学位論文

Ghrelin acts in the brain to block colonic hyperpermeability in response to lipopolysaccharide　through the vagus nerve.

（グレリンは脳に作用して迷走神経を介して LPS で誘導される腸管透過性亢進を抑制する.）

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Ghrelin acts in the brain to block colonic hyperpermeability in response to lipopolysaccharide through the vagus nerve

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HIGHLIGHTS

- Ghrelin acts centrally to improve colonic hyperpermeability induced by LPS.
- The improvement by central ghrelin is mediated by vagal cholinergic pathway.
- Brain orexin is also involved in the change of intestinal barrier function by ghrelin.
- Ghrelin may be useful for diseases associated with brain-gut axis and leaky gut.

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ABSTRACT

Brain ghrelin plays a role in gastrointestinal functions. Among them, ghrelin acts centrally to stimulate gastrointestinal motility and induce visceral antinociception. Intestinal barrier function, one of important gastrointestinal functions, is also controlled by the central nervous system. Little is, however, known about a role of central ghrelin in regulation of intestinal permeability. The present study was performed to clarify whether brain ghrelin is also involved in regulation of intestinal barrier function and its mechanism. Colonic permeability was estimated in vivo by quantifying the absorbed Evans blue in colonic tissue in rats. Intracisternal injection of ghrelin dose-dependently abolished increased colonic permeability in response to LPS while intraperitoneal injection of ghrelin at the same dose or intracisternal injection of des-acyl-ghrelin failed to block it. Carbachol potently attenuated LPS-induced intestinal hyperpermeability, and atropine or bilateral subdiaphragmatic vagotomy prevented the improvement of intestinal hyperpermeability by central ghrelin. Intracisternal (D-Lys3)-GHRP-6, a selective ghrelin receptor antagonist, significantly blocked improvement of intestinal barrier function by intravenously administered 2-deoxy-D-glucose, central vagal stimulant. Intracisternal injection of orexin 1 receptor antagonist, SB-334867 blocked intracisternal ghrelin-induced improvement of colonic hyperpermeability. These results suggest that exogenously administered or endogenously released ghrelin acts centrally to improve a disturbed intestinal barrier function through orexinergic signaling and the vagal cholinergic pathway. Central ghrelin may be involved in the pathophysiology and be a novel therapeutic option in not only gastrointestinal diseases such as irritable bowel syndrome but also non-gastrointestinal diseases associated with the altered intestinal permeability.

1. Introduction

Leaky gut that is a condition reflecting intestinal barrier dysfunction has been attracting attention for its relations with many diseases (Camilleri, 2019). A disturbed intestinal barrier function has been described in many human diseases such as irritable bowel syndrome (IBS), inflammatory bowel disease, asthma, autism, Parkinson's disease, multiple sclerosis, eczema, psoriasis, eosinophilic esophagitis, environmental enteropathy, kwashiorkor, fibromyalgia, depression, chronic fatigue syndrome, non-alcoholic fatty liver disease, alcoholic

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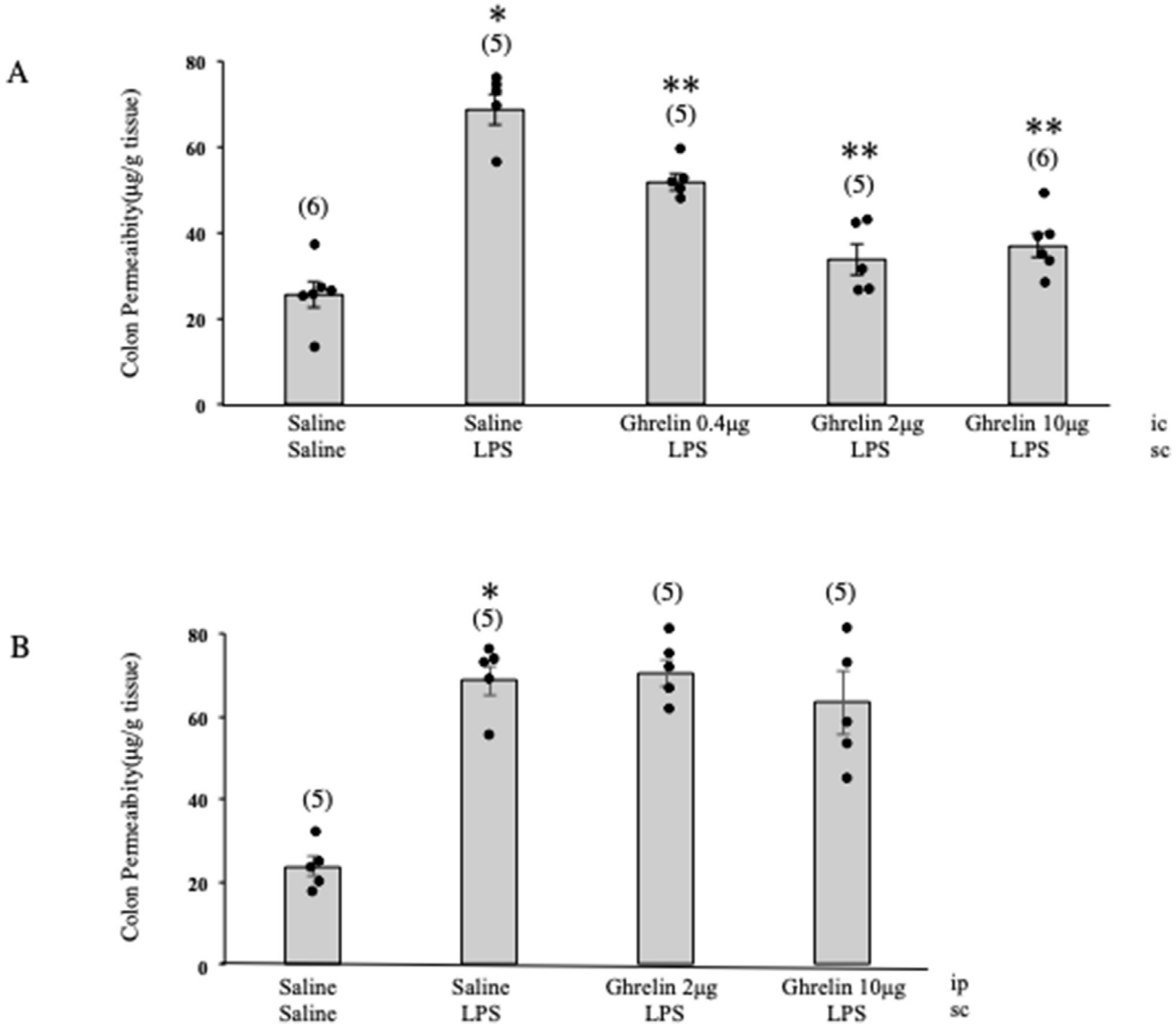

