

Role of sympathetic nervous system in rat model of chronic visceral pain

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Key Messages

- Treatments that decrease sympathetic outflow or block activation of adrenergic receptors on sensory nerves could be beneficial in generalized pain syndromes such as irritable bowel syndrome (IBS).
- The sympathetic nervous system (SNS) exacerbates pain sensation when central pain inhibition is reduced; this observation is highly relevant to chronic pain disorders.
- This study was conducted using recordings of electromyographic responses to colorectal distension in a rat colon irritation model of chronic visceral hypersensitivity with hallmarks of IBS.
- Clonidine—alpha2-adrenergic agonist—and prazosin—alpha1-adrenergic antagonist—reduced the visceral hypersensitivity. Chemical sympathectomy with guanethidine and surgical sympathectomy resulted in a loss of the chronic visceral hypersensitivity.

Abstract

Background Changes in central pain modulation have been implicated in generalized pain syndromes such as irritable bowel syndrome (IBS). We have previously demonstrated that reduced descending inhibition unveils a role of sympathoneuronal outflow in decreasing peripheral sensory thresholds, resulting in stress-induced hyperalgesia. We investigated whether

sympathetic nervous system (SNS) exacerbation of pain sensation when central pain inhibition is reduced is relevant to chronic pain disorders using a rat colon irritation (CI) model of chronic visceral hypersensitivity with hallmarks of IBS. **Methods** Rats were treated to a series of colorectal balloon distensions (CRD) as neonates resulting in visceral and somatic hypersensitivity and altered stool function that persists into adulthood. The visceral sensitivity was assessed by recording electromyographic (EMG) responses to CRD. Somatic sensitivity was assessed by paw withdrawal thresholds to radiant heat. The effects on the hypersensitivity of (i) inhibiting sympathoneuronal outflow with pharmacological and surgical interventions and (ii) enhancing the outflow with water avoidance stress (WAS) were tested. **Key Results** The alpha2-adrenergic agonist, clonidine, and the alpha1-adrenergic antagonist, prazosin, reduced the visceral hypersensitivity and WAS enhanced the pain. Chemical sympathectomy with guanethidine and surgical sympathectomy resulted in a loss of the chronic visceral hypersensitivity. **Conclusions & Inferences** The results support a role of the SNS in driving the chronic visceral and

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Received: 14 April 2015

Accepted for publication: 31 October 2015

somatic hypersensitivity seen in CI rats. The findings further suggest that treatments that decrease sympathetic outflow or block activation of adrenergic receptors on sensory nerves could be beneficial in the treatment of generalized pain syndromes.

Keywords *alpha-adrenergic receptors, animal model, behavior, chronic pain, irritable bowel syndrome, sympathetic nervous system, visceral pain.*

INTRODUCTION

Although the etiology of chronic generalized pain syndromes is not well understood, altered central and peripheral modulation of pain has been implicated. Experimental pain studies demonstrate that conditions such as fibromyalgia and irritable bowel syndrome (IBS) are characterized by changes in central pain processing. Fibromyalgia patients¹ and a subset of IBS patients² exhibited increased wind-up of the intensity of their C-fiber mediated 'second pain' response to repetitive heat pulses compared with control subjects, consistent with altered central pain modulation. Evidence of descending facilitation was apparent in another study of fibromyalgia patients in which sustained muscle contractions resulted in lower pressure pain thresholds distal to the affected muscle.³ Functional magnetic resonance imaging studies have also provided evidence of altered central modulation of pain in fibromyalgia patients⁴ and IBS patients.⁵ For instance, in fibromyalgia patients, a prolonged response to intramuscular sensitization with PGE₂ in an acid solution is associated with more widespread brain activation in a fMRI study.⁶

As in the clinical disorders, central pain processing is altered in animal models of chronic pain. For instance, changes in the rostral ventral medulla resulting in descending pain facilitation have been described in the spinal nerve ligation model.⁷ Capsaicin-induced analgesia, which involves central modulation in response to pain, is perturbed in rats that were previously treated with oxaliplatin to induce neuropathic pain 15 days earlier or were exposed to an ethanol diet for 3 weeks to induce alcoholic neuropathy. Short-term exposures to pain, however, do not affect capsaicin-induced analgesia.⁸ It is interesting that in the oxaliplatin and alcohol-induced neuropathic pain models, the altered pain processing is dependent on the sympathoadrenal axis, providing evidence that sympathetic hyperactivity can contribute to chronic pain. Like chronic pain, a 4-day exposure of rats to unpredictable noise stress perturbs capsaicin-induced analgesia by a sympathoadrenal-

dependent mechanism.⁸ Thus, the chronic pain is acting as a chronic stressor.

