Research article

Brain orexin improves intestinal barrier function via the vagal cholinergic pathway

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\begin{abstract}
Orexins are neuropeptides that are implicated in a number of functions. With regard to the gastrointestinal functions, orexin acts centrally to regulate gastric secretion, gastrointestinal motility and visceral sensation. Little is however known about a role of central orexin in the control of intestinal barrier function. The present study was performed to clarify whether brain orexin plays a role in the control of intestinal permeability. Colonic permeability was estimated in vivo by quantifying the absorbed Evans blue in colonic tissue in rats. Intracisternally administered orexin-A but not orexin-B dose-dependently blocked the increased intestinal permeability by lipopolysaccharide (LPS) or corticotropin-releasing factor while intraperitoneally injected orexin-A failed to block it. Intracisternally administered orexin-A but not orexin-B dose-dependently blocked the increased intestinal permeability by lipopolysaccharide (LPS) or corticotropin-releasing factor while intraperitoneally injected orexin-A failed to block it. Atropine or vagotomy abolished the action by central orexin-A. Intracisternally administered orexin-A but not orexin-B dose-dependently blocked the increased intestinal permeability by lipopolysaccharide (LPS) or corticotropin-releasing factor while intraperitoneally injected orexin-A failed to block it. Atriptine or vagotomy abolished the action by central orexin-A. Intravenous injection of 2-deoxy-D-glucose (2-DG), a central vagal stimulant, significantly blocked the LPS-induced increase in intestinal permeability and atropine prevented the action of 2-DG. Intracisternal injection of SB-334687, a selective orexin 1 receptor antagonist, significantly blocked the action of 2-DG-induced improvement of intestinal hyperpermeability. These results suggest that exogenously administered or endogenously released orexin acts centrally to improve the intestinal hyperpermeability by LPS via the vagal cholinergic pathway. The findings also suggest for the first time that the brain could control intestinal permeability. The neuronal rapid protective advantage to the host by improving the intestinal barrier function by the neuropeptide may help us understand the brain-gut interaction in stress sensitive gastrointestinal disorders like irritable bowel syndrome associated with the altered intestinal permeability.
\end{abstract}

1. Introduction

The intestinal barrier is one of the largest interfaces between the outer world and the human internal milieu. The ability to protect from harmful luminal content and to control the mucosal permeability is defined as intestinal barrier function. A disturbed intestinal barrier function has been described in many human diseases such as irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD) [1]. Several other non-gastrointestinal (GI) diseases have been associated with leaky gut. These include asthma, autism, Parkinson’s disease, multiple sclerosis, eczema, psoriasis, eosinophilic esophagitis, depression, chronic fatigue syndrome, multiorgan failure syndrome, non-alcoholic fatty liver disease, alcoholic cirrhosis, obesity, metabolic syndrome, pancreatitis and rheumatoid arthritis [1]. Thus, improvement of disturbed intestinal barrier function may contribute to control of activity of several GI and non-GI 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 [1,2]. Little is known however how the brain controls intestinal permeability.

Orexins are neuropeptides that are localized in neurons in the lateral hypothalamus [3,4]. Despite their highly restricted origin, orexin nerve fibers are identified widely throughout the central nervous system (CNS) [5] and orexins are implicated in a number of functions. These include feeding, sleep/awake, anxiety/depression or energy balance [6]. In addition, orexin acts centrally to regulate gastrointestinal functions such as gastric secretion, gastrointestinal motility and visceral sensation [7–11]. We do not know however at this moment...
whether orexin is involved in the regulation of intestinal barrier function. The present study was therefore performed to clarify a hypothesis that brain orexin may play a role in the control of intestinal permeability in rats. Here we provide an evidence that brain orexin improves colonic hyperpermeability through the vagal cholinergic pathway, suggesting for the first time intestinal barrier function is regulated by the CNS.

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 orexin-A, orexin-B and rat/human corticotropin-releasing factor (CRF) were purchased from Peptide Institute Inc., Osaka, Japan and dissolved in normal saline. Lipopolysaccharide (LPS) obtained from Escherichia coli with the serotype O55:B5, 2-deoxy-D-glucose (2-DG) and atropine (Sigma-Aldrich, St. Louis, MO) were dissolved in normal saline. Selective orexin 1 receptor (OX1R) antagonist, SB-334867 (40 μg), a selective OX1R antagonist, was obtained from Toxor Pharmaceuticals, Ellisville, MO) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

