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Tsukasa Nozu [1], Saori Miyagishi [2], Shima Kumei [3], Rintaro Nozu [1], Kaoru Takakusaki [4], Toshikatsu Okumura [2]

[1] Department of Regional Medicine and Education, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido, 078-8510, Japan

[2] Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido, 078-8510, Japan

[3] Department of General Medicine, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido, 078-8510, Japan

[4] Research Center for Brain Function and Medical Engineering, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido, 078-8510, Japan

Address for corresponding:

Tsukasa Nozu, MD, PhD, FACP, FJSIM

Department of Regional Medicine and Education, Asahikawa Medical University,
2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido, 078-8510, Japan

Ph; +81-166-68-2844

Fax; +81-166-68-2846

e-mail; tnozu@sea.plala.or.jp

Disclosure statement

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Background and Aim: A glucagon-like peptide-1 (GLP-1) analog, liraglutide, has been reported to block inflammatory somatic pain. We hypothesized that liraglutide attenuates lipopolysaccharide (LPS)- and repeated water avoidance stress (WAS)-induced visceral hypersensitivity and tested the hypothesis in rats.

Methods: The threshold of the visceromotor response (VMR) induced by colonic balloon distention was measured to assess visceral sensation. Colonic permeability was determined in vivo by quantifying the absorbed Evans blue spectrophotometrically, which was instilled in the proximal colon for 15 min. The interleukin-6 (IL-6) level in colonic mucosa was also quantified using ELISA.

Results: Subcutaneously (s.c.) injected LPS (1 mg/kg) reduced the VMR threshold after 3 h. Liraglutide (300 μg/kg s.c.) at 15 h and 30 min before injecting LPS eliminated LPS-induced allodynia. It also blocked the allodynia induced by repeated WAS for 1 h for 3 consecutive days. Neither vagotomy nor naloxone altered the antinociceptive effect of liraglutide, but N^G-nitro-L-arginine methyl ester, a nitric oxide (NO) synthesis inhibitor, blocked it. LPS increased colonic permeability and the IL-6 level, and the analog significantly inhibited these responses.

Conclusions: This study suggests that liraglutide blocked LPS-induced visceral allodynia, which may be a NO-dependent response, and was probably mediated by inhibiting proinflammatory cytokine production and attenuating the increased gut permeability. Because the LPS-cytokine system is considered to contribute to altered visceral sensation in irritable bowel syndrome, these results indicate the possibility that liraglutide can be useful for treating this disease.

Key words: Liraglutide, Visceral pain, Colonic permeability, lipopolysaccharide, Water avoidance stress

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by the presence of recurrent or chronic abdominal pain with altered bowel habits without any organic cause.[1] Disturbed gut motility and altered visceral sensory function play important roles in IBS pathophysiology.[2]

The importance of the immune system activation in the development of IBS is well recognized.[3, 4] There is evidence that plasma proinflammatory cytokines and serum lipopolysaccharide (LPS) levels are elevated,[5, 6] and increased gut permeability with minor mucosal inflammation has been identified in IBS.[7, 8]

LPS-induced stimulation of cytokine release from peripheral blood mononuclear cells is enhanced in IBS, and greater severity of symptoms, such as urgency and diarrhea, is associated with a higher cytokine response induced by LPS.[9] We also recently demonstrated that LPS induced visceral allodynia via the interleukin (IL)-1 and IL-6 pathways in rats. Therefore, LPS-cytokine pathways may contribute to visceral hypersensitivity in IBS, and thus, we advocated that this visceral sensory response by LPS could be used for an experimental animal model of IBS.[10]

Glucagon-like peptide-1 (GLP-1), a gut-derived hormone, is released from intestinal L cells and potentiates glucose-dependent insulin release by activating GLP-1 receptors located in pancreatic β cells.[11] GLP-1 receptors are expressed in various tissues such as neurons and gastrointestinal tract[12, 13] and display a wide variety of physiological activities.

Activating GLP-1 receptors in immune cells reduces the production of proinflammatory cytokines.[14, 15] In addition, a GLP-1 analog was demonstrated to attenuate inflammation- and peripheral nerve injury-induced somatic pain, among others.[16, 17] In this context, a GLP-1 analog may be beneficial for treating IBS via its antinociceptive effect and antiinflammatory activity.

In this study, we attempted to determine the effect of liraglutide, a GLP-1 analog, on LPS- and repeated water avoidance stress (WAS)-induced visceral allodynia, which are considered to be features appropriate for establishing experimental animal models of IBS[10, 18] to examine the above possibility.

Materials and Methods

Animals. Adult male Sprague Dawley rats (Charles River Laboratory, Atsugi, Japan) weighing approximately 300 g were used. The animals were housed in groups under controlled conditions of illumination (12-h light/dark cycle starting at 7 a.m.) and temperature at 23-25°C with food (Solid rat chow, Oriental Yeast, Tokyo, Japan) and water available ad libitum.

Chemicals. LPS obtained from Escherichia coli with the serotype 055:B5 (Sigma-Aldrich, St. Louis, MO, USA); liraglutide (Novo Nordisk, Bagsvaerd, Denmark), naloxone hydrochloride, an opioid receptor antagonist; N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthesis inhibitor; and IL-1β (Wako Pure Chemical Industries, Osaka,

Japan) were dissolved in normal saline. The chemical doses were determined according to previous studies.[10, 19, 20]

Measuring visceral sensation. Visceral sensation was assessed by colonic distention-induced abdominal muscle contractions (visceromotor response; VMR) using electromyogram (EMG) in conscious rats.[10, 21, 22]

Implanting electrodes and placing colonic distention balloon. Under brief ether anesthesia, the electrodes (Teflon-coated stainless steel, 0.05-mm diameter, MT Giken, Tokyo, Japan) were inserted approximately 2 mm into the left external oblique musculature via a small skin incision. They were fixed to the musculature by cyanoacrylate instant adhesive together with the incised skin. The electrode leads were directly externalized through this closed incision without a subcutaneous tunnel. A distension balloon (6-Fr disposable silicon balloon urethral catheter, JU-SB0601; Terumo Corporation, Tokyo, Japan) was intra-anally inserted, with the distal end positioned 2 cm proximal to the anus. Analgesics were not administered after the surgery.