We have previously investigated the role of sympathoneuronal outflow via postganglionic sympathetic nerves in pain exacerbation. In rodents without any nerve injury, we have shown that the sympathetic nervous system (SNS) can exacerbate pain sensation under conditions in which descending inhibition is reduced and sympathetic outflow is dysregulated.⁹ The reduced descending inhibition unveils a role of the SNS in decreasing peripheral sensory thresholds. This SNS effect is enhanced by sympathetic overflow that occurs when feedback autoinhibition of norepinephrine release from sympathetic postganglionic nerve terminals is altered. Such conditions, created by treatment with alpha₂-adrenergic antagonists or in alpha_{2A}-adrenergic receptor knockout mice, lead to stress-induced hyperalgesia instead of stress-induced analgesia following an acute stress. Interestingly, the hyperalgesia persists with repeated stress exposures.

The results raise the question whether SNS exacerbation of pain sensation when central pain inhibition is reduced is relevant to chronic pain disorders. Other than in nerve ligation models, in which there is sprouting of sympathetic postganglionic nerves,^{10,11} and models of complex regional pain syndrome (CRPS) that involve traumatic tissue injury,¹² there has been no demonstration of sympathetically driven pain in a chronic pain model. Using a rat colon irritation (CI) model of chronic visceral hypersensitivity with hallmarks of IBS including visceral and somatic hypersensitivity and altered stool function, we investigated whether sympathoneuronal outflow can drive the chronic pain state. In this model, rats that are treated to a series of colonic balloon distensions as neonates develop visceral hypersensitivity to the colonic balloon distension that persists into adulthood.^{13,14} This hypersensitivity is coupled with a change in descending modulation from being predominantly inhibitory to predominantly facilitatory.¹⁵

If SNS neural outflow drives the visceral hypersensitivity in this model, the pain should exhibit adrenergic pharmacology and be exacerbated by acute stress. We demonstrate that the alpha₂-adrenergic agonist, clonidine, and the alpha₁-adrenergic antagonist, prazosin, reduce the visceral hypersensitivity and that water avoidance stress (WAS) enhances the pain. We confirm the involvement of the SNS by using pharmacological and surgical approaches to ablate the peripheral sympathetic innervations and demonstrate a loss of the visceral hypersensitivity. The preliminary results of this study were reported earlier in abstract form.^{16,17}

METHODS

Animals

Experiments were done using adult male Sprague-Dawley rats weighing 250–300 g. The rats were obtained as preweaning neonates (younger than 6 days) from Harlan Sprague-Dawley Inc. (Indianapolis, IN, USA). They were housed in plastic cages containing corn chip bedding (Sani-Chips; PJ Murphy Forest Products, Montville, NJ, USA) and maintained on a 12 : 12 h light:dark cycle (lights on at 7 am). Neonatal CI was applied using colorectal distension (CRD) during postnatal development. Briefly, rats (8 days old) were divided into two groups for purposes of different treatments. Group 1 (CI rats) received CRD once daily on postnatal (PN) days 8, 10, and 12. The distention was applied using an angioplasty balloon (Advanced Polymers Inc., [Carlstadt, NJ, USA] length: 20.0 mm; diameter: 3 mm), inserted rectally into the descending colon in awake neonates. The balloon was distended with 0.3 mL of water, exerting a pressure of 60 mmHg (as measured with a sphygmomanometer) for 1 min and then deflated and withdrawn. Group 2 (which served as control) was handled in a way similar to Group 1 except that no colonic insertion was made. Rats in this group were gently held and touched on the perineal area on a schedule similar to that described for group 1. No treatment, procedure, or further intervention was done by the investigator until the rats were adult. The CI rats used in this study were hypersensitive to CRD and selected with no signs of altered fecal output (for additional details on animal preparation, see Wang *et al.*¹⁴). All studies were performed 2.5–3 months later on the neonatally treated rats in accordance with the proposals of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences in accordance with the guidelines provided by the National Institutes of Health, USA.

