotoxin-induced intestinal vascular damage is known to be mediated by cytokines such as nitric oxide, platelet-activating factor and thromboxane A<sub>2</sub> (14). Alternatively, the LPS-induced gastric disturbance may be the result of an action of this

bacterial endotoxin on specific areas of the CNS which control some of the gastric functions via the vagus nerve. Several studies have demonstrated that certain hypothalamic nuclei, e.g., the paraventricular (12) and dorsomedial nuclei (15), can modulate the motor activity of the gastrointestinal tract. In addition, several reports have provided evidence for a vagal and central action of LPS. For instance, endotoxin from *E. coli* can induce 1) a fever response in rabbits when administered intracerebroventricularly (16), and 2) activation of hypothalamic histaminergic neurons in rats leading to ACTH release (17). In addition, a recent study has demonstrated that subdiaphragmatic vagotomy can suppress the endotoxin-induced activation of hypothalamic corticotropin-releasing hormone and ACTH secretion (18).

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