Colonic distention and measuring abdominal muscle contractions. After completing electrode implantation and balloon placement, the rats were placed in Bollmann cages and acclimated to experimental conditions for 30 min before testing. The electrode leads were then connected to an EMG amplifier, and EMG signals were amplified, filtered (3000 Hz),

digitized by a PowerLab system (AD Instruments, Colorado Springs, CO, USA) and recorded using a computer software (LabChart 7; AD Instruments). Colonic distension was performed 30 min after the surgery, as previously described,[10, 22] namely, the ascending method of limits paradigm with phasic distensions was applied by manually inflating the balloon with water using a syringe, and the distention increased progressively in 0.1 ml steps for 5 s until significant sustained abdominal muscle contractions, i.e., VMR, were detected (Fig. 1a). The VMR threshold was defined as the distended balloon volume (ml) that induced VMR. Tang et al.[23] previously demonstrated that colorectal distention-induced pain threshold, assessed by observing abdominal withdrawal reflex using a balloon quite similar to ours, could be determined as the distended balloon volume in rats and could also show that intracolonic pressure was linearly associated with intraballoon volume. The threshold was assessed twice (2-min interval), and the threshold mean was calculated as the data of the animals. The percentage change threshold, i.e., the threshold value after drug administration divided by the basal threshold value and multiplied by 100, was also calculated.

Experimental procedures. First, the basal VMR threshold was measured. The electrodes and distention balloon were then removed, and either the vehicle or LPS at a 1-mg/kg dose was subcutaneously (s.c.) injected. The rats were returned to their home cages, and after 2.5 h, they again underwent surgery for electrode implantation and balloon placement. The second measurement of threshold was performed 3 h after the injection. The vehicle or

liraglutide at a 300-μg/kg dose was s.c. injected twice at 15 h and 30 min before injecting LPS (Fig. 1b).

Next to explore the mechanisms of action by liraglutide, the effects of vagotomy, naloxone (1 mg/kg s.c.) and L-NAME (10 mg/kg intraperitoneally) were examined. These drugs were administered twice together with liraglutide or the vehicle.

The effect of liraglutide on repeated WAS-induced allodynia was also assessed. First, the basal threshold was measured, and either WAS or sham stress was applied for 1 h. The animals were daily subjected to a 1-h stress session for 3 consecutive days. The threshold was again measured at 24 h after undergoing the last stress session. This repeated WAS protocol previously successfully induced visceral allodynia in rats.[22] Liraglutide or the vehicle was s.c. injected twice at 15 h and 30 min before measuring the second threshold (Fig. 1c).

The effect of liraglutide on IL-1β (10 μg/kg s.c.)-induced allodynia was also evaluated. The basal VMR threshold was measured, and either the vehicle or IL-1β was injected. The second threshold measurement was performed 3 h after the injection. We recently showed that this protocol consistently induced visceral allodynia in rats.[10] Liraglutide or the vehicle was s.c. injected twice at 15 h and 30 min before injecting IL-1β.

Vagotomy. Subdiaphragmatic vagotomy was performed by circular seromuscular myotomy of the esophagus at 2 cm proximal from the gastroesophageal junction under ether

anesthesia.[24] Sham-vagotomized rats underwent laparotomy without esophagus myotomy. After 5-6 days, the rats were subjected to the study.

Stress protocol. Exposure to WAS was performed as previously described.[25] Rats were individually placed on a plastic platform (height, 8 cm; length, 6 cm; width, 6 cm) positioned in the middle of a plastic cage filled with water up to 7 cm of the platform height. Control animals were also placed in the same plastic cage, but the cage was not filled with water (sham stress).

Measuring colonic permeability. Colonic permeability measurement was performed as previously described with minor modifications.[26] The permeability was determined 5 h after injecting LPS.

The rats were anesthetized, and laparotomy was performed. The colon was ligated at the junction with the cecum, and an open-tipped catheter was inserted in the proximal colon and secured by a ligature. Using a catheter, the colon was gently flushed with phosphate-buffered saline (PBS) until all stools were washed out. Then, another ligation was added on the colon at approximately 4 cm from the junction with the cecum, and 1 ml of 1.5 % Evans blue (Sigma-Aldrich) in PBS was instilled into the colon. After15 min, the rats were killed, and the colons were excised and washed with PBS. Then, the colons were opened and placed in 2 ml of N,N-dimethylformamide for 12 h. Permeability was

calculated by measuring the Evans blue concentration in the supernatant using a spectrophotometer at 610 nm.

Quantifying IL-6 in the colon using enzyme-linked immunosorbent assay. The rats were killed, and a 2-cm length of the proximal colon was excised. The sample was flushed by cold PBS and cut along the antimesenteric border. Then, the mucosa was carefully scraped using glass slides and homogenized in ice-cold lysis buffer (RayBiotech, Norcross, GA, USA) with the protease inhibitor cocktail (RayBiotech). Homogenates were centrifuged at 4°C for 15 min at 2000 g, and the resulting supernatant was then obtained. Protein determination was performed using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). For measuring IL-6 level, ELISA kits (RayBiotech) were used as per the manufacturer's protocols. The cytokine levels were expressed as pg/mg protein and determined 4 h after injecting LPS.

Statistical analysis. Data are expressed as means ± standard error. Multiple comparisons were performed by two-way analysis of variance followed by Tukey's honestly significant difference test. Comparisons between two groups were performed using Student's t- or paired t-test. The SYSTAT 13 software (Systat Software, Chicago, IL, USA) was used for the study.

Ethical considerations. For all studies, approval was obtained from the Research and Development and Animal Care Committees at Asahikawa Medical University (#15132, approved on April 1, 2015).

Results

Liraglutide eliminated LPS-induced visceral allodynia. Liraglutide treatment per se did not induce any effect on the basal threshold (ml), i.e., before injecting LPS or the vehicle (0.53 ± 0.020, $n = 13$ for liraglutide vs. 0.53 ± 0.022, $n = 12$ for the vehicle; $p > 0.05$). LPS significantly reduced the VMR threshold ($p < 0.05$), while the vehicle did not alter the threshold. Liraglutide per se did not modify the threshold change but blocked LPS-induced visceral allodynia (Fig. 2a).

After calculating the percentage change threshold, liraglutide reversed the decreased threshold by LPS without altering the threshold change in vehicle-treated rats (effect of LPS: $F = 18.1$, $p < 0.05$; effect of liraglutide: $F = 16.3$, $p < 0.05$; interaction between LPS and liraglutide: $F = 8.1$, $p < 0.05$; Fig. 2b).

Liraglutide blocked repeated WAS-induced visceral allodynia. WAS reduced the threshold, and injecting liraglutide after a stress session blocked this response without affecting the threshold change in sham-stressed rats (effect of WAS: $F = 5.4$, $p < 0.05$; effect of liraglutide: $F = 22.1$, $p < 0.05$; interaction between WAS and liraglutide: $F = 22.7$, $p < 0.05$; Fig. 3).