Water avoidance stress

Water avoidance stress was applied using a Plexiglas tank (25 × 25 × 45 cm) with a block (8 × 8 × 10 cm) affixed to the center of the floor (island). The tank was filled with fresh tap water (25 °C) to within 1 cm of the top of the island block. Rats were placed individually on the block for a period of 45 min prior to behavioral testing. This well-characterized test represents a potent psychological stressor with large elevations of adrenocorticotropic hormone and corticosterone.¹⁸

Sympathectomy

To examine the role of the SNS in the sensitized responses to visceral stimulation, adult rats were given either a chemical or a surgical sympathectomy (SSx). For the chemical sympathectomy, rats were injected with guanethidine (50 mg/kg; i.p.) or saline, 2 days before testing. The SSx was made under sterile conditions by removing the sympathetic paravertebral ganglia (L1–L6) bilaterally; the sympathetic chain was identified through a transperitoneal approach under isoflurane anesthesia. The surgical procedure consists of an incision made at the ventral midline surface of the body, between the diaphragm and the bladder. The intestines are carefully externalized and gently placed on sterile gauze soaked in sterile saline. The lumbar sympathetic paravertebral ganglia can be visualized after retract-

ing the superficial muscle layers and by careful separation of the psoas and the quadratus lumborum muscles from the vertebral column and the transverse processes. Sham surgery consists of a ventral midline incision and externalization of the intestines. Carprofen (5 mg/kg i.p.) is given once immediately after surgery, and the animals are allowed to recover for 2 weeks prior to further testing.

Pharmacological treatment

The following agonists or antagonists of alpha-adrenergic receptors were given to CI and control rats with or without a SSx: prazosin (alpha1 antagonist, 0.1–20 µg/kg, i.p.) and clonidine (alpha2 agonist, 0.01–0.5 mg/kg, i.p.). Electromyographic (EMG) responses to CRD were recorded before drug injection and starting at 30 min afterward. The effect of vehicle (saline) was similarly tested. Responses recorded after the injection were compared with those recorded before.

Assessing sensitivity to somatic and visceral stimuli

All behavioral tests were performed in a quiet room by a single examiner blinded to the treatment of the rats. The somatic stimulus consisted of a thermal sensitivity test using the UGO Basile Plantar apparatus. Animals were placed in Plexiglas cubicles on a clear glass plate maintained at 30 °C and allowed to acclimate for 10 min. The radiant heat source was placed under the glass plate directly beneath the hind paw and calibrated to a temperature of 48 °C at the source. The glass surface temperature was maintained at 30 ± 0.1 °C. Withdrawal latencies were defined as the time from the activation of the heat source until hind paw withdrawal with a 15-s cutoff to avoid tissue damage. The test was repeated five times for each paw with 15 min between testing the same paw. The average of the withdrawal latencies for all repetitions was calculated for each animal.

The visceral stimulus consisted of CRD produced by inflating a balloon, inserted through the anus, inside the descending colon and rectum. The balloon is 4 cm in length (made from the finger of a latex glove), attached to polyethylene tubing. Distension was produced by rapidly inflating the balloon to the desired pressure (20, 40, 60, or 80 mmHg) for duration of 20 s with a 10-min interval between distensions. Visceral sensitivity was assessed by recording the EMG responses to CRD in rats mildly sedated with isoflurane (0.5%). The EMG electrode (Teflon-coated silver wire) was inserted into the external oblique muscle superior to the inguinal ligament and connected to an amplifier. The signal was displayed on an oscilloscope and fed into a computer using CED 1401 plus and was recorded using Spike 6 software. The raw EMG signal was rectified offline for analysis; baseline activity was subtracted, and the area of the rectified response was divided by the duration of the CRD. All EMG responses are reported as area under the curve.

Statistical analysis

All responses are reported as averages ± SEM. Statistical analysis was done using SigmaStat software (Systat Software Inc., San Jose, CA, USA). In all studies involving repetitive visceral stimulation (CRD), a repeated-measures analysis of variance (RM ANOVA) was conducted to examine variation between groups. A one-way RM ANOVA was used when analysis involved a single factor such as the variation in the response to CRD within one group of rats. For example, an RM ANOVA was applied to each group data (CI and control) to measure the extent

to which changes before and after treatment (e.g. sympathectomy) in the CI group differed from those in the control group. A two-way RM ANOVA was used when the effects of more than one factor (two factors) were looked at (for instance, treatment with a drug or a surgical procedure). The RM ANOVA was followed by a statistical test to determine significant differences between treated groups. Where the data were normally distributed, a paired *t*-test was used to look at the effect of a given treatment (drug or surgery) at a single time point before and after the treatment in the same animal. However, when the data were not normally distributed, the Wilcoxon test was used for pairwise comparisons.