Fig. 1. Effect of intracisternal (A) or intraperitoneal (B) injection of ghrelin on the increased colonic permeability by LPS in conscious rat. Each column represents the mean ± S.E. The number of rats is shown in the parentheses. *P < 0.01, when compared with saline + saline. **P < 0.01, when compared with saline + LPS (+). ic, intracisternal; sc, subcutaneous; ip, intraperitoneal.

cirrhosis, obesity, metabolic syndrome, pancreatitis and rheumatoid arthritis (Camilleri, 2019). Thus, improvement of disturbed intestinal barrier function may contribute to control of activity of several gastrointestinal and non-gastrointestinal diseases. Intestinal barrier function is regulated by a number of factors such as tight junction proteins in the epithelium, peripheral neuroimmune-related molecules and microbiota (Camilleri, 2019; Keita and Soderholm, 2018). However, the mechanisms how the brain controls intestinal barrier function still remain to be fully elucidated. We have very recently demonstrated that exogenously administered or endogenously released orexin in the brain blocked colonic hyperpermeability in rats, suggesting for the first time that the brain indeed plays a role in regulation of intestinal barrier function (Okumura et al., 2020).

Ghrelin, a 28-amino acid peptide acts as an endogenous ligand for the growth hormone secretagogue receptor type (GHSR), which is expressed throughout the brain and mainly in the pituitary and hypothalamus (Kojima et al., 1999). In peripheral organs, the main source of ghrelin is the stomach but it is also synthesized in the small intestine, pancreas, heart, kidney, and the gonads (Kojima and Kangawa, 2010). In the brain, ghrelin is produced by hypothalamic neurons in the arcuate nucleus, lateral hypothalamus and paraventricular nucleus (Cowley et al., 2003). Ghrelin has a wide variety of physiological functions, such as stimulating appetite, promoting secretion of growth hormone, modulating metabolism or regulating cardiovascular and gastrointestinal functions (Korbonits et al., 2004). With regard to the gastrointestinal functions, it has been reported that ghrelin acts centrally to regulate gastrointestinal motility (Goyal et al., 2019). Centrally administered ghrelin acts as a modulator stimulating colonic motility via hypothalamic nuclei including the arcuate nucleus, dorsomedial hypothalamic nucleus, and paraventricular nucleus in rats (Chen and Tsai, 2012; Fujino et al., 2003; Root and Root, 2002). Furthermore, administration of ghrelin directly into the paraventricular nucleus accelerated small intestinal transit in rats that was competitively inhibited by a GHSR antagonist (Wang et al., 2015a). Thus, ghrelin acts in the hypothalamic neurons to regulate gastrointestinal motility. In addition, we have recently demonstrated that ghrelin acts in the brain to enhance antinociceptive response to colonic distension, suggesting brain ghrelin regulates not only gastrointestinal motility but visceral sensation (Okumura et al., 2018). The present study was performed to clarify whether brain ghrelin is also involved in regulation of intestinal barrier