Vagotomy or naloxone did not modify the antinociceptive effect of liraglutide. Vagotomy per se did not change the basal threshold (ml; 0.53 ± 0.027 for vagotomy, n = 10 vs. 0.53 ± 0.027 for sham vagotomy, n = 11; $p > 0.05$) and the response to LPS (effect of vagotomy: $F = 0.0$, $p > 0.05$; effect of LPS: $F = 16.0$, $p < 0.05$; interaction between vagotomy and LPS: $F = 0.003$, $p > 0.05$; % change 71.9 ± 7.0 for sham vagotomy + LPS, n = 6 vs. 72.3 ± 7.8 for vagotomy + LPS, n = 5; $p > 0.05$).

Next, we determined the effect of vagotomy on the antinociceptive effect of liraglutide on LPS-induced allodynia. Vagotomy did not alter the effect of liraglutide (effect of vagotomy: $F = 0.04$, $p > 0.05$; effect of liraglutide: $F = 21.0$, $p < 0.05$; interaction between vagotomy and liraglutide: $F = 1.82$, $p > 0.05$; Fig. 4a).

Naloxone also did not alter the basal threshold (ml; 0.53 ± 0.013 for naloxone, n = 12 vs. 0.52 ± 0.021 for vehicle, n = 12; $p > 0.05$). Moreover, it did not modify the response to LPS (effect of naloxone: $F = 0.013$, $p > 0.05$; effect of LPS: $F = 29.8$, $p < 0.05$; interaction between naloxone and LPS: $F = 0.39$, $p > 0.05$; % change 71.0 ± 5.6 for vehicle + LPS, n = 7 vs. 68.1 ± 5.1 for naloxone + LPS, n = 7; $p > 0.05$).

In the following experiment, the impact of naloxone on the antinociceptive effect of liraglutide was explored, which was not altered (effect of naloxone: $F = 0.012$, $p > 0.05$; effect of liraglutide: $F = 19.1$, $p < 0.05$; interaction between naloxone and liraglutide: $F = 0.012$, $p > 0.05$; Fig. 4b).

L-NAME reversed the antinociceptive effect of liraglutide. L-NAME did not change the basal threshold (ml; 0.53 ± 0.021 for L-NAME, n = 10 vs. 0.53 ± 0.017 for vehicle, n = 13; $p > 0.05$), and it did not modify the LPS response (effect of L-NAME: $F = 0.11$, $p > 0.05$; effect of LPS: $F = 45.6$; $p < 0.05$, interaction between L-NAME and LPS: $F = 0.0010$, $p > 0.05$; % change 67.2 ± 5.2 for vehicle + LPS, n = 6 vs. 68.7 ± 4.7 for L-NAME + LPS, n = 5; $p > 0.05$).

Next, we assessed the impact of L-NAME on the antinociceptive effect of liraglutide, which was eliminated by the drug (effect of L-NAME: $F = 6.13$, $p < 0.05$; effect of liraglutide: $F = 9.3$, $p < 0.05$; interaction between L-NAME and liraglutide: $F = 6.8$, $p < 0.05$; Fig. 5).

Liraglutide did not alter IL-1β-induced visceral allodynia. IL-1β induced visceral allodynia at 3 h after injection, and liraglutide did not alter this response (effect of IL-1β: $F = 44.6$, $p < 0.05$; effect of liraglutide: $F = 0.074$, $p > 0.05$; interaction between IL1-β and liraglutide: $F = 0.0020$, $p > 0.05$; Fig. 6).

Liraglutide attenuated LPS-induced increased colonic permeability. LPS significantly increased colonic permeability (μg/g tissue) and liraglutide attenuated this response without affecting the basal permeability (effect of liraglutide: $F = 4.2$, $p < 0.05$; effect of LPS: $F = 54.0$, $p < 0.05$; interaction between liraglutide and LPS: $F = 6.6$, $p < 0.05$; Fig. 7).

Liraglutide inhibited LPS-induced increased IL-6 levels in colon. LPS significantly increased colonic IL-6 levels (pg/mg protein) and liraglutide inhibited this response (effect of liraglutide: $F = 7.7$, $p < 0.05$; effect of LPS: $F = 7.0$, $p < 0.05$; interaction between liraglutide and LPS: $F = 6.7$, $p < 0.05$; Fig. 8).

Discussion

Several studies have indicated that a GLP-1 analog provokes antinociceptive effect against somatic pain.[16, 17] Conversely, evidence showing the effect of such an analog on visceral sensation is scarce. One study very recently demonstrated that exendin-4, a GLP-1 analog, attenuated neonatal visceral hyperalgesia induced by intracolonic infusion of acetic acid in rats.[27] In the current study, liraglutide eliminated both LPS- and repeated WAS-induced visceral allodynia, further confirming the antinociceptive effect of a GLP-1 analog on visceral sensation.

Likewise, the mechanisms behind the antinociceptive effect of a GLP-1 analog on visceral pain have not been well determined. The analog reportedly stimulates the release of β-endorphin from spinal microglia, thereby inducing antinociception against somatic pain.[16, 17] Incidentally, GLP-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms.[28] Therefore, the effects of vagotomy and naloxone were evaluated in this study, but they did not modify the effect of liraglutide.

In contrast, L-NAME eliminated the effect of liraglutide. Several studies have demonstrated that NO exerts an antinociceptive effect. Peripheral or central injection of a

NO donor, such as sodium nitroprusside, or L-arginine, the substrate for NO generation, induced an analgesic effect on paw hyperalgesia and in the acetic acid abdominal constriction test in rodents.[29, 30] The mechanism of antinociceptive effect by NO has been suggested by electrophysiological studies that indicate that NO induces cyclic guanosine monophosphate generation to open ATP-sensitive K^+ channels, thereby hyperpolarizing nociceptive neurons.[31]

Incidentally, GLP-1 inhibits the electrically evoked cholinergic contractions of colonic circular smooth muscles in mice, which is reduced by L-NAME;[32] moreover, liraglutide ameliorates renal injury in diabetic rats by activating endothelial nitric oxide synthase (NOS).[33] These lines of evidence suggest that activating GLP-1 receptors produce NO, thereby exerting an antinociceptive effect.

