学位論文

Centrally administered orexin prevents lipopolysaccharide and colchicine induced lethality via the vagal cholinergic pathway in a sepsis model in rats.
（オレキシン中枢投与は,リポポリサッカライドとコルヒチンによるラット敗血症死をコリン作動性迷走神経路を介して改善する）に関する研究

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Centrally administered orexin prevents lipopolysaccharide and colchicine induced lethality via the vagal cholinergic pathway in a sepsis model in rats

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ABSTRACT

Orexins are neuropeptides implicated in several physiological functions. Accumulating findings suggest a relationship between orexin and sepsis. A recent study demonstrated that orexin acts centrally to improve conditions in sepsis. The present study aims to clarify the precise mechanisms by which central orexin could induce a protective action against septic conditions. We established a new septic model by treating rats with lipopolysaccharide (LPS) and colchicine and used this to examine the effect of brain orexin on survival. Observation of survival was stopped three days after the chemicals injection or at death. We established a lethal model (rats died within 24 h) by injecting subcutaneously a combination of 1 mg/kg LPS and 1 mg/kg colchicine. A Toll-like receptor 4 (TLR4) inhibitor completely blocked lethality, suggesting a vital role of LPS-TLR4 signaling in the process. Intracisternal orexin-A dose-dependently reduced lethality in the sepsis model while neither intracisternal orexin-B nor intraperitoneal orexin-A changed the mortality rate. Vagal stimulation with carbachol or 2-deoxy-D-glucose improved survival and atropine potently blocked the protection by carbachol or 2-deoxy-D-glucose. The orexin-A-induced reduction of lethality was significantly blocked by atropine or surgical vagotomy. Intracisternal injection of an OX_1 receptor antagonist blocked the improvement of survival by intracisternal injection of orexin-A, carbachol, or 2-deoxy-D-glucose. These results suggest that orexin acts centrally to reduce the lethality in our septic model treated (LPS and colchicine). Activation of the vagal cholinergic pathway may mediate the action of orexin, and the OX_1 receptor in the brain might play a role in the process. Since the efferent vagus nerve mediates anti-inflammatory mechanisms, we speculate that the vagal cholinergic anti-inflammatory pathway is implicated in the mechanisms of septic lethality reduction by brain orexin.

1. Introduction

Sepsis is one of the oldest and most elusive syndromes in medicine [1]. Sepsis and septic shock are associated with high mortality and substantial morbidity. More than 25%–30% of patients with sepsis die, with hospital mortality for septic shock approaching 40%–60% [2]. A novel therapeutic option should be developed to improve the outcome of septic conditions.

Orexins are neuropeptides that are produced in neurons in the lateral hypothalamus (LHA) [3]. Despite their highly restricted origin, orexin nerve fibers are spread throughout the central nervous system (CNS) [4] and orexins are implicated in several physiological functions. These include feeding, sleep/wake cycle, anxiety/depression, and energy balance [4].

Accumulating findings suggest a relationship between orexin and sepsis. A couple of reports showed that infusing lipopolysaccharide (LPS), an endotoxin, reduced the number of orexin-immunoreactive neurons [5–7]. Although they did not show a relationship between orexin and lethality or toxicity caused by LPS, we speculate that decreased orexin signaling in the brain plays a role in the pathophysiology of LPS-induced sepsis. Deutschman et al. have shown that intracerebroventricular (ICV) administration of orexin modulates heart rate

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and body temperature, and increases adrenocorticotropic hormone levels in a murine sepsis model (induction by cecal ligation and puncture), suggesting that the orexinergic system modulates the sepsis-induced changes in vital signs and pituitary hormone levels [8]. Ogawa et al. [9] recently demonstrated that peripherally administered orexin-A improved survival in LPS-induced septic mice. They also showed that when mice were treated with LPS to increase the permeability of the blood brain barrier, peripherally administered orexin-A was able to act centrally. Based on this, they suggested that peripherally injected orexin-A would be able to reduce lethality in LPS-treated mice. These findings suggest that brain orexin signaling may contribute to a novel therapeutic approach to treat sepsis. Little is known however about the precise mechanism by which central orexin could induce its protective action against septic conditions. This study aims to clarify the possible mechanisms of orexin-induced improvement of survival in septic conditions.

In the present study, we first tried to establish a new septic model in rats treated with a combination of LPS and colchicine because the latter synergistically increases the toxicity of LPS in rats [10]. Experiments were performed in the novel sepsis model by LPS and colchicine.

2. Materials and 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 at a room temperature of 23 °C–25 °C.

2.3. Chemicals

LPS (obtained from Escherichia coli with the serotype O55:B5 (Sigma-Aldrich, St. Louis, MO, USA), colchicine (Wako Pure Chemical Corp., Osaka, Japan), carbachol (Sigma-Aldrich, St. Louis, MO, USA), orexin-A and orexin-B (Peptide Institute Inc., Osaka, Japan), 2-deoxy-D-glucose (2-DG) and atropine (Sigma-Aldrich, St. Louis, MO, USA) were all dissolved in normal saline. SB-334867 (Tocris Bioscience, Ellisville, MO, USA) or TAK-242 (Cayman Chemical, Ann Arbor, MI, USA) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA).