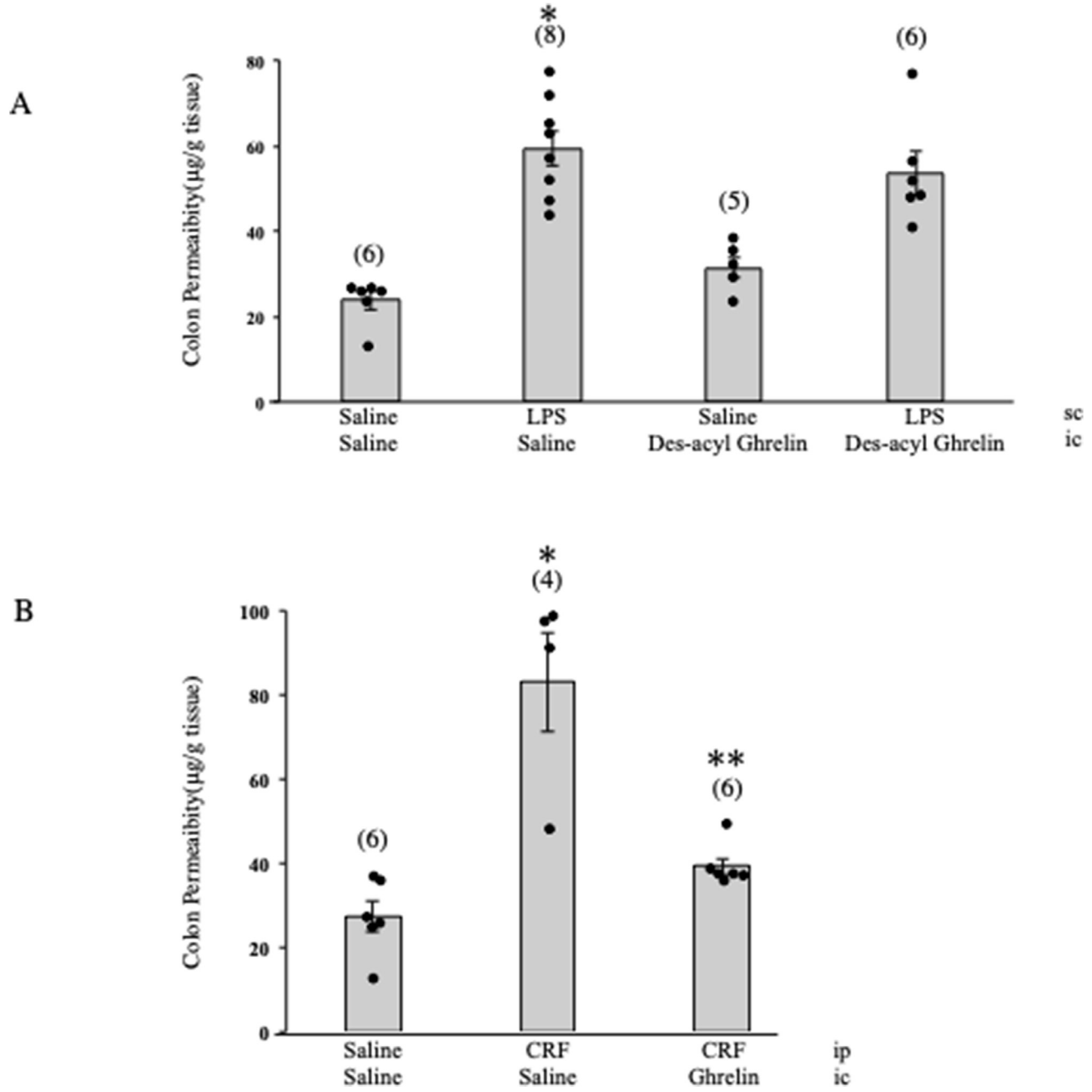

Fig. 2. (A) Effect of intracisternal injection of des-acyl-ghrelin on the increased colonic permeability by LPS in conscious rat. Each column represents the mean ± S.E. The number of rats is shown in the parentheses. *P < 0.01, when compared with saline + saline. sc, subcutaneous; ic, intracisternal. (B) Effect of intracisternal injection of ghrelin on the increased colonic permeability by CRF in conscious rat. *P < 0.01, when compared with saline + saline. **P < 0.01, when compared with saline + CRF. ic, intracisternal; ip, intraperitoneal.

function and its mechanism.

2. Methods

2.1. Ethical considerations

Approval was obtained from the Research and Development and Animal Care committees at Asahikawa Medical University (No. 13030) for all of the experiments conducted in this study.

2.2. Animals

Male Sprague-Dawley rats (Charles River Laboratory, Atsugi, Japan) weighing about 200 g were housed under controlled light/dark conditions (lights on: 07:00–19:00), and the room temperature was regulated at 23–25 °C. Rats were allowed free access to standard rat chow (solid rat chow; Oriental Yeast Co., Tokyo, Japan) and tap water. All of the experiments were performed using conscious animals, which had been deprived of food for 24 h but with free access to water until the initiation of the experiments.

2.3. Chemicals

Synthetic human ghrelin, des-acyl-ghrelin and rat/human corticotropin-releasing factor (CRF) were purchased from Peptide Institute Inc., Osaka, Japan and dissolved in normal saline. Lipopolysaccharide