There is accumulating evidence that compromised gut barrier function manifested by increased gut permeability, resulting from impaired tight junction (TJ), is observed in at least a proportion of patients with IBS.[34] Several studies have shown that TJ proteins such as zonula occludens-1 were reduced in the gut of patients with IBS,[35, 36] and LPS could mimic this change, resulting in increased gut permeability,[37] thereby inducing bacterial translocation and mucosal inflammation with increased proinflammatory cytokines.[38] This is considered to be an important aspect of IBS pathophysiology and associated visceral hypersensitivity.[2, 10, 39] Animal models have shown that increased intestinal permeability induces visceral hypersensitivity,[40] and patients with IBS having somatic and visceral hypersensitivity exhibit increased intestinal permeability.[41]

In this study, liraglutide attenuated LPS-induced increased colonic permeability, which has been demonstrated for the first time. Although the mechanisms of this action were not determined, it might be one of the factors evoking the antinociceptive effect of liraglutide, according to the above mentioned evidence.

Several studies have shown that GLP-1 exhibits antiinflammatory activity. GLP-1 receptors are expressed in monocytes/macrophages,[14] and He et al.[42] demonstrated that an increased production of proinflammatory cytokines in peripheral blood mononuclear cells was observed in type 2 diabetes, which was suppressed by exendin-4. Moreover, this analog was also demonstrated to reduce the production of proinflammatory cytokines by activated intestinal intraepithelial lymphocytes, which have GLP-1 receptors.[15] Because both LPS- and repeated WAS-induced visceral allodynia are IL-1- and IL-6-dependent responses,[10, 22] liraglutide may inhibit cytokine release from immune cells, thereby evoking the antinociceptive effect.

This hypothesis might be supported by the finding that IL-1β-induced visceral allodynia was not modified by liraglutide in this study, suggesting that it acted upstream of proinflammatory cytokines to modulate visceral sensation. Furthermore, we also showed that increased IL-6 levels in colonic mucosa by LPS were eliminated by liraglutide, which is also consistent with the hypothesis.

This study has several limitations. Our method required minor surgery, which is inevitable for assessing visceral sensation by EMG. However, repeated surgery might have some influence on the immune system, which could modify the results. Although the antinociceptive effect of liraglutide was blocked by L-NAME, we did not directly show that

NO synthesis was increased by the analog. In addition, the NOS isoform responsible for the effect was not determined. Because the sources of proinflammatory cytokines contributing to LPS-induced allodynia have not yet been evaluated, the suppression of LPS-induced IL-6 release in the colon by liraglutide is not direct evidence indicating that it is related to its antinociception. Thus, further investigations are required to clarify these issues.

Despite the above limitations, our results suggest that liraglutide is a promising tool for treating IBS. One report demonstrated that ROSE-010, a GLP-1 analog, reduced acute IBS exacerbation in a clinical trial.[43] The results of that study may support the validity of our data, and the mechanisms behind the clinical utility of this approach may be explained by our results.

In summary, liraglutide blocked LPS-induced visceral allodynia, which may be a NO-dependent response, and was probably mediated by inhibiting proinflammatory cytokine production and attenuating increased gut permeability. Therefore, liraglutide may be useful for IBS treatment.

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467

468

Figure legends

Figure 1

a An EMG recording is depicted. The threshold of visceromotor response (VMR) was determined by the distended balloon volume (ml) inducing apparent sustained abdominal muscle contractions. The threshold was 0.5 ml in this animal. **b** Schematic representation of the experimental protocol. The basal VMR threshold was measured at 30 min after the surgery for implanting EMG electrodes and placing the balloon, and LPS (1 mg/kg) or the vehicle was administered. Later, the surgery and balloon placement were performed again, and the threshold was measured at 3 h after the injection. Liraglutide at 300 μg/kg or the vehicle was injected twice at 15 h and 30 min before injecting LPS. **c** The basal threshold was measured, and the rats were subjected to either water avoidance or sham stress for 1 h daily for 3 consecutive days. The second threshold measurement was performed at 24 h after the last stress session. Liraglutide or the vehicle was injected twice at 15 h and 30 min before the second measurement.

Figure 2

a Effect of liraglutide on LPS-induced reduced threshold of visceromotor response (VMR) induced by colonic distention. Liraglutide (300 μg/kg twice) blocked LPS (1 mg/kg)-induced visceral allodynia, but the analog per se did not alter the threshold. * $p < 0.05$ vs. basal threshold by paired t-test. **b** Percentage change threshold of VMR was significantly reduced in the vehicle + LPS group compared with that in the vehicle + vehicle group. Liraglutide

eliminated this response by LPS. * $p < 0.05$ vs. vehicle + vehicle, # $p < 0.05$ vs. vehicle + LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

Figure 3

Effect of liraglutide on repeated water avoidance stress (WAS)-induced visceral allodynia. WAS for 1 h daily for 3 consecutive days significantly reduced the threshold, and liraglutide blocked this response. * $p < 0.05$ vs. sham + vehicle, # $p < 0.05$ vs. WAS + vehicle by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses. Sham, sham stress.

Figure 4

Effect of vagotomy or naloxone on the antinociceptive effect of liraglutide against LPS-induced visceral allodynia. **a** Vagotomy modified neither the response to LPS nor the antinociceptive effect of liraglutide. * $p < 0.05$ vs. sham vagotomy + vehicle + LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. **b** Naloxone (1 mg/kg twice) did not alter the reduced threshold by LPS and it did not change the effect by liraglutide either. * $p < 0.05$ vs. vehicle + vehicle + LPS by two-way analysis of variance

followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

Figure 5

Blockade of nitric oxide synthesis by L-NAME (10 mg/kg twice) reversed the antinociceptive effect of liraglutide against LPS-induced visceral allodynia without altering the response by LPS. * $p < 0.05$ vs. vehicle + vehicle + LPS, # $p < 0.05$ vs. vehicle + liraglutide + LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

Figure 6

IL-1β (10 μg/kg) reduced the threshold, and liraglutide did not modify this response. * $p < 0.05$ vs. vehicle + vehicle by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

