■ PAIN AND REGIONAL ANESTHESIA

Peripheral Nerve Injury Sensitizes the Response to Visceral Distension but Not Its Inhibition by the Antidepressant Milnacipran

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Background: Manipulations that cause hypersensitivity to visceral stimuli have been shown to also result in hypersensitivity to somatic stimuli coming from convergent dermatomes, but the converse has not been examined. The authors tested whether lumbar spinal nerve ligation in rats, a common model of neuropathic pain that results in hypersensitivity to somatic stimuli, also leads to hypersensitivity to visceral stimuli coming from convergent dermatomes and whether pharmacology of inhibition differed between these two sensory modalities.

Methods: Female Sprague-Dawley rats were anesthetized, and the left L5 and L6 spinal nerves were ligated. Animals received either intrathecal saline or milnacipran (0.1–3 µg), and withdrawal thresholds to mechanical testing in the left hind paw, using von Frey filaments, and visceral testing, using balloon colorectal distension, were determined.

Results: Nerve ligation resulted in decreases in threshold to withdrawal to somatic mechanical stimulation (from 13 ± 1.8 g to 2.7 ± 0.7 g) and also in decreases in threshold to reflex response to visceral stimulation (from 60 mmHg to 40 mmHg). Intrathecal milnacipran increased withdrawal threshold to somatic stimulation in a dose-dependent manner but failed to alter the response to noxious visceral stimulation.

Conclusions: Injury of nerves innervating somatic structures enhances nociception from stimulation of viscera with convergent input from nearby dermatomes, suggesting that somatic neuropathic pain could be accompanied by an increased likelihood of visceral pain. Lack of efficacy of the antidepressant milnacipran against visceral stimuli suggests that visceral hypersensitivity may not share the same pharmacology of inhibition as somatic hypersensitivity after nerve injury.

HYPERSENSITIVITY, reflected in increased pain to a normally noxious stimulus (hyperalgesia) and pain to a normally innocuous stimulus (allodynia), frequently ac-

companies spontaneous pain in patients with injury to the peripheral nervous system, whether the injury stems from a traumatic, a metabolic, or a chemotherapeutic cause. Because such neuropathic pain is often chronic, difficult to treat, and leads to prolonged suffering, considerable research efforts are expended to examine its underlying causes and potential new treatments.

Chronic visceral pain has attracted less basic research interest than neuropathic pain. Chronic inflammation of the gut, uterus, bladder, or ureters results in hypersensitivity of these organs to distension.[1–5] In addition, there is also hypersensitivity to distension of nearby, uninflamed visceral organs that share an overlapping dermatomal innervation in the spinal cord to the experimentally inflamed viscus. This cross-reactivity is thought to reflect sensitization of the spinal cord neurons, which receive convergent inputs from several visceral structures.[6] In some cases, there is also evidence for cross-reactivity to somatic structures with convergent input to the same dermatomes as the injured visceral organ, as evidenced by interstitial extravasation or hypersensitivity to mechanical or thermal somatic stimulation.[3,7]

The current study examines the converse of these observations. We tested the hypothesis that injury to a peripheral nerve that results in hypersensitivity to somatic stimulation also induces hypersensitivity to stimulation of viscera with innervation that converges on dermatomes in the spinal cord near the injured nerve. We used two commonly employed methods: tight ligation of the left L5 and L6 spinal nerves in the rat, which results in a long lasting reduction in withdrawal threshold to light touch in the paw at the L4,[8] and balloon distension of the descending colon and rectum, which stimulates afferents entering the spinal cord at the L6–S2 dermatomes.[9] Thus, the somatic stimulation was one dermatome cephalad to the injured nerves, and the visceral stimulation was with two dermatomes caudad to the injured nerves.

In normal animals and humans, noxious somatic or visceral input increases the release of norepinephrine and serotonin in the spinal cord,[10,11] reflecting activation of descending inhibition in response to acute nociception. One might predict, therefore, that intrathecal injection of monoamine reuptake inhibitors would produce antinociception to acute nociception, but this has not been uniformly observed.[12–14] In contrast, these agents are usually active in settings of chronic hypersen-

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sitivity to somatic stimuli.[15-18] A secondary purpose was to test whether intrathecal injection of the monoamine reuptake inhibitor, milnacipran, would inhibit responses to both somatic and visceral stimulation after spinal nerve injury. *Milnacipran* is a nontricyclic antidepressant, dual reuptake inhibitor that preferentially blocks the reuptake of norepinephrine over that of serotonin by a ratio of approximately 3:1; this compound is also pharmacologically characterized by weak *N*-methyl-D-aspartate receptor antagonist activity.[19] Therefore, the overall pharmacologic profile is similar to that of amitriptyline, a commonly used tricyclic antidepressant. However, unlike amitriptyline, milnacipran exhibits a minimal affinity to postsynaptic adrenergic, muscarinic, and histamine receptors, and therefore produces fewer side effects compared with amitriptyline and other tricyclic antidepressants.[20] In addition, in the rat formalin test, a model of persistent pain, milnacipran shows a moderate antinociceptive effect.[21]

Materials and Methods

After approval from the Animal Care and Use Committee (Wake Forest University School of Medicine, Winston-Salem, North Carolina), virgin female Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 237–302 g, aged 14–18 weeks, were studied. Rats were housed at 22°C with a 12 h light–dark cycle and with free access to food and water. The stage of estrus cycle was not determined at any time throughout the experiment.

Spinal Nerve Ligation

Rats were anesthetized with 1–3% halothane in oxygen, and spinal nerve ligation was performed as previously described.[8] Briefly, a midline incision was made in the lower back, the paraspinous muscles were incised and retracted, and the L5 and L6 spinal nerves were visualized *via* small laminotomies. The spinal nerves were tightly ligated with 6-0 sutures, and the wound was covered in layers. Animals were monitored closely for normal recovery from anesthesia and thereafter for any evidence of autotomy (which was not observed in any animal).