RESULTS

Alpha2-adrenergic agonist and alpha1-adrenergic antagonist reduce hypersensitivity in CI rats

Since SNS postganglionic outflow of norepinephrine is inhibited by presynaptic autoinhibitory alpha2-adrenergic receptors,¹⁹ we investigated whether the alpha2-adrenergic agonist clonidine inhibited the enhanced EMG response to CRD in the hypersensitive (CI) rats. Clonidine inhibited in a dose-dependent manner the responses to CRD in CI rats ($n = 8$) with a significant effect starting at 50 $\mu\text{g}/\text{kg}$. At this dose, the response to CRD (80 mmHg) was reduced from 6.0 ± 0.5 recorded following treatment with vehicle (saline) to 4.2 ± 0.4 after clonidine (Fig. 1A), which is similar to the response that is measured in non-CI control rats. The 50- $\mu\text{g}/\text{kg}$ dose of clonidine had no significant effect on CRD in the non-CI control rats (data not shown). Norepinephrine released from sympathetic nerves often activates postsynaptic alpha1-adrenergic receptors. To further verify the role of the SNS in visceral hypersensitivity, the effect of an alpha1-adrenergic receptor antagonist (prazosin, P) was tested on the responses to visceral stimulation in CI rats ($n = 8$). Prazosin inhibited in a dose-dependent manner the EMG responses to CRD recorded in CI rats with a significant effect obtained with a dose of 20 $\mu\text{g}/\text{kg}$ (Fig. 1B). At this dose, the average response to CRD (80 mmHg) dropped from 6.1 ± 0.9 before treatment with prazosin to 3.0 ± 0.9 , a value equivalent to those recorded in non-CI control

rats. Prazosin at this dose had no effect on the non-CI control rats (data not shown).

Stress-induced hyperalgesia in response to WAS

Acute stressors can increase SNS norepinephrine outflow, so the effect of WAS on behavioral responses to visceral (CRD) or somatic (radiant heat) stimulation was recorded in CI and non-CI control rats. In each group, WAS was given for 45 min. The CRD responses were significantly facilitated after WAS at all intensities of CRD in control (Fig. 2A, $n = 8$) and CI (Fig. 2B, $n = 8$) rats. For example, in control rats, the average response to CRD (80 mmHg) augmented from 2.74 ± 1.0 before WAS to 3.48 ± 1.0 after WAS. In CI rats, the average response to CRD (80 mmHg) augmented from 4.72 ± 1.5 before WAS to 6.4 ± 1.4 after WAS. WAS had no significant effect on the average paw withdrawal latency (PWL) to radiant heat in control rats (Fig. 2C, $n = 8$). However, in CI rats, WAS caused a significant decrease in PWL (Fig. 2C, $n = 8$), indicating an increased sensitivity to radiant heat. For example, the average PWL of the hind paws was 5.48 ± 0.3 s before WAS and 4.27 ± 0.2 s after WAS.

Guanethidine sympathectomy reduces hypersensitivity in CI rats and blocks the pronociceptive effect of WAS

In order to test the role of SNS postganglionic norepinephrine outflow in the hypersensitivity seen in CI rats (both basal and stress induced), the rats were treated with guanethidine. Guanethidine reduces the release of norepinephrine, by displacing it from the presynaptic release vesicles, resulting in a chemical sympathectomy. Guanethidine (G; 50 mg/kg, i.p., 48 h prior to testing) significantly reduced the average EMG responses to CRD in CI rats ($n = 8$) but had no significant effect on the average responses similarly

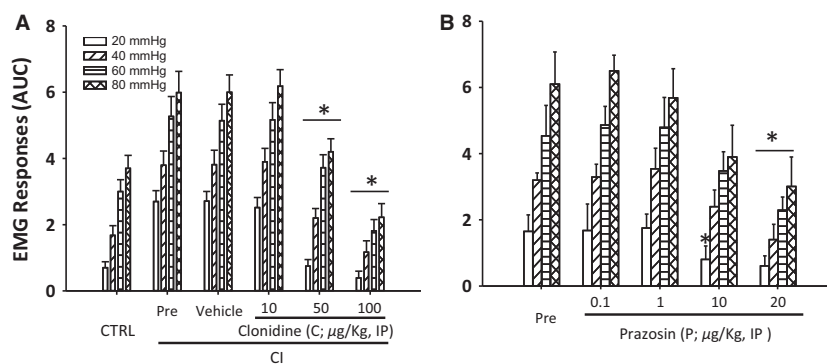


Figure 1 (A) Bar graphs (\pm SEM) illustrate the EMG responses to graded colorectal distension (CRD; 20, 40, 60 or 80 mmHg) in control rats (CTRL) or in CI rats before (Pre) and after treatment with vehicle (Vehicle) or clonidine (C; 10, 50, 100 $\mu\text{g}/\text{kg}$, IP). * $p < 0.05$ when comparing the drug effect with vehicle for a given CRD. (B) Bar graphs (\pm SEM) illustrate the EMG responses to CRD in CI rats before (Pre) and after treatment with Prazosin (P; 0.1, 1, 10, 20 $\mu\text{g}/\text{kg}$, IP). * $p < 0.05$ when comparing the drug effect with Pre.