2.4. Survival experiments

We first established a novel and rapid lethality septic model in rats. We had incidentally and surprisingly observed in previous experiments that almost all rats treated with a subcutaneous injection of 1 mg/kg LPS and 1 mg/kg colchicine died within 24 h (unpublished observation), while each chemical alone did have this effect. We therefore developed a novel and rapid lethality rat model by administering subcutaneous LPS and colchicine. We assessed survival as the primary endpoint. The observation was stopped three days after injection or at death. Survival rates were represented as Kaplan–Meier curves. Survival time was defined as period (h) until death or 72 h when rats did not die within 3 days. First, we examined the effect of subcutaneous injection of either LPS (1, 3, 10, 30, or 50 mg/kg) or colchicine (1, 3, or 10 mg/kg) on survival. Next, the effect of the combination of LPS (1 mg/kg) and colchicine (0, 0.3, 1, 3, or 10 mg/kg) on the survival period was investigated. Subcutaneous injection of LPS at a dose of 1 mg/kg and colchicine at a dose of 1 mg/kg consistently induced death within 24 h. We used this sepsis model induced by subcutaneous administration of both LPS (1 mg/kg) and colchicine (1 mg/kg) in later experiments of this study. In this model, 3 mg/kg of TAK-242, a TLR4 inhibitor, was injected intraperitoneally to examine the role of LPS-TLR4 signaling. To examine the effect of central orexin on survival in our sepsis model, we injected saline, orexin-A (2, 5, or 10 μg) or orexin-B (2, 5, or 10 μg) intracisternally, or saline or orexin-A (10 μg) intraperitoneally, immediately followed by subcutaneous injection of LPS and colchicine, and survival period was monitored. Intraperitoneal carbachol (0.02 mg/kg) or intravenous 2-DG (200 mg/kg) were injected to clarify whether the vagal cholinergic pathway is involved in the lethality of the model. Then, the effect of intraperitoneal atropine on the action of carbachol or 2-DG was examined. The effect of either intraperitoneal atropine or surgical bilateral subdiaphragmatic vagotomy on the change of lethality by central administration of orexin-A (10 μg/10 μl) was examined to clarify if the vagal cholinergic pathway mediates the orexin-induced decrease in lethality. Surgical bilateral subdiaphragmatic vagotomy was performed as previously described [11]. Intracisternal injection of SB-334867 (40 μg/10 μl), an OX_1 receptor antagonist [12], was performed immediately before intracisternal orexin-A (10 μg), intravenous 2-DG (200 mg/kg), or intraperitoneal carbachol (0.02 mg/kg). The intracisternal injection was performed under brief isoflurane anesthesia using a 10-μl Hamilton microsyringe after the rats were mounted on a stereotaxic apparatus (David Kopf Instruments, Tijunga, CA), as reported previously [13]. The doses and injection routes of TAK-242, carbachol, 2-DG, atropine, or SB-334867 were selected according to previous reports [14–17]. Injection volume was 0.2 ml for subcutaneous, intraperitoneal, or intravenous administration.

2.5. Serum analysis

The serum was obtained from the inferior vena cava and biochemical analysis was performed by Oriental Yeast Co., Ltd, Japan.

2.6. Measuring colonic permeability

Colonic permeability measurement with Evans blue was performed as previously described [18–20] with minor modifications. The permeability was determined 3 h after injection of the tested chemicals, as shown in our recent reports [14,16].

2.7. Statistical analysis

Survival rates were represented as Kaplan–Meier curves and comparisons of survival curves were performed using a Mantel–Cox log-rank test using EZR [21]. All values are expressed as means ± SEM. Unpaired Student *t*-test was used for comparisons between two groups. 1-way ANOVA was used for comparisons between more than two groups, and individual group means were then compared using a Bonferroni's test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. A novel lethality model was developed by administering LPS and colchicine subcutaneously to rats

Since LPS is capable of inducing death as endotoxin shock/sepsis, we first examined the dose–response effect of the subcutaneous injection of LPS alone on survival. As shown in Fig. 1A and 1B, rats did not die within 72 h when LPS was administered at doses lower than 30 mg/kg. At 50 mg/kg, half the rats died within 24 h.

Fig. 1C and 1D shows the dose–response effect of the subcutaneous injection of colchicine on survival. Rats treated with a dose of 1 mg/kg did not die at all until 72 h after injection. In contrast, 3 or 10 mg/kg of colchicine dose-dependently shortened the survival period. All rats treated with 3 mg/kg or more died within 72 hrs.

