Journal of Gastroenterology (2015.3) :1-9.

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Running head; colorectal distention and visceral pain

Word count; **4622**

Abstract

Background. Most of the studies evaluating visceral sensation measure visceromotor response (VMR) to colorectal distention (CRD). However, CRD itself induces visceral sensitization, and little is known about the detailed characteristics of this response. The present study tried to clarify this question. ***Methods.*** VMR was determined by measuring abdominal muscle contractions to CRD in rats. CRD set consisted of twice isobaric distentions (60 mmHg for 10 min, twice with a 30 min rest), and the CRD set was submitted on two separate days, i.e., day 1 and 3, or 8. ***Results.*** On day 1, VMR to the second CRD was increased as compared to that to the first CRD, which is the acute sensitization. VMR to the first CRD on day 3 returned to the same level as that to the first CRD on day 1, and total VMR, i.e., whole response to CRD set was not different between day 1 and 3. Meanwhile, total VMR was significantly increased on day 8 as compared to that on day 1, suggesting CRD induced the delayed sensitization. Intraperitoneal (ip) astressin (200 μg/kg), corticotropin-releasing factor receptor antagonist at the end of the first CRD blocked the acute sensitization, but anakinra (20 mg/kg, ip), interleukin-1 receptor antagonist did not modify it. Astressin (200 μg/kg, twice before CRD on day 8) did not alter, but anakinra (20 mg/kg, twice) abolished the delayed sensitization. ***Conclusions.*** CRD induced both acute and delayed sensitization, which was mediated through peripheral corticotropin-releasing factor and interleukin-1 pathways, respectively.

Key words: colorectal distention, visceral sensitization, IL-1, CRF

Introduction

Irritable bowel syndrome (IBS) displays chronic abdominal pain or discomfort with altered defecation, and abnormality of gut motility and visceral sensation play an important role in the generation of symptoms [1]. Meanwhile, stress has been recognized as an important factor in the pathophysiology. Namely, it alters the colonic functions [2] and frequently exacerbates the symptoms of IBS [3]. Corticotropin-releasing factor (CRF) is a main mediator of the stress responses [4], and central and peripheral CRF receptors are involved in the stress-induced alterations of colonic functions [2].

Many studies have been conducted to evaluate the visceral sensation in order to explore the pathogenesis of IBS so far. The method adopted by the most of these studies relies on monitoring visceromotor response (VMR) to colorectal distention (CRD). However, CRD itself alters VMR [5-7], even though it is submitted for the purpose of measuring VMR. A lot of studies demonstrated that the stress such as restraint, water avoidance stress, etc. modifies VMR [8, 9], but it is important to note that the changes of VMR detected in these stress models may include those induced by CRD itself.

Therefore, to know the precise mechanisms and characteristics of CRD-induced altered visceral sensation is fundamental for conducting the experiments measuring VMR to CRD. Although it was reported that repetitive CRD induces enhanced VMR, which is mediated through peripheral CRF receptors [6, 10], little is known about precise mechanisms and it is not clear how long it continues.

In the present study, first we tried to determine the duration of CRD-induced hypersensitivity in rats. CRD was submitted to the same

animals in two separate days, i.e., day 1 and 3, 8 or 15 in order to clarify it. In these experiments, we obtained another new finding that CRD also induced delayed onset of hypersensitivity. In other words, CRD induced two different types of sensitization, such as acute and delayed sensitization. Then we tried to determine the mechanisms of these responses.

As described above, CRD may activate peripheral CRF signaling. Several studies demonstrated that peripheral CRF increases colonic permeability [11, 12], thereby contributing to the development of inflammatory processes [13]. Meanwhile, circulating level of proinflammatory cytokines including interleukin-1β (IL-1β) are increased in IBS patients [14], and peripheral administration of IL-1β induces visceral allodynia in rats [15]. Therefore, peripheral CRF and IL-1 signaling may contribute to visceral sensitization and the pathophysiology of IBS. In this context, we evaluated the role of peripheral CRF and IL-1 signaling in these responses.

Materials and methods

Animals

Experiments were conducted in adult male Sprague-Dawley rats weighing about 250 g. Rats were housed in group cages (3–4 rats/cage) in a temperature-regulated room (23–25 ºC) under controlled light/dark conditions (lights on 07:00–19:00) with free access to standard rat chow (Solid rat chow, Oriental Yeast, Tokyo, Japan) and tap water. Experiments started between 8 AM–3 PM and finished no later than 4 PM.

Chemicals

Recombinant human IL-1 receptor antagonist, anakinra (Swedish Orphan Biovitrum, Stockholm, Sweden) and IL-1β (Wako Pure Chemical Industries, Osaka, Japan) were dissolved in normal saline. Astressin, CRF receptor antagonist (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in double-distilled water. All drugs were administered though intraperitoneal (ip) route. Girard et al. [16] reported that lipopolysaccharide (LPS)-induced cytokine expression in rat placenta was dose-dependently inhibited by ip anakinra at doses of 2–20 mg/kg. Moreover, we previously showed that ip anakinra (20 mg/kg) blocked LPS-induced suppressed gastric contractility in rats [17]. In addition, we demonstrated that astressin (200 μg/kg, ip) successfully blocked CRD-induced visceral sensitization, and IL-1β (10 μg/kg, ip) are known to induce visceral allodynia in rats [15]. The doses of chemicals used in the present study were selected according to the above evidence.

Measurement of visceral sensation

Visceral pain in response to CRD was assessed by abdominal muscle contractions in conscious rats. In the present study, the electrodes for measuring the muscle contractions electrophysiologically were acutely implanted on the day of the experiment.

Implantation of electrodes and balloon placement

The rats were trained to the experimental conditions by placing them singly in Bollmann cages for 3 h per day for 3 consecutive days before the study. On the day of the experiment, under brief ether anesthesia, skin incision about 5 mm in length was made in non-fasted rats. Then the electrodes (Teflon coated stainless steel, 0.05 mm diameter, MT Giken, Tokyo, Japan) were inserted approximately 2 mm

into left side external oblique musculature through the incision and secured by cyanoacrylate instant adhesive (Aron Alpha, TOAGOSEI, Tokyo, Japan) together with the incised skin. The electrode leads were externalized through this closed incision and threaded through a urethane tube. The distension balloon (a 6 cm long latex balloon tied around a 4-Fr polyvinyl chloride catheter, Atom, Tokyo, Japan) was inserted through anus with the distal end positioned 1 cm proximal to the anus. The balloon was fixed in place by taping the catheter to the tail.