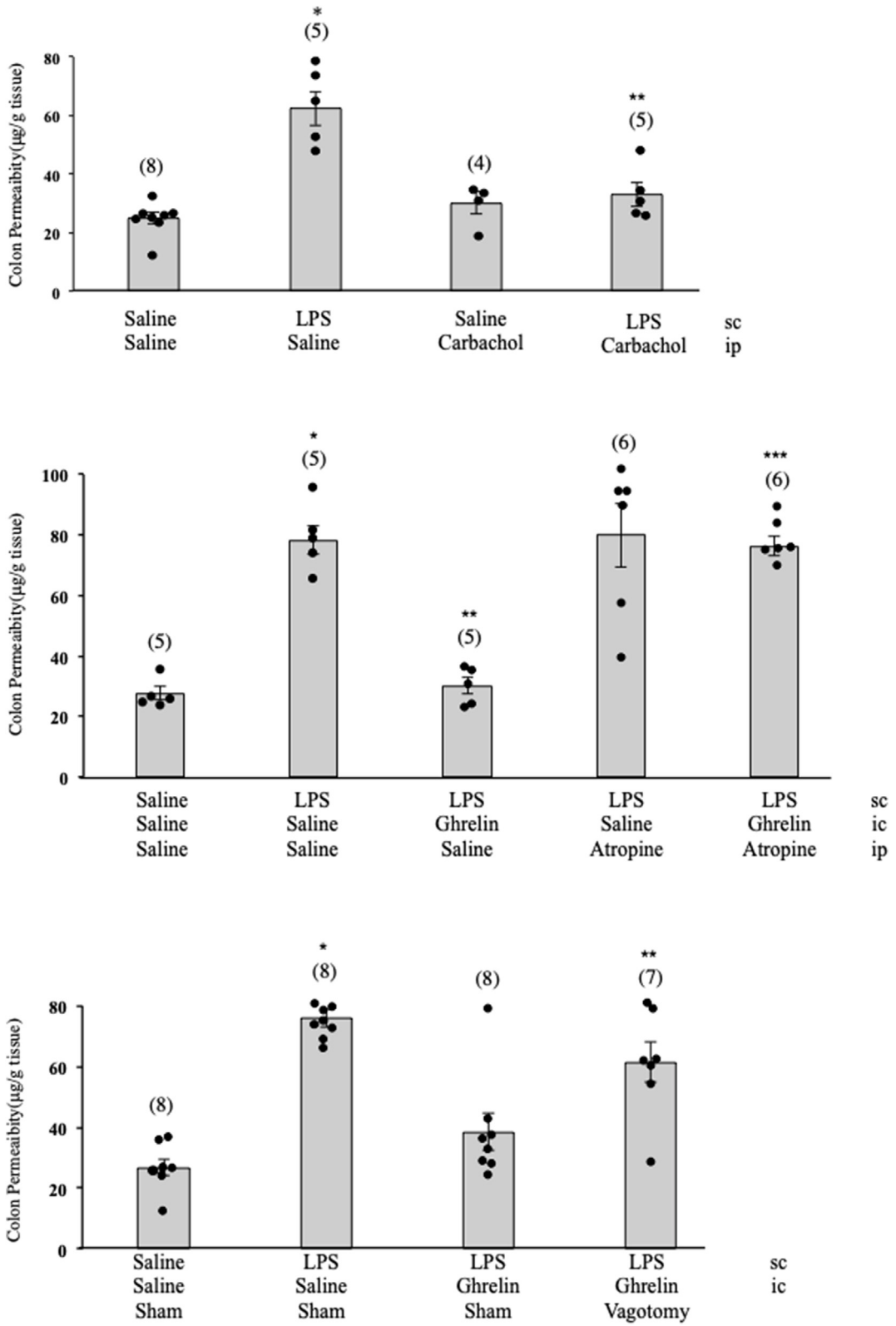

Fig. 3. A) Effect of intrapertoneal injection of carbachol on the increased colonic permeability by LPS in conscious rat. Each column represents the mean ± S.E. The number of rats is shown in the parentheses. *P < 0.01, when compared with saline + saline. **P < 0.01, when compared with LPS (sc) + saline (ip). sc, subcutaneous; ip, intraperitoneal. (B) Effects of atropine on the ghrelin-induced blockade of increased colonic permeability by LPS. P < 0.01, when compared with saline sc, ic and ip. **P < 0.01, when compared with LPS (sc) + saline (ic) + saline (ip). ***P < 0.01, when compared with LPS (sc) + ghrelin (ic) + saline (ip). ic, intracisternal. (C) Effects of vagotomy on the ghrelin-induced blockade of increased colonic permeability by LPS. *P < 0.01, when compared with saline. **P < 0.01, when compared with LPS + ghrelin + sham. Sham, sham operation.

Table 1
Effects of intracisternal injection of ghrelin antagonist on colonic permeability.

Treatment	N	AWR threshold volume (ml)		
Saline	4	28.8	±	2.8
(D-Lys3)-GHRP-6	4	32.1	±	3.8

(D-Lys3)-GHRP-6 was intracisternally injected. Colonic permeability was estimated in vivo by quantifying the absorbed Evans blue in colonic tissue in rats. Each data represents the mean ± S.E.

(LPS) obtained from *Escherichia coli* with the serotype 055:B5, 2-deoxy-D-glucose (2-DG), atropine or carbachol (Sigma-Aldrich, St. Louis, MO) were dissolved in normal saline. (D-Lys3)-GHRP-6, the classic ghrelin receptors, growth hormone secretagogue (GHSR) antagonist (Tocris Bioscience, Ellisville, MO) was dissolved in saline. Selective orexin 1 receptor (OX1R) antagonist, SB-334867 (Tocris Bioscience, Ellisville, MO) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

2.4. Measuring colonic permeability

We examined colonic permeability in LPS or CRF-stimulated intestinal permeability models as we have shown in several recent studies (Nozu et al., 2018b; Okumura et al., 2020). LPS administration was performed as a model of gram-negative bacterial translocation through a leaky gut (Dlugosz et al., 2015; Moriez et al., 2005). Exogenous CRF is considered as a model of stress-mediated permeability (Taché et al., 2009). Colonic permeability measurement with Evans blue was performed according to previous studies (Dai et al., 2012; Kitajima et al., 1999; Lange et al., 1994; Ukena et al., 2007) with minor modification. The permeability was determined 3 h or 4 h after injecting LPS or CRF, respectively. The rats anesthetized by intraperitoneal administration of the mixture of medetomidine hydrochloride (Orion Pharma Ltd., Dhaka, Bangladesh, 0.15 mg/kg), midazolam (Sandoz, Tokyo, Japan, 2 mg/kg) and butorphanol tartrate (Meiji Seika Pharma, Tokyo, Japan, 2.5 mg/kg) were placed in a supine position on a heating pad, and laparotomy was performed. The colon was ligated at the junction with the cecum, and the small hole was made by a puncture using 18 G needle at the 1 cm from the ileocecal junction. Then an open-tipped catheter (3-Fr, Atom, Tokyo, Japan) was inserted into the proximal colon through the hole and fixed by purse-string sutures. The colon was gently flushed with phosphate buffered saline (PBS, 37 °C) using the catheter until all stools were washed out. Generally, the required volume of PBS was approximately 10 ml and the perfusion rate was 5 ml/min. Then another ligation was added on the colon at approximately 4 cm from the proximal ligation, and 1 ml of 1.5% Evans blue in PBS was instilled into the colon segment between ligations through the catheter. Fifteen min later, the animals were killed and the colons were excised. Later they were washed with PBS and 1 ml of 6 mM N-acetylcysteine, and were opened and placed in 2 ml of N, N-dimethylformamide for 12 h. The permeability was calculated by measuring the Evans blue concentration in the supernatant using a spectrophotometer at 610 nm.

2.5. Experimental procedures

Initially, we examined the dose-dependent effects of intracisternal injection of ghrelin on the subcutaneous LPS-induced colonic