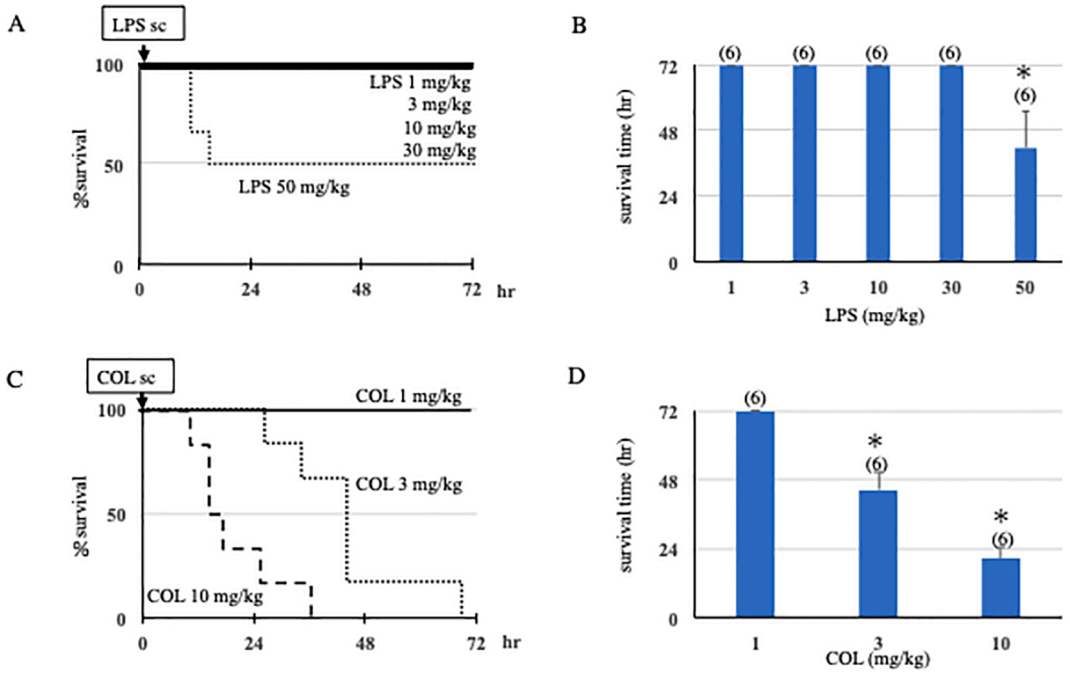

Fig. 1. Dose-response effect of subcutaneous injection of LPS (A, B) or colchicine (COL) (C, D) on survival. Kaplan-Meier survival curves (A, C) and survival time (B, D). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. (A) *P < 0.01, when compared with either 1, 2, 10, or 30 mg/kg of LPS. (B) *P < 0.01, when compared with 1 mg/kg of COL.

Next, we examined the effect of LPS and colchicine combined on survival. Fig. 2A and 2B show the dose–response effect of colchicine with 1 mg/kg of LPS. Colchicine dose-dependently shortened the survival in rats treated with 1 mg/kg LPS. All rats died within 24 h after subcutaneous injection of the combination of 1 mg/kg of LPS and 1 mg/kg of colchicine while all rats treated with either 1 mg/kg of LPS or 1 mg/kg colchicine survived more than 72 h (Fig. 1), suggesting a synergistic effect. The most significant effect was observed in rats treated with 1 mg/kg of colchicine or more along with 1 mg/kg LPS. Thus, the combination of 1 mg/kg LPS and 1 mg/kg colchicine was selected as our sepsis model.

To examine possible factors for the lethality, a biochemical analysis was performed. Four hours after administration of LPS, colchicine, or both, serum biochemistry was evaluated. As shown in Fig. 3, the combination of LPS and colchicine increased serum levels of AST, ALT, and Cre, but not LDH and CK when compared with control or LPS alone,

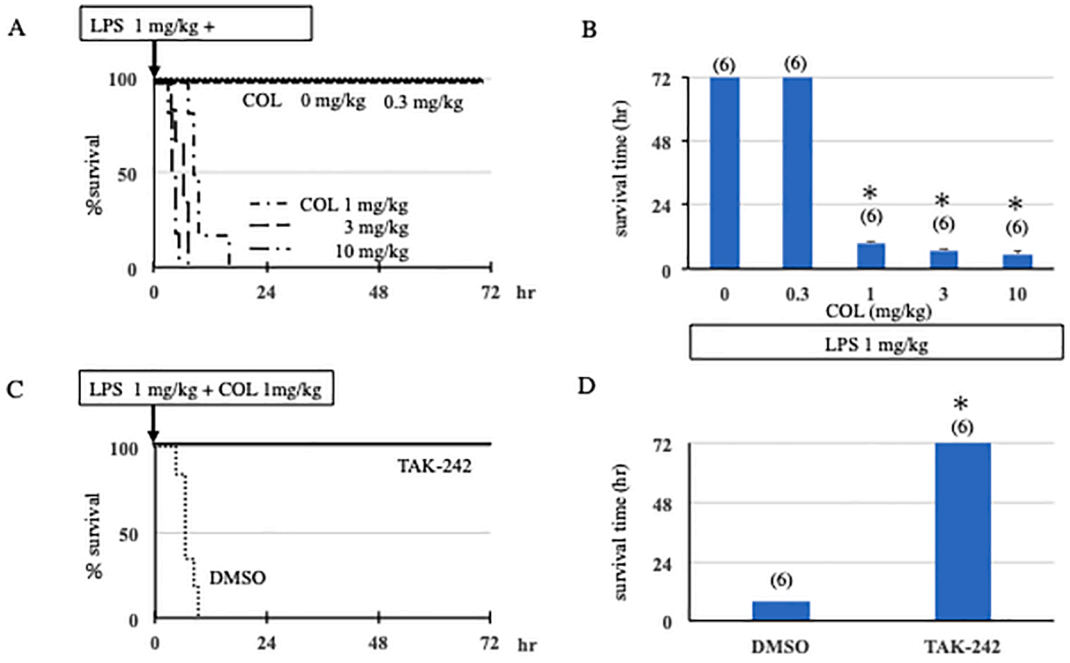

Fig. 2. Dose-response effect of colchicine on the survival of rats treated with LPS (1 mg/kg). Kaplan-Meier survival curve (A) and survival time (B). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *P < 0.01, when compared with 0 mg/kg COL together with 1 mg/kg LPS. (B) Effect of TAK-242 on the lethality of rats treated with subcutaneous injection of 1 mg/kg LPS and 1 mg/kg COL. Kaplan-Meier survival curve (C) and survival time (D). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *P < 0.01, when compared with DMSO.