Figure 7

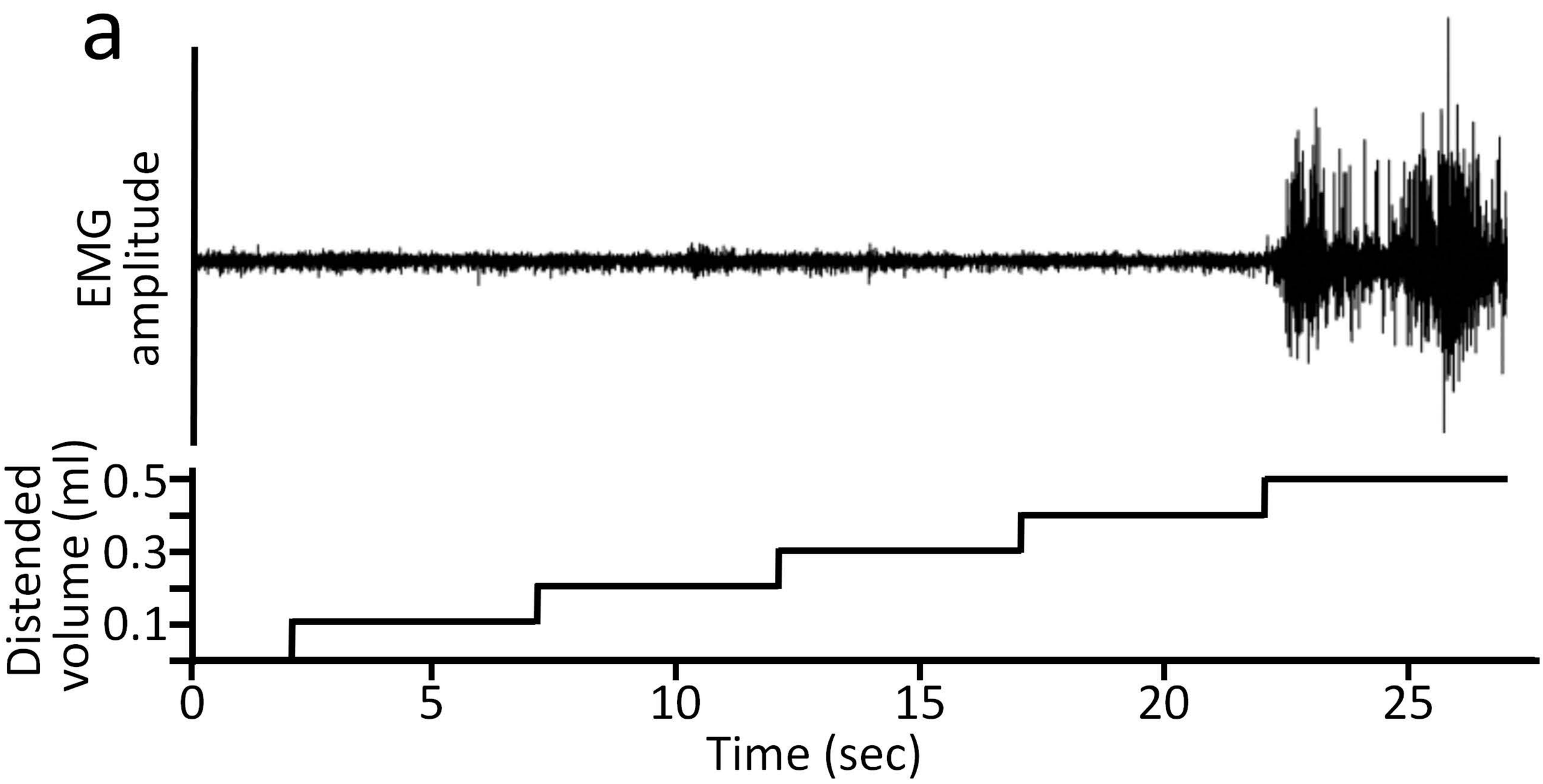
Effect of liraglutide on colonic permeability. LPS significantly increased the permeability, and liraglutide attenuated this response. * $p < 0.05$ vs. vehicle + vehicle, # $p < 0.05$ vs. vehicle +

LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

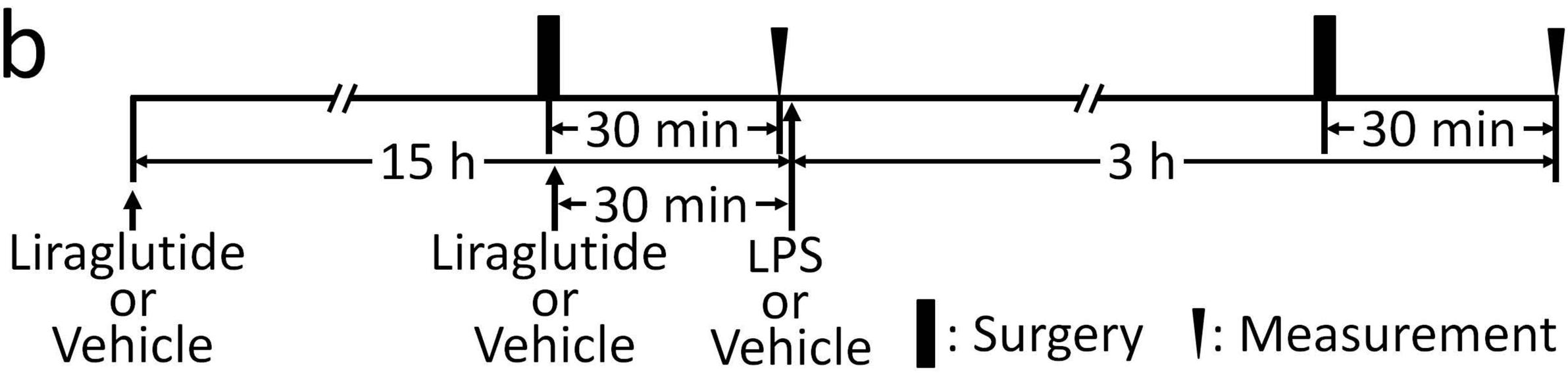
Figure 8

LPS increased the IL-6 level in colonic mucosa, and liraglutide eliminated this response. * $p < 0.05$ vs. vehicle + vehicle, # $p < 0.05$ vs. vehicle + LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