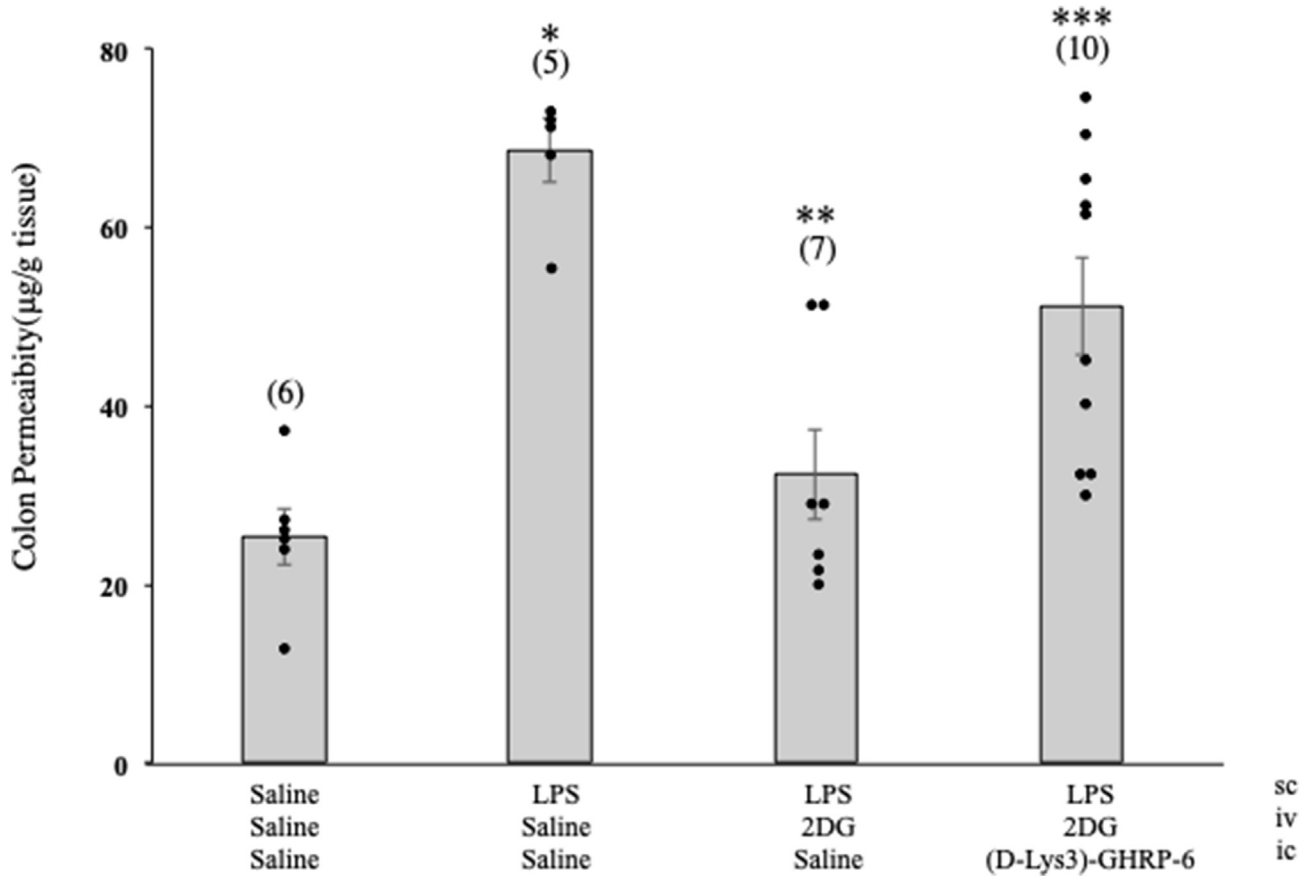

Fig. 4. Effects of intracisternal (D-Lys3)-GHRP-6, a specific ghrelin receptor antagonist on the 2-deoxy-D-glucose (2-DG) -induced blockade of increased colonic permeability by LPS. Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. P < 0.01, when compared with saline sc, ic and ip. **P < 0.01, when compared with LPS sc + saline iv + saline ic. ***P < 0.01, when compared with LPS + 2-DG + saline (ic). sc, subcutaneous; iv, intravenous; ic, intracisternal.

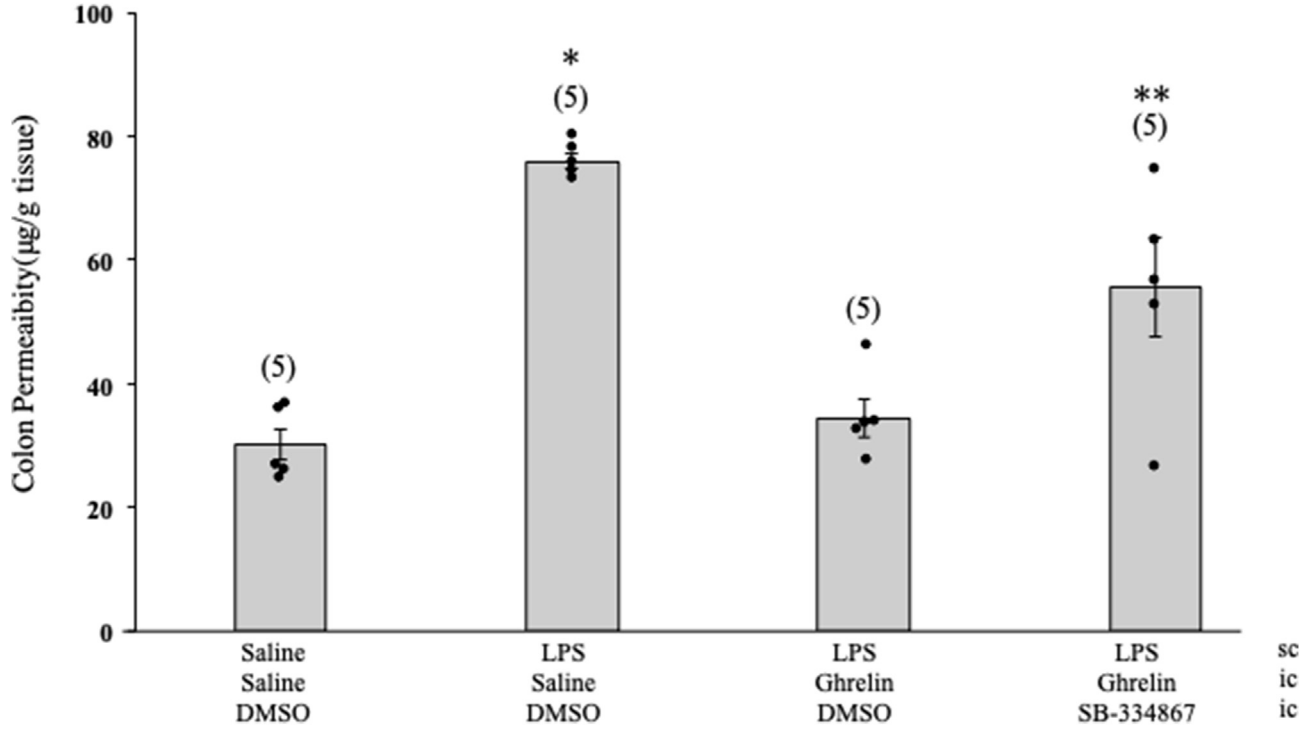

Fig. 5. Effect of intracisternal injection of SB-334867, an OX1R antagonist, on the intracisternally administered ghrelin-induced improvement of colonic hyperpermeability in conscious rats. Each column represents the mean ± S.E. The number of rats examined is shown parenthese. *P < 0.01, when compared with saline (sc) + saline (ic) + DMSO (ic). **P < 0.01, when compared with LPS (sc) + ghrelin (ic) + DMSO (ic). SB; SB-334867. sc, subcutaneous; ic, intracisternal.

