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Endogenous orexin-A in the brain mediates 2-deoxy-D-glucose-induced stimulation of gastric motility in freely moving conscious rats

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Running head; Orexin and gastric motility

Abstract

Background. Increasing evidence has indicated that brain orexin plays a vital role in the regulation of gastrointestinal (GI) physiology such as gastric acid secretion and GI motility. The aim of this study was to elucidate the effects and mechanisms of orexin on gastric motility in non-fasted rats. ***Methods***. In this study, we recorded intraluminal gastric pressure waves in freely moving conscious rats by manometric catheter located in antrum. We assessed area under the manometric trace as motor index (MI), and compared it for 1 h before and after drug administration. ***Results.*** Intracisternal (ic) injection of orexin-A (10 μg) significantly increased MI, but intraperitoneal (ip) injection did not have any effect. Pretreatment of ip injection of atropine significantly blocked the orexin-A-induced stimulation of gastric motility. Intravenous injection of 2-Deoxy-D-glucose (2-DG, 200 mg/kg), a central vagal stimulant, significantly increased MI. Ic injection of SB-334687 (40 μg), a selective orexin-A antagonist, did not modify basal MI, but the antagonist significantly suppressed this stimulated action of 2-DG. ***Conclusions.*** These results suggest that endogenous orexin-A in the brain is involved in the vagal-dependent stimulation of gastric contractions.

Key words; orexin, brain, stomach, motility, intraluminal pressure wave

Introduction

Orexins/hypocretins are novel neuropeptides that are localized in neurons in the lateral hypothalamus [1, 2]. Orexins may be implicated in a wide variety of physiological functions. These include feeding [2, 3], behavioral activity [4], sleep/awake [5], anxiety [6], energy balance [7], neuroendocrinological response [8] and cardiovascular functions [9]. In addition to these functions, we suggested for the first time that orexin-A is involved in central regulation of gastric acid secretion [10–12].

It has been so far shown that orexins bind to two specific receptors, named the orexin-1 (OX1R) and orexin-2 (OX2R) receptors. According to in vitro binding and functional assays, OX1R is selective for orexin-A and OX2R is non-selective for orexin-A and -B [2]. The effect of intracisternal (ic) injection of orexin-A or -B on gastric acid secretion was examined and it was clearly demonstrated that the acid stimulation was induced by orexin-A but not orexin-B [11], suggesting orexin-A-induced stimulation of gastric functions may be mediated by OX1R.

Meanwhile, recently several researchers reported orexin-A mediates gastrointestinal (GI) motility. Bülbül et al. demonstrated intracerebroventricular (icv) orexin-A stimulated gastric contractions in non-fasted conscious rats [13]. Kobashi et al. [14] showed that ic administration of orexin-A enhanced phasic contractions of the distal stomach, and this action was blocked by vagotomy in anesthetized rats. On the other hand, it has been reported that orexin-A accelerates gastric emptying through peripheral and vagal independent mechanisms [15]. Thus,

the effects and mechanisms of orexin-A on GI motility have not been fully studied.

In this study, to investigate the role of brain orexin-A in regulation of gastric motility, we studied 1) whether central or peripheral administration of orexin-A affects gastric motility and 2) whether endogenously released orexin-A in the brain indeed plays a role in the regulation of gastric contractions in freely moving conscious rats.

Materials and methods

Animals

Male Sprague-Dawley rats weighing about 250 g were housed under controlled light/dark conditions (lights on: 07:00–19:00) with the room temperature regulated to 23–25°C. Rats were allowed free access to standard rat chow (Solid rat chow, Oriental Yeast Co., Tokyo, Japan) and tap water.

Chemicals

Synthetic orexin-A (human/bovine/rat/mouse) was purchased from Peptide Institute Inc., Osaka, Japan and were dissolved in normal saline just before the experiments. Selective orexin-A antagonist, SB-334867 (Tocris Bioscience, Ellisville, MO) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO). 2-Deoxy-D-glucose (2-DG) and atropine (Sigma-Aldrich, St. Louis, MO) was dissolved in normal saline.

Implantation of catheter for manometric recordings

The rats were anesthetized with ether, and open-tipped catheter (3-Fr, 1 mm ID; Atom, Tokyo, Japan) for manometric measurement was placed at the gastric antrum, fixed by purse-string sutures. The catheter was brought out through the abdominal wall musculature, and tunneled subcutaneously to exit at the back of the neck and secured to the skin. The rats were allowed to recover in individual cages for 2–5 days before the experiments.

Manometric recordings

Gastric motility was measured by manometric methods described in previous study [16]. Conscious animals without fasting were put in wire-bottom and non-restraint polycarbonate cages. Manometric catheter from each animal was threaded through a flexible metal sheath to protect from biting and connected to the infusion swivel (Instech Laboratories, Plymouth Meeting, PA) to allow free movement. It was connected to a pressure transducer (TP-400T; Nihon Koden Kogyo, Tokyo, Japan) and was continuously infused with degassed distilled water at a rate of 1.5 ml/h by a heavy-duty pump (CVF-3100; Nihon Koden). Pressure signals from the transducer were digitized and stored using a PowerLab system (AD Instruments, Colorado Springs, CO). First, the basal state of the gastric pressure wave was measured for 1 h after 1-h of stabilization. Then, the manometric catheter was disconnected and the rats were removed from polycarbonate cages. Peptide, compound or saline was injected intracisternally in 10-μl volume,

intravenously through the jugular vein in 200-µl volume and/or intraperioneally in 300-µl volume in rats under brief ether anesthesia. Ic was performed with a 10-µl-Hamilton microsyringe after rats were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) as reported previously [17]. After that, the rats were put in the cages again and the catheter was re-connected to a pressure transducer. The pressure wave was monitored up to 2 h after injection. Using the recordings, we evaluated motor index (MI) as below.

Evaluation of MI

MI was assessed by area under the manometric trace (AUT). AUT was calculated using a data-acquisition software (LabChart v7, AD instruments, Colorado Springs, CO). The baseline drifting and recording noise due to movement of the animals was very minor. To avoid any baseline drifting, we selected the analysis points with stable baseline. Basal MI was determined by calculating AUT for 1-h period before drug administration. The %MI was determined by calculating following the formula; (AUT for each 1-h period after drug administration)/(basal MI) × 100. In this experiment, pressure signals were continuously recorded up to 4 h (1 h for stabilization, 1 h for basal MI and 2 h for determining the changes induced by drugs), but the measurement was temporally stopped in order to perform injection [ic, intraperitoneal (ip) or intravenous (iv)] at the middle of the recordings. In relation to injection, we also need the time for recovery from the anesthesia and re-stabilization of baseline of manometric pressure in order to obtain the

adequate recordings for the analysis. Therefore, the manometric data during the recovery and re-stabilization period for approximately 5 min was excluded from later analysis. Since atropine was administered 10 min before ic orexin-A, the data during these preparations for approximately 15 min was excluded in that experiment.

Statistical analysis

Data were expressed as means ± S.E. Comparison of %MI was performed using the Student's t test. Statistica (StatSoft Inc. Tulsa, Okla., USA) was used throughout the study.

Ethical considerations

The approval of the Research and Development and Animal Care committees at the Asahikawa Medical University (#11042, approved on March 7, 2011) was obtained for all studies.

Results

First, we examined the effect of orexin-A on gastric contractions in freely moving conscious rats. As demonstrated in Figure 1, ic injection of orexin-A (10 μg) stimulated gastric contractions, while saline administration did not affect the gastric motility. The stimulatory action of orexin-A was observed immediately after ic injection and persisted about 60 min. Therefore, we examined the MI change before and after orexin-A or saline

injection for the first 1-h period. Figure 2 showed the MI change induced by ic and ip injection of saline or orexin-A. Ic injection of saline did not change MI (107.3 ± 4.78 %), while orexin-A significantly increased the MI (169.6 ± 13.2 %, $p < 0.05$). On the other hand, ip injection of orexin-A did not have any effect (94.1 ± 6.4 % for saline vs. 99.5 ± 8.1 % for orexin-A, $p > 0.05$), suggesting that orexin-A acts in the brain to stimulate gastric motility.

As demonstrated in Figure 3 and 4, pretreatment with atropine (1 mg/kg, ip, 10 min before ic orexin-A) potently blocked the ic orexin-A-induced stimulation of gastric contractions and MI change (170.7 ± 10.3 % for saline + orexin-A vs. 117.3 ± 16.4 % for atropine + orexin-A, $p < 0.05$), suggesting that the vagal cholinergic pathway plays a vital role in the stimulation of gastric motility by orexin-A.

Figure 5 shows the representative recordings of antral motility in rats treated with saline or 2-DG. As demonstrated, 2-DG (200mg/kg) stimulated gastric motility immediately after iv injection and the stimulation lasted more than 60 min. The MI was significantly increased in rats treated with 2-DG when compared with saline control (183.0 ± 27.0 % for 2-DG, $n = 5$, vs. 100.0 ± 5.9 % for saline, $n = 6$, $p < 0.05$).

Next, to clarify a hypothesis that endogenous orexin-A may be involved in stimulation of gastric motility, we tested the effect of SB-334867, a selective OX1R antagonist in the 2-DG stimulation of gastric motility model. Ic injection of the antagonist (40 μg) by itself did not change gastric contractions (Figure 6) and MI (119.8 ± 9.6 % for SB-334867, $n = 7$, vs. 107.1 ± 7.4 % for DMSO, $n = 5$, $p > 0.05$). On the other hand, ic SB-334867 (40 μg,

immediately before iv 2-DG) potently blocked the 2-DG (200mg/kg)-induced stimulation of gastric contractions (Figure 7) and MI change (222.1 ± 28.8 % for DMSO + 2-DG vs. 122.9 ± 12.3 % for SB-334867 + 2-DG, $p < 0.05$, Figure 8).

Discussion

Gastric motility pattern in rats can be divided into the interdigestive and postprandial states. In fasted rats, injection of orexin-A into the cerebrospinal fluid changes the gastric motor pattern from interdigestive to postprandial in conscious rats [18]. On the other hand, in fed rats, administration of orexin-A into the cerebrospinal fluid stimulated gastric contractility in conscious rats [13]. Thus exogenously injected orexin-A into the brain has an effect on gastric motility. However, the physiological relevance of brain orexin-A in regulation of gastric motility remains to be clarified. We therefore tried to investigate this issue using freely moving conscious rats in this study.

First, we clarified that central but not peripheral injection of orexin-A stimulated gastric motility. Bülbül et al. [13] have recently demonstrated that icv administration of orexin-A increased gastric motility, being in good agreement with the present data. However, whether peripherally injected orexin-A has any effect on gastric contractility has not been investigated. It was reported that continuous iv administration of orexin-A showed an inhibitory effect on small intestinal migrating motility

complex in the rat [19]. Moreover, Bülbül et al. also demonstrated that endogenous orexin-A accelerates gastric emptying through peripheral mechanisms [15]. These results suggest that orexin-A may also mediate GI motor function through a peripheral pathway. The orexin immunoreactivity is distributed in various regions of alimentary tract [20], moreover, it was also demonstrated that orexin induced dose-dependent contractions of intestinal segment in vitro [21]. Thus orexin-A might act peripherally to have an effect on GI function. Because there is a possibility that centrally injected orexin-A leaks into the periphery, we tried to exclude the speculation that orexin-A acts peripherally but not centrally to enhance gastric motility when orexin-A was injected centrally. The present data clearly demonstrated that ip injection of orexin-A failed to stimulate gastric motility, strongly confirming that ic orexin-A acts centrally in the brain to stimulate gastric motility in freely moving conscious rats. We have very recently demonstrated that ic injection of orexin-A significantly enhanced colonic motor function in conscious rats [22]. The present study therefore provides evidence that orexin-A in the brain stimulates not only colonic but also gastric contractility in conscious rats.

Studies have demonstrated that the vagal system completely mediates the 2-DG-stimulated gastric acid secretion [23, 24]. As demonstrated in this study, pharmacological suppression of the vagal system by atropine potently blocked the ic orexin-A-induced stimulation of gastric motility, suggesting that orexin-A acts centrally in the brain to increase gastric motility through the vagal cholinergic pathway. Since the cells of

origin innervating the stomach through the vagus nerve are located in the dorsal motor nucleus of the vagus (DMN) nerve [25], the DMN neurons must be excited when orexin-A is injected into the cisterna magna. In fact, injection of orexin-A into the cerebrospinal fluid induced c-fos expression in a number of neurons including the DMN in rats [26], furthermore supporting that the DMN neurons are excited when orexin-A is injected into the cerebrospinal fluid as in this study. With regard to whether the DMN neurons are activated directly or indirectly by centrally administered orexin-A, a couple of electrophysiological studies [27, 28] have demonstrated that orexin-A directly activates the DMN neurons in rat medullary slices. For instance, Grabauskas et al. [27] have demonstrated using whole-cell patch cramp recordings in rat brainstem slices that application of orexin-A produced a slow depolarization dose-dependently, accompanied by increased firing of the DMN neurons that projected their axon terminals to the stomach. Although the excitatory responses were persisted even after application of tetrodotoxin, they were completely abolished by an application of Cd^{2+}, indicating that the orexin-effects are mediated by direct postsynaptic excitation of the DMN neurons via orexin receptors. In addition, immunocytochemical studies have shown that OX1R was highly expressed in neurons in the DMN [29]. These findings suggest that orexin-A directly excites the DMN neurons that project to the stomach. According to the findings by Krowicki et al. [29], microinjection of orexin-A into the DMN in anesthetized rats increased intragastric pressure and antral motility. Based upon these results, we could conclude that injection of orexin-A into the

cerebrospinal fluid activates directly the DMN neurons, followed by increasing the vagal tone, thereby stimulating gastric antral motility as shown in this study using freely moving conscious rats.

2-DG has been used as a tool for central activation of the vagal pathway. It is known that 2-DG stimulates the glucoprivic receptors in the lateral hypothalamic area (LHA), which in turn initiates and sustains the vagus-mediated gastric function excitation [30]. With regard to the effect of 2-DG on gastric motility, Quintana et al. [31] demonstrated iv 2-DG (200 mg/kg) stimulated gastric contractions in urethane-anesthetized rats. So far, whether 2-DG is capable of stimulating gastric contractility also in conscious rats has never been reported. The present study clearly showed for the first time that iv injection of 2-DG potently stimulated gastric motility in conscious rats.

Since a part of orexin-A-positive neurons in the LHA project to the DMN [32], 2-DG activates orexin-A neurons in the LHA [33], possibly to induce the excitation of the DMN neurons, leading to activation of the vagal outflow to the stomach. It was suggested that both 2-DG and orexin-A stimulate gastric contractions through central activation of the vagal pathway [14, 31]. Based on these findings, we therefore made a hypothesis that orexin-A in the brain might be involved in the 2-DG-induced stimulation of gastric contractions. Next, we used the model of 2-DG-stimulated gastric motility in conscious rats, and tried to clarify the above hypothesis.

SB-334867 is a non-peptide selective OX1R antagonist [34], and it made it possible to examine a physiological role of orexin-OX1R pathways in

the biological actions. Bülbül et al. [13, 18] reported that icv injection of SB-334867 (16 μg) enhanced gastric spontaneous phase-III like contractions in fasted rats and suppressed postprandial gastric contractions in non-fasted rats, suggesting endogenous orexin-A changes GI motor pattern from interdigestive to postprandial. However, in our study, the antagonist (40 μg) by itself did not change basal MI. This discrepancy may be explained by the reasons as follows. The first, the route of administration was different, i.e., icv and ic. The second, dose of antagonist was also different and 2.5 times higher in the present study. Diffusion of chemicals into the cerebrospinal fluid in rats treated with icv or ic injection would be different simply because of the difference of the injection site, i.e., 3rd ventricle (icv) vs 4th ventricle (ic), which may make a difference of action of chemicals in time- and dose-dependent manners. In fact, Stengel et al. [35] have recently demonstrated that icv but not ic injection of nesfatin-1 at the same dose inhibited gastric emptying in rats. They suggested that icv or ic injection of the chemical would reach easier to the forebrain or hindbrain, respectively, leading to the difference of the effect of nesfatin-1 on gastric emptying. Thus, the route of difference might, at least in part, explain the discrepancy of action and dose of orexin antagonist to exert its activity. Our tested dose (40 μg) administered by ic injection increased the frequency of phase-III like contractions of the colon in non-fasted rats, while 16 μg of antagonist did not significantly change colonic motility (unpublished data). These data revealed this dose (40 μg) administered by ic injection was thought to be adequate for examining a physiological role of endogenous orexin on GI motility in rats.

As clearly shown in this study, ic injection of SB334867 (40 μg) significantly blocked the 2-DG-evoked stimulation of gastric motility. This result demonstrated for the first time that endogenous orexin-A in the brain mediates the 2-DG-stimulated gastric motility in conscious rats possibly through acting to OX1R on the DMN neurons.

Based upon these lines of evidence, Figure 9 reveals the schematic illustration of our hypothesis on the mechanism by which orexin-A acts in the brain to stimulate gastric motility. Orexin-A containing neurons in the LHA project their terminals to the DMN and release orexin-A, and activates neurons in the DMN through acting to OX1R on the DMN neurons, followed by activation of the vagal efferent pathway, thereby stimulating gastric motility. Glucoprivation by 2-DG might stimulate gastric motility through this signaling pathway.

The cephalic phase of GI response produces coordinated GI alterations that prime the gut to assist digestion of the impending meal. Since the alterations in response to cephalic stimulation in stomach and pancreas were mimicked by orexin-A [11, 36], i.e., stimulation of gastric acid and pancreas exocrine secretion, this peptide is thought to be a candidate molecule which triggers the process of cephalic phase stimulation [37]. Meanwhile, the cephalic phase stimulation also mediates gastric motility, such as fundic accommodation and stimulation of antral contractions [38, 39]. The present study revealed that endogenous orexin-A in the brain enhances antral contractions in conscious rats, furthermore supporting our hypothesis that orexin-A in the brain mediates the response to cephalic phase

stimulation. This speculation may be supported by a different point of view. 2-DG is known as a compound that inhibits glucose utilization (glucoprivation) and causes intracellular glucopenia [40], followed by stimulation of central vagal pathway. As shown in this study, endogenously released orexin-A induced by central glucoprivation as hunger state evoked stimulation of gastric motility, supporting the hypothesis that orexin-A in the brain triggers the cephalic phase stimulation (Figure 9).

Studies have identified several neurotransmitters and neuromodulators that activate or inhibit orexin neurons [41]. Among them, ghrelin is reported to be a peptide that activates orexin neurons. With regard to the relation between orexin and ghrelin, Toshinai et al. [42] have demonstrated immunohistochemically the synaptic contact of ghrelin-containing axons with orexin-producing neurons in the rat hypothalamus. In addition, the authors also showed there was a functional interaction between ghrelin and orexin in the brain as following. Icv administration of ghrelin stimulated food intake, and this action of ghrelin was attenuated by pretreatment with anti-orexin-A antibody. It was furthermore showed that ghrelin-induced feeding was also suppressed in orexin knockout mice. These data suggest that ghrelin increases food intake through the action of the orexin system in the brain. Since ghrelin is involved in the regulation of GI functions [43], further studies should be performed to clarify a relationship between the both peptides in the brain to control gastric motility.

Functional gastrointestinal disorders (FGIDs) are characterized as

chronic or recurrent GI symptoms, which are not explained by structural or biochemical abnormalities. Brain-gut interaction plays an important role in the pathophysiology of FGIDs [44]. Based upon reported actions of central orexin-A on body functions such as GI function, appetite, sleep/awake cycle and mood state, we made and provided a hypothesis that decreased orexin signaling may be involved in the pathophysiology in patients with FGID [45]. The patients with functional dyspepsia (FD), which is one of the FGIDs frequently complain of GI symptoms after meals, and postprandial antral hypomotility and reduced fundic accommodation was reported to be one of the physiological mechanisms of FD [46, 47]. Since fundic accommodation [14] and hypermotility in the distal stomach as shown in this study were observed in rats treated with central orexin-A, decreased orexin signaling may contribute to the pathophysiology of FD. In this context, to explore the physiological role of orexin on GI motility may pave the way for further understanding the pathophysiology and therapy of FD.

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Figure legends

Figure 1.

Representative recordings of the gastric contractions in rat injected intracisternally with either orexin-A (10 μg) or saline. * indicated the period which was needed for intracisternal injection (ic) and re-stabilization of baseline. The manometric data during the period were omitted from the analysis.

Figure 2.

The effect of orexin-A (10 μg) on gastric motility. Intracisternal injection (ic) of orexin-A significantly increased the motor index as compared to saline treatment. However, this effect was not observed by intraperitoneal injection (ip) of orexin-A. Each column represents the mean ± S.E. Number of rats was shown in the parenthesis. *$p < 0.05$ vs. saline-treated group.

Figure 3.

Representative recordings showing the effects of atropine (1 mg/kg) on intracisternal (ic) injection of orexin-A (10 μg)-induced stimulation of gastric contractions. Pretreatment with atropine potently blocked this stimulation. Intraperitoneal (ip) injection of either saline or atropine was performed 10 min before ic orexin-A. * indicated the period which was needed for the treatment and re-stabilization of baseline.

Figure 4.

Intraperitoneal injection (ip) of atropine (1 mg/kg) blocked the increased motor index induced by intracisternal injection (ic) of orexin-A (10 μg). Each column represents the mean ± S.E. Number of rats was shown in the parenthesis. *$p < 0.05$ vs. saline + orexin-treated group.

Figure 5.

The effect of 2-Deoxy-D-glucose (2-DG) on gastric contractions. Intravenous injection (iv) of 2-DG (200 mg/kg) immediately enhanced gastric contractions and this action lasted more than 60 min. * indicated the period which was needed for iv and re-stabilization of baseline.

Figure 6.

Gastric contractions in response to either intracisternal injection (ic) of dimethyl sulfoxide (DMSO) or SB-334867 (40 μg). SB-334867 by itself did not modify gastric contractions. * indicated the period which was needed for ic and re-stabilization of baseline.

Figure 7.

Gastric contractions in response to either intracisternal injection (ic) of dimethyl sulfoxide (DMSO) or SB-334867 immediately followed by intravenous injection (iv) of 2-Deoxy-D-glucose (2-DG). SB-334867 (40 μg) significantly suppressed 2-DG (200 mg/kg)-induced stimulation of gastric contractions. * indicated the period which was needed for ic and iv, and

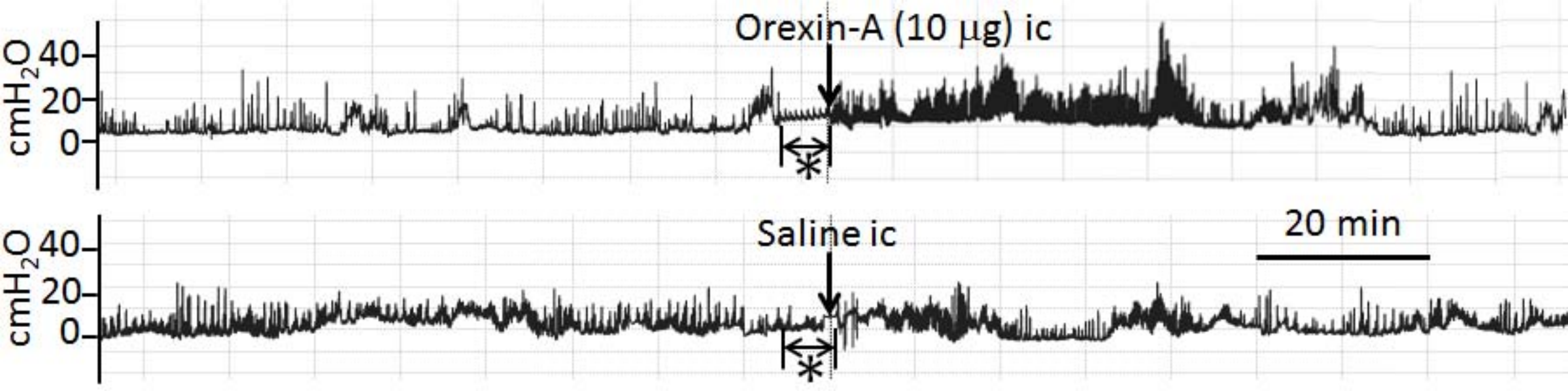
re-stabilization of baseline.

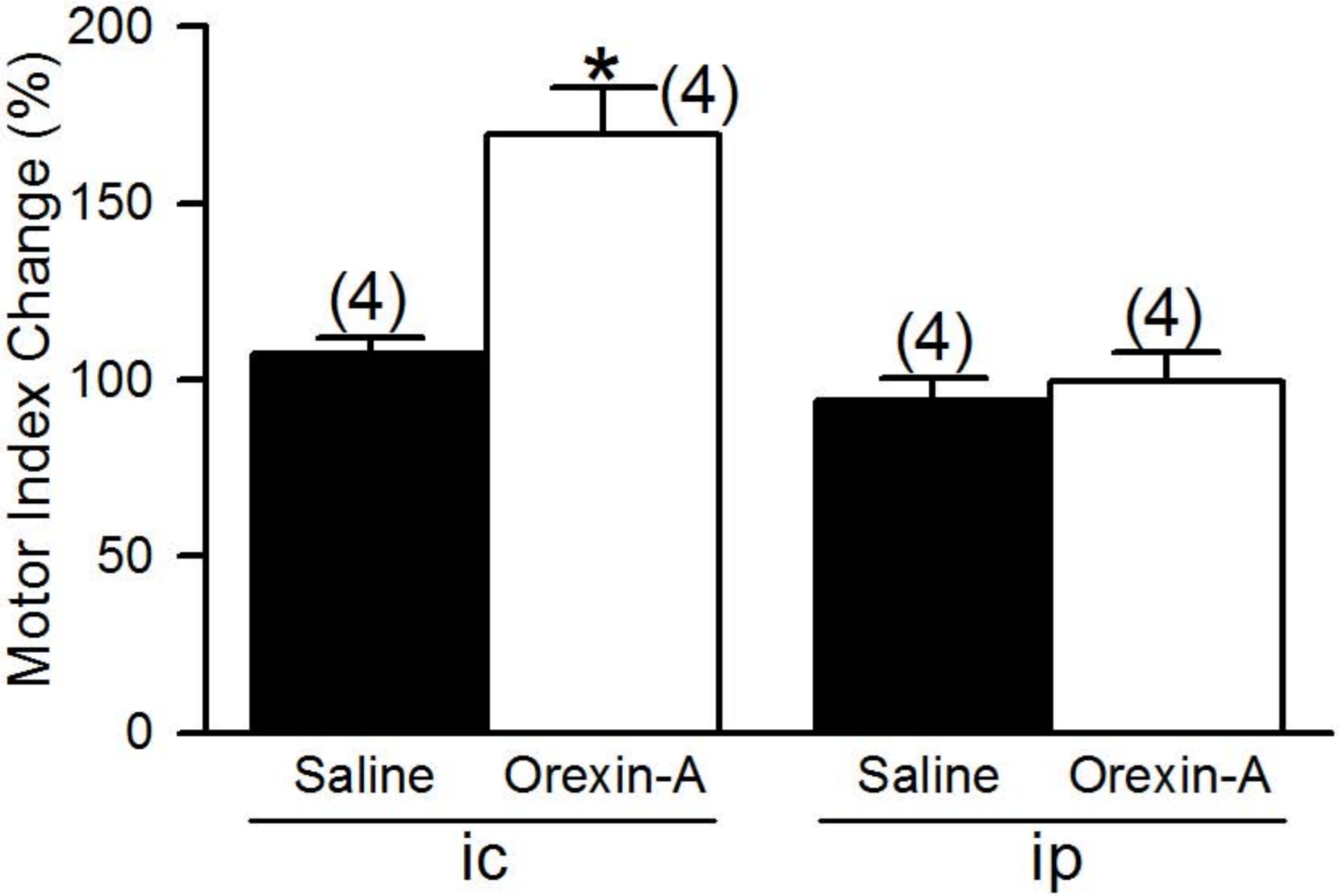
Figure 8.

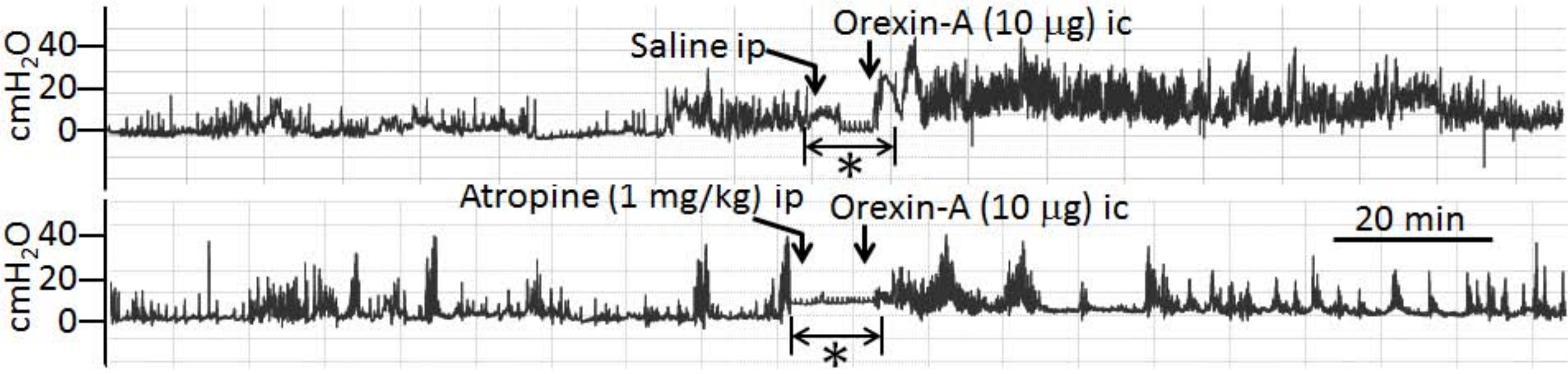
Effect of SB-334687 on increased motor index induced by intravenous injection (iv) of 2-Deoxy-D-glucose (2-DG, 200 mg/kg). Intracisternal injection (ic) of SB-334687 (40 μg) significantly suppressed the motor index as compared to dimethyl sulfoxide (DMSO) treatment. Each column represents the mean ± S.E. Number of rats was shown in the parenthesis. *p < 0.05 vs. DMSO + 2-DG-treated group.

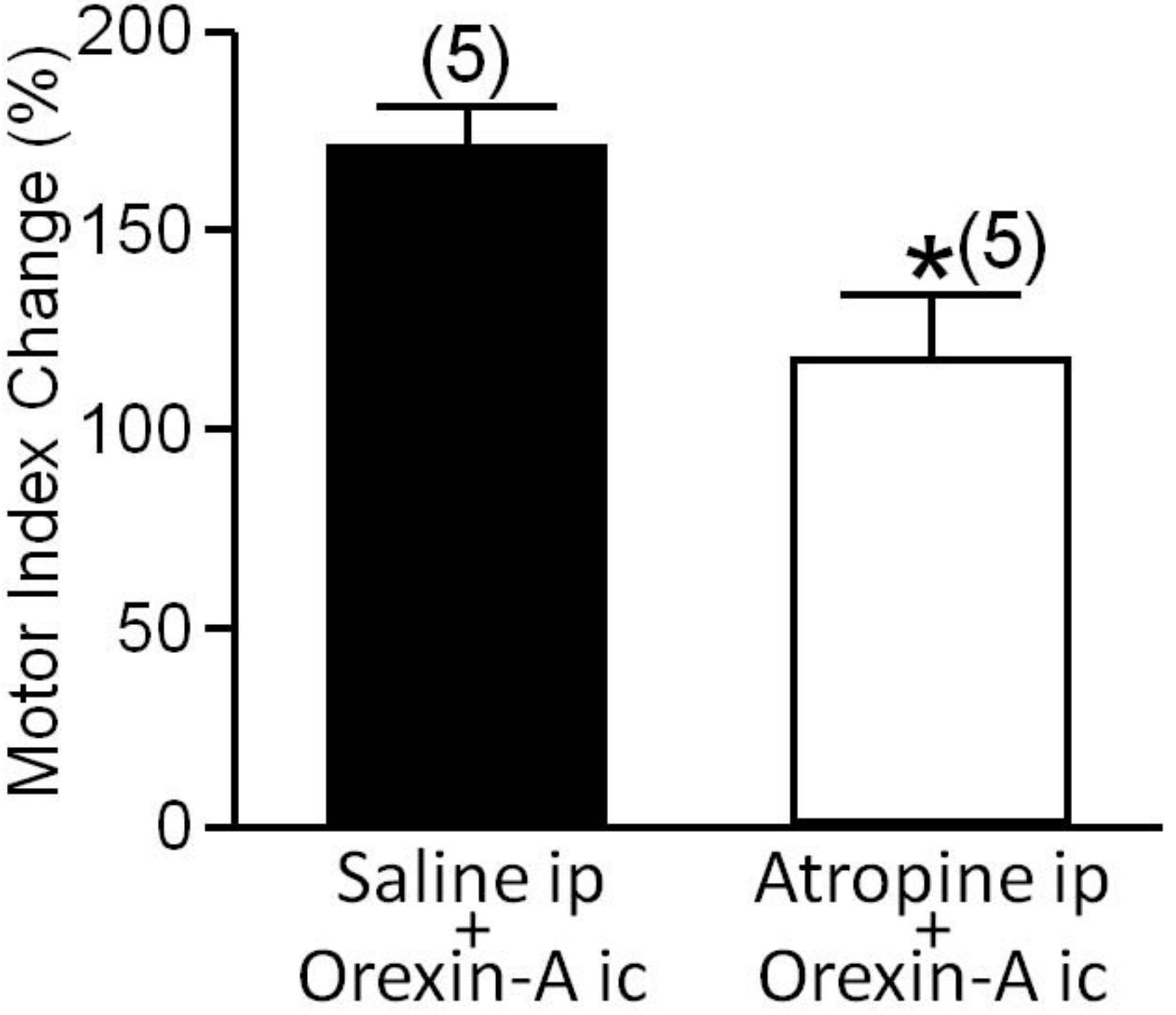
Figure 9.

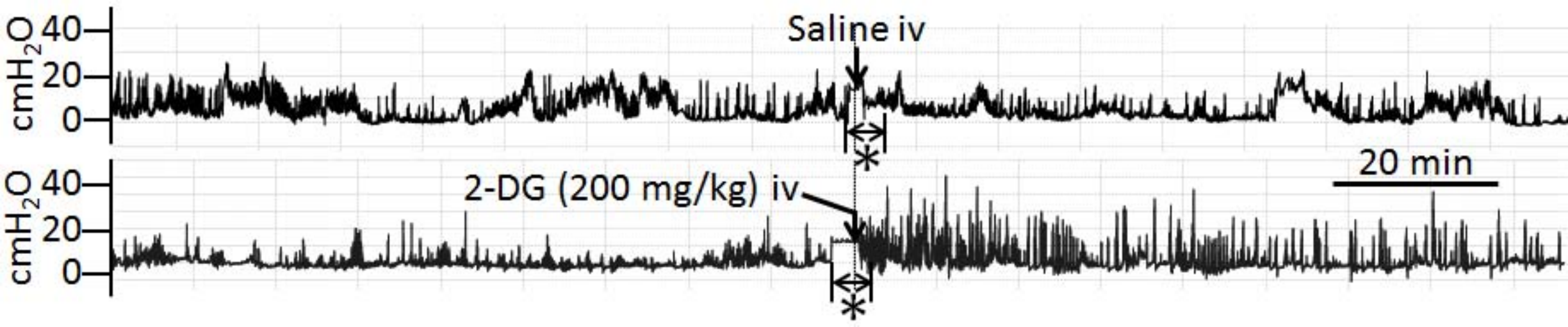
Schematic illustration of our hypothesis on the mechanism by which orexin-A acts in the brain to stimulate gastric motility. Orexin-A containing neurons in the lateral hypothalamic area (LHA) project their terminals to the dorsal motor nucleus (DMN) and release orexin-A, and activates neurons in the DMN in the medulla through acting to OX1R on the DMN neurons, followed by activation of the vagal efferent pathway, thereby stimulating gastric motility. Glucoprivation by 2-Deoxy-D-glucose or cephalic phase stimulation might stimulate gastric motility through this signaling pathway.

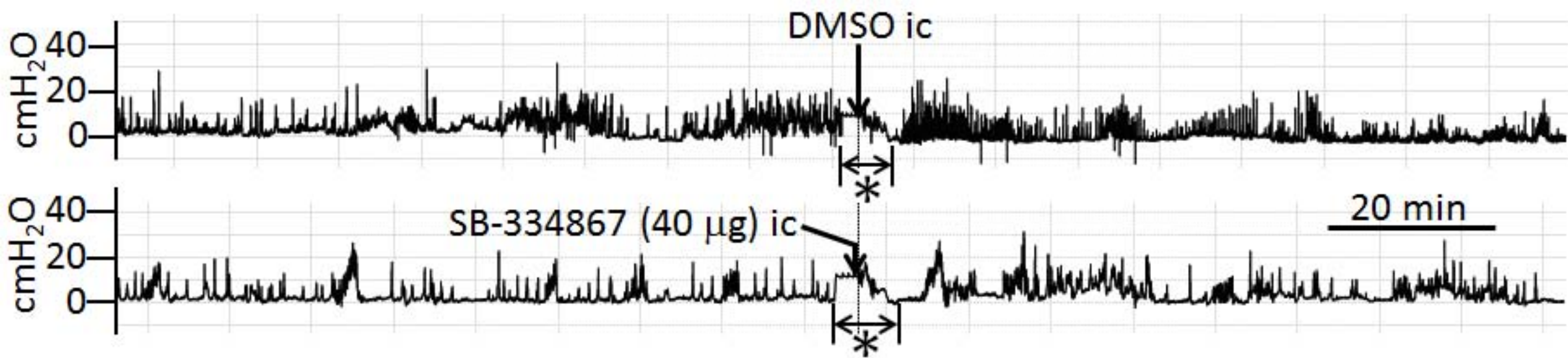

Orexin-A (10 µg) ic
cmH2O
40
20
0
Saline ic
20 min

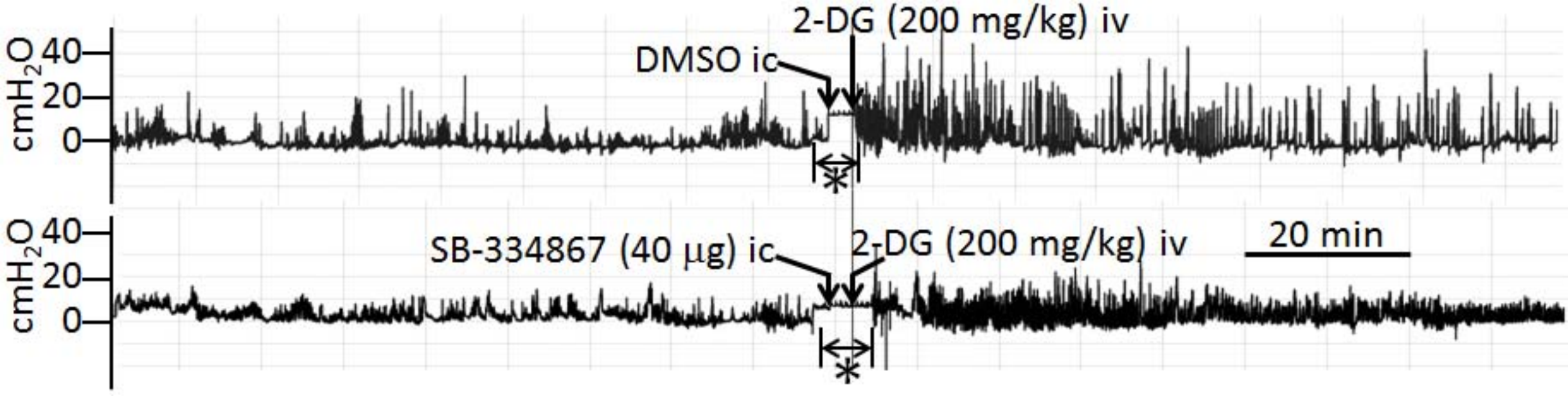

Motor Index Change (%)
200
150
100
50
0
*
(4)
(4)
(4)
(4)
Saline
Orexin-A
Saline
Orexin-A
ic
ip

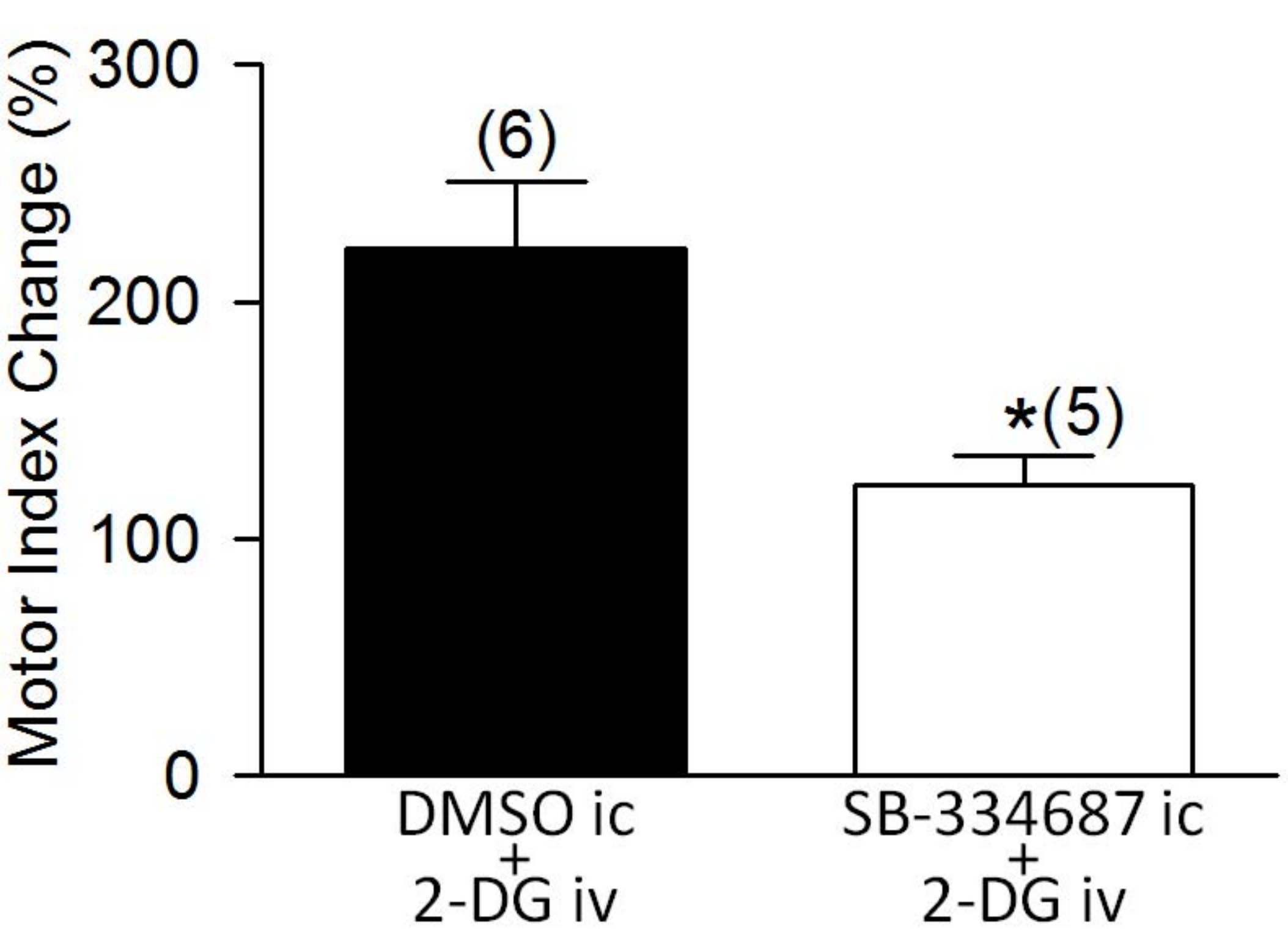

Saline ip
Orexin-A (10 μg) ic
cmH2O 40
20
0
*
Atropine (1 mg/kg) ip
Orexin-A (10 μg) ic
20 min
cmH2O 40
20
0
*

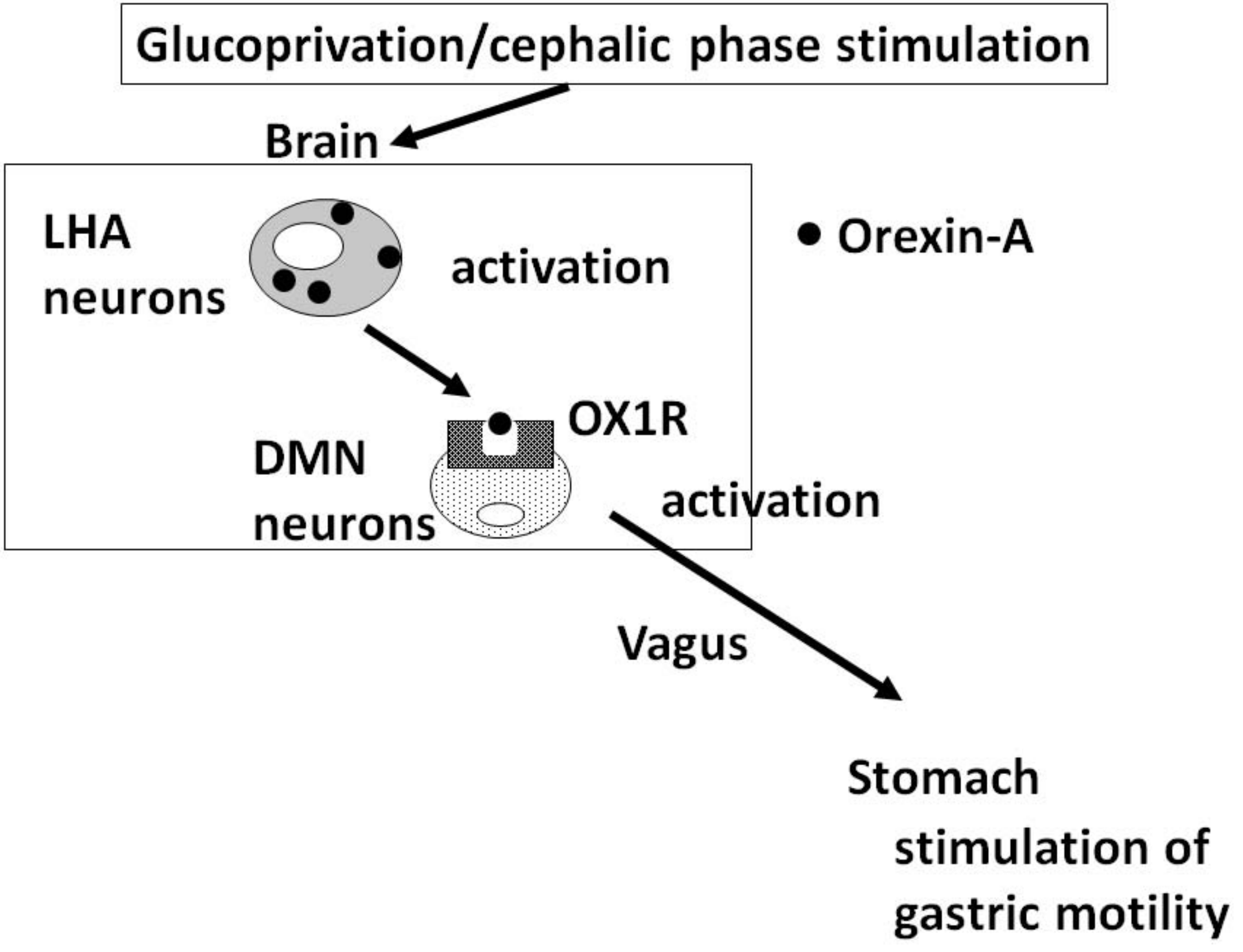

Motor Index Change (%)
200
150
100
50
0
(5)
*(5)
Saline ip
+
Orexin-A ic
Atropine ip
+
Orexin-A ic


cmH2O
40
20
0
Saline iv
*
cmH2O
40
20
0
2-DG (200 mg/kg) iv
*
20 min


DMSO ic
cmH2O
40
20
0
SB-334867 (40 µg) ic
20 min


2-DG (200 mg/kg) iv
DMSO ic
cmH2O
40
20
0
SB-334867 (40 μg) ic
2-DG (200 mg/kg) iv
20 min
cmH2O
40
20
0


Motor Index Change (%)
300
200
100
0
(6)
*(5)
DMSO ic
+
2-DG iv
SB-334687 ic
+
2-DG iv


Glucoprivation/cephalic phase stimulation
Brain
LHA
neurons
activation
● Orexin-A
OX1R
DMN
neurons
activation
Vagus
Stomach
stimulation of
gastric motility