CRD and monitoring VMR

After completing the manipulation for electrodes implantation and balloon placement, the animals were put in Bollmann cages. Then electrode leads were connected to a custom made electromyogram (EMG) amplifier. EMG signals were amplified, filtered (3000 Hz) and digitized by a PowerLab system (AD Instruments, Colorado Springs, CO, USA), and stored by computer software (LabChart 7, AD Instruments). The distention balloon catheter was connected to a pressure amplifier (AP-641G, Nihon Kohden, Tokyo, Japan) via a pressure transducer (TP-400T, Nihon Kohden), and balloon pressure signals were digitized by a PowerLab system. The balloon catheter was also connected to an air-filled 50-ml syringe. After a 60 min stabilization period of recovery and stabilization in the cages, they were submitted to isobaric CRD by inflating the balloon using the syringe manually. Such an acute preparation was previously validated to study visceral hyperalgesia induced by CRD in rats [6, 10, 18]. Basal area under the curve (AUC) was determined by calculating the AUC of EMG signal trace for the 10 min period immediately preceding each CRD using LabChart 7 software. The VMR (μV×min) was calculated by subtracting the basal AUC from the AUC during distension period.

168 Experimental protocols (Fig. 1)

169 In the present study, we adopted the distention protocol as follows.
170 Single CRD set consisted of twice isobaric distentions (60 mmHg for 10
171 min, twice with a 30 min rest), which was shown to induce visceral
172 sensitization [5, 6], i.e., VMR to the second CRD is increased as
173 compared with that to the first CRD.

174 First, we determined how long this acute sensitization continues
175 and whether delayed onset of sensitization occurs. In this experiment,
176 the CRD set was loaded to the same animals on two separate days, i.e.,
177 day 1 and 3, 8 or 15, and VMR on each day was compared. Next, the
178 effect of drugs on CRD-induced sensitization was determined in order to
179 elucidate the mechanisms of the response.

180 In the experiment to reveal the mechanisms of acute sensitization,
181 single CRD set was submitted, and drug or vehicle was administered at
182 the end of the first CRD. % change in VMR between the first and second
183 CRD [(VMR to the second CRD)/(VMR to the first CRD) × 100] was
184 calculated and the effect of drug was determined.

185 In addition, in the experiment regarding the delayed sensitization,
186 CRD set was loaded on two separate days. Total VMR, i.e., summation of
187 VMR to the first and the second CRD in each CRD set and % change in
188 total VMR to CRD set between day 1 and the later day [(total VMR to
189 the CRD set on later day)/(total VMR to the CRD set on day 1) × 100]
190 were calculated. Drug or vehicle was administered twice, 18 h and 1 h
191 prior to the later CRD set in order to reveal the mechanisms.

192

193 Colonic tissue damage assessment

194 In order to assess whether repeated CRD induces colonic tissue
195 damage, four rats underwent two CRD sets on day 1 and 8, and control

196 rats subjected to balloon placement but without CRD were prepared for the
197 analysis. The animals underwent whole perfusion fixation before tissue
198 sampling as described previously [19] with minor modification. The animals
199 were anesthetized with ketamine/xylazine mixture and the heart was exposed
200 by thoracotomy. Perfusion needle was inserted into the ascending aorta
201 through an apical left ventricle puncture, and the right atrium was incised.
202 Then the animals were perfused with 300 ml of 4 % paraformaldehyde
203 phosphate buffer solution (Wako Pure Chemical Industries, Osaka, Japan) for
204 about 15 min at room temperature. Next, the distal colon tissues were removed
205 and further fixed by overnight immersion in the fixative at 4 °C. They were
206 embedded in paraffin wax, sectioned (4 μm), stained with hematoxylin and
207 eosin, and examined by light microscopy. Presence of colonic wall damage and
208 inflammatory cells were assessed.
209
210 Statistical analysis
211 Data were expressed as means ± S.E. Multiple comparison was
212 performed by one-way repeated measures analysis of variance or one-way
213 analysis of variance followed by Fisher's Least-Significant-Difference Test.
214 Comparison between two groups was performed using the Student's t or paired
215 t test. SYSTAT 13 software (Systat Software, Chicago, IL, USA) was used
216 throughout the study.
217
218 Ethical considerations
219 Approval by the Research and Development and Animal Care
220 Committees at the Asahikawa Medical University (#11042, approved on March
221 7, 2011) was obtained for all studies.
222
223

Results

CRD-induced acute sensitization was no longer observed after 48 h (Fig. 2A)

On day 1, initial CRD set was loaded, and VMR to the second CRD was significantly higher than that to the first CRD ($F = 8.2$, $p < 0.05$, 67.8 ± 3.5 for first CRD vs., 98.0 ± 4.7 for second CRD, $n = 8$, $p < 0.05$), which is consistent with the previous studies demonstrating that CRD induces acute sensitization in rats [5, 6]. The same CRD set was loaded to the same animals again on day 3, i.e., 48 h later from the initial CRD set, and this acute sensitization was observed again (58.3 ± 8.2 for the first CRD vs., 85.3 ± 13.7 for the second CRD, $p < 0.05$). However, VMR to the first CRD on day 3 was significantly reduced as compared with that to the second CRD on day 1, and was returned to the same level as that to the first CRD on day 1. These results indicated that acute sensitization no longer continued after 48 h from the last CRD set.

In separate experiment, VMR to the second CRD was significantly increased as compared to that to the first CRD on day 8 ($F = 12.2$, $p < 0.05$, 103.6 ± 10.4 for the first CRD, vs., 134.0 ± 6.4 for the second CRD, $n = 12$, $p < 0.05$). Moreover, VMR to the first CRD on day 8 was also greater as compared to that to the first CRD on day 1 (vs., 67.0 ± 12.3 for the first CRD on day 1, $p < 0.05$). On the other hand, the acute sensitization was not detected on day 15 (83.1 ± 16.3 for the first CRD, vs., 86.8 ± 15.7 for the second CRD, $n = 8$, $p > 0.05$).