2.4. Measuring colonic permeability

The permeability was determined 3 h or 4 h after injecting LPS or CRF, respectively according to our previous reports [12,13]. Colonic permeability measurement was performed as previously described [12]. The rats anesthetized by intraperitoneal injection of atropine (1 mg/kg) or surgical vagotomy on the intrinsic medially administered orexin (10 μg/10 μl)-induced change of colonic hyperpermeability by LPS. The surgical bilateral vagotomy was performed as previously described [7]. To examine whether endogenous orexin in the brain mediates the brain orexin-induced alteration of colonic hyperpermeability, we tested the effect of intracisternal injection of orexin-A at a dose of 10 μg by itself did not change the basal permeability measurement was performed as previously described [12]. The rats anesthetized by intraperitoneal injection of atropine (1 mg/kg) or surgical vagotomy on the intrinsic medially administered orexin (10 μg/10 μl)-induced change of colonic hyperpermeability by LPS. The surgical bilateral vagotomy was performed as previously described [7]. To examine whether endogenous orexin in the brain mediates the brain orexin-induced alteration of colonic hyperpermeability, we tested the effect of intracisternal injection of orexin-A at a dose of 10 μg by itself did not change the basal

2.5. Experimental procedures

Initially, we examined the dose-dependent effects of intracisternal injection of orexin-A on the subcutaneous LPS-induced colonic hyperpermeability. Rats received intracisternal or intraperitoneal injections of several doses of orexin-A or orexin-B, and then LPS or CRF was injected. 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 [14]. Next, to clarify whether the vagal system is involved in the central orexin-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 orexin (10 μg/10 μl)-induced change of colonic hyperpermeability by LPS. The surgical bilateral vagotomy was performed as previously described [7]. To examine whether endogenous orexin in the brain mediates the brain orexin-induced alteration of colonic hyperpermeability, we tested the effect of intracisternal injection of orexin-A at a dose of 10 μg by itself did not change the basal

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

We first examined the effects of central orexin on the intestinal permeability. As shown in Fig. 1A, subcutaneously administered LPS at a dose of 1 mg/kg significantly increased the colonic permeability, being in good agreement with our previous study [12]. Intracisternal injection of orexin-A at a dose of 10 μg by itself did not change the basal

Fig. 1. Effects of intracisternal injection of orexin-A on the increased colonic permeability by LPS (A) or CRF (B). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *P < 0.01, when compared with saline ic + LPS sc. # P < 0.01, when compared with saline ic + LPS sc (A) or saline ic + CRF sc (B), respectively. ic, intracisternal; sc, subcutaneous.
To change the colonic permeability (Table 1), suggesting that orexin-A or intracisternal injection of orexin-B at a dose of 10 μg/g tissue increased the colonic permeability, as shown in our previous study [13]. In this condition, intracisternal injection of orexin-A at the similar doses could potently block the colonic hyperpermeability (Fig. 1B), confirming the improvement of intestinal barrier function by brain orexin in an another model.

Next, we examined the mechanisms of orexin-A-induced improvement of colonic hyperpermeability. Orexin acts centrally in the dorsal motor nucleus of the vagus (DMN) to increase the vagal tone [15,16] and the proximal colon evaluated in this study is innervated by the vagus nerve. We therefore examined the role of the vagal system in the improvement of intestinal barrier function by brain orexin. Although intraperitoneal injection of atropine by itself could not change the basal colonic permeability (29.7 ± 2.1 in saline vs 29.7 ± 2.1 in atropine, μg/g tissue) or the LPS-induced colonic hyperpermeability, atropine completely reversed the blockade of orexin-A on the LPS-evoked colonic hyperpermeability (Fig. 2A). Vagotomy also completely reversed the blockade of orexin-A on the LPS-evoked colonic hyperpermeability (Fig. 2B), suggesting that pharmacological or surgical blockade of the vagal cholinergic pathway canceled the protective action of central orexin on the LPS-evoked colonic hyperpermeability. These results suggest the vagal cholinergic pathway is involved in the orexin-induced improvement of intestinal barrier function.

As illustrated in Fig. 3, intravenous 2-DG, a central vagal stimulant [17], significantly blocked the stimulated colonic permeability by LPS. Atropine blocked the effect of 2-DG, supporting that the vagal cholinergic stimulation mediates the improvement of colonic hyperpermeability by 2-DG. Next, to clarify a hypothesis that endogenous orexin may be involved in the 2-DG-induced blockade of stimulation of colonic permeability, we used SB-334867, a selective OX1R antagonist [18]. While intracisternal injection of the antagonist by itself did not change the colonic permeability stimulated by LPS, SB-334867 completely reversed the 2-DG-induced blockade of stimulation of colonic permeability, suggesting that endogenous orexin in the brain plays a vital role in the improvement of intestinal barrier function.