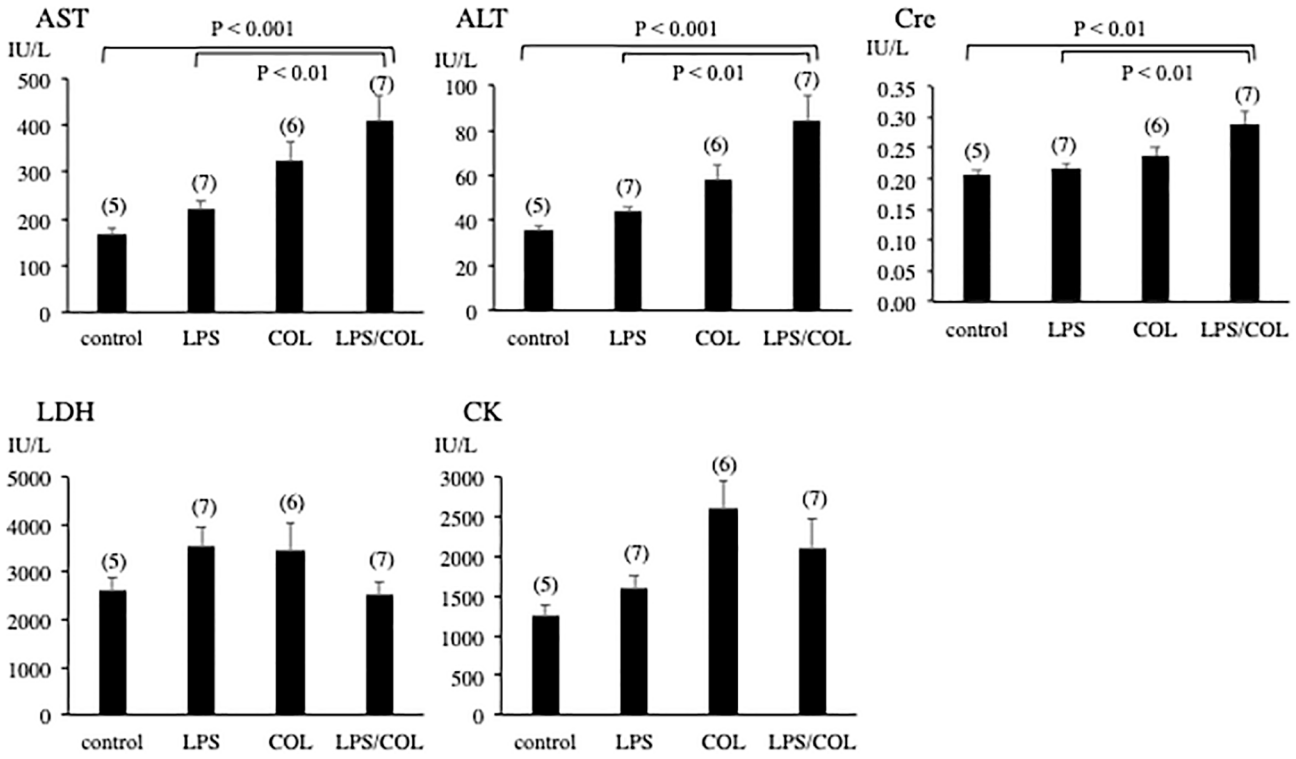

Fig. 3. Effect of subcutaneous LPS, colchicine (COL), or both on serum biochemistry. Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses.

suggesting that hepatic and renal toxicity might at least in part contribute to the lethality in this model.

We examined the effect of TAK-242 on survival. As illustrated in Fig. 2C and 2D, TAK-242 drastically increased the survival period, suggesting that the LPS-TLR4 signaling plays a key role in the LPS and colchicine induced sepsis model.

3.2. Intracisternal injection of orexin-A improved survival in the sepsis model

Next, we examined the effect of orexin-A on survival in the sepsis model. Intracisternal injection of orexin-A dose-dependently improved the survival period in rats treated with LPS and colchicine (Fig. 4A and 4B). In contrast, neither intraperitoneal injection of orexin-A at 10 μg (Fig. 4C and 4D) nor intracisternal injection of orexin-B at 2, 5, or 10 μg (Fig. 4E and 4F) changed the survival of rats treated with LPS and colchicine, suggesting that orexin-A acts specifically in the brain to prevent the death process in this sepsis model.

3.3. Improvement of survival by central orexin-A was blocked by atropine or vagotomy

Next, we examined the role of the vagal cholinergic pathway in the sepsis model. Fig. 5A and 5B show how intraperitoneal carbachol potently improved the survival period. It was also shown that the lethality of the LPS and colchicine combination was significantly attenuated by intravenous 2-DG, a central vagal stimulant (Fig. 5C and 5D) [22]. As shown in Figure 5, 0.3 mg/kg atropine significantly blocked the improvement of survival by 0.02 mg/kg carbachol, and 2 mg/kg atropine blocked the 2-DG (200 mg/kg)-induced reduction of lethality, suggesting that carbachol or 2-DG improves survival through the vagal cholinergic pathway. Since orexin-A activates the vagal efferent pathway [23], we hypothesized that the vagal cholinergic pathway mediates the central orexin-A-induced prevention of the death process in the LPS and colchicine model. We therefore investigated the effect of blockade of the vagal cholinergic pathway by atropine or surgical vagotomy on the orexin-A-induced improvement of survival. Although 0.3 mg/kg atropine alone did not change survival, it significantly blocked the orexin-A-induced improvement of survival (Fig. 6A and 6B). Besides, surgical vagotomy blocked the orexin-induced reduction of lethality (Fig. 6C and 6D), suggesting that the vagal cholinergic pathway may play a key role in the improvement of survival by central orexin-A.

3.4. Orexin-A-, 2-DG-, or carbachol-induced improvement of survival was blocked by OX_1 receptor antagonist

To assess whether the OX_1 receptor is involved in the improvement of lethality in our model, we examined the effect of SB-334867 on the orexin-A-induced improvement of survival. While SB-334867 alone did not affect survival (Fig. 7A and 7B), it significantly attenuated the central orexin-A-induced improvement of survival (Fig. 7C and 7D), suggesting that OX_1 receptor mediates the action of orexin-A. We then evaluated the effect of SB-334867 on the carbachol- or 2-DG-induced improvement of survival in the LPS and colchicine model. As clearly illustrated in Fig. 7E, 7F, 7G, and 7H, SB-334867 significantly blocked the 2-DG- or carbachol-induced improvement of survival, once again suggesting that the OX_1 receptor is involved in the protection against lethality and additionally that endogenously released orexin plays a vital role in the improvement of survival our model.