CRD induced the delayed sensitization 7 days later from the last CRD (Fig. 2B)

The total VMR was not different between day 1 and 3 (165.8 ± 8.3 for day 1 vs., 143.7 ± 21.8 for day 3, $p > 0.05$). On the other hand, it was significantly increased on day 8 as compared with that on day 1 ($157.1 \pm$

16.5 for day 1 vs., 237.6 ± 26.9 for day 8, $p < 0.05$). Because VMR to the first CRD was significantly higher on day 8 than that on day 1 as described above (Fig. 2A), increased total VMR on day 8 did not result from enhanced response of the acute sensitization, suggesting that CRD induced another type of visceral hypersensitivity response, such as delayed sensitization. We also determined VMR on day 1 and 15, and total VMR was not different between these days (150.7 ± 24.7 for day 1 vs., 169.9 ± 31.7 for day 15, $p > 0.05$), indicating that this response disappeared within two weeks.

Manipulation related to the measuring VMR did not induced the delayed sensitization (Fig. 3)

Next, in order to further confirm that CRD induces the delayed sensitization indeed, we prepared the animals underwent only manipulation related to the measuring VMR, i.e., anesthesia, skin incision, electrodes implantation and balloon insertion without CRD on day 1 and measured VMR on day 8. These rats were placed in Bollmann cages for 3 h per day for 3 consecutive days before the manipulation on day 1 and day 8-measurement similar to controls. Controls underwent two CRD sets on day 1 and 8.

Total VMR of the manipulation only animals was 136.2 ± 18.8 ($n = 6$), which was significantly smaller than that of respective controls on day 8 ($F = 10.0$, $p < 0.05$, 214.4 ± 12.8, $n = 8$, $p < 0.05$) and comparable to that of controls on day 1 (140.9 ± 11.8). These results showed that the manipulation did not contribute to the delayed sensitization and CRD definitely induced this response.

The delayed sensitization was abolished by anakinra but not by astressin (Fig. 4)

Next, the mechanisms of the delayed sensitization was evaluated. Astressin (200 μg/kg, twice before day 8-CRD) did not modify this response (% change in total VMR between day 1 and 8, 153.3 ± 18.2 for vehicle, n = 7, vs., 138.0 ± 6.5 for astressin, n = 5, $p > 0.05$).

On the other hand, anakinra (20 mg/kg, twice before day 8-CRD) abolished the response (% change in total VMR between day 1 and 8, 147.8 ± 17.9 for vehicle, n = 7, vs., 102.1 ± 6.7, n = 7, $p < 0.05$), suggesting that IL-1 pathways contribute to the delayed sensitization.

IL-1β increased VMR (Fig. 5)

We also tested the effect of IL-1β on VMR to CRD. IL-1β (10 μg/kg) or vehicle was injected 1 h prior to CRD set. Total VMR was significantly increased as compared to that of vehicle-treated group (144.2 ± 13.0 for vehicle, n = 11 vs., 199.8 ± 16.9 for IL-1β, n = 8, $p < 0.05$).

The acute sensitization was blocked by astressin but not by anakinra (Fig. 6)

Finally, we determined the role of CRF and IL-1 signaling on the acute sensitization. Astressin (200 μg/kg) administered at the end of the first CRD abolished the acute sensitization (% change in VMR between the first and the second CRD, 120.1 ± 7.3 for vehicle, n = 6, vs., 95.9 ± 13.2 for astressin, n = 5, $p < 0.05$), which is consistent with our previous study [6].

Meanwhile, anakinra (20 mg/kg) administered at the end of first CRD did not altered the sensitization (% change in VMR between the first and the second CRD, 122.7 ± 7.9 for vehicle, n = 6, vs., 121.4 ± 4.6 for anakinra, n = 9, $p > 0.05$). Moreover, anakinra at the same dose, twice 18 h and 1 h prior to CRD set did not alter the response either (% change in VMR between the first and the second CRD, 132.0 ± 8.1 for

vehicle, n = 9, vs., 128.2 ± 9.0 for anakinra, n = 10, $p > 0.05$). Total VMR was not different between anakinra and vehicle group (159.0 ± 12.2 for vehicle, vs., 153.7 ± 20.0 for anakinra, $p > 0.05$).

Repeated CRD did not produce colonic tissue damage (Fig. 7)

Histological analysis did not detect any differences in colonic wall structure and the presence of inflammatory cells.

Discussion

We reconfirmed the finding that CRD induces the acute sensitization and showed that it disappeared within 48 h. Meanwhile, it is of interest, VMR was enhanced again after 7 days from the last CRD set, i.e., delayed sensitization. Since manipulation associated with the placement of EMG electrodes did not induce this sensitization, this response was thought to result from CRD itself. We only measured VMR on day 1 and 3, 8 or 15, therefore additional experiments to determine more accurate onset or duration of the delayed sensitization are needed in future. In any event, to our knowledge, this is the first report showing CRD induces the delayed visceral hypersensitivity. There have been several studies adopting the experimental protocols submitting several CRD sets in different days in order to evaluate the mechanisms of chronic or repeated stress-induced altered visceral sensation [20, 21]. Our results may raise caution in interpreting the results obtained by these experiments, because CRD itself may induce the delayed hypersensitivity.

The present finding that ip astressin having poor penetrance into brain [22] blocked the acute sensitization strongly suggested that CRD activates peripheral CRF signaling to induce this response. A couple of studies have

335 shown that peripheral injection of cortagine, which is CRF receptor
336 subtype 1 agonist, induces visceral hyperalgesia within 30 min of this
337 peptide injection in rats [6, 12], indicating stimulating peripheral CRF
338 pathways display rapid response, which is consistent with the notion
339 above. With regard to the mechanisms, peripheral CRF signaling is
340 thought to modulate visceral sensation directly through acting visceral
341 afferent neurons [23] and/or indirectly through stimulating the release of
342 mediators such as serotonin, etc. from enterochromaffin cells [24] and
343 mast cells [25], leading to activating afferents to induce acute
344 sensitization. As described above, astressin does not penetrate to the
345 brain but it may affect brain through circumventricular organs, which
346 are relatively unprotected by the blood-brain barrier. In this context, the
347 contribution of central CRF signalings to CRD-induced visceral
348 sensitization cannot be denied completely.
349 The present study also showed that the acute sensitization
350 occurred not only on day 1, but also on day 3 and 8. While it was not
351 detected on day 15, of which reason was not known. Aging of rats may
352 alter the responsiveness of stress [26], which may one of the possible
353 explanations.
354 On the other hand, administration of astressin before the day 8-
355 measurement did not block the delayed sensitization, suggesting a CRF-
356 independent mechanism of the delayed sensitization. The most
357 important point of the present study is this delayed sensitization was
358 completely blocked by the pretreatment of anakinra before CRD set on
359 day 8. This result indicates that it may be mediated through IL-1
360 pathways, suggesting that inflammatory process may engage in this
361 phenomenon. We microscopically evaluated the colonic tissue of rats
362 underwent CRD, but neither tissue damage nor inflammatory changes