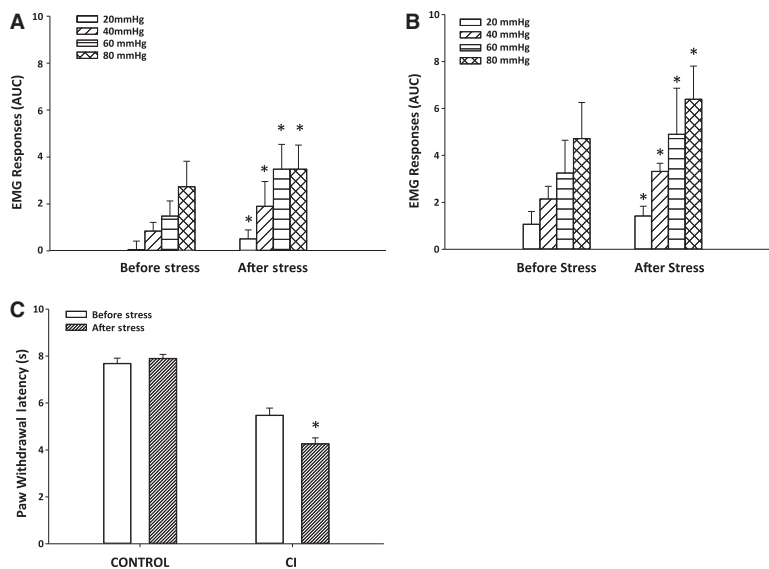


Figure 2 Bar graphs (\pm SEM) illustrate the effect of water avoidance stress (Stress) on the EMG responses to graded colorectal distension (CRD; 20, 40, 60 or 80 mmHg) in control (A) or CI (B) rats. (C) It illustrates the effect of WAS on the paw withdrawal latency to radiant heat in control and CI rats. * $p < 0.05$ by comparison to Before Stress.

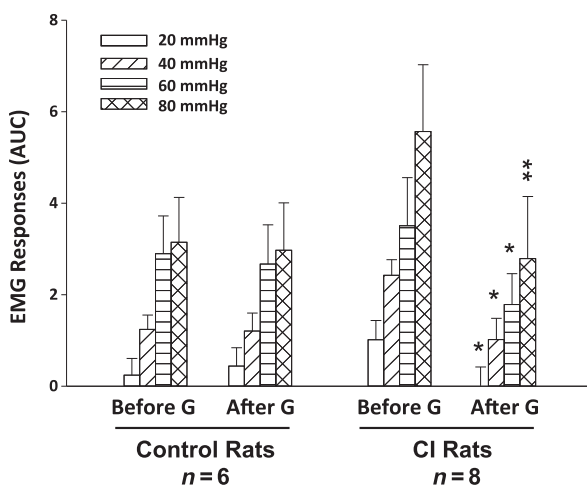


Figure 3 Bar graphs (\pm SEM) illustrate the EMG responses to graded colorectal distension (CRD; 20, 40, 60, or 80 mmHg) in control rats ($n = 6$) or in CI rats ($n = 8$) before (Before G) and after treatment with guanethidine (After G). * $p < 0.05$ compared with Before G; ** $p < 0.01$ compared with Before G.

recorded in non-CI control rats ($n = 6$; Fig. 3). For example, the average EMG response to CRD (80 mmHg) in CI rats was 5.57 ± 1.5 before treatment with guanethidine and was reduced to 2.79 ± 1.4 afterward. On the other hand, the response to the same stimulus in control rats was 3.15 ± 0.9 before guanethidine and 2.97 ± 1.0 afterward.

Furthermore, guanethidine sympathectomy seems to block the exacerbation of visceral hypersensitivity by WAS. Guanethidine pretreatment prevented the enhanced EMG responses to CRD (40, 60, and 80 mmHg) in CI rats ($n = 8$), similar to the results in

Fig. 3 for a different group of CI rats, and subsequent exposure of guanethidine-treated rats to WAS had no significant effect on their responses to visceral stimulation (Fig. 4A). Similarly, guanethidine seems to lessen the somatic hypersensitivity observed in CI rats in response to radiant heat (Fig. 4B). The resulting PWL in CI rats ($n = 8$) is not significantly different from preguanethidine PWL; however, it is not significantly different from the PWL seen in non-CI control rats either (Fig. 2C), indicating a partial reduction of the somatic sensitivity. For example, the average PWL in CI rats after guanethidine was 6.98 ± 0.34 s compared with 7.3 ± 0.25 s in control rats. There was also no significant enhancement of the somatic sensitivity following WAS in the guanethidine-treated CI rats (Fig. 4B).

