Regulatory Peptides 190–191 (2014) 12–17

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Urocortin 2 blocks the suppression of gastric antral contractions induced by lipopolysaccharide in freely moving conscious rats

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ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form 2 April 2014
Accepted 22 April 2014
Available online 2 May 2014

Keywords:
Lipopolysaccharide
IL-1
Urocortin 2
CRF receptor
Peripheral
Gastric contractions

ABSTRACT

Lipopolysaccharide (LPS) inhibits gastric antral contractions in conscious rats. Since LPS regulates corticotropin-releasing factor type 2 receptor (CRF2) expression in the rat stomach, and activation of peripheral CRF2 alters gastric motility, we tried to determine the role of peripheral CRF2 in the LPS-induced suppression of gastric antral contractions. Intraluminal gastric pressure waves were measured in freely moving conscious non-fasted rats using the perfused manometric method. We assessed the area under the manometric trace as the motor index (MI), and compared this result with those obtained 1 h before and after intraperitoneal injection of drugs. LPS (0.2 mg/kg) significantly decreased MI. Indomethacin (10 mg/kg) itself did not alter MI but blocked this inhibitory action by LPS. Astressin 2-B (200 μg/kg), a selective CRF2 antagonist, modified neither the basal MI nor the action by LPS. Meanwhile, urocortin 2 (30 μg/kg), a selective CRF2 agonist, reversed the suppression by LPS without affecting the basal MI. This action by urocortin 2 was blocked by pretreatment with astressin 2-B. In conclusion, LPS inhibited gastric antral contractions possibly through a prostaglandin-dependent pathway. Peripheral CRF2 stimulation reversed this response by LPS.

1. Introduction

Bacterial lipopolysaccharide (LPS) is known to be a causative factor of sepsis, which induces systemic inflammatory response syndrome and disturbance of gastrointestinal (GI) motility [1]. One major issue during sepsis is delayed GI motility, clinically recognized as paralytic ileus. Systemic administration of LPS delays gastric emptying (GE) [2–4] and we recently have demonstrated that LPS suppresses gastric antral contractions in conscious rats [5], suggesting that LPS is thought to be one of the possible causes of this GI disturbance.

Stress including infection alters GI functions [6,7] and corticotropin-releasing factor (CRF) is the main mediator of these responses to stress [8]. CRF exerts its action through the activation of two receptors, CRF receptor type 1 (CRF1) and type 2 (CRF2) in not only the central nervous system but also the peripheral tissues including GI tract [9,10]. Stimulation of each CRF receptor induces distinct changes in GI functions, i.e., inhibiting GE and stimulation of colonic motility by CRF2 and CRF1, respectively [7]. In addition to CRF, urocotins (Ucns; Ucn 1, Ucn 2 and Ucn 3), which are also capable of binding to CRF receptors with distinct affinities for each CRF receptor subtype, were shown to be prominently expressed in peripheral tissues where they mediate visceral stress responses [11,12]. Meanwhile, infection and inflammation affect the expression of CRF receptors in GI tract [13], and it has been also demonstrated that LPS increases Ucns mRNA and regulates CRF2 receptor expression in the rat stomach [4], suggesting that LPS induces peripheral CRF2 signaling.

These lines of evidence raise the possibility that LPS-induced suppression of gastric contractions is mediated through peripheral CRF2 signaling. In the present study, we measured gastric antral contractions using the perfused manometric method in freely moving conscious rats in order to test this possibility and the mechanisms of LPS action were also discussed. We studied 1) whether indomethacin blocks this LPS action to clarify the role of prostaglandin (PG) and 2) whether peripheral CRF2 agonist/antagonist modifies the action by LPS.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing about 200–250 g were housed under controlled light/dark conditions (lights on 07:00–19:00) with the room temperature regulated to 23–25 °C. Rats were allowed free access to standard rat chow (Solid rat chow, Oriental Yeast, Tokyo, Japan) and tap water. Experiments started between 8 and 10 AM and finished no later than 3 PM.