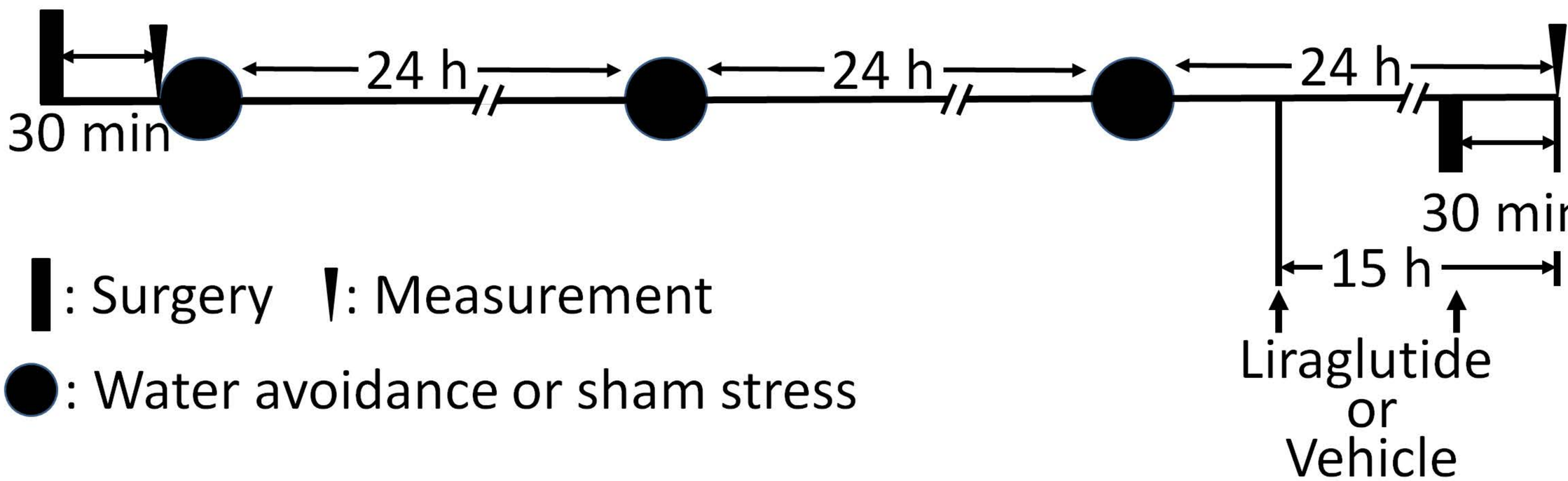
a


EMG amplitude
Distended volume (ml)
0.5
0.3
0.1
0
5
10
15
20
25
Time (sec)

b


30 min
15 h
30 min
3 h
30 min
Liraglutide or Vehicle
Liraglutide or Vehicle
LPS or Vehicle
: Surgery
: Measurement

C


24 h
24 h
24 h
30 min
30 min
15 h
: Surgery
: Measurement
: Water avoidance or sham stress
Liraglutide or Vehicle

a

Basal

After vehicle injection

After LPS injection

VMR threshold (mL)

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

(5) (5) (7) (8)

*

Vehicle Liraglutide Vehicle Liraglutide

b

% change threshold

120
100
80
60
40
20
0

(5) (5) (7) (8)

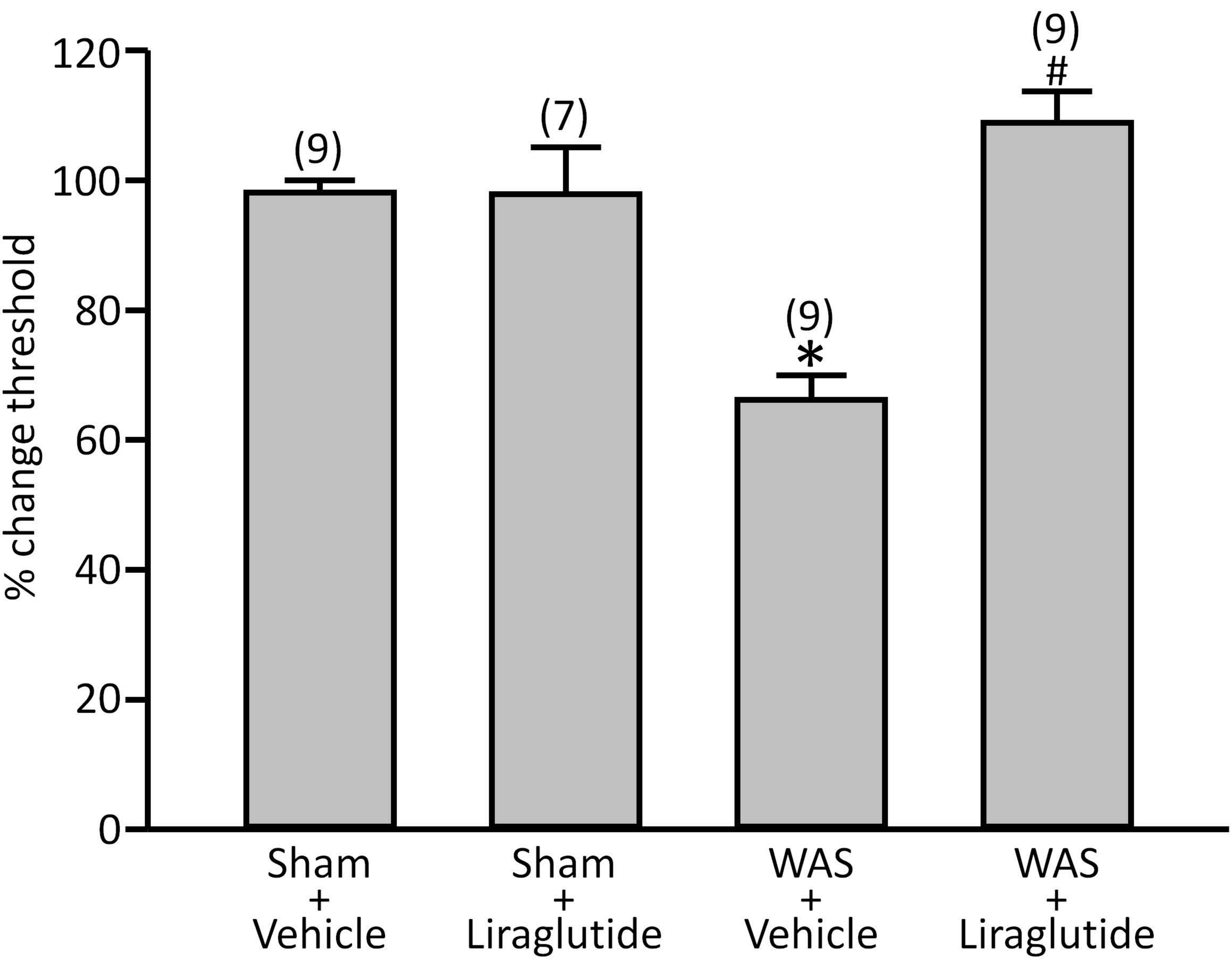
* #

Vehicle + Vehicle

Liraglutide + Vehicle

Vehicle + LPS

Liraglutide + LPS


120
100
80
60
40
20
0
% change threshold
(9)
(7)
(9)
*
(9)
#
Sham + Vehicle
Sham + Liraglutide
WAS + Vehicle
WAS + Liraglutide

a

Sham vagotomy

Vagotomy

% change threshold

120

100

80

60

40

20

0

(6)