Surgical sympathectomy reduces hypersensitivity in CI rats

Guanethidine sympathectomy could potentially have additional actions, so we further investigated the effect of SSx on visceral hypersensitivity. Surgical sympathectomy also reduced significantly the average EMG responses to CRD in CI rats ($n = 10$) but not in non-CI control rats ($n = 10$). The average responses to CRD (80 mmHg) recorded in CI rats dropped from 5.1 ± 0.6 before the SSx to 3.1 ± 0.4 recorded 3.5 months afterward (Fig. 5B), a value comparable to that recorded in control rats 4 months after the SSx (2.5 ± 0.6 ; Fig. 5A). Sham SSx performed in CI rats ($n = 10$) or in control rats ($n = 10$) had no effect on the EMG responses to CRD (data not shown).

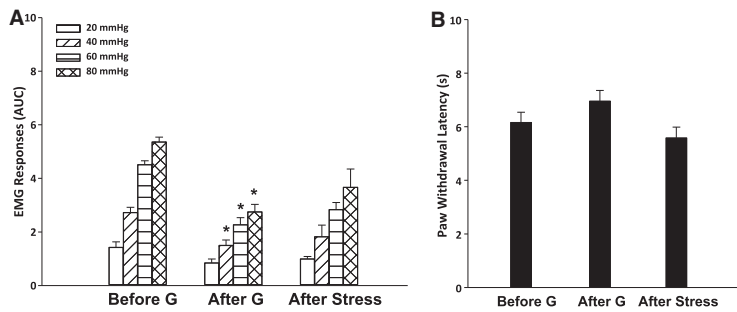


Figure 4 Bar graphs (\pm SEM) illustrate the effect of treatment with guanethidine (G) on the EMG responses of CI rats to graded colorectal distension (A) or on the paw withdrawal latency (B) before and after water avoidance stress (Stress). * $p < 0.05$ by comparison to *Before G*. Stress had no significant effect on visceral or somatic responses recorded following treatment with G.

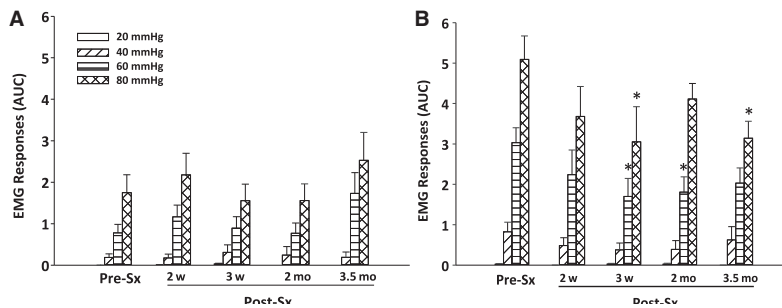


Figure 5 Bar graphs (\pm SEM) illustrate the EMG responses to graded colorectal distension (CRD; 20, 40, 60, or 80 mmHg) in control rats (A) or in CI rats (B) before (*Pre-Sx*) and after surgical sympathectomy (*Post-Sx*). The *Post-Sx* responses were recorded at 2 weeks (2w), 3 weeks (3w), 2 months (2 mo), and 3.5 months (3.5 mo) following surgery. * $p < 0.05$ compared with *Pre-Sx*.

To test whether the reduction of visceral hypersensitivity by the α_2 -adrenergic agonist clonidine (Fig. 1A) was via presynaptic α_2 receptors located on sympathetic nerve endings, the same doses of clonidine were tested in CI rats with SSx. At 50 and 100 $\mu\text{g}/\text{kg}$, clonidine had no significant effect on the responses to CRD (Fig. 6).

DISCUSSION

Clinical studies have provided evidence that generalized pain disorders such as IBS and fibromyalgia are characterized by changes in central pain processing and SNS activity. In an experimental acute pain model,⁹ pharmacological and genetic manipulations that decreased spinal descending inhibition and caused sympathetic overflow resulted in lowered pain thresholds and stress-induced hyperalgesia that was mediated by SNS neuronal outflow. We have now demonstrated SNS-driven hyperalgesia in a chronic pain model. The chronic CI model was selected because it is induced without direct nerve injury and the visceral hypersensitivity persists for many months after it is induced.^{13,14} The rats have altered water content in their fecal output, a marker consistent with symptoms of constipation or diarrhea seen in patients with IBS. They also show significantly shorter withdrawal latencies in response to heat stimulation of the hind paws, an indication of referred somatic hypersensitivity.