were found. Whereas, CRD with higher intensity (80 mmHg for 30 seconds with a 90 seconds rest for 2 h or 80 mmHg for 20 seconds with a 60 seconds rest, 15 times for 6 consecutive days) was reported to induce colonic plasma extravasation, or increase numbers of neutrophils, eosinophils, and intraepithelial lymphocytes in muscularis mucosae suggesting colonic inflammation [7, 27]. This fact suggests that significant level of CRD intensity is needed to induce histological changes, but even lower intensity of CRD as in this study might induce minor inflammation without histological abnormalities, leading to activating IL-1 signaling. Although the mechanisms of CRD-induced inflammatory processes remain unknown, repeated CRD might induce ischemia and reperfusion of colonic wall, which is known to increase the production of IL-1β [28]. That may be one of the possible explanations.

With regard to the mechanisms by which IL-1 signaling plays a role in the control of visceral sensation, increasing evidence has been reported as following. Coelho et al. demonstrated that ip IL-1β induces rectal allodynia in rats [15], and we also showed in this study that it induced hyperalgesia. There are several studies suggesting the possible mechanisms of IL-1-induced visceral sensitization. IL-1 immunoreactive nerve fiber afferents are located in the abdominal visceral organs and celiac-superior mesenteric ganglion complex of rat [29]. In addition, peripheral administration of IL-1β stimulates abdominal visceral afferents [30]. It follows from these lines of evidence that peripheral IL-1 may activate visceral afferents, causing visceral sensitization.

Although peripheral administration of anakinra blocked the delayed sensitization, involvement of central IL-1 pathways is not able to be denied according to the following reasons. Several studies showed that anakinra has brain penetrance [31, 32]. Greenhalgh et al. [31] demonstrated that a single subcutaneous injection of anakinra (100 mg/kg) increased concentration of cerebrospinal fluid. It is also known that LPS induces rectal allodynia

391 mediated through brain IL-1β and moreover, intracerebroventricular IL-
392 1β induces rectal allodynia in rats [33]. Thus, not only peripheral but
393 central IL-1 may be involved in the control of visceral sensation.
394 Our study has several limitations. The barostat system with the
395 high-compliance polyethylene bag which can provide a constant pressure
396 is thought to be reliable to measure visceral sensitivity, but we used
397 latex balloon which was inflated by syringe. There is a report indicating
398 the different rectal thresholds between the bag and balloon in humans
399 [34]. This issue, therefore might modify the main results presented in
400 our study. Additionally, CRD did not induce significant tissue damage or
401 inflammatory response, but hematoxylin and eosin staining may not be a
402 perfect staining to detect immune activation. Moreover, the delayed
403 sensitization was not blocked by astressin, but there is a possibility that
404 the dose and the timing of the antagonist administration might be
405 inappropriate for blocking CRF signaling. Further studies are warranted
406 to clarify these issues.
407 There is growing evidence suggesting the importance of gut
408 immune system on the pathogenesis of IBS. In particular, recent clinical
409 studies demonstrated that subset of IBS patients displays low-grade
410 inflammation in the intestinal mucosa without macroscopic abnormal
411 findings [35, 36]. Moreover, circulating level of proinflammatory
412 cytokines such as IL-1β, IL-6 and TNF-α are increased [14]. Therefore,
413 the delayed hypersensitivity induced by CRD, which is IL-1 dependent
414 without pathological abnormality of colon, might be a new stress model
415 mimicking IBS pathogenesis.
416 In summary, we demonstrated that CRD induced both acute and
417 delayed visceral sensitization, which was mediated through peripheral
418 CRF and IL-1 pathways, respectively. The delayed visceral sensitization

without apparent pathological changes might help us understand the pathophysiology of post-infectious IBS patients with visceral hypersensitivity at late onset [37].

Acknowledgments

This work was supported in part by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan [C-23590252 (TN), and C-22590753 (TO)].

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583 **Figure Legends**

584

585 Figure 1

586 The rats were submitted to colorectal distention (CRD) set, which consisted of
587 twice isobaric distentions (60 mmHg, 10 min twice with a 30 min rest) on two
588 separate days such as day 1 and 3, 8 or 15. The abdominal contractions were
589 electrophysiologically measured and visceromotor response (VMR) was
590 determined by calculating area under the curve of the trace of electromyogram
591 (EMG). Total VMR, i.e., summation of VMR to the first and the second CRD
592 was also calculated.