3.5. LPS and colchicine induced colonic hyperpermeability were attenuated by centrally administered orexin-A

The effect of intracisternal orexin-A on colonic permeability was examined to clarify whether central orexin may affect the intestinal barrier in rats treated with LPS and colchicine. Subcutaneous LPS at 1 mg/kg potently increased colonic permeability but this was not further increased by subcutaneous injection of colchicine at 1 mg/kg. Intracisternal orexin-A at 10 μg significantly improved the colonic hyperpermeability caused by LPS and colchicine (Fig. 8).

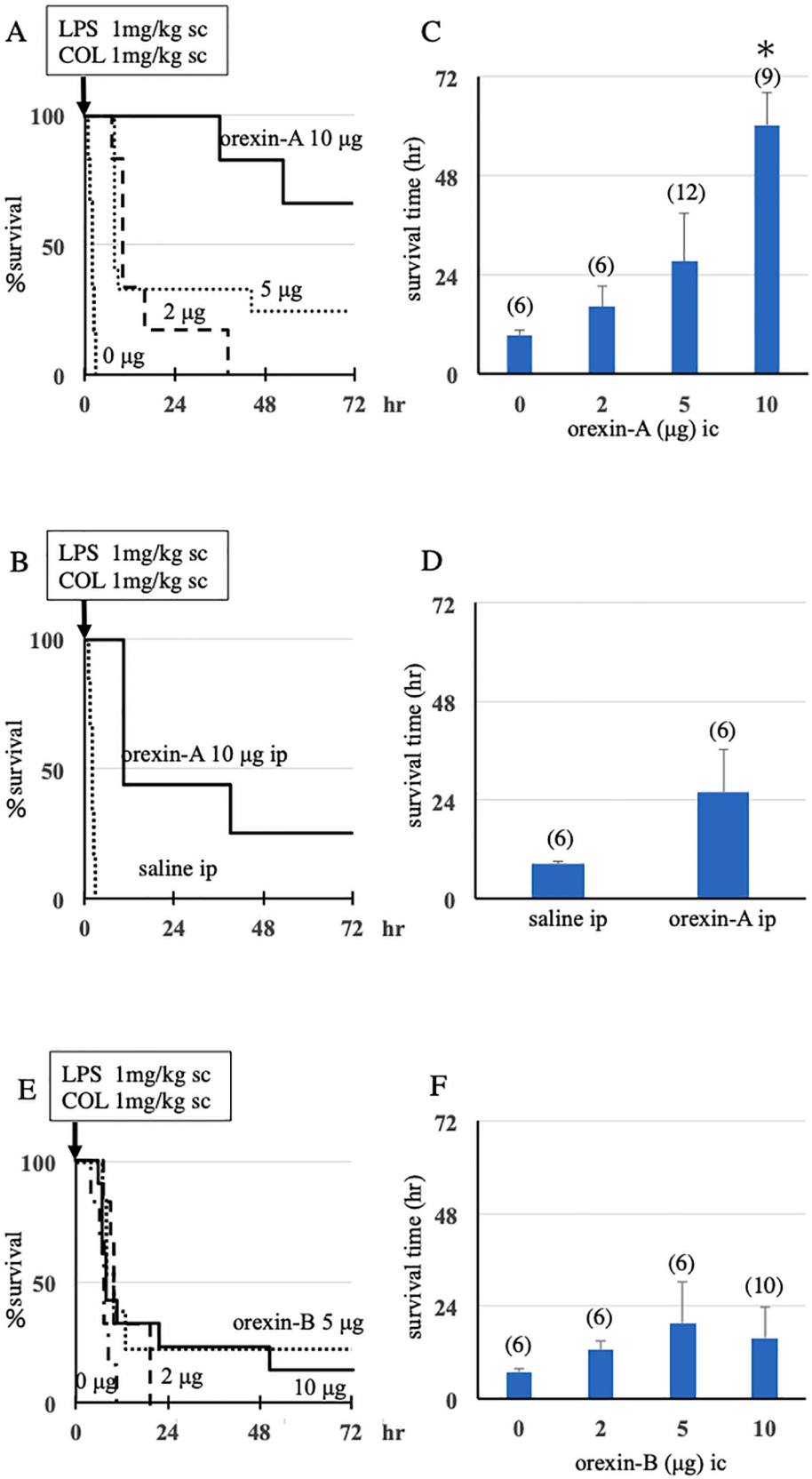

Fig. 4. Effect of orexin on lethality by LPS and colchicine (COL). (A, B) Dose-dependent effect of intracisternal orexin-A on the survival of rats treated with LPS and COL. Kaplan-Meier survival curve (A) and survival time (B). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *P < 0.01, when compared with 0 μg. (C, D) Effect of intraperitoneal injection of orexin-A at a dose of 10 μg on the survival of rats treated with LPS and COL. Kaplan–Meier survival curve (C) and survival time (D). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. (E, F) Dose-dependent effect of intracisternal orexin-B on the survival of rats treated with LPS and COL. Kaplan–Meier survival curve (E) and survival time (F). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses.