hyperpermeability. Rats received intracisternal (0, 0.4, 2 or 10 μg) or intraperitoneal (0, 2 or 10 μg) injections of ghrelin or des-acyl-ghrelin (10 μg), and then LPS (1 mg/kg) or CRF (50 μg/kg) was injected subcutaneously or intraperitoneally, respectively. Intracisternal injection was performed under brief isoflurane anesthesia using a 10-μl Hamilton microsyringe after the rats were mounted in a stereotaxic apparatus (David Kopf Instruments, Tijunga, CA), as reported previously (Okumura et al., 1994). To test a role of the vagal cholinergic pathway in the LPS-induced colonic hyperpermeability, we examined the effect of intraperitoneal injection of carbachol (0.1 mg/kg) on the colonic hyperpermeability by LPS. Next, to clarify whether the vagal system is involved in the central ghrelin-induced changes of LPS-evoked intestinal hyperpermeability, we examined the effect of the intraperitoneal injection of atropine (1 mg/kg) or surgical vagotomy on the intracisternally administered ghrelin (10 μg/10 μl)-induced change of colonic hyperpermeability by LPS. The surgical bilateral subdiaphragmatic vagotomy was performed as previously described (Takahashi et al., 1999). To examine whether endogenous ghrelin in the brain mediates the brain orexin-induced alternation of colonic hyperpermeability, we tested the effect of intracisternal (D-Lys3)-GHRP-6, GHSR antagonist on the 2-DG-induced improvement of colonic hyperpermeability by LPS. SB-334867 (Smart et al., 2001), an OX1R antagonist, at a dose of 40 μg/10 μl was injected intracisternally to examine a role of orexin in the ghrelin-induced improvement of colonic hyperpermeability. The doses of LPS, CRF, atropine, carbachol, 2-DG and SB-334867 were selected according to previous publications (Bugajski et al., 2007; Nozu et al., 2018a; Okumura et al., 2020). The dose of (D-Lys3)-GHRP-6 was selected according to the previous report (Sibilia et al., 2012; Szakács et al., 2015).

2.6. Statistical analysis

The data were expressed as means ± standard error (SE). The data were compared with Student's t-test or one-way analysis of variance followed by Tukey's Multiple Comparison Test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of intracisternal ghrelin on increased colonic permeability by LPS

Subcutaneously administered LPS at a dose of 1 mg/kg significantly stimulated the colonic permeability (Fig. 1) as reported previously (Nozu et al., 2018b, 2019). Intracisternal injection of ghrelin dose-dependently inhibited the increased colonic permeability [$F(4, 22) = 30.15$, $P < 0.01$] (Fig. 1A). The significant effects were observed when ghrelin at 0.4 μg or more was administered. In contrast, intraperitoneal injection of ghrelin at the same doses failed to block the stimulated colonic permeability [$F(3, 16) = 21.67$, $P < 0.01$] (Fig. 1B), suggesting that ghrelin acts centrally in the brain to improve colonic barrier function. Because ghrelin (acyl-ghrelin) acts in the GHSRs while des-acyl-ghrelin is not capable of binding to the specific receptors (Hosoda et al., 2000), we examined the effects of intracisternal injection of des-acyl-ghrelin on colonic permeability to test the hypothesis that the specific receptors may mediate the brain ghrelin-induced improvement of colonic permeability. As shown in Fig. 2A [$F(3, 21) = 19.94$, $P < 0.01$], intracisternal injection of des-acyl-ghrelin at a dose of 10 μg could not block the LPS-evoked stimulation of colonic hyperpermeability, strongly suggesting that GHSRs mediate the effect of ghrelin. In other model of colonic hyperpermeability, the effect of ghrelin was examined. As shown in Fig. 2B [$F(3, 16) = 23.46$, $P < 0.01$], intraperitoneally administered CRF at a dose of 50 μg/kg significantly increased colonic permeability as shown previously (Nozu et al., 2018b; Okumura et al., 2020). In this CRF-induced colonic hyperpermeability model, intracisternal injection of ghrelin at a dose of 10 μg significantly blocked the hyperpermeability.