593

594 Figure 2

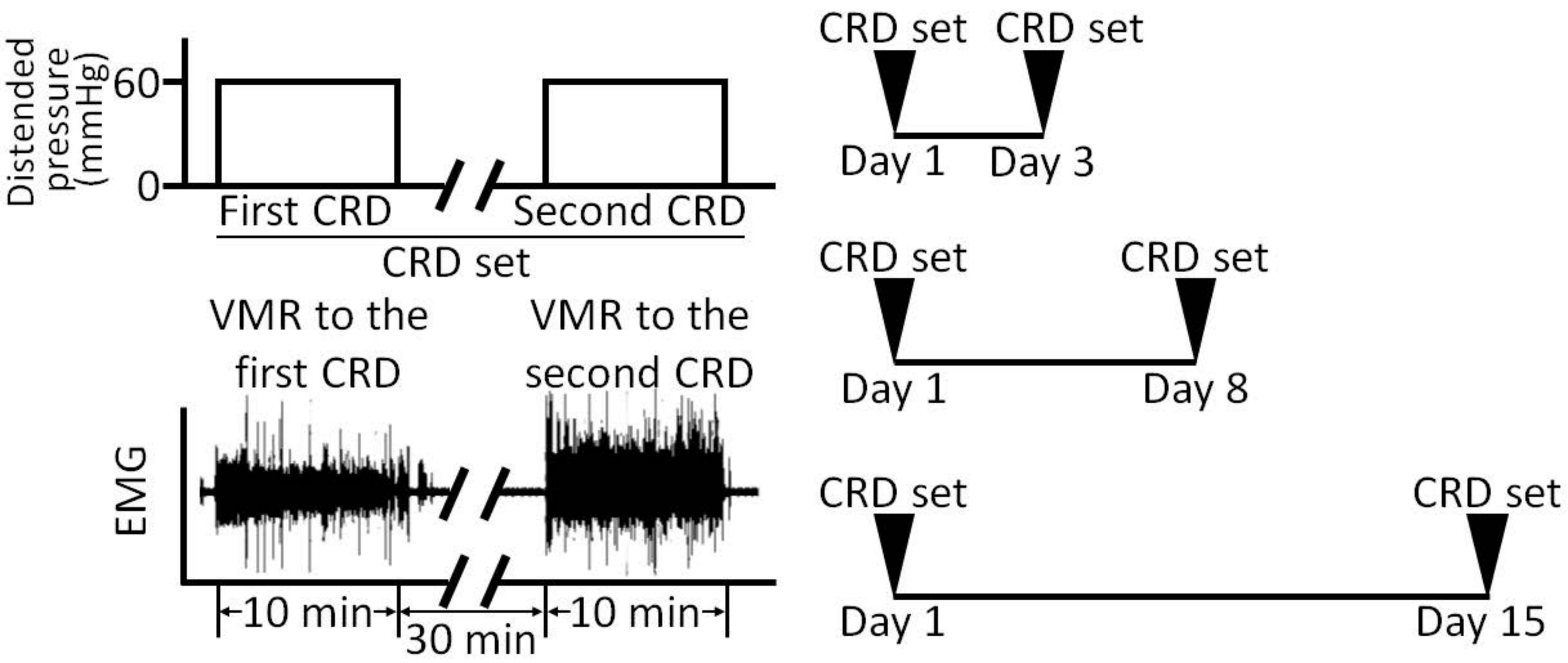
595 **a**; Visceromotor response (VMR) to the second colorectal distention (CRD) on
596 day 1 was significantly higher as compared to that to the first CRD on day 1,
597 which was the acute sensitization. However, VMR to the first CRD on day 3
598 was returned to the same level as that to the first CRD on day 1, indicating
599 that this acute response disappeared within 2 days. Meanwhile, the acute
600 sensitization was also detected on day 3 and 8. Each column represents the
601 mean ± S.E. *$p < 0.05$ vs., VMR to the respective first CRD. #$p < 0.05$ vs., VMR
602 to the second CRD on day 1. +$p < 0.05$ vs., VMR to the first CRD on day 1. **b**;
603 Total VMR was not different between day 1 and 3, but it was increased on day
604 8. Since VMR to the first CRD on day 8 was significantly higher than that on
605 day 1 (see Fig. 2a), increased total VMR on day 8 did not result from enhanced
606 response of the acute sensitization, indicating that CRD induced delayed
607 sensitization. This response was no longer observed on day 15. *$p < 0.05$ vs.,
608 total VMR on day 1.

609

610 Figure 3

611 Manipulation related to measuring visceromotor response (VMR) to colorectal
612 distention (CRD) on day 1 did not induce the delayed sensitization on day 8.
613 Each column represents the mean ± S.E. *p < 0.05 vs., total VMR on day 1. #p
614 < 0.05 vs., total VMR on day 8 in controls.
615
616 Figure 4
617 Anakinra abolished the delayed sensitization but astressin did not modify the
618 response. Colorectal distention (CRD) set was loaded to the same animals on
619 day 1 and 8, and % change in total visceromotor response to CRD set was
620 determined. Vehicle or drug was administered twice, 18 h and 1 h prior to the
621 CRD set on day 8. Each column represents the mean ± S.E. Number of rats
622 examined is shown in the parenthesis. *p < 0.05 vs., vehicle-treated group.
623
624 Figure 5
625 IL-1ß (10 μg/kg, 1 h prior to colorectal distention set) significantly increased
626 total visceromotor response. Each column represents the mean ± S.E. Number
627 of rats examined is shown in the parenthesis. *p < 0.05 vs., vehicle-treated
628 group.
629
630 Figure 6
631 Astressin abolished the acute sensitization but anakinra did not modify it.
632 Single colorectal distention (CRD) set was loaded and vehicle or drug was
633 administered at the end of first CRD. % change in visceromotor response
634 between the first and the second CRD was determined. Each column
635 represents the mean ± S.E. Number of rats examined is shown in the
636 parenthesis. *p < 0.05 vs., vehicle-treated group.
637
638 Figure 7

639 Photomicrographs of distal colon tissue (**a**; balloon placed into the colorectum
640 but no distention, **b**; two colorectal distention sets loaded on two different days
641 with 7 days interval). Magnification × 100. Colorectal distention did not induce
642 significant tissue damage or inflammatory response.
643


Distended pressure (mmHg)
60
0
First CRD
Second CRD
CRD set
VMR to the first CRD
VMR to the second CRD
EMG
10 min
30 min
10 min
CRD set
CRD set
Day 1
Day 3
CRD set
CRD set
Day 1
Day 8
CRD set
CRD set
Day 1
Day 15

A

Visceromotor Response (μV × min)

160
120
80
40
0

First CRD
Second CRD

*
*
\#
*
\+
*
*

Day 1 Day 3
n = 8

Day 1 Day 8
n = 12

Day 1 Day 15
n = 8

B

Total Visceromotor Response (μV × min)