Abbreviations: LPS, lipopolysaccharide; IL-1, interleukin-1; CRF, corticotropin-releasing factor; Ucn, urocortin; GE, gastric emptying; GI, gastrointestinal; AUT, area under the manometric trace; MI, motor index.

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http://dx.doi.org/10.1016/j.regpep.2014.04.004
0167-0115/

2.2. Chemicals

LPS (from *Escherichia coli*, serotype 055:B5, Sigma-Aldrich, St. Louis, MO, USA), interleukin-1β (IL-1β, Wako Pure Chemical Industries, Osaka, Japan) and human Ucn 2 (Bachem AG, Bubendorf, Switzerland) were dissolved in sterile saline. Astressin 2-B (Sigma-Aldrich) and indomethacin (Sigma-Aldrich) were dissolved in sterile water and 1% sodium bicarbonate-saline solution, respectively. The doses of the chemicals were determined according to the previous reports [5, 14–16].

2.3. Animal preparation

Intraluminal gastric pressure waves were measured in freely moving conscious non-fasted rats using the perfused manometric method, which had been already well established to measure GI contractions as described in the recent publications [5,15,17–20]. After overnight fasting, an open-tipped catheter (3-Fr, 1 mm internal diameter, Atom, Tokyo, Japan) for manometric measurement was inserted through a small hole produced by an 18-G needle into the gastric antrum (recording point). The catheter was fixed by purse-string sutures at the point of exit from the gastric wall, and it passed through the abdominal wall musculature and a subcutaneous (sc) tunnel to exit at the back of the neck. The rats were allowed to recover in individual cages for 4–6 days before the experiments.

2.4. Manometric recordings and study design

Non-fasted conscious rats were placed in the wire-bottom and non-restraint polycarbonate cages. The manometric catheter from each animal was threaded through a flexible metal sheath to protect it from biting and connected to an infusion swivel (Instech Laboratories, Plymouth Meeting, PA, USA) to allow free movement. The catheter was infused continuously with degassed distilled water at a rate of 1.5 ml/h using a heavy-duty pump (CVF-3100, Nihon Kohden, Tokyo, Japan) and was connected to a pressure transducer (TP-400 T, Nihon Kohden). Pressure signals from the transducer were amplified, filtered (20 Hz) and digitized by a PowerLab system (AD Instruments, Colorado Springs, CO, USA), and stored by computer software (LabChart 7, AD Instruments). First, the basal state of the gastric pressure waves was measured for 1 h after 1 h of stabilization period. Then, the catheter was disconnected and the rats were taken out from polycarbonate cages to receive chemicals. The rats were anesthetized with ether and received intraperitoneal (ip) injection(s) of each chemical in a 0.2-ml volume. In order to determine the mechanisms of LPS or IL-1β-induced suppressed gastric antral contractility, chemical was administered 10 min [vehicle, astressin 2-B (200 μg/kg) or Ucn 2 (15, 30, 60 μg/kg)] or 60 min [vehicle or indomethacin (10 mg/kg)] prior to ip LPS (0.2 mg/kg) or IL-1β (2 μg/kg). Meanwhile, to test the effect of indomethacin on the basal contractions, the rats received single ip injection. After the administration, the animals were returned to the cages again and the catheter was re-connected to a pressure transducer. The pressure waves were monitored for up to 2 h after administration of drug. Using the recordings, we evaluated the motor index (MI) to assess gastric motor activity as described below.