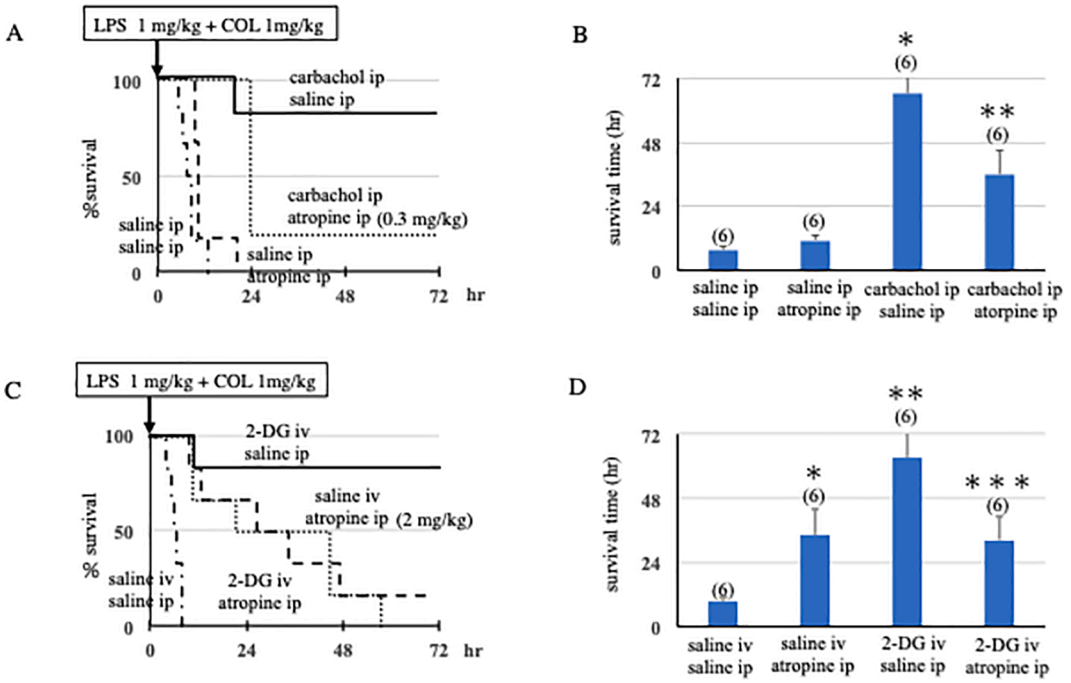

Fig. 5. Effect of intraperitoneal carbachol (A, B) or intravenous 2-DG (C, D) on the survival of rats treated with LPS and colchicine (COL). Effect of intraperitoneal 0.02 mg/kg carbachol with or without intraperitoneal 0.3 mg/kg atropine on the survival of rats treated with LPS and colchicine. Kaplan–Meier survival curve (A) and survival time (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 + saline. **P < 0.01, when compared with carbachol + atropine. Effect of intravenous 2-DG at 200 mg/kg with or without intraperitoneal atropine at 2 mg/kg on the survival of rats treated with LPS and COL. Kaplan-Meier survival curve (C) and survival time (D). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *P < 0.01, when compared with saline + saline (B) or saline + saline (D). **P < 0.01, when compared with carbachol + saline (B) or 2-DG + saline (D).

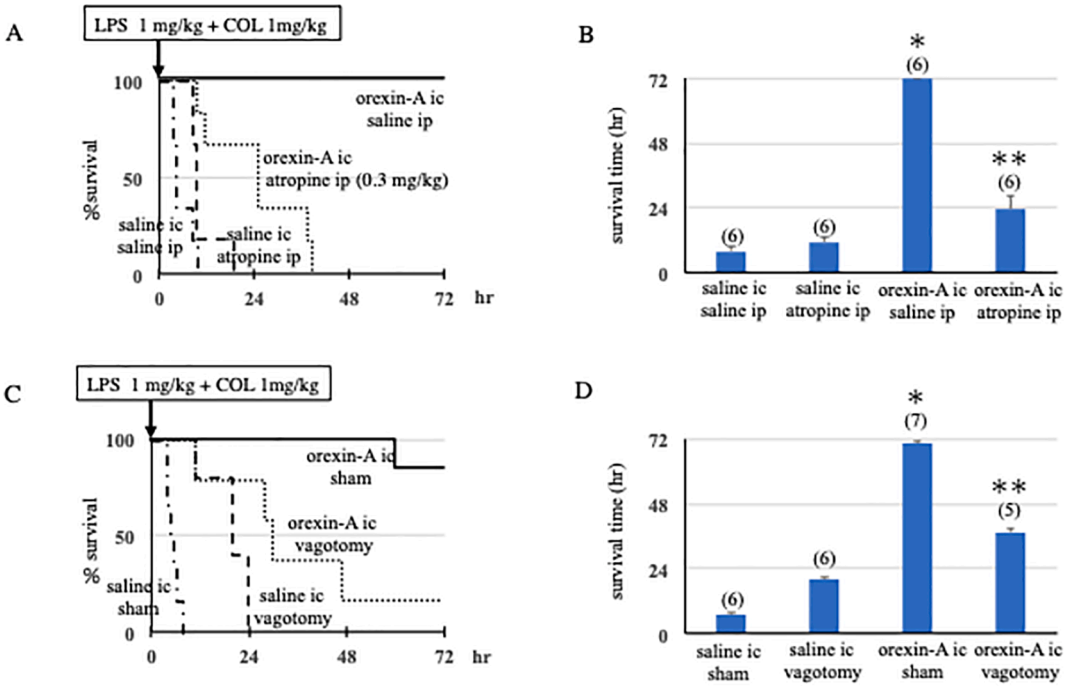

Fig. 6. Effect of atropine (A, B) or surgical vagotomy (C, D) on the orexin-A-induced improvement of lethality in rats treated with LPS and colchicine (COL). Kaplan–Meier survival curve (A, C) and survival time (B, D). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *P < 0.01, when compared with saline + saline (B) or saline + sham (D). **P < 0.01 when compared with orexin-A + saline (B) or orexin-A + sham (D).

4. Discussion

Ogawa et al. [9] showed that ICV infusion of orexin-A at 0.3 mg/24 h significantly reduced the lethality of intraperitoneal injection of LPS in mice. A significant effect was obtained approximately 60 h after the infusion. They needed to observe the survival for over 7 days to obtain a

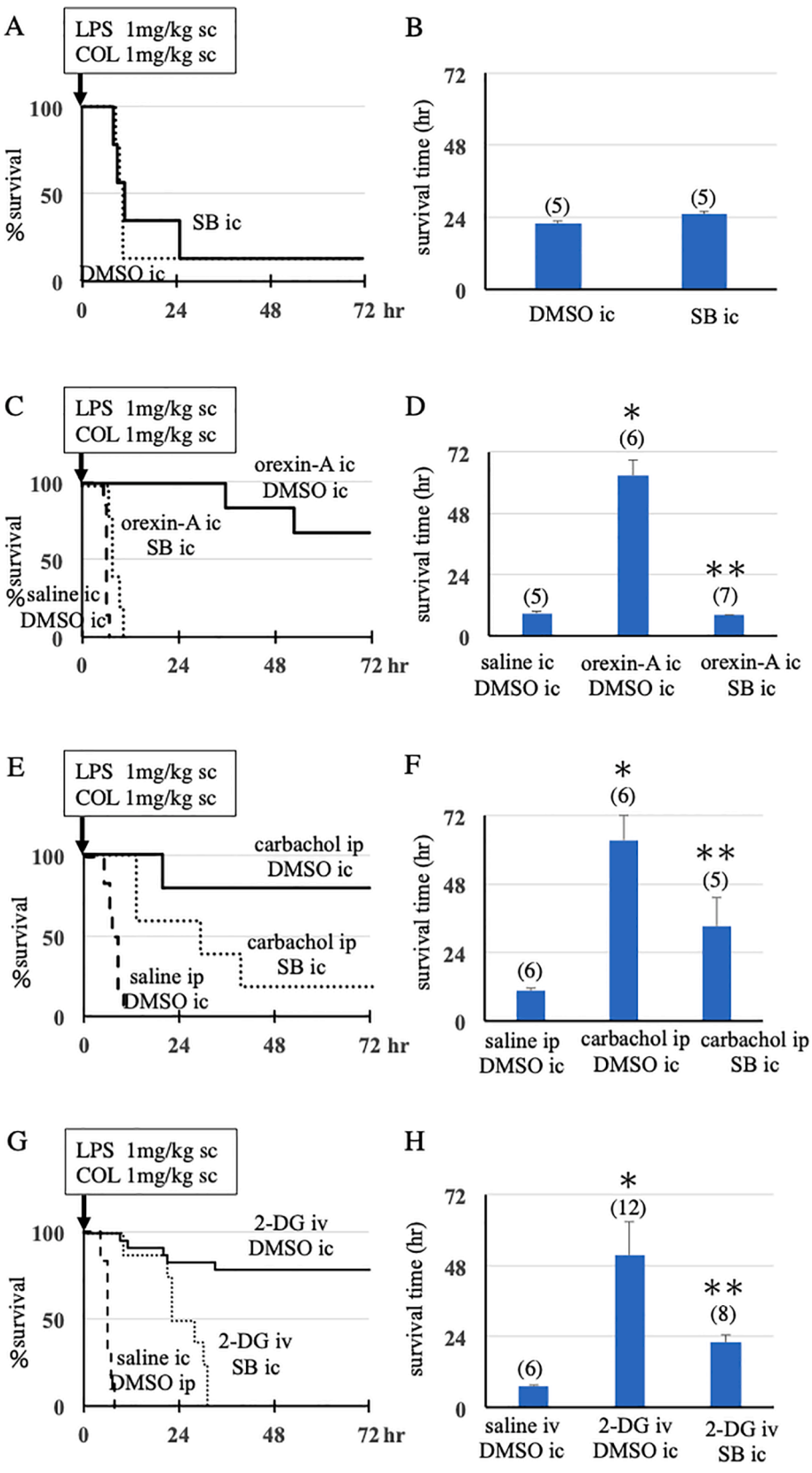

Fig. 7. (A, B) Effect of intracisternal SB-334867 (SB) alone on the survival of rats treated with LPS and colchicine (COL). Effect of intracisternal SB-334867 on intracisternal orexin-A- (C, D), carbachol- (E, F) or 2-DG-induced (G, H) improvement of lethality in rats treated with LPS and COL. Kaplan–Meier survival curve (A, C, E, G) and survival time (B, D, F, H). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. (D) *P < 0.01, when compared with saline (ic) + DMSO (ic), ** P < 0.01, when compared with orexin-A (ic) + DMSO (ic). (F) * P < 0.01, when compared with saline + DMSO (ic). ** P < 0.01, when compared with carbachol + DMSO (ic). (H) * P < 0.01, when compared with saline + DMSO (ic). ** P < 0.01, when compared with 2-DG + DMSO (ic).

significant result. <0.01 mg of centrally administered orexin-A in rats significantly changed bodily functions such as gastrointestinal secretion and motility in previous studies [11,24–26]. In the study by Ogawa et al., mice received a much higher dose (0.3 mg/mouse vs 0.01 mg/rat), which explains the long period of over 60 h needed to observe an effect. To examine the effect of one shot of the central injection of orexin on the lethality in rats, we needed a faster sepsis model. First, we established a novel and rapid sepsis rat model using subcutaneous LPS and colchicine.

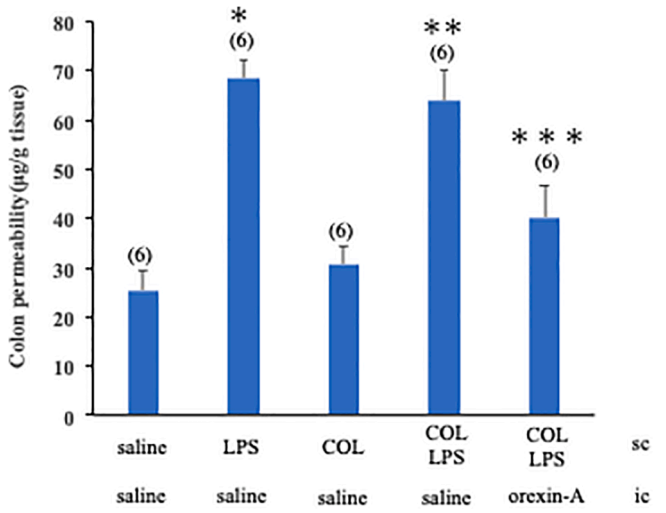

Fig. 8. Effect of intracisternal orexin-A on colonic permeability in rats treated with LPS and colchicine (COL). Each column represents the mean ± S.E.M. The number of rats examined is shown in parentheses. *, ** P < 0.01, when compared with saline + saline. ***P < 0.01, when compared with LPS + COL (sc) + saline ic.

Colchicine possesses anti-inflammatory activity and is used therapeutically in Bechet's disease, familial Mediterranean fever, and gout [27]. However, Colchicine has many potentially toxic properties. For example, colchicine overdose induces shock, hypoventilation, bone marrow depression, coagulation, and acute renal and liver failure [28,29]. It has been reported that acute ingestion of 7–12 mg of colchicine is fatal in humans [30]. Wiesenfeld et al. [10] have shown the acute oral toxicity of colchicine and examined the effect of pre-exposure to LPS on its toxicity in rats. Intraperitoneal injection of LPS (83 μg/kg) produced limited toxicity that normalized within 2 days after administration. However, LPS pretreatment at the same dose 1 h before colchicine administration significantly potentiated colchicine toxicity particularly regarding lethality, suggesting a synergistic effect. Pretreatment with a minimally toxic dose of LPS (to activate the early induced innate immune system) modulated colchicine's toxic potential. They however did not show the mechanism by which LPS increased colchicine-related lethality. It has been recently demonstrated that orally administered colchicine increased serum endotoxin levels and that a TLR4 inhibitor improved the survival of mice who received oral colchicine, suggesting that LPS-TLR4 signaling may play a role in acute colchicine poisoning [31]. Based on these results, LPS and colchicine synergistically induce lethality through a septic condition. Considering these findings, we first tried to develop a sepsis model by injecting both LPS and colchicine. As clearly shown in the present study, the combination of subcutaneous injection of 1 mg/kg LPS and 1 mg/kg colchicine to rats could surprisingly lead to death within 24 h while either LPS or colchicine alone at the same dose failed to be lethal, establishing a novel and rapid sepsis model. The present study directly demonstrated that a TLR4 inhibitor completely blocked the effect of LPS and colchicine on survival, strongly suggesting that TLR4 signaling plays a vital role in the toxicity of LPS and colchicine.

The present study has shown that activation of the vagal cholinergic pathway improved survival outcome in this sepsis model because vagal stimulation with either carbachol or 2-DG potently increased survival and atropine could reverse the action. We suggest the following mechanism for the improvement of survival via the activation of the vagal cholinergic pathway. Many studies confirmed that the release of cytokines from immune cells is partially regulated by the autonomic nervous system [32–35]. Tracey and coworkers described anti-inflammatory mechanisms mediated by the efferent vagus nerve [36]. Stimulation of the vagus nerve significantly reduced inflammation in rats with endotoxemia, and this effect was blocked by vagotomy and atropine administration [37]. We therefore speculate that the vagal cholinergic anti-inflammatory pathway is implicated in the mechanisms of reduction of septic lethality by carbachol or 2-DG in the present model.

Accumulating evidence shows that centrally injected orexin can increase vagal efferent nerve discharge as follows. The cells innervating the vagus nerve are located in the dorsal motor nucleus (DMN) of the vagus nerve [38]. Injection of orexin-A into the cerebrospinal fluid-induced c-fos expression in several neurons in the DMN in rats [39], supporting the assumption that the DMN neurons are excited when orexin-A is injected into the cerebrospinal fluid as in this study. A couple of electrophysiological studies [40,41] have demonstrated that orexin-A directly activates the DMN neurons in rat medullary slices. Also, OX_1 receptor mRNA is highly expressed in neurons in the DMN [42]. These findings suggest that orexin-A directly excites the DMN neurons that project through the vagal efferent nerves. We could therefore conclude that injecting orexin-A into the cerebrospinal fluid directly activates the DMN neurons, which then increase the vagal efferent tone. This pathway plays an important role in the regulation of gastrointestinal functions such as gastric acid secretion, gastric motility, and intestinal permeability [11,14,25]. Atropine treatment or vagotomy canceled the orexin-induced improvement of survival in our model. These findings suggest that orexin acts centrally to activate the vagal cholinergic pathway, inducing an anti-inflammatory response. We suggest that the induction of an anti-inflammatory response by central orexin via the vagal cholinergic pathway contributes to the improvement of survival under septic conditions.

Leaky gut is one of the factors contributing to the development of sepsis [43,44]. We recently demonstrated that orexin improves colonic hyperpermeability in rats by acting centrally through the vagal cholinergic pathway [14]. We therefore speculated that improvement of the intestinal barrier function by brain orexin mediates the potent blockade of lethality in this LPS and colchicine sepsis model. In other words, orexin stimulates vagal tone, which inhibits the intestinal hyperpermeability. This improvement of the leaky gut then activates the peripheral immune system, effectively promoting an anti-inflammatory response which counteracts the septic condition. The present study shows that colonic permeability is enhanced by LPS and colchicine, and intracisternal orexin-A significantly inhibits the colonic hyperpermeability. We therefore speculate that improvement of the intestinal barrier function is one of the factors that reduce lethality in septic rats treated with orexin.

In summary, the present study suggests that orexin acts centrally through the vagal cholinergic pathway to reduce septic lethality. Activation of orexinergic signaling in the brain, followed by stimulation of the vagal cholinergic pathway appears to be a novel therapeutic avenue for systemic inflammation such as sepsis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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