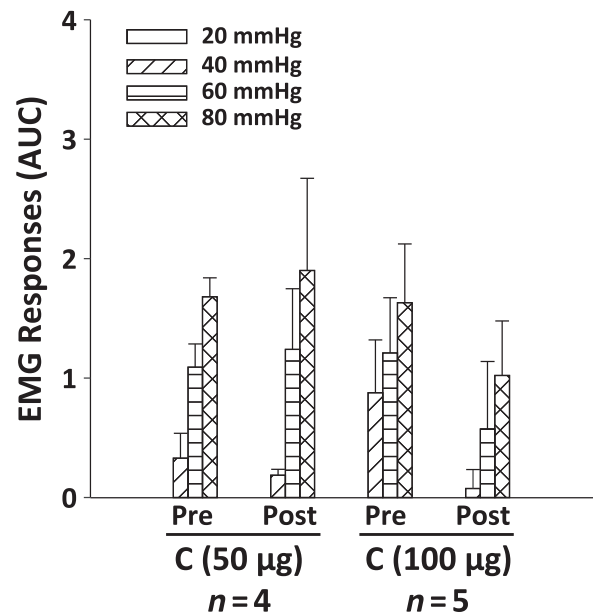


Figure 6 Bar graphs (\pm SEM) illustrate the EMG responses to graded colorectal distension (CRD; 20, 40, 60, or 80 mmHg) in CI rats with a surgical sympathectomy treated with clonidine (C; 50 or 100 $\mu\text{g}/\text{kg}$, IP). The responses are shown for each dose before (*Pre*) and 30 min after (*Post*) clonidine injection. No significant effect of clonidine was seen on the responses to CRD in these rats.

These behavioral and metabolic changes correlate with broad plastic changes in the peripheral and central nervous systems. In CI rats, descending control onto spinal nociceptive circuits switches from being

predominantly inhibitory (as the case is in control animals) to predominantly facilitatory. Thalamic stimulation in control rats causes inhibition of the response to CRD in a majority of spinal cord neurons, whereas the same stimulation, when given in CI rats, causes facilitation of the responses to CRD in most viscerosensitive neurons.¹⁵ The visceral hypersensitivity seen in CI rats is also associated with microglial activation. Fractalkine (a chemokine involved in neuron-to-microglia signaling) facilitates EMG responses to noxious CRD and induces thermal hyperalgesia in control rats.²¹ On the other hand, inhibition of microglial activation by minocycline reduces the visceral hypersensitivity and the neural responsiveness to visceral stimulation^{21–23} indicating a significant neuroimmune factor in these outcomes.

We used pharmacological studies and both chemical and SSx to demonstrate that the chronic hypersensitivity is dependent on postganglionic SNS neuronal outflow. Alpha-adrenergic receptors play an important modulatory role in the activity of the SNS. Postsynaptic alpha1-adrenergic receptors typically mediate the excitatory actions of the sympathetic neurotransmitter norepinephrine, and presynaptic alpha2-adrenergic receptors are inhibitory autoreceptors that mediate feedback inhibition of norepinephrine release. If SNS norepinephrine outflow is a peripheral driver of generalized pain, agonists of the presynaptic alpha2-adrenergic receptor would be expected to decrease pain. In the chronic CI rat, clonidine at a dose of 50 µg/kg reduced the visceral hyperalgesia back to control levels (Fig. 1A). At this dose, clonidine has no effect on CRD in non-CI control rats and is non-sedating, suggesting primarily a peripheral site of action. The absence of clonidine activity in the sympathectomized rats (Fig. 6) is evidence that the site of action is on the sympathetic nerve endings. Alpha1-adrenergic antagonists would also be expected to reduce the visceral hyperalgesia if the SNS mediates the pain, and this was the case in the chronic CI rat. Prazosin reduced the hypersensitivity back to control levels (Fig. 1B). Thus, the pharmacology is consistent with the SNS driving the chronic visceral hypersensitivity in the CI rats.

