Possible involvement of adrenomedullin in lipopolysaccharide-induced small-intestinal motility changes in conscious rats

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Introduction

Gastrointestinal motility disturbances such as vomiting and diarrhea are common manifestations of sepsis. Some in vitro studies have suggested that an endotoxin, lipopolysaccharide (LPS), which is a constituent of the cell wall of gram-negative bacteria, is mainly responsible for these symptoms. It has been shown that adrenomedullin (AM) production is highly augmented in the blood vessels, lung, and intestine by the intravenous administration of LPS and during polymicrobial sepsis. LPS is found to cooperatively augment the gene transcription of AM and inducible nitric oxide synthase in vascular cells, resulting in marked elevation of AM and nitric oxide (NO) production.

Shoji et al. reported that plasma AM concentration in rats was markedly elevated after the intravenous infusion of 0.008 to 5 mg/kg LPS, in a dose-dependent manner. AM is a peptide isolated from human pheochromocytoma tissue, and, structurally, is thought to be a member of the calcitonin gene-related peptide (CGRP) superfamily. Extensive experimental data show AM synthesis in vascular smooth muscle cells. Immunoreactive AM (ir-AM) has been detected in different regions of the gastrointestinal (GI) system: stomach, jejunum, ileum, cecum, and colon. The results of mRNA blot analysis reported 10 years ago and more recently imply that rat AM mRNA can also be expressed in visceral smooth muscle cells of the GI tract.

Shoji et al. reported that plasma AM concentration in rats was marked elevated after the intravenous infusion of 0.008 to 5 mg/kg LPS, in a dose-dependent manner. AM might be responsible for a variety of effects likely to occur in endotoxia, such as hypotension and vasorelaxation. However, in contrast to vascular muscle, there has been little research on the contribution of AM to the regulation of contractile responses in the visceral muscle. Only a few in vitro reports indicate that AM is able to inhibit muscle contraction in the rat colon and ileum.

The present study was carried out (i) to evaluate the effects of AM on the intestinal motor profile in fasted conscious rats.
conscious rats, and (ii) to determine whether the release of endogenous AM induced by LPS contributes to the genesis of intestinal motor disturbances in rats, using AM (22–52), a specific AM receptor antagonist. Contractions were monitored mechanically utilizing miniature strain-gauge force transducers developed specifically for motility studies in rats.

Methods

Materials and animal preparation

Male Wistar rats weighing 250 to 300g were anaesthetized with intraperitoneal sodium pentobarbital (Nembutal; 35 mg/kg, Abbott Laboratories, USA). Laparotomy was performed via a midline incision, and a miniature strain-gauge force transducer (FT-08; 4 x 8 mm; Star Medical, Tokyo, Japan) was sutured to the serosal surface of the small intestine 5 cm distal to the ligament of Treitz along the circular axis. Lead wires extending through the abdominal wall were exteriorized through a subcutaneous tunnel, exiting on the top of the head through a skin incision to terminate in a metallic spring sutured to the scalp. An antibiotic was given subcutaneously (cefmetazole, 100 mg/kg; Sankyo, Tokyo, Japan) after the operation. During the 7-day recovery period, rats were fed on laboratory chow and received water ad libitum. The transducer lead wires were then connected to an amplifier. Electrical signals were fed into a computer through an analog/digital converter board and were analyzed using a software package (Star Medical). Each rat was used in only one experiment, and AM or LPS was never given twice to the same rat.

Study protocol 1

Rats were fasted for 30 h prior to each experiment, and kept separately in special cages with wire mesh to facilitate fecal disposal and prevent coprophagy. They were then placed in cylindrical enclosures to maintain placement of an intravenous line inserted in the tail vein. To examine the effect of AM on the motility of the small intestine, either AM (Sigma, St. Louis, MO, USA; 1, 3, 6, and 10 μg/kg per min) or a control solution (0.9% NaCl) was given intravenously over 30 min after 3 h of prerecording. Small-intestinal contractions were recorded for 5 h after giving AM. Six rats were used for each dose of drugs.

Study protocol 2

To confirm that the possible motility changes induced by AM were mediated by AM receptors, AM (22–52) (Bachem, Bubendorf, Switzerland), a selective AM receptor antagonist, was given 5 min before AM intravenous administration.

Study protocol 3

The aim of protocol 3 was to determine whether AM directly affects intestinal motility or whether it acts through possible systemic hypotension resulting in motility changes. First, the mean blood pressure of rats was continuously monitored by tail-artery catheterization, while rats were given either AM (3 and 10 μg/kg per min) or a control solution (0.9% NaCl). Secondly, to investigate small-intestinal motility during hypotension, three conscious rats were subjected to hypovolemic shock, with a 60-mmHg decrease in mean arterial blood pressure brought about by the aspiration of blood from the tail artery. Intestinal motility was simultaneously recorded by the same method as that described in study protocol 1.

Study protocol 4

The aim of study protocol 4 was to investigate the possible involvement of AM in the small-intestinal motility changes induced by LPS. Four rats received LPS (from Escherichia coli 0111: B4; Sigma; 50 μg/kg), while other rats were pretreated with either a 50-μg/kg intravenous injection of AM (22–52) (n = 6), a selective AM receptor antagonist, or CGRP (8–37) (Peptide Institute, Osaka, Japan; 3 nmol/kg), a selective antagonist of the CGRP1 receptors (n = 6), followed by an intravenous injection of LPS 5 min later.

Data analysis

The recording of small-intestinal contractions was modified by computer software to exclude artifacts such as respiration and body movements. The number of contractions, the mean amplitude, and the motility index (MI) were determined from these modified recordings. The MI was defined as the area under contraction curves in each 30-min recording and was expressed as the ratio to the MI of the preadministration period, which was the mean MI obtained during the 1-h period prior to drug administration. Motility parameters obtained after drug administration were expressed as ratios to those obtained prior to drug administration. Effects of drugs on motility were also evaluated, by measuring the time elapsed from the injection to the recovery of a normal pattern, i.e., the migrating motor complex (MMC) pattern, by three individual experts who were unaware of the doses of the drugs. Data values were expressed as means ± SE, and statistical analy-