Intrathecal Catheter Insertion

One week after spinal nerve ligation, rats were reanesthetized with 1–3% halothane in oxygen, and an intrathecal catheter was inserted as previously described.[22] Briefly, the atlanto-occipital membrane was exposed, and a 30-gauge polyethylene catheter was advanced intrathecally in a caudal direction 7.5 cm, such that its tip lay in the lower thoracic/upper lumbar region. Only rats without motor deficits were studied, and a minimum of 5 days passed after intrathecal catheter insertion before experimental study. The catheter tip location was verified at the end of each experiment by complete blockade of the response to colorectal distension from injection of 10 µl lidocaine, 2%, through the catheter.

Nociceptive Testing

To examine the response to acute somatic mechanical stimulation, animals were placed in a clear box with an elevated nylon mesh floor and allowed to acclimate to the environment. Then, a calibrated von Frey filament was pressed to the point of bending on the plantar surface of the left or right paw. The withdrawal threshold was determined using an up–down method,[23] with testing performed at 5-min intervals. To examine the response to acute visceral mechanical stimulation, animals were anesthetized with 1–2% halothane and a balloon inserted in the descending colon and rectum through the anus. The balloon was fixed to rigid plastic tubing and was inflated manually over 1–2 s to a fixed distension force, monitored using a force transducer. Electrodes were inserted in the rectus abdominus muscle for recording of the visceromotor reflex elicited by colorectal distension. The threshold sensitivity for electromyographic recording was set to each experiment such that there was no recorded activity in the absence of stimulation. Colorectal distension forces of 20, 40, 60, 80, and 100 mmHg were applied for 20 s, with a 5-min interstimulus interval. To examine the effect of intrathecal drugs, a fixed stimulus of 75 mmHg was applied at 5-min intervals before and after drug injection. Previous studies have shown that this stimulus paradigm results in stable responses, without evidence for sensitization.[9] The integrated electromyographic response over the 20 s of stimulation was recorded for each stimulus.

Experimental Groups

A total of 23 animals were studied. To determine whether injury of a mixed nerve (spinal nerve ligation) that results in hypersensitivity to somatic stimuli in adjacent dermatomes also alters response to visceral stimuli in adjacent dermatomes, a stimulus response from 20 to 100 mmHg distension force was determined in nine normal animals and seven animals with spinal nerve ligation.

To determine the effect of intrathecal milnacipran on somatic stimulation after nerve injury, seven animals with spinal nerve ligation were studied on four occasions, with experiments separated by a minimum of 4 days. Withdrawal threshold was determined, the animals then received a single intrathecal injection of saline or 0.1, 0.3, or 1 µg milnacipran, and withdrawal thresholds were determined at 30, 60, 80, 120, 150, 210, and 300 min after injection.

To determine the effect of intrathecal milnacipran on visceral stimulation after nerve injury, nine normal animals and seven animals with spinal nerve ligation received 1.0 µg intrathecal milnacipran. General behavior,

including grooming and ambulation, was observed in these animals after intrathecal injection. Electromyographic response was determined to colorectal distension, 75 mmHg, applied at 5-min intervals before and for 60 min after intrathecal injection.

Drugs

Halothane was from Halocarbon Laboratories (River Edge, NJ), and lidocaine hydrochloride was from Abbott laboratories (North Chicago, IL). Milnacipran (a gift from Cypress Pharmaceuticals, San Diego, CA) was diluted in normal saline. All intrathecal injections were administered in a 10-μl volume followed by a 10-μl flush with normal saline.

Statistics

Data were normally distributed and are presented as mean ± SEM. Single observations between groups were compared by means of the Student t test. Electromyographic responses and withdrawal thresholds were tested by means of repeated-measures one-way analysis of variance within groups and two-way analysis of variance between groups, followed by the Dunnett test compared with predrug injection control. $P < 0.05$ was considered significant.

Results

Animals recovered uneventfully from intrathecal catheter insertion. All animals with spinal nerve ligation exhibited a reduced withdrawal threshold, from 12 ± 1.8 g before surgery to less than 4 g after surgery. Normal animals weighed slightly more at the time of testing (280 ± 9.3 g) compared with those with spinal nerve ligation (260 ± 4.7 g; $P < 0.05$).

Effect of Nerve Injury on Response to Visceral Nociception

There was no difference in the electronic filtering threshold required to subtract baseline electromyographic activity in animals with or without spinal nerve ligation. The concentration of inspired halothane during testing for colorectal distension did not differ between normal animals (0.8 ± 0.07%) and those with spinal nerve ligation (0.7 ± 0.04%). Colorectal distension produced a stimulus-dependent increase in electromyographic activity in the abdominal musculature in both groups. However, the stimulus response was shifted to the left in spinal nerve–ligated animals, which exhibited a lower threshold (40 mmHg distension force) compared with normal animals (60 mmHg distension force; fig. 1).

Effect of Intrathecal Milnacipran on Somatic Stimulation

Intrathecal milnacipran produced a dose-dependent increase in withdrawal threshold to mechanical stimula-
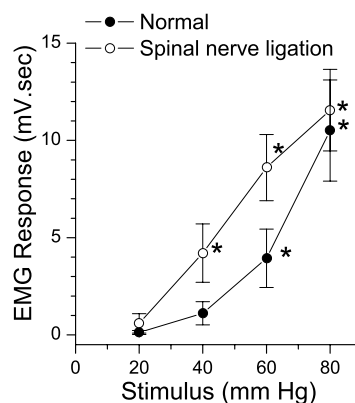

Fig. 1. Electromyographic (EMG) