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Water-avoidance stress enhances gastric contractions in freely moving conscious rats: role of peripheral CRF receptors

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Running head; Stress and gastric motility

Abstract

Background. Stress alters gastrointestinal motility through central and peripheral corticotropin-releasing factor (CRF) pathways. Accumulating evidence has demonstrated that peripheral CRF is deeply involved in the regulation of gastric motility, and enhances gastric contractions through CRF receptor type 1 (CRF1) and delays gastric emptying (GE) through CRF receptor type 2 (CRF2). Since little is known whether water-avoidance stress (WAS) alters gastric motility, the present study tried to clarify this question and the involvement of peripheral CRF receptor subtypes in the mechanisms. ***Methods.*** We recorded intraluminal gastric pressure waves using a perfused manometric method. The rats were anesthetized and the manometric catheter was inserted into the stomach 4–6 days before the experiments. We assessed the area under the manometric trace as the motor index (MI), and compared this result with those obtained 1 h before and after initiation of WAS in non-fasted conscious rats. Solid GE for 1 h was also measured. ***Results.*** WAS significantly increased gastric contractions. Intraperitoneal (ip) administration of astressin (100 μg/kg, 5 min prior to stress), a non-selective CRF antagonist, blocked the response to WAS. On the other hand, pretreatment (5 min prior to stress) with neither astressin2-B (200 μg/kg, ip), a selective CRF2 antagonist, nor urocortin 2 (30 μg/kg, ip), a selective CRF2 agonist, modified the response to WAS. These drugs did not alter the basal MI. WAS did not change GE. ***Conclusions.*** WAS may activate peripheral CRF1 but not CRF2 signaling and stimulates gastric contractions

without altering GE.

Key words: CRF receptor type 1, stress, gastric contractions, intraluminal gastric pressure, gastric emptying

Introduction

Stress alters gastrointestinal (GI) functions, such as motility and sensation [1, 2]. Corticotropin-releasing factor (CRF) is the main mediator of these responses to stress [3]. CRF exerts its action through the activation of two receptors, CRF receptor type 1 (CRF1) and type 2 (CRF2) in not only the central nervous system but peripheral tissues [4, 5], and stimulation of each CRF receptor induces distinct changes in GI functions. Restraint stress stimulates defecation and delays gastric emptying (GE) through central and peripheral CRF1 and CRF2, respectively [6-8]. Moreover, stimulation of each CRF receptor subtype by administration of the selective ligands, mimics these stress-induced alterations [6, 9, 10]. According to these lines of evidence, it has been thought so far that CRF1 exclusively mediates colonic motility response and CRF2 solely contributes to alteration of gastric motility induced by stress.

Water-avoidance stress (WAS) is a conventional psychological stress protocol, and is known to stimulate defecation through activating CRF1 signaling [11, 12]. However, it has not been demonstrated whether this stress alters gastric motility. We have very recently demonstrated that peripheral administration of CRF stimulates gastric contractions through peripheral CRF1 in conscious rats [13], suggesting WAS may also stimulate gastric contractions.

In the present study, the effects of WAS on gastric motility were evaluated. We measured gastric contractions using a perfused manometric method in freely moving conscious rats and the role of peripheral CRF

receptors was evaluated. We also determined whether GE was changed by WAS.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing about 200–250 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, Tokyo, Japan) and tap water. Experiments started between 8–10 AM and finished no later than 3 PM.

Chemicals

A rat/human CRF (Peptide Institute, Osaka, Japan) and human urocortin 2 (Ucn 2; Bachem AG, Bubendorf, Switzerland) were dissolved in normal saline. Astressin and astressin2-B (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in double-distilled water.

Stress protocol

Exposure to WAS was performed as described previously [14] with minor modification. Rats were placed individually on a plastic platform (height, 8 cm; length, 6 cm; width, 6 cm) positioned in the middle of a plastic cage filled with water up to 7 cm of the height of the platform. To avoid

contact with water, rats stood on a platform during the entire experimental period but the tail was immersed into the water. To avoid exposure to cold stress, the temperature of water was kept around 30 °C. Control animals were also put on the same plastic platform in a plastic cage but not filled with water. However, the animals immediately jumped down to the bottom of the cage, and they stayed there for the rest of experimental period.

Animal preparation

After overnight fasting, the rats were anesthetized with ether. An open-tipped catheter (3-Fr, 1 mm internal diameter, Atom, Tokyo, Japan) for manometric measurement was inserted through a small hole produced by an 18-G needle in the gastric body. The tip of the catheter was placed at the gastric antrum (recording point) and the catheter was fixed by purse-string sutures at the point of exit from the gastric wall. Then it passed through the abdominal wall musculature and a subcutaneous tunnel to exit at the back of the neck, and was secured to the skin. The rats were allowed to recover in individual cages for 4–6 days before the experiments.

Manometric recordings and study design

The method used in the present study had already established well to measure GI contractions [13, 15-18]. Non-fasted rats were placed in a wire-bottom and non-restraint polycarbonate cage. The manometric catheter from each animal was threaded through a flexible metal sheath to protect it from biting and connected to an infusion swivel (Instech Laboratories,

Plymouth Meeting, PA, USA) to allow free movement. The catheter was infused continuously with degassed distilled water at a rate of 1.5 ml/h using a heavy-duty pump (CVF-3100, Nihon Koden, Tokyo, Japan) and was connected to a pressure transducer (TP-400T, Nihon Koden). Pressure signals from the transducer were digitized by a PowerLab system (AD Instruments, Colorado Springs, CO, USA) and stored by computer software (LabChart 7, AD Instruments). First, the basal state of the gastric pressure waves was measured for 1 h after 1 h of stabilization period. Then, the catheter was disconnected and the rat was taken out from polycarbonate cage. The animal was subjected to WAS or put in a plastic cage without water as controls, and the catheter was re-connected to a pressure transducer. The pressure waves were monitored for up to 2 h after initiation of these manipulations. In a different experiment, after measuring basal state of gastric contractions for 1 h, CRF receptor(s) agonist, antagonist or vehicle was injected intraperitoneally in a 0.2-ml volume in rat under brief ether anesthesia. After injection, the rat was returned to polycarbonate cage again in order to wait for recovery from the anesthesia. Five min later (all the rats came out of the anesthesia within 5 min), the catheter was re-connected to a pressure transducer and the animal was subjected to the stress (WAS or control manipulation). Moreover, in order to determine the effect of these above drugs on basal gastric contractions, the rat was returned to the cage again after injection. Five min later, the recording of pressure waves was re-started but the animal was not subjected to the stress.

Using the recordings, we evaluated the motor index (MI) to assess gastric motor activity as described below.

Evaluation of the MI

The MI ($cmH_2O \cdot sec$) was determined by the area under the manometric trace (AUT). AUT was calculated using software (LabChart 7, AD Instruments). The drawback of this manometric method is that pressure data is able to be modified by moving of animals. Indeed the animals moved frequently at the start of measuring, but usually within 15 min, they stopped moving. After that, the baseline drifting and recording noise due to movement of the animals were minor. Meanwhile, when the rats were subjected to WAS, they could not move significantly because they placed on a small platform surrounded by water. Control animals also stably stayed in the bottom of the plastic cage. Therefore, the recording noise was minor during the experimental period.

The basal MI was determined by calculating AUT for the 1 h period before the stress or drug administration. The % MI was determined by calculating following the formula: (AUT for the first 1 h or the second 1h period after initiation of stress or drug administration)/(basal MI) × 100. In this experiment, pressure signals were recorded continuously for up to 4 h (1 h for stabilization, 1 h for the basal MI and 2 h for determining the changes induced by the stress or drug), but the measurements were stopped briefly in order to perform intraperitoneal (ip) injection and/or initiate the stress protocol. In relation to these manipulations, time for re-stabilization of

baseline of manometric trace was required in order to obtain adequate recordings for the analysis. Therefore, the manometric data during this period for approximately 5−10 min were excluded from later analysis.

Measurement of solid GE

GE of a solid meal was measured with the method previously described by Nakade et al [7]. First, preweighed pellets (1.5 g, Solid rat chow, Oriental Yeast) were given for 10 min to 24-h fasted rats. If the rats did not eat all of the 1.5-g meal within 10 min, they were excluded from the experiment. Immediately after the end of feeding, the rats were subjected to WAS. Control animals were put in the same plastic cage but not filled with water. Sixty or 90 min later, the rats were euthanized by CO_2 inhalation, and the stomach was surgically isolated and removed. The gastric content was recovered from the stomach, dried, and weighed. Solid GE was calculated according to the following formula.

GE (%) = [1 – (dried weight of food recovered from stomach/weight of food intake)] × 100.

Statistical analysis

Data were expressed as means ± S.E. Statistical analysis was performed with Student's t test. A multiple-group comparison was performed by ANOVA followed by the least significant difference test. An α cutoff level (P value) of < 0.05 was used throughout the study. Statistica software (StatSoft, Tulsa, OK, USA) was used throughout the study.

Ethical considerations

Approval by 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 tested the effect of WAS on gastric contractions for 2 h. As shown in demonstrable recordings of Fig. 1, WAS immediately enhanced gastric contractions. The stress significantly increased the MI for the first 1 h period as compared to that before the stress (ANOVA, $F = 5.02$, $P < 0.05$, $n = 20$, 48985 ± 7216 for the basal MI for 1 h, vs. 88793 ± 11018 for the first 1 h period of WAS, $P < 0.05$, Fig. 2A). However, this stimulation was no longer observed in the next 1 h period of WAS (72891 ± 8161 for 1-2 h period of WAS, vs. basal MI, $P = 0.064$). On the other hand, control group did not show any significant changes of the MI (ANOVA, $F = 1.43$, $P > 0.05$, $n = 5$, 48879 ± 4807 for the basal MI for 1 h, 50234 ± 8078 for the first 1 h period, and 49774 ± 4856 for 1-2 h period, Fig. 2A). As compared to controls, the % MI for the first 1 h period was significantly greater in WAS group (101.3 ± 8.3 for controls, vs. 187.0 ± 20.2 for WAS, $P < 0.05$, Fig. 2B). However, in 1-2 h period of WAS, the % MI was not significant different from that of controls (102.1 ± 3.7 for controls, vs. 165.2 ± 19.2 for WAS, $P = 0.11$). On the basis of these results, the % MI change for the first 1 h period of WAS was analyzed for later experiments.

Next, we evaluated the effect of ip astressin, a non-selective CRF antagonist, on the WAS-induced stimulation of gastric contractions. Pretreatment with ip astressin (100 μg/kg, 5 min prior to stress) completely blocked this stimulation (representative recordings in Fig. 3A, % MI, 184.2 ± 36.3 for vehicle + WAS, n = 7, vs. 94.4 ± 6.7 for astressin + WAS, n = 8, $P < 0.05$, Fig. 3B). This dose of astressin did not change basal gastric contractions (% MI for 1 h after injection, 102.5 ± 9.3 for vehicle, n = 5, vs. 97.9 ± 10.0 for astressin, n = 5, $P > 0.05$).

Then to determine the role of each CRF receptor subtype, the effect of ip astressin2-B, a selective CRF2 antagonist, was tested. Astressin2-B (200 μg/kg, ip) itself did not change basal gastric contractions (% MI for 1 h after injection, 99.5 ± 10.3 for vehicle, n = 5, vs. 98.9 ± 13.0 for astressin2-B, n = 5, $P > 0.05$). Ip Astressin2-B (200 μg/kg, 5 min prior to stress) failed to block the WAS-induced stimulation (representative recordings in Fig. 4A. % MI, 174.3 ± 40.3 for vehicle + WAS, n = 4, vs. 174.6 ± 16.7 for astressin2-B + WAS, n = 5, $P > 0.05$, Fig. 4B).

We have very recently shown that peripheral administration of CRF enhanced gastric contractions through stimulation of peripheral CRF1. And we also demonstrated that Ucn 2, a selective CRF2 agonist, itself did not modify basal gastric contractions but abolished the stimulatory response induced by CRF in conscious rats, suggesting activation of peripheral CRF2 suppresses CRF1 signaling [13]. Since our results in the present study also suggest that WAS-induced stimulation of gastric contractions is probably mediated through peripheral CRF1, the effect of Ucn 2 was examined in order to know this counter regulatory mechanism of CRF2 is also observed in this stress model.

Ucn 2 (30 μg/kg, ip) itself did not change basal gastric contractions (% MI for 1 h after injection, 105.1 ± 20.0 for vehicle, n = 5, vs. 102.0 ± 27.0 for Ucn 2, n = 4, P > 0.05). This peptide did not alter the WAS-induced stimulation either (representative recordings in Fig. 5A. % MI, 183.0 ± 26.5 for vehicle + WAS, n = 5, vs. 178.0 ± 21.0 for Ucn 2 + WAS, n = 5, P > 0.05, Fig. 5B).

Since previous study by Nakade et al. [7], showed restraint stress for 90 min delayed solid GE significantly, we firstly tested GE for 90 min with WAS. As shown in Table 1, the stress did not modify it. As we described above, significant stimulation of gastric contractions induced by WAS was only observed during the first 60 min, then GE for 60 min was also determined. However, the stress did not modify it either (Table 1).

Discussion

Previous studies have demonstrated that WAS stimulates defecation and induces visceral hyperalgesia [11, 12]. However, little is known about the effects of WAS on GI contractility, probably because direct recording of GI contractions in conscious rats under WAS had been very difficult. The present study therefore provided the solid evidence for the first time that WAS increased gastric contractions.

On the other hand, we also provided a novel finding that GE was not modified by WAS. This finding seems conflict with the result of gastric contractions. However, GE and gastric contractions do not always correspond. For example, restraint stress delays GE but enhances gastric contractions [7,

19]. Gastric contraction is one of the determinant factors of GE, and GE is also regulated by activity of the fundus and coordination of the antrum, pylorus and duodenum [20, 21]. Nakade et al, showed delayed GE induced by restraint stress results from the impairment of antropyloric coordination [19]. These results suggest that GE and contractility do not correspond during stress and that different mechanisms are involved in WAS or restraint stress-induced altered gastric motility because GE was not changed by WAS and was inhibited by restraint stress, although gastric contractility was stimulated by the both stress protocols as seen in this study and a previous experiment [7, 19].

We also demonstrated that the stimulation of gastric contractions by WAS was blocked by ip astressin, which has poor penetrance into brain [1], but not modified by ip astressin2-B, indicating that it is probably mediated through peripheral CRF1. It was very recently demonstrated by us, activating peripheral CRF1 signaling stimulates gastric contractions in conscious rats [13], which is consistent with the result in the present study. The speculation that WAS activates peripheral CRF1 signaling may also be supported by the following evidence. Stimulation of peripheral CRF2 induces delayed GE [6, 22], but activating CRF1 does not modify it [10]. Meanwhile, activation of peripheral CRF2 does not alter basal gastric contractions but CRF1 stimulation enhances them [13]. In this context, WAS may exclusively activate CRF1 signaling because it enhanced gastric contractions without modifying GE.

Although the above discussion supports the importance of CRF1

signaling in the stimulation of gastric contractions by WAS, that does not necessarily exclude the possibility that CRF2 signaling may play a role in the mechanism as following. Recent studies including ours demonstrated that peripheral CRF2 signaling has modulatory and inhibitory action to CRF1 in peripheral CRF-induced altered GI motility [13, 23]. Peripheral administration of astressin2-B enhances but Ucn 2 suppresses peripheral CRF-induced stimulation of gastric contractions, although the both peptides failed to change basal gastric contractions [13]. From these results, we have proposed the concept that gastric contractions are stimulated by CRF1 signaling and CRF2 signaling could inhibit the tone of CRF1 signaling, followed by modulating gastric contractions [13]. The activity balance of peripheral CRF1 and CRF2 signaling would decide the changes of GI functions during stress. In the present study, enhanced gastric contractions by WAS were mediated through peripheral CRF pathway, because ip astressin completely blocked the WAS-induced increase in gastric contractions, but modifying CRF2 signaling by astressin-2B or Ucn 2 did not change this increase, which is incompatible with the concept. However, the stimulatory action of gastric contractions by WAS seemed to be greater as compared to that induced by peripheral administration of CRF at a dose that could induce the maximal response, i.e., the % MI was around 180 by WAS and 150 by CRF in the previous our study [13], suggesting that activation of CRF1 signaling by WAS is too strong to be inhibited by CRF2 signaling.

In addition to CRF, urocotins (Ucns; Ucn 1, Ucn 2 and Ucn 3) that are capable of binding to CRF receptors with distinct affinities for each CRF

receptor subtype were shown to be prominently expressed in peripheral tissues where they mediate visceral stress responses [24, 25]. Meanwhile, several recent reports demonstrated that various stress such as psychological, physical stress, infection and inflammation affect the expression of CRF receptors in the GI tract [26-28]. Each CRF receptor subtype is recruited or diminished by stress and the expression profile is varied depending on the stress [27]. These lines of evidence suggest the released peptides such as Ucns and the expression of CRF receptors in GI tract under stress may determine the activity balance of peripheral CRF1 and CRF2 signaling.

Several issues remain to be clarified. We should determine the effect of CRF1 antagonist in order to reveal directly the role of peripheral CRF1 on the WAS-induced stimulation of gastric contractions. However, all available selective CRF1 antagonists have been designed to cross the blood-brain barrier [29], and central administration of CRF suppresses gastric contractions [19], which is opposite response to peripheral CRF [13]. In this context, the role of genuine peripheral CRF1 cannot be clarified at present. Stress-induced alterations of gastric motility are mediated by both peripheral and central CRF receptors [9], and there is no data whether and how central CRF signaling interacts with peripheral one. Moreover, there is evidence that peripheral CRF acts on myenteric neurons that determines the activity of colonic functions during stress [23], but the precise mechanism of altered gastric motility induced by peripheral CRF or stress has not been clarified. Further studies are needed to elucidate these issues.

In summary, we measured gastric contractions during WAS by the perfused manometric method in freely moving conscious rats. WAS significantly stimulated gastric contractions via peripheral CRF1 without affecting GE. Stress alters gastric motility, but GE and contractions do not always correspond during stress. These new findings may contribute to further understanding the mechanisms of stress-related alterations of gastric motility.

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Table 1 Effect of water-avoidance stress (WAS) on solid gastric emptying (GE) in rats.

		N	GE (%)
GE for 90 min	Controls	5	69.5 ± 7.1
	WAS	6	70.6 ± 3.3
GE for 60 min	Controls	5	50.5 ± 5.9
	WAS	6	55.3 ± 5.8

N; The number of animals

Figure legends

Figure 1.

Representative recordings of the gastric contractions in rats subjected to water-avoidance stress and controls. * indicated the period required for the treatment and re-stabilization of baseline. The period * was excluded from evaluation. The stress immediately and significantly enhanced gastric contractions.

Figure 2.

Effect of water-avoidance stress (WAS) on gastric contractions. A. The motor index for the first 1 h period of WAS was significantly greater than that before subjection to stress. But this enhancement was no longer observed in the next 1 h period of WAS. The motor index was not changed in controls. B. The % motor index for the first 1 h period but not for the next 1 h period was significantly greater in WAS group as compared to that of controls. Each point or column represents the mean ± S.E. Number of rats examined is shown in the parenthesis. *$P < 0.05$ vs. basal motor index or controls. Base; Before stress, 1 h; The first 1 h period, 2 h; 1–2 h period.

Figure 3.

Effect of intraperitoneal (ip) astressin (100 μg/kg) on the water-avoidance stress (WAS)-induced stimulation of gastric contractions. A. Representative recordings. The pretreatment with astressin (5 min prior to WAS) abolished

the WAS-induced stimulation. * indicated the period required for the treatment and re-stabilization of baseline. The period * was excluded from evaluation. B. Astressin blocked the increased motor index induced by WAS. Each column represents the mean ± S.E. Number of rats examined is shown in the parenthesis. *P < 0.05 vs. vehicle + WAS group.

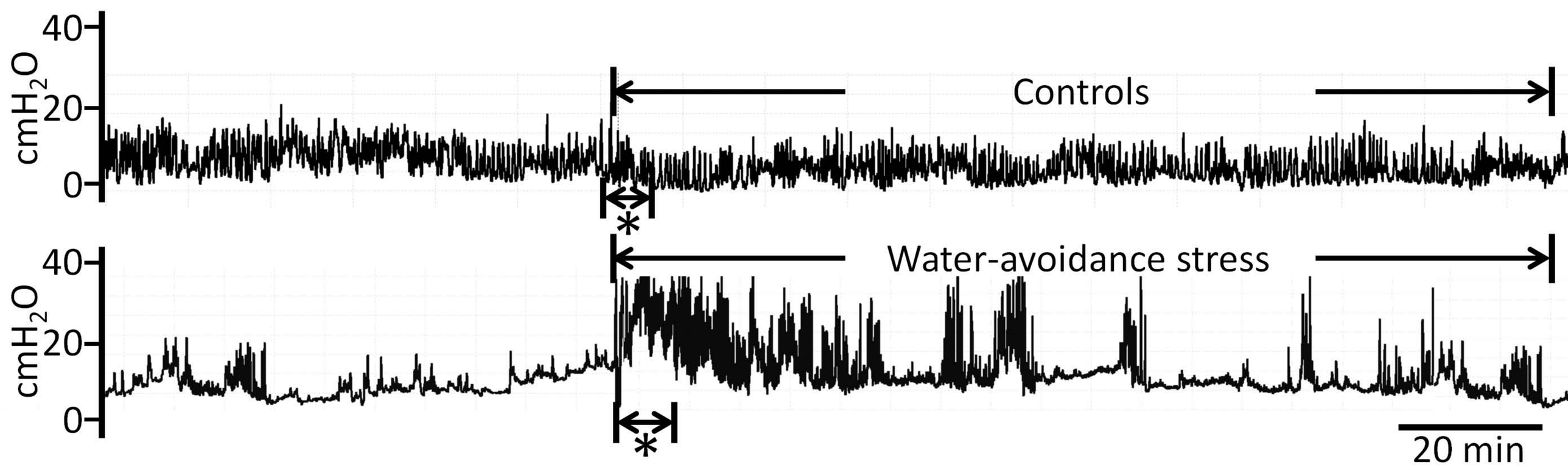
Figure 4.

Effect of intraperitoneal (ip) astressin2-B (200 μg/kg) on the water-avoidance stress (WAS)-induced stimulation of gastric contractions. A. Representative recordings. The pretreatment with astressin2-B (5 min prior to WAS) did not modify the WAS-induced stimulation. * indicated the period required for the treatment and re-stabilization of baseline. The period * was excluded from evaluation. B. Astressin2-B did not alter the increased motor index induced by WAS. Each column represents the mean ± S.E. Number of rats examined is shown in the parenthesis.

Figure 5.

Effect of intraperitoneal (ip) urocortin 2 (30 μg/kg) on the water-avoidance stress (WAS)-induced stimulation of gastric contractions. A. Representative recordings. Urocortin 2 (5 min prior to WAS) did not alter the WAS-induced stimulation. * indicated the period required for the treatment and re-stabilization of baseline. The period * was excluded from evaluation. B. Urocortin 2 did not change the increased motor index induced by WAS. Each column represents the mean ± S.E. Number of rats examined is shown in the

parenthesis.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WAS (20)
Controls (5)

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Motor Index Change (%)

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WAS (20)
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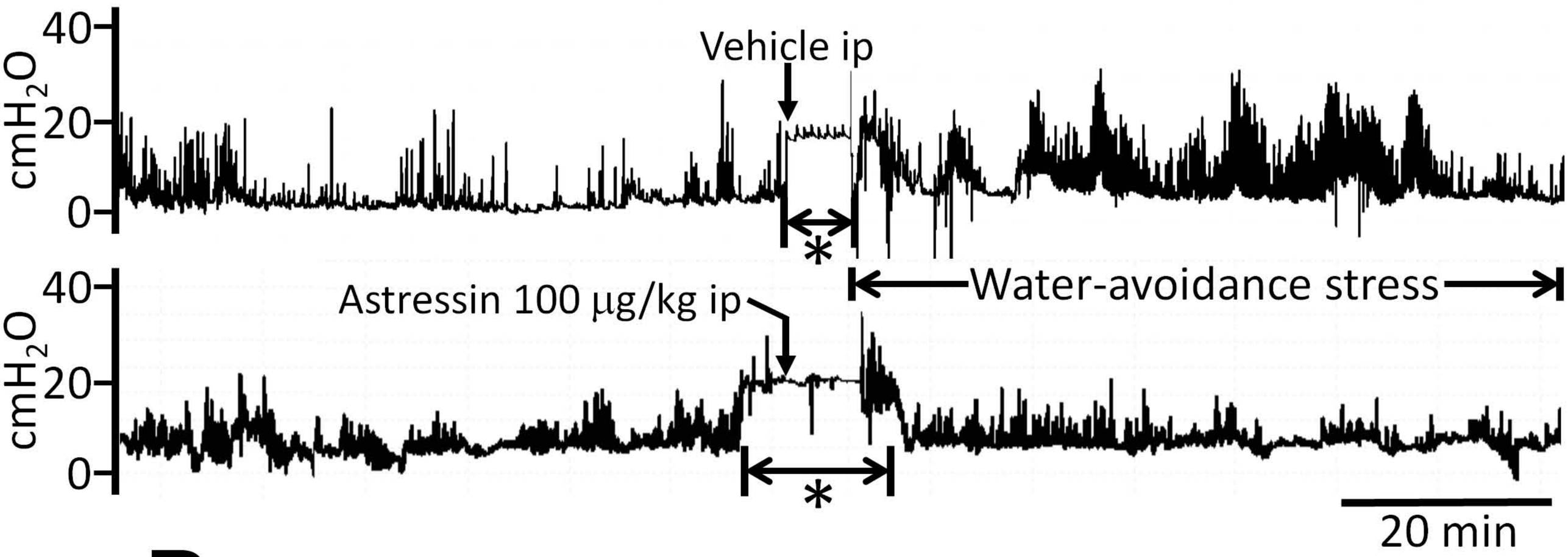
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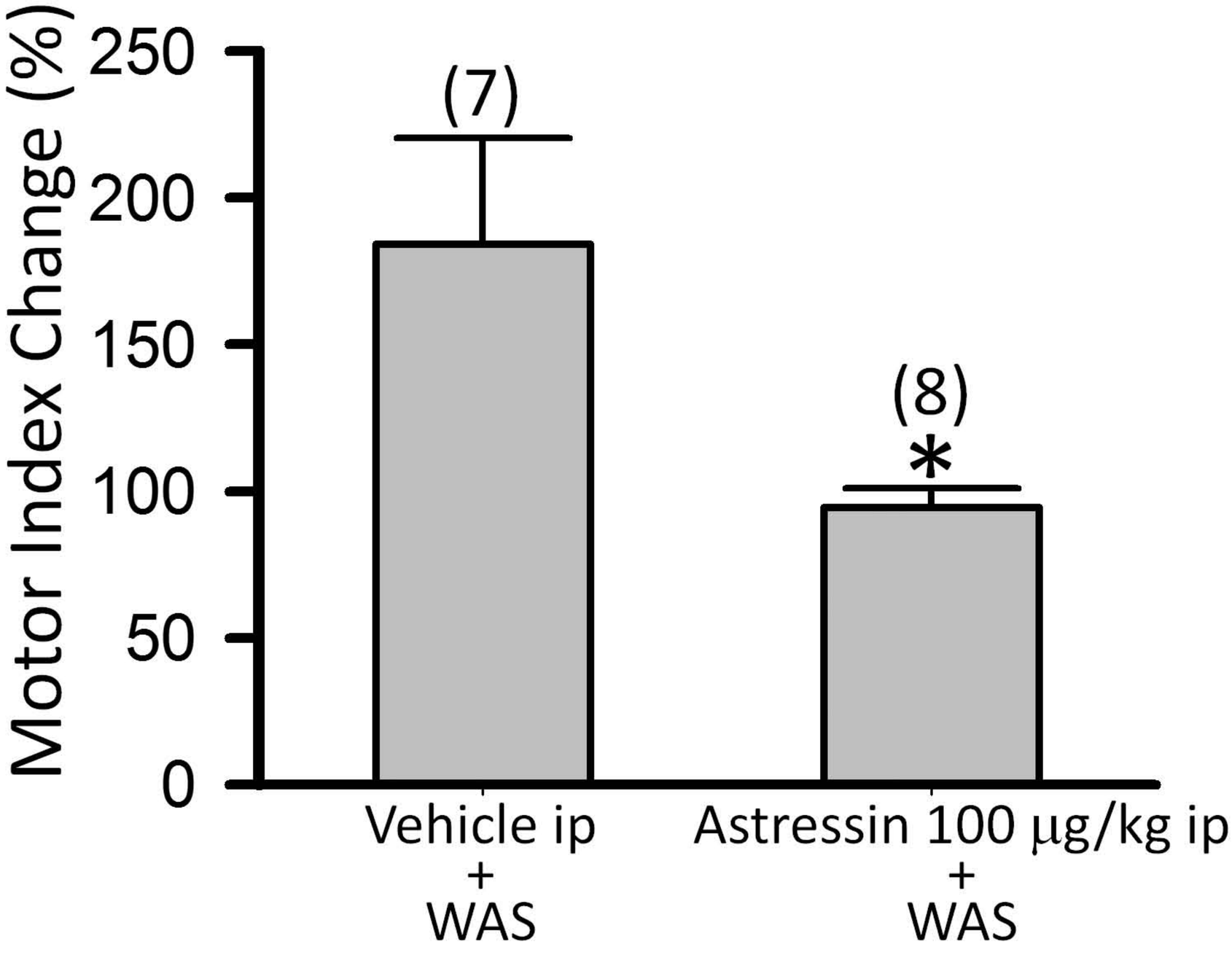
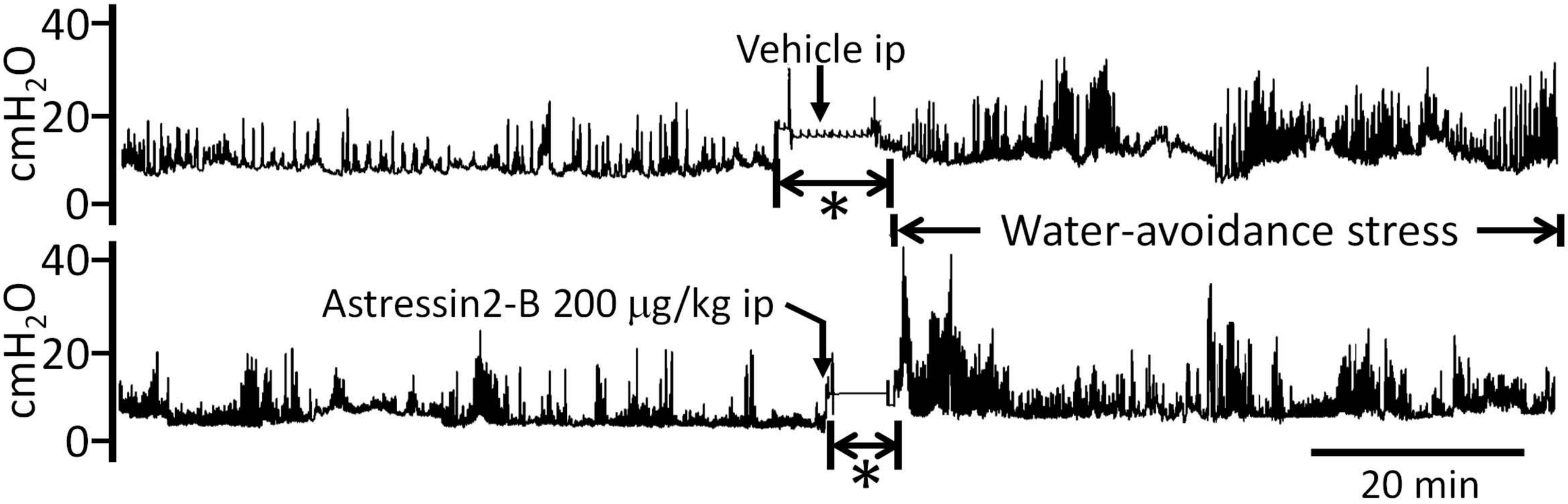
20
0
cmH2O
Vehicle ip
*
Water-avoidance stress
Astressin 100 µg/kg ip
20 min

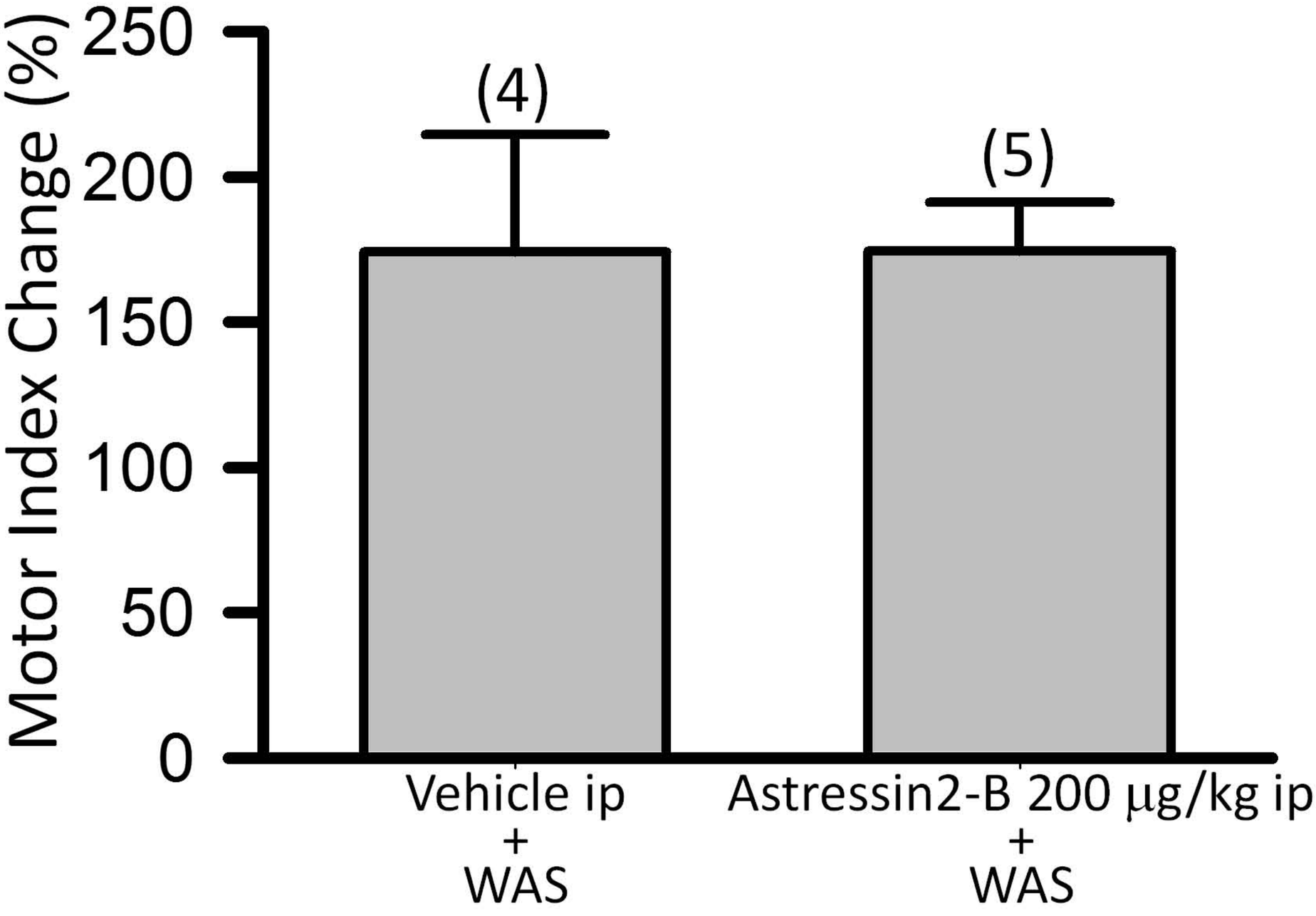
B


Motor Index Change (%)
250
200
150
100
50
0
(7)
(8)
*
Vehicle ip
+
WAS
Astressin 100 µg/kg ip
+
WAS

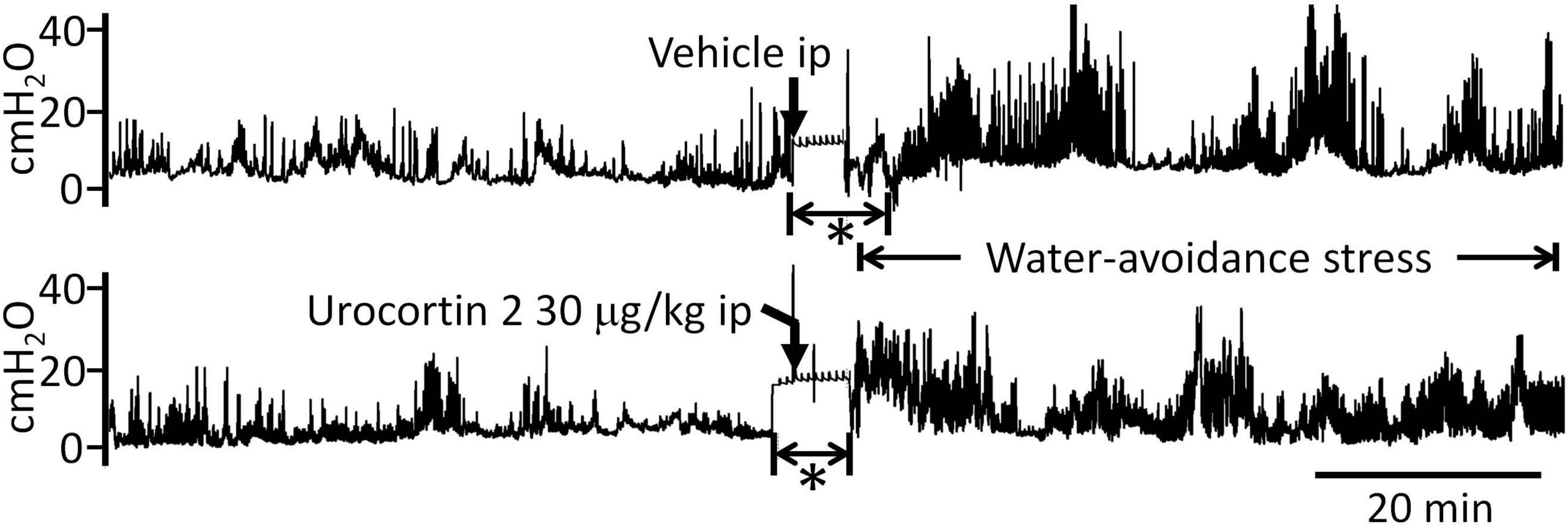
A


40
20
0
cmH2O
Vehicle ip
*
Water-avoidance stress
40
20
0
cmH2O
Astressin2-B 200 µg/kg ip
*
20 min

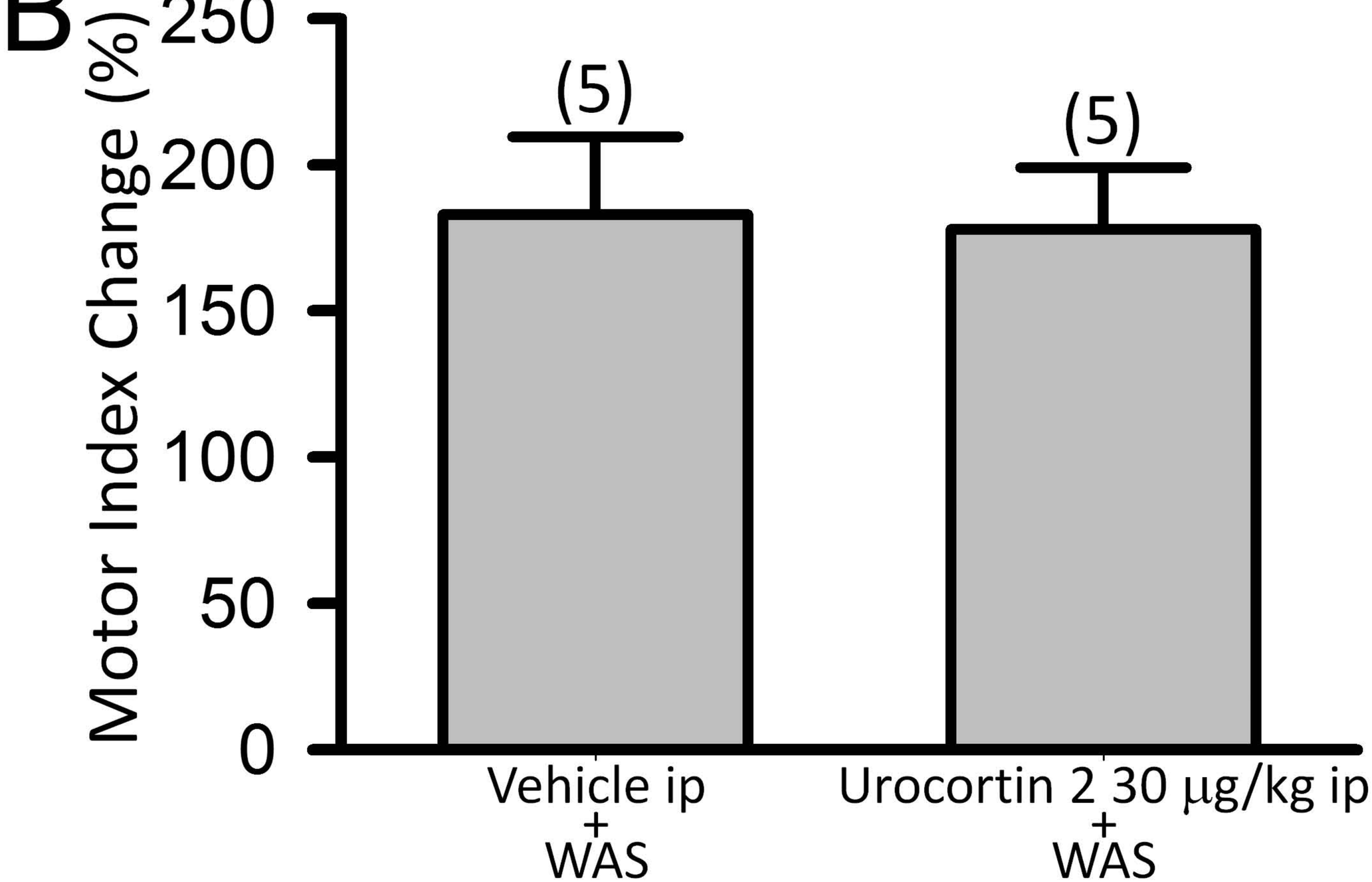
B


Motor Index Change (%)
250
200
150
100
50
0
(4)
(5)
Vehicle ip
+
WAS
Astressin2-B 200 µg/kg ip
+
WAS

A


40
20
0
cmH2O
Vehicle ip
*
Water-avoidance stress
40
20
0
cmH2O
Urocortin 2 30 μg/kg ip
*
20 min

B


Motor Index Change (%)
250
200
150
100
50
0
(5)
(5)
Vehicle ip
+
WAS
Urocortin 2 30 μg/kg ip
+
WAS