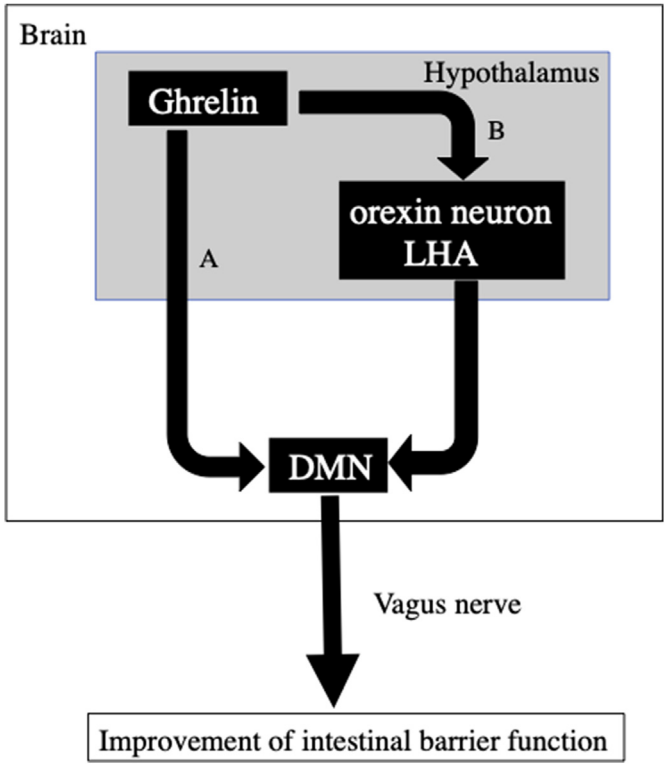

Fig. 6. Schematic illustration of the mechanisms by which central ghrelin improves intestinal barrier function based upon the present study. Ghrelin acts in the DMN, cells of origin innervating the gastrointestinal tract through the vagus nerve, and activates the vagal efferent pathway to improve intestinal hyperpermeability (A). On the other hand, ghrelin activates orexin neurons in the lateral hypothalamic area and the orexin acts in the DMN neurons, thereby improving intestinal hyperpermeability through activation of the efferent vagal pathway (B). Dual pathways may mediate the improvement of intestinal barrier function by brain ghrelin. DMN, the dorsal motor nucleus of the vagus in the medulla. LHA, the lateral hypothalamic area.

3.2. Effect of atropine or vagotomy on ghrelin-induced improvement of colonic hyperpermeability

To examine a role of the vagal cholinergic pathway in the LPS-induced colonic hyperpermeability, we examined the effect of carbachol on the LPS-evoked colonic hyperpermeability. While carbachol by itself failed to change the basal colonic permeability, the LPS-induced altered colonic barrier function was significantly improved by intraperitoneal injection of carbachol [$F(3, 15) = 19.72$, $P < 0.01$] (Fig. 3A), suggesting that the vagal cholinergic pathway plays a role in improvement of altered colonic permeability by LPS. Fig. 3B illustrates the effect of atropine on the ghrelin-induced improvement of colonic hyperpermeability [$F(4, 22) = 19.67$, $P < 0.01$]. While atropine by itself did not change the LPS-induced colonic hyperpermeability, atropine intraperitoneally administered potently blocked the ghrelin-induced inhibition of LPS-induced colonic hyperpermeability. As shown in Fig. 3C [$F(3, 27) = 21.34$, $P < 0.01$], surgical bilateral subdiaphragmatic vagotomy significantly blocked the ghrelin-induced improvement of colonic hyperpermeability in response to LPS, suggesting that the vagal cholinergic pathway is involved in the improvement of colonic hyperpermeability by central ghrelin.

3.3. Effect of ghrelin receptor antagonist on 2-DG-induced improvement of colonic hyperpermeability

As we recently demonstrated, intravenous 2-DG, a central vagal stimulant (Kadekaro et al., 1975), significantly blocked the increased colonic permeability by LPS and atropine blocked the effect of 2-DG, supporting that the vagal cholinergic stimulation mediates the improvement of colonic hyperpermeability by 2-DG (Okumura et al., 2020). We therefore made a hypothesis that endogenous brain ghrelin may be involved in the 2-DG-induced blockade of stimulation of colonic permeability. To test the hypothesis, we used (D-Lys3)-GHRP-6, a selective ghrelin receptor antagonist (Traebert et al., 2002). While intracisternal injection of the antagonist by itself did not change the colonic permeability (Table 1), (D-Lys3)-GHRP-6 significantly reversed the 2-DG-induced blockade of stimulation of colonic permeability [$F(3, 24) = 12.46$, $P < 0.01$] (Fig. 4), suggesting that endogenous brain ghrelin may play an important role in the improvement of intestinal barrier function. However, we could not completely exclude the possibility that circulating ghrelin may also improve intestinal barrier function because ghrelin and its antagonist are capable of crossing the blood-brain barrier (Mosa et al., 2018; Rhea et al., 2018).

3.4. Effect of orexin receptor antagonist on ghrelin-induced improvement of colonic hyperpermeability

We have very recently demonstrated that brain orexin could block the LPS-induced colonic hyperpermeability in rats (Okumura et al., 2020). To clarify whether orexin may mediate the action by ghrelin, effect of intracisternal OX1R antagonist, SB-334867 on the central ghrelin-induced improvement of colonic hyperpermeability was examined. As illustrated in Fig. 5 [$F(3, 16) = 21.37$, $P < 0.01$], intracisternal injection of SB334867 significantly blocked the ghrelin-induced improvement of colonic barrier function.

4. Discussion

Orexin in the brain improved the intestinal barrier function in rats (Okumura et al., 2020). The finding indicated for the first time that intestinal permeability is regulated by the brain. Some reports have shown that peripherally administered ghrelin reduced intestinal hyperpermeability by traumatic brain injury, intracerebral hemorrhage, cecal ligation and puncture-induced sepsis or whole body irradiation (Bansal et al., 2010; Cheng et al., 2016; Wang et al., 2015b; Wu et al., 2009). The present study showed that brain ghrelin could also improve the colonic hyperpermeability in LPS-treated rats. In addition to orexin, the present study provided that ghrelin acts centrally to improve colonic hyperpermeability, suggesting that intestinal barrier function is also regulated by ghrelin in the brain.

Shimizu et al. have demonstrated that stimulation of ghrelin receptors in the spinal cord initiates propulsive activity in the colon of the rat (Shimizu et al., 2006). Thus, the spinal cord is one of sites of action of ghrelin in the central nervous system (CNS) to regulate gastrointestinal functions. We could not therefore exclude the possibility that the spinal cord might be a site of action of centrally injected ghrelin to change colonic permeability as observed in this study. The present study however strongly suggest that the vagal cholinergic pathway plays a vital role in the mechanisms by which central ghrelin blocked the LPS-induced colonic hyperpermeability. While the human colon is vagally innervated for the ascending and transverse segments, the entire rat colon is vagally innervated in both the proximal and distal aspects (Gonella et al., 1987). In the present study, colonic permeability was evaluated in the proximal colon which is consistently innervated by the vagus nerve across species. Sato et al. have suggested that 2-DG promotes hypothalamic ghrelin secretion (Sato et al., 2005). It has been shown that central administration of ghrelin stimulates the vagal efferent nerve in anesthetized rats (Sato et al., 2003). These findings suggest that ghrelin released from the hypothalamic neurons by 2-DG stimulates the vagal efferent pathway. Swartz et al. have demonstrated that ghrelin acts in the dorsal motor nucleus (DMN) in the medulla to activate vagal cholinergic pathway innervating the gastrointestinal tract in rats (Swartz et al., 2014). It has been reported that endogenous