2.5. Evaluation of the MI

The MI ($cmH_2O \cdot sec$) was determined by the area under the manometric trace (AUT). AUT was calculated using software (LabChart 7, AD Instruments). The basal MI was determined by calculating AUT for the 1 h period before drug administration. The % MI was calculated by the following formula: (AUT for the first 1 h or the second 1 h period after drug administration) / (basal MI) × 100. For multiple injections, the basal MI was set as AUT for the 1 h period before the first drug administration. The drawback of this manometric method is that pressure data is able to be modified by movements of the animals. Indeed the animals moved frequently at the start of measuring, but usually within 15 min, they stopped moving and stably stayed in the bottom of the cages. After that, the baseline drifting and recording noise due to movements of the animals were minor. In this experiment, pressure signals were recorded continuously until the end of the experiment, but the measurements were stopped briefly in order to perform ip injection(s) under ether anesthesia. In relation to injection(s), time for re-stabilization of baseline of manometric trace was required in order to obtain adequate recordings for the analysis. Therefore, the manometric data during this period for approximately 5–10 min were excluded from later analysis.

2.6. Statistical analysis

Data were expressed as means ± S.E. Multiple comparison was performed by one-way analysis of variance (ANOVA) followed by Tukey's Honestly-Significant-Difference Test. Comparison between two groups was performed using the Student's t or paired *t* test. SYSTAT 13 software (Systat Software, Chicago, IL, USA) was used throughout the study.

2.7. Ethical considerations

Approval by the Research and Development and Animal Care Committees at the Asahikawa Medical University (#11042, approved on March 7, 2011) was obtained for all studies.

3. Results

Before examining the effect of indomethacin on LPS-induced suppressed gastric antral contractions, we tested whether indomethacin itself modifies the basal contractions. Indomethacin (10 mg/kg, ip) did not alter them significantly for the first 1 h period after injection (% MI 95.5 ± 7.3% for vehicle, n = 8, vs. 105.2 ± 11.1% for indomethacin, n = 11, $p > 0.05$), and moreover, it did not change MI in the next 1 h period either (100.1 ± 5.3% for vehicle, vs. 99.2 ± 8.1% for indomethacin, $p > 0.05$). % MI was not different between the first 1 h and the next 1 h period in both indomethacin and vehicle-treated rats ($p > 0.05$).

We had already shown that ip LPS at a dose of 0.2 mg/kg suppresses gastric antral contractions, and this effect is observed immediately after injection and continues more than 60 min [5]. Therefore, % MI change for the first 1 h period after administration of LPS was selected for all the following experiments and analyses similar to our previous study [5]. In order to test the effect of indomethacin, we injected it 1 h prior to ip LPS according to the previous report [21] and the above result that indomethacin did not change MI in the first and the next 1 h period after injection.

Indomethacin blocked the LPS-induced suppressed antral contractions. Demonstrable recordings are shown in Fig. 1A. LPS decreased MI (Fig. 1B, ANOVA: $F = 16.4$, $p < 0.05$, % MI 97.7 ± 5.4% for vehicle + vehicle, n = 7, vs. 51.8 ± 6.4% for vehicle + LPS, n = 9, $p < 0.05$), and indomethacin itself did not change it (100.1 ± 5.8% for indomethacin + vehicle, n = 9, vs. vehicle + vehicle, $p > 0.05$). However, indomethacin reversed the reduced MI induced by LPS (102.7 ± 6.2% for indomethacin + LPS, n = 11, vs. vehicle + LPS, $p < 0.05$).

Next, we tested the effect of astressin 2-B (200 μg/kg, ip), a selective CRF2 antagonist. This antagonist neither altered the basal contractions nor LPS-induced suppressed antral contractions. Demonstrable recordings are shown in Fig. 2A and B shows % MI change (ANOVA: $F = 10.4$, $p < 0.05$, % MI 104.1 ± 7.8% for vehicle + vehicle, n = 6, vs. 99.0 ± 6.4% for astressin 2-B + vehicle, n = 5, $p > 0.05$, 52.0 ± 4.4% for vehicle + LPS, n = 7, vs. 54.8 ± 5.1% for astressin 2-B + LPS, n = 6, $p > 0.05$).

Then, we tested the effect of a selective CRF2 agonist, Ucn 2 on LPS-induced suppressed antral contractions. This peptide (60 μg/kg, ip) itself