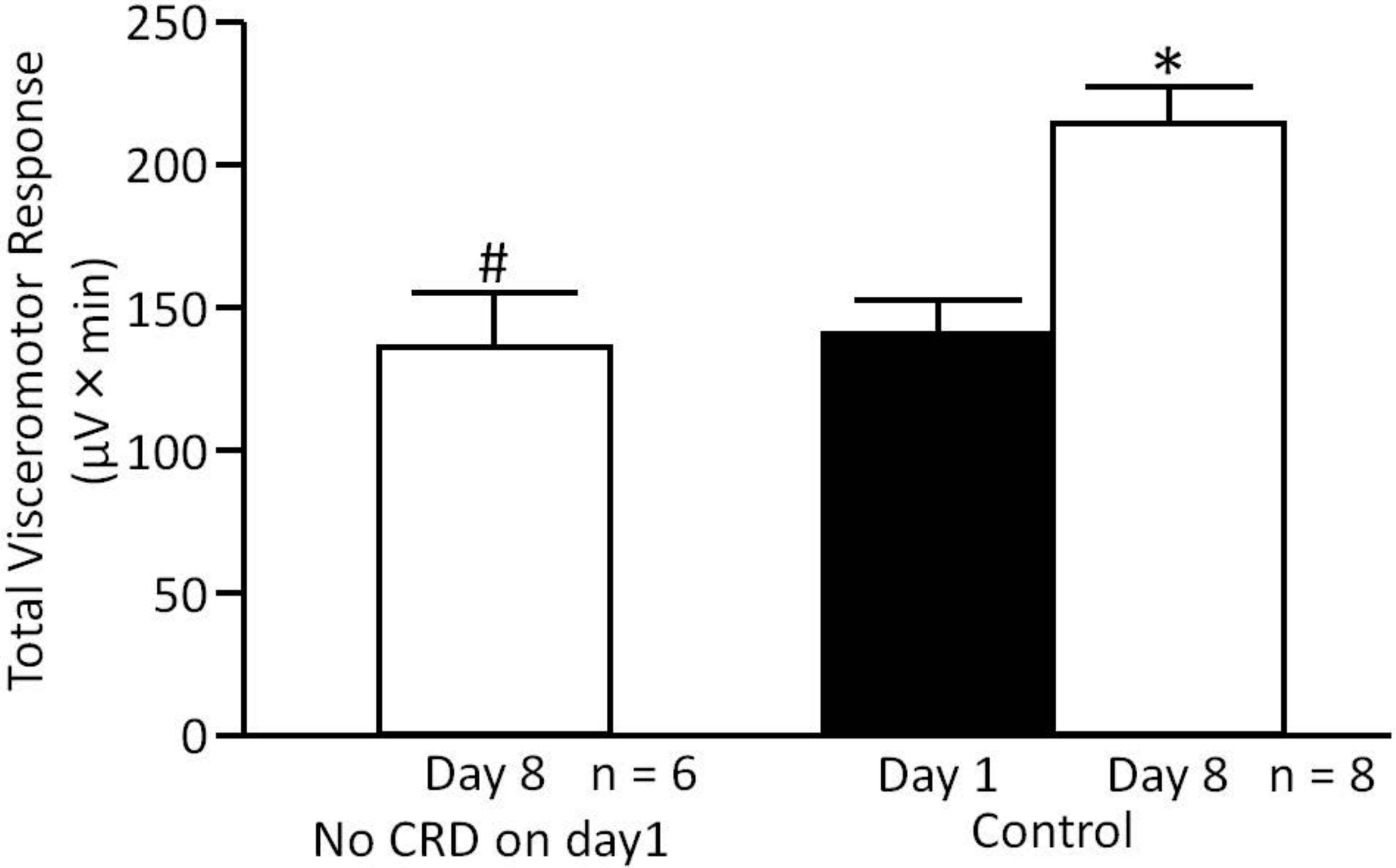
300
250
200
150
100
50
0

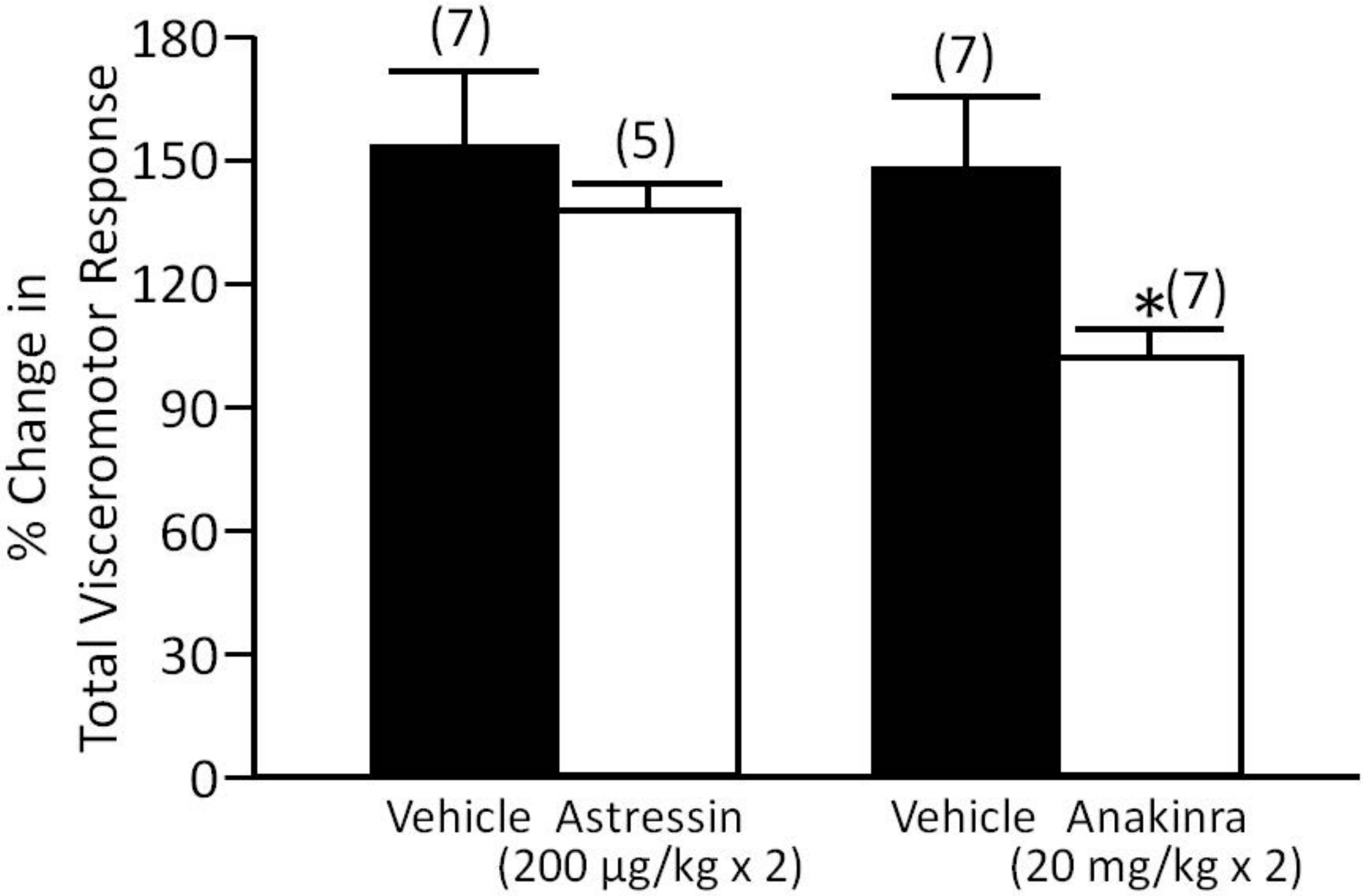
*

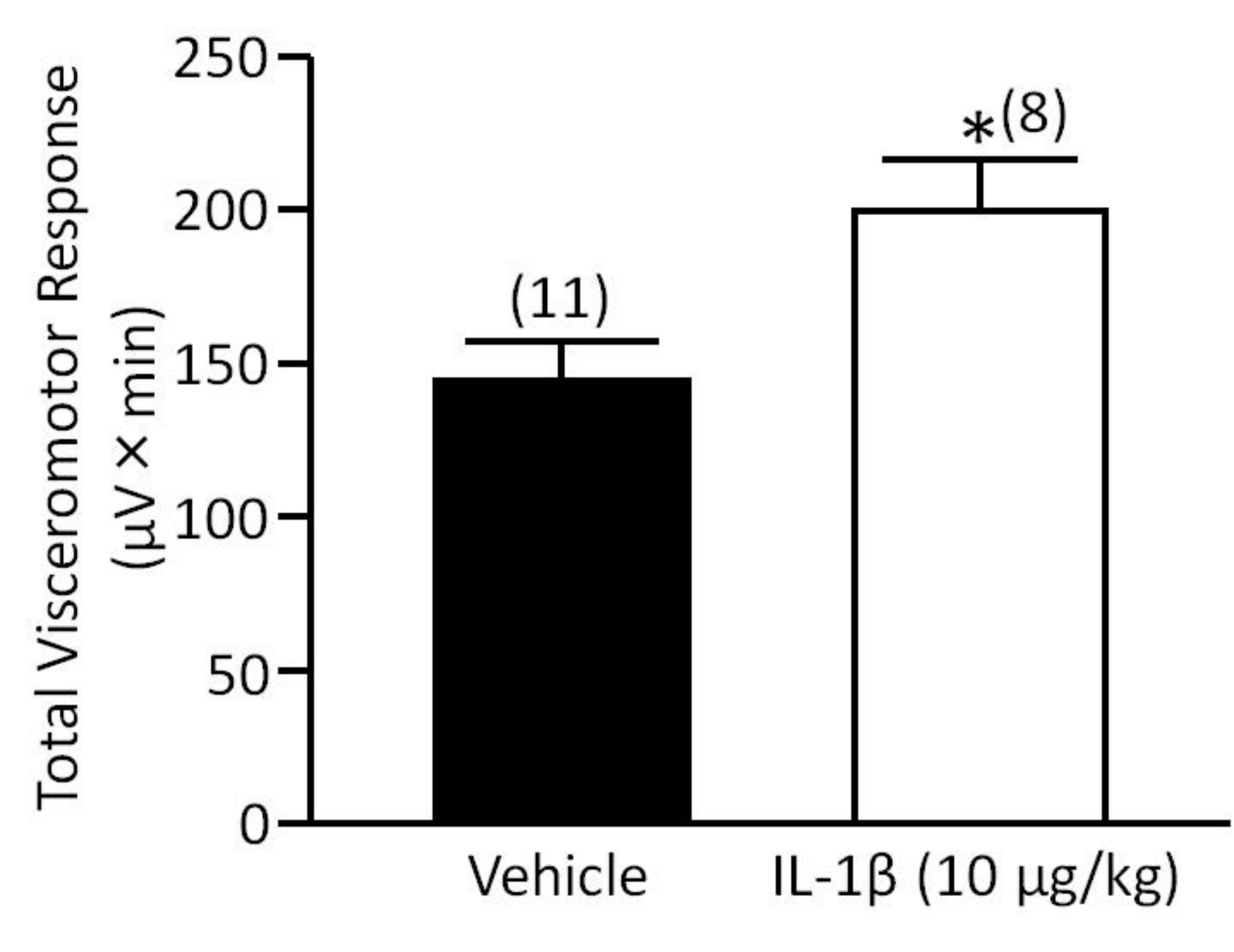
Day 1 Day 3

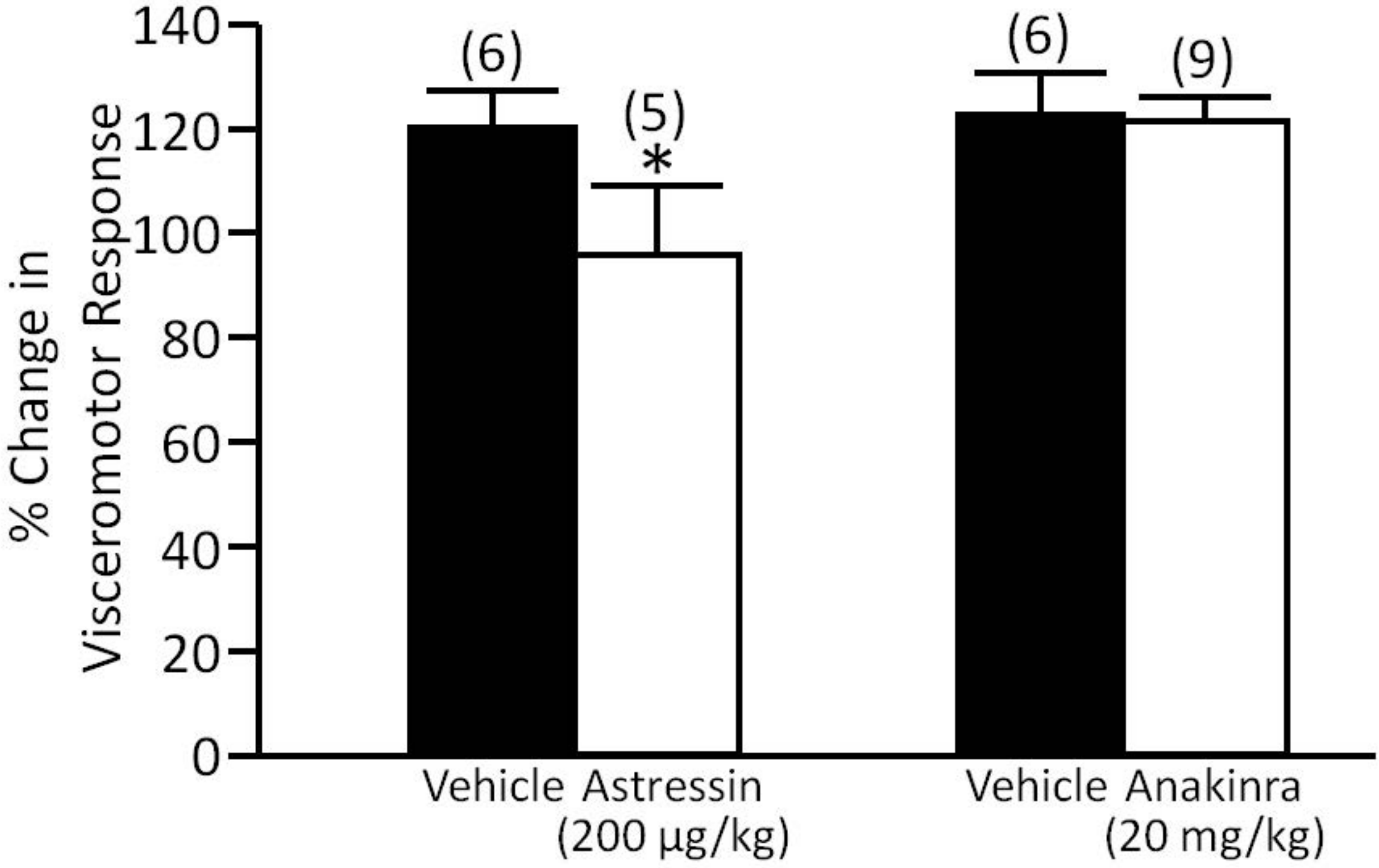
Day 1 Day 8

Day 1 Day 15

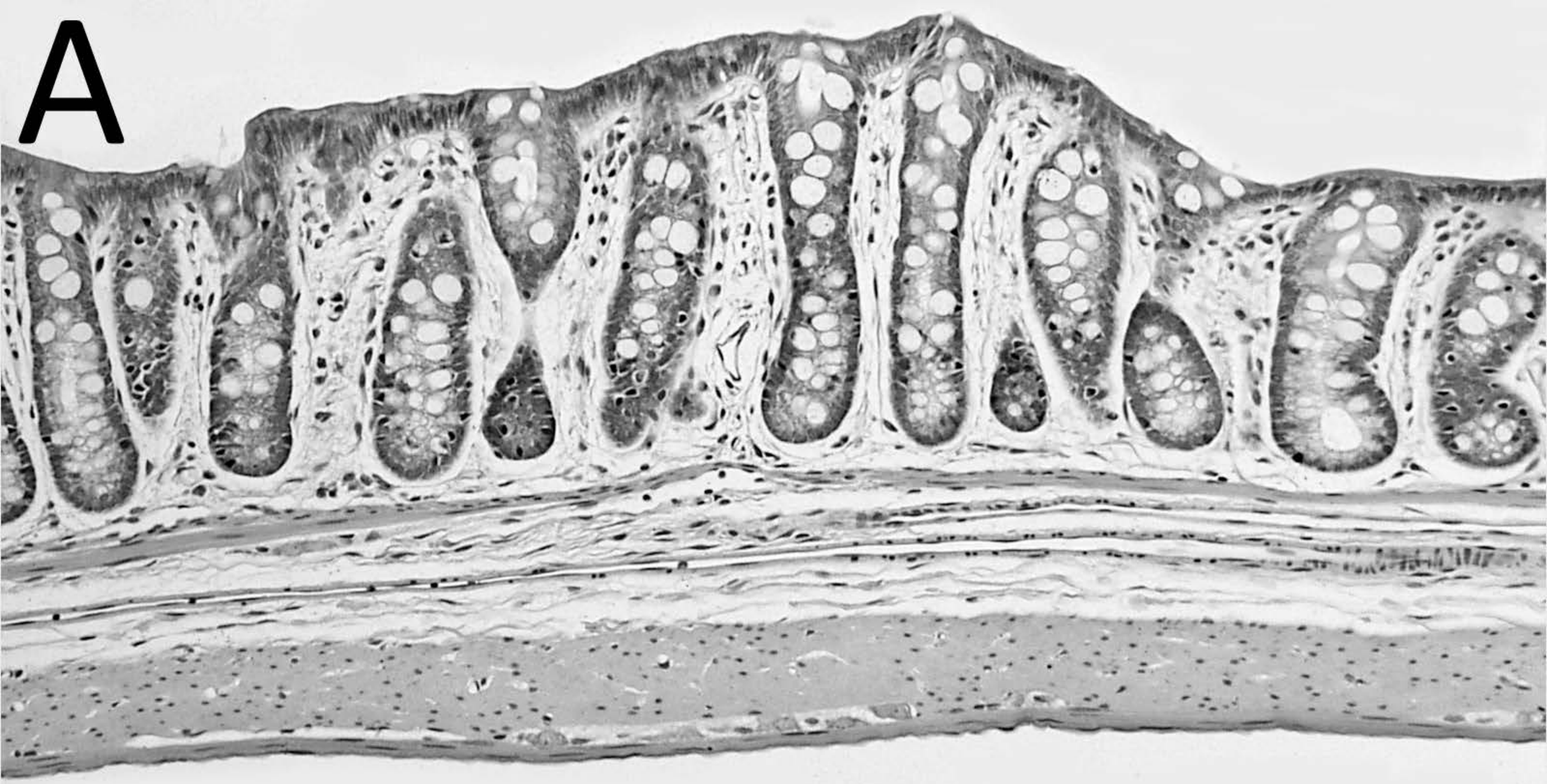

Total Visceromotor Response
(μV × min)
250
200
150
100
50
0
#
*
Day 8
n = 6
Day 1
Day 8
n = 8
No CRD on day1
Control


% Change in
Total Visceromotor Response
180
150
120
90
60
30
0
(7)
(5)
(7)
*(7)
Vehicle
Astressin
(200 µg/kg x 2)
Vehicle
Anakinra
(20 mg/kg x 2)


Total Visceromotor Response
(μV × min)
250
200
150
100
50
0
(11)
*(8)
Vehicle
IL-1β (10 μg/kg)


% Change in Visceromotor Response
140
120
100
80
60
40
20
0
(6)
(5)
*
(6)
(9)
Vehicle
Astressin (200 µg/kg)
Vehicle
Anakinra (20 mg/kg)

A



B