Pain exacerbation by psychological stress is a hallmark of IBS, fibromyalgia, and other generalized pain syndromes.^{24,25} Induction of thermal hyperalgesia by an acute psychological stressor (noise stress) was an indicator of SNS overflow enhancing pain in our acute pain experimental model.⁹ Similarly, the CI rats exhibited increased sensitivity to radiant heat applied to their paws following WAS. The baseline PWLs of the CI rats were already lower than baseline values in control rats. The PWLs were further reduced by WAS

so that they were approximately half of the PWL values in control, non-stressed rats (Fig. 2C). The control rats did not exhibit lower PWLs following WAS. Interestingly, WAS enhanced visceral response to CRD in non-CI control rats (Fig. 2A). Stress-induced visceral hyperalgesia has been well-characterized (see 26,27) with the exception of Laruche *et al.* who reported stress-induced analgesia in response to CRD at 40 mmHg only, a near threshold intensity for nociceptive visceral stimulation.²⁸ Water avoidance stress further enhanced visceral response to CRD in CI rats (Fig. 2B) so that there was a 2–3× increase in EMG intensity to CRD in stressed CI rats compared with unstressed control rats. Acute WAS but not chronic WAS is reported to induce visceral hyperalgesia in female Wistar rats, although no additional effect was noticed in rats with inflammation-induced visceral hyperalgesia.²⁹

The sympathectomy experiments confirmed that the chronic hypersensitivity and the exacerbation by WAS were dependent on postganglionic SNS outflow. Guanethidine sympathectomy, which depletes norepinephrine from the postganglionic SNS nerve endings, completely eliminated the visceral hypersensitivity to CRD (Fig. 3) and at least some of the somatic hypersensitivity to radiant heat in CI rats (Fig. 4). The WAS exacerbation of visceral and somatic hypersensitivity in CI rats was also inhibited (Fig. 4), demonstrating the involvement of the SNS in the chronic hypersensitivity. Since guanethidine could have other non-selective actions, the effect of SSx on visceral response to CRD was tested. The chronic hypersensitivity was significantly inhibited for at least 3.5 months, the duration of our postsurgical testing.

Several lines of evidence suggest that the findings in the CI rats are relevant to generalized pain syndromes. Clonidine treatment resulted in increased pain thresholds to colonic balloon distension in human experimental pain studies on IBS patients.³⁰ As mentioned above, the pain in IBS and fibromyalgia can also be exacerbated by psychological stress.^{24,25} Several studies have demonstrated dysregulation of the hypothalamic-pituitary-adrenal axis.^{31,32} There is also evidence in generalized pain syndromes of autonomic nervous system dysfunction. Some clinical studies of heart rate variability, a measure of the balance of sympathetic and parasympathetic regulation of the sinus node, have demonstrated that patients with fibromyalgia³³ and IBS³⁴ have excess sympathetic activity, although these findings are not always consistent.

Clinical investigations on patients with IBS and fibromyalgia have also demonstrated the role of peripheral stimuli in maintaining these chronic pain

syndromes. Rectal administration or muscle tender point injections, respectively, of lidocaine gel significantly reduced secondary heat hyperalgesia in these patients.^{35,36} Based on the results reported here, post-ganglionic SNS neuronal outflow could be the source of this peripheral drive. In fact, the authors of the lidocaine studies suggested that IBS and fibromyalgia may share a peripheral mechanism with CRPS, as local anesthetic injection can also alleviate pain and allodynia at widespread loci in CRPS patients.³⁷

Pain is a chronic stressor, and chronic stress studies suggest several potential mechanisms for the increase in SNS activity in chronic pain including decreased alpha2-adrenergic receptor expression and changes in adrenergic receptor signaling. Chronic stress results in decreased expression of alpha2-adrenergic receptors in various brainstem regions of the tree shrew including the locus coeruleus (LC), which is associated with upstream regulation of SNS outflow.³⁸ Decreased alpha2-adrenergic receptor expression can in turn lead to reduced feedback inhibition of LC activity. In the rat, chronic cold stress leads to increased firing of LC neurons, and this is associated with reduced inhibition of LC neuron activity by the alpha2-adrenergic receptor agonist clonidine.³⁹ The 4-day unpredictable sound stress paradigm, which alters central pain processing, also results in altered peripheral nociceptor beta-adrenergic receptor signaling, due to corticosterone

and epinephrine released from the adrenal medulla. A 10-fold lower concentration of the beta-adrenergic agonist epinephrine decreased pain thresholds in the stressed rats compared with control rats.⁴⁰ A similar effect could be anticipated for norepinephrine released from SNS nerves.

The results support a role of the SNS in driving the chronic visceral and somatic hypersensitivity seen in CI rats. Follow-up studies in a chronic somatic hypersensitivity model are needed. The findings further suggest that sympatholytic treatments that decrease sympathetic outflow or block activation of adrenergic receptors on sensory nerves could be beneficial in the treatment of generalized pain syndromes.

FUNDING

This study was funded by a grant from Allergan.

DISCLOSURE

DWG, JED and SC are employees of Allergan.

AUTHOR CONTRIBUTION

DWG, JED, and ACE designed the research study; JW, CG, and SC performed the research; ACE and JW analyzed the data; and DWG and ACE wrote the article.

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