(6)

(5)

*

(7)

*

Vehicle + LPS

Liraglutide + LPS

b

Vehicle

Naloxone

% change threshold

120

100

80

60

40

20

0

(7)

(7)

(8)

*

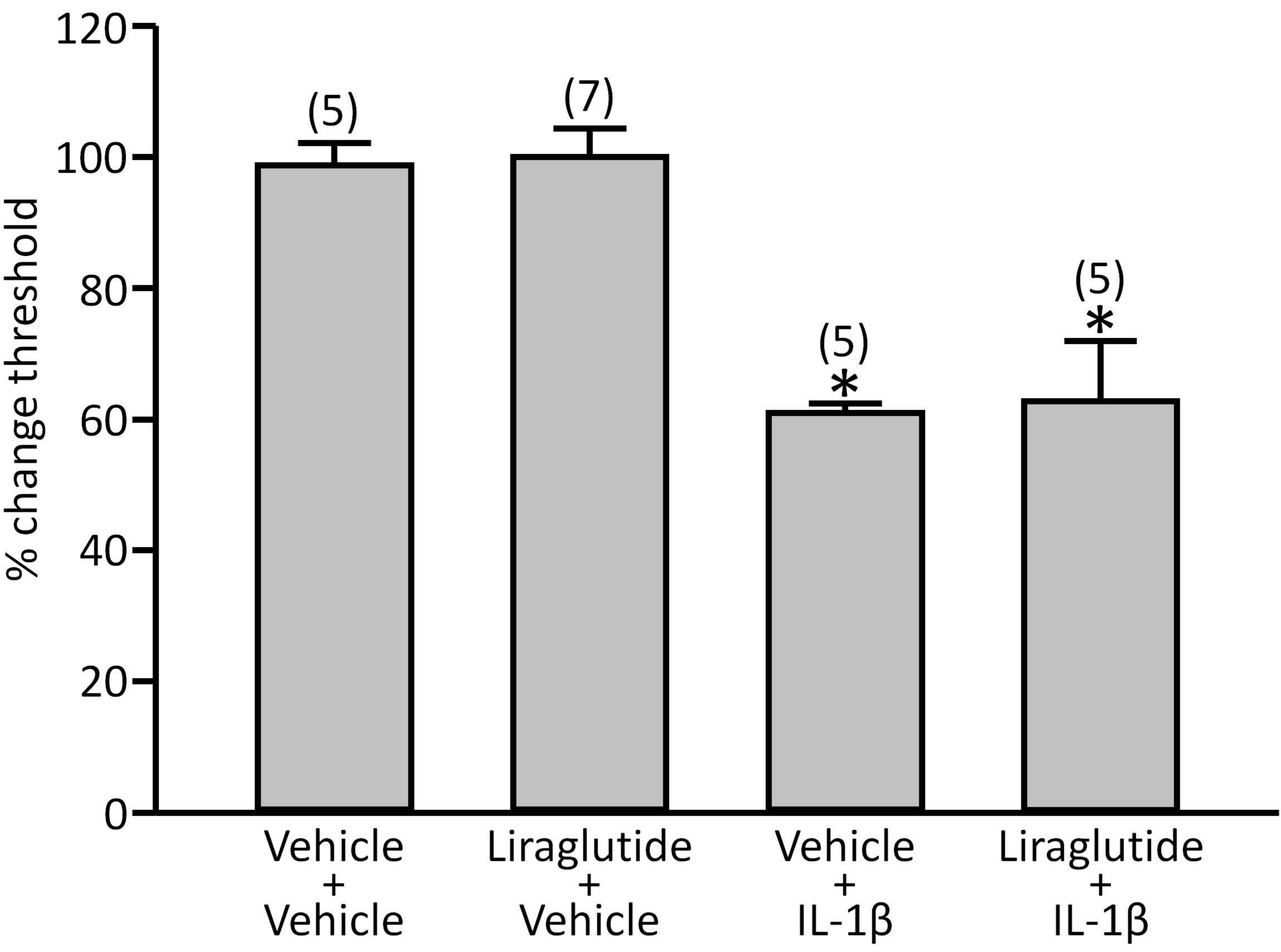
(5)

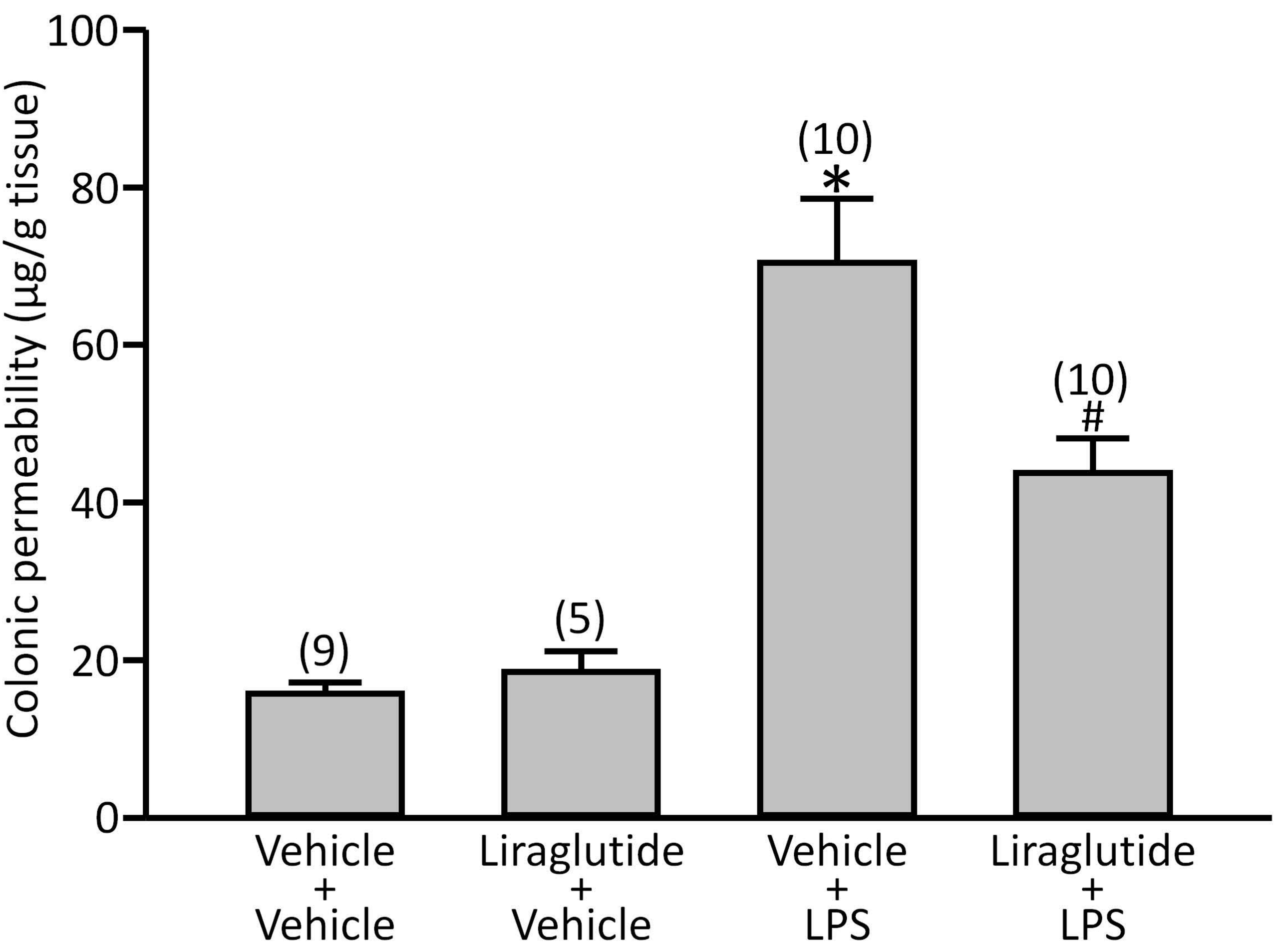
*

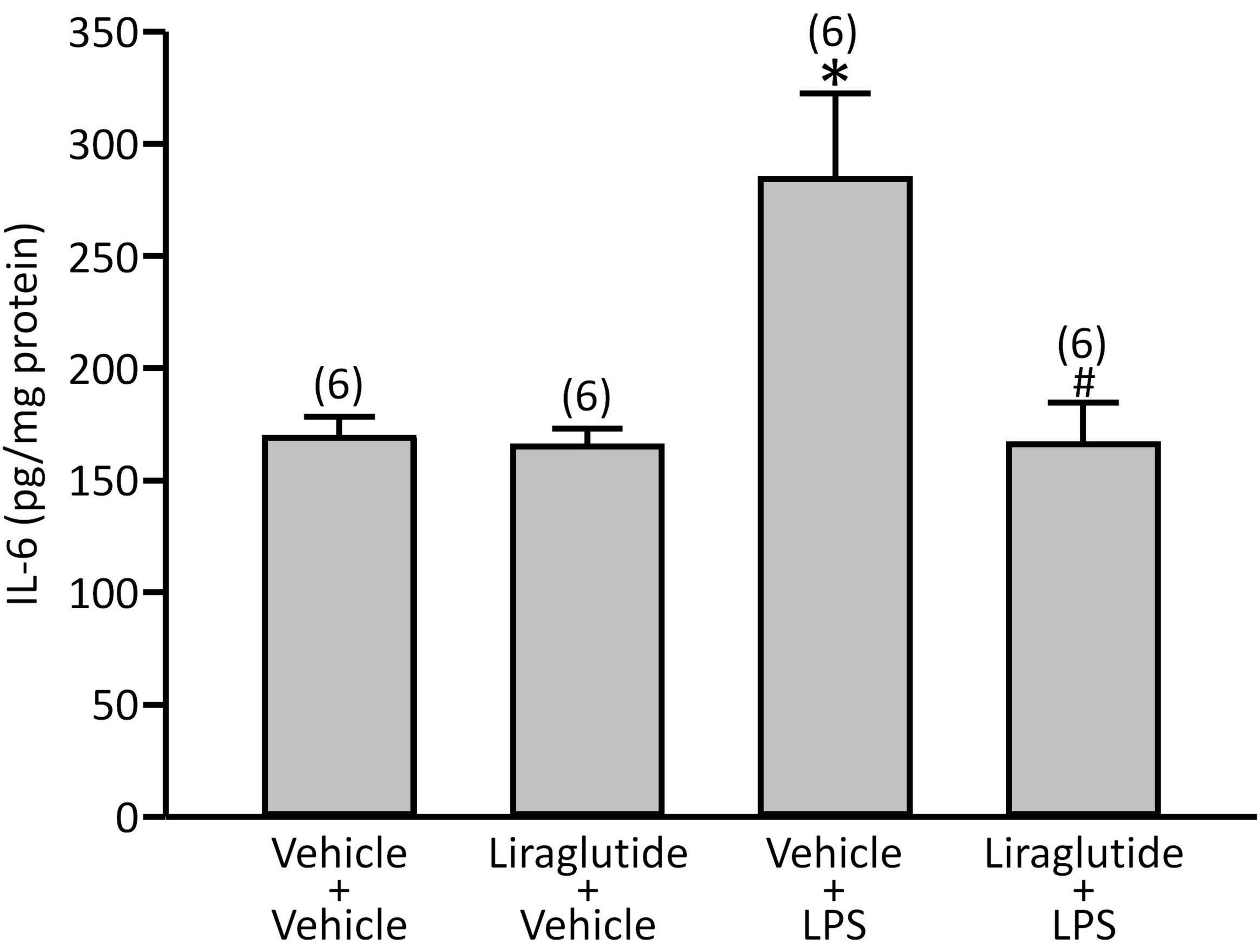
Vehicle + LPS

Liraglutide + LPS

Vehicle
L-NAME
% change threshold
120
100
80
60
40
20
0
(6)
(5)
(5)
*
(6)
#
Vehicle + LPS
Liraglutide + LPS


120
100
80
60
40
20
0
% change threshold
(5)
(7)
(5)
*
(5)
*
Vehicle + Vehicle
Liraglutide + Vehicle
Vehicle + IL-1β
Liraglutide + IL-1β


Colonic permeability (µg/g tissue)
100
80
60
40
20
0
(9)
(5)
(10)
*
(10)
#
Vehicle + Vehicle
Liraglutide + Vehicle
Vehicle + LPS
Liraglutide + LPS


IL-6 (pg/mg protein)
350
300
250
200
150
100
50
0
(6)
(6)
(6)
*
(6)
#
Vehicle + Vehicle
Liraglutide + Vehicle
Vehicle + LPS
Liraglutide + LPS