ghrelin receptor is expressed on the neurons in the DMN (Zigman et al., 2006), the cells of origin innervating the gastrointestinal tract through the vagus nerve (Okumura and Namiki, 1990). These findings suggest that ghrelin acts in the DMN to increase the vagal cholinergic pathway. We would therefore strongly suggest that ghrelin acts in the DMN to improve the LPS-induced colonic hyperpermeability through the vagal cholinergic pathway (Fig. 6A). To further clarify the speculation that the DMN is the site of action of ghrelin in the CNS, a microinjection study with much less doses of ghrelin or its antagonist when compared with intracisternal injection should be performed.

While intracisternal SB-334867, a specific OX1R antagonist, by itself did not alter colonic permeability as shown in our previous report (Okumura et al., 2020), SB-334867 injected intracisternally significantly blocked the ghrelin-induced improvement of colonic hyperpermeability, suggesting that the orexin signaling mediates the brain ghrelin-induced improvement of intestinal barrier function via OX1Rs. Accumulating evidence has suggested that there is a functional relationship between ghrelin and orexin in the CNS. Toshinai et al. (2006) have shown that ghrelin-immunoreactive nerve terminals made direct synaptic contacts with orexin-producing neurons and intracerebroventricular administration of ghrelin induced Fos expression in orexin-producing neurons in the lateral hypothalamus (LHA), indicating that ghrelin acts in the orexin neurons, followed by the stimulation of the orexinergic pathway. The neuroanatomical and neuropharmacological findings may support the present data that the orexin signaling mediates the brain ghrelin-induced improvement of colonic hyperpermeability. Thus, hypothalamic ghrelin activates orexin neurons in the LHA and orexin acts in the DMN to increase the vagal tone, thereby improving colonic hyperpermeability (Fig. 6B). On the other hand, ghrelin may activate directly neurons in the DMN to improve intestinal permeability through stimulation of the vagal cholinergic pathway as we suggested above (Fig. 6A). From these findings, we would suggest that dual pathways (Fig. 6A and B) are involved in the ghrelin-induced improvement of intestinal barrier function.

Increased colonic permeability by LPS tested in this model was mediated via TLR4 and cytokine system (Nozu et al., 2018b). Many studies confirmed that the release of cytokines from immune cells is partially regulated through autonomic nervous system (Elenkov et al., 2000; Pavlov and Tracey, 2004; Pavlov et al., 2003; Tracey, 2002). Tracey and coworkers demonstrated anti-inflammatory mechanisms mediated by efferent vagus nerve (Tracey, 2007). Stimulation of vagus nerve significantly reduced inflammation in endotoxemic rats while this effect was blocked by vagotomy or atropine administration (Borovikova et al., 2000). We would therefore speculate that the vagal cholinergic anti-inflammatory pathway may be implicated in the peripheral mechanisms by which LPS-induced intestinal hyperpermeability was blocked. In other words, ghrelin acts centrally in the brain to activate the vagal cholinergic pathway, followed by induction of anti-inflammatory response though the vagus nerve, thereby protecting intestinal hyperpermeability by inhibition of LPS-TLR4-cytokine signaling.

Creekmore et al. have shown that the level of stress-associated visceral hyperalgesia directly correlates with the magnitude of altered colon epithelial permeability, suggesting a strong correlation between visceral sensation and intestinal permeability (Creekmore et al., 2018). We have recently showed that ghrelin acts in the brain to evoke a visceral hyposensitivity in rats (Okumura et al., 2018) and the present study clearly demonstrated that ghrelin improved the intestinal hyperpermeability by LPS. Based on these findings, we would suggest that the improvement of intestinal barrier function by brain ghrelin would be tightly associated with the changed visceral sensation. In other words, ghrelin may act centrally to improve intestinal permeability, followed by inducing a visceral antinociception. Since visceral hypersensitivity and altered intestinal barrier function are two major gastrointestinal conditions in IBS (Camilleri et al., 2012), it is suggested that ghrelin could have a therapeutic capability for IBS through improving the visceral sensation and intestinal permeability. From a different point of view, we may be allowed to speculate that decreased ghrelin signaling in the brain may be capable of inducing leaky gut and visceral hypersensitivity, thereby generating IBS. IBS is a disorder that is closely associated with stress (Whitehead et al., 1992). Since stress is known to have an association with the ghrelin system (Bali and Jaggi, 2016), the present finding furthermore suggests that the ghrelin signaling in the brain may have a beneficial impact on IBS, stress-related disease, through improvement of intestinal barrier function and intestinal sensation. The neuronal rapid improvement in intestinal barrier function by central ghrelin observed in this study may help us understand the brain-gut interaction in stress sensitive gastrointestinal disorders like IBS associated with the altered intestinal permeability (Camilleri et al., 2012).

5. Conclusions

The present study demonstrated that ghrelin acts centrally to improve a disturbed intestinal barrier function through orexinergic signaling and the vagal cholinergic pathway. Central ghrelin may be involved in pathophysiology and a novel therapeutic option in not only gastrointestinal diseases such as irritable bowel syndrome but also non-gastrointestinal diseases associated with the altered intestinal permeability.

Declaration of competing interestCOI

The authors declare no competing financial interests.

CRediT authorship contribution statement

Masatomo Ishioh: Writing - original draft, Formal analysis. **Tsukasa Nozu:** Writing - original draft. **Sho Igarashi:** Formal analysis. **Hiroki Tanabe:** Formal analysis. **Shima Kumei:** Formal analysis. **Masumi Ohhira:** Formal analysis. **Toshikatsu Okumura:** Writing - original draft, Formal analysis.

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