4. Discussion

With regard to the role of central orexin in digestive functions, we have previously showed that orexin act centrally to stimulate gastric secretion or gastrointestinal motility, and induce a visceral atinomicceptive action [7–11]. In addition, the present study has shown that exogenously administered or endogenously released orexin improves the intestinal hyperpermeability, suggesting that orexin also plays a role in regulation of intestinal barrier function. To our knowledge, there is no data how the brain controls the intestinal barrier function. The present findings therefore provided a solid evidence for the first time that intestinal permeability is regulated by the CNS. This neuronal protective advantage conferred to the host by the neuropeptide, through the improvement in intestinal barrier function, may help us understand the brain-gut interaction in stress sensitive gastrointestinal disorders like IBS associated with the altered intestinal permeability because stress induces intestinal hyperpermeability and can influence the onset of symptoms and predict the clinical outcome in IBS [19–21].

The present results strongly suggest the vagal cholinergic pathway is involved in the orexin-induced improvement of intestinal

| N | Colonic permeability (μg/g tissue) |
|---|----------------------------------|
| 3 | 23.5 ± 1.1                       |
| 3 | 56.1 ± 3.2                       |
| 3 | 52.4 ± 3.8                       |
| 3 | 25.5 ± 1.4                       |
| 3 | 63.9 ± 4.2                       |
| 3 | 68.2 ± 4.7                       |

Table 1: Effects of intraperitoneal orexin-A or intracisternal orexin-B on the LPS-induced colonic hyperpermeability.

The present results strongly suggest the vagal cholinergic pathway is involved in the orexin-induced improvement of intestinal

![Fig. 2.](image-url) Effects of atropine (A) or vagotomy (B) on the orexin-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. †P < 0.01, when compared with LPS sc + orexin ic + saline ip (A) or LPS sc + orexin ic + sham, respectively. sc, subcutaneous; ic, intracisternal; ip, intraperitoneal.

![Fig. 3.](image-url) Effects of intracisternal orexin 1 receptor antagonist (SB-334867, SB) 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.

The present results strongly suggest the vagal cholinergic pathway is involved in the orexin-induced improvement of intestinal

Visceral sensation was evaluated by colonic distension-induced abdominal withdrawal reflex (AWR). Each data represents the mean ± S.E.
hyperpermeability because pharmacologically or surgically blockade of the vagal cholinergic pathway completely abolished the action of orexin to improve intestinal barrier function. The cells of origin innervating the gastrointestinal tract through the vagus nerve are located in the DMN [22]. Injection of orexin-A into the cerebrospinal fluid induced c-fos expression in DMN neurons in rats [23]. A couple of studies [15,16] have shown that orexin-A directly activates the DMN neurons and OX1R was highly expressed in the DMN neurons [24]. These findings suggest that orexin-A directly acts on the OX1R and excites the DMN neurons that project to the proximal colon evaluated in the present study. Based upon these results, we could conclude that central injection of orexin-A activates directly the DMN neurons, followed by increasing the vagal tone, thereby improving intestinal permeability as shown in this study.

Next, we tried to clarify whether endogenous orexin in the brain play a role in the control of intestinal permeability. As shown in this study, we have demonstrated that 2-DG, a central vagal stimulant, potently improved the increased intestinal permeability and atropine prevented the action, suggesting that intestinal barrier function is maintained by the 2-DG-induced stimulation of the vagal cholinergic pathway. Since the present study suggested that both 2-DG and orexin-A blocked the increased intestinal hyperpermeability through the vagal pathway, we made a hypothesis that orexin-A in the brain might be involved in the 2-DG-induced improvement of intestinal barrier function. As clearly shown in the present study, SB-334867, a specific OX1R antagonist, canceled the 2-DG-induced improvement of intestinal hyperpermeability, suggesting that endogenous orexin mediates the blockade of increased intestinal hyperpermeability by 2-DG through OX1Rs probably expressed in the DMN neurons.

Stress is associated with increased intestinal permeability and visceral hyperalgesia [19]. However, it is unknown whether enhanced visceral pain and permeability are intrinsically linked and correlate. Creekmore et al. [25] have recently demonstrated that the level of stress-associated visceral hyperalgesia directly correlates with the magnitude of altered colon epithelial permeability, suggesting a tight correlation between visceral sensation and intestinal permeability. We have previously shown that orexin induced a visceral hypopersensitivity in rats [11] and the present study clearly demonstrated that orexin improved the intestinal permeability. These findings suggest that the improvement of intestinal barrier function by orexin would be deeply associated with the changed visceral sensation. In other words, orexin may act centrally to improve intestinal permeability, followed by inducing a visceral antinoceception.

We have recently presented the hypothesis that decreased central orexin signaling may be involved in the development of IBS [11,24,26]. This is because decreased orexin signaling has been shown to induce a number of characteristics observed in patients with IBS, including, and disorders. The increase in intestinal permeability by orexin demonstrated in this study further supports the above hypothesis since intestinal hyperpermeability is also an important pathogenic feature of IBS [1,19].

In conclusion, brain orexin plays a vital role the regulation of intestinal barrier function, and the orexin signal is considered to be a target to control the altered intestinal barrier function especially in IBS.

Declaration of competing interest

The authors declare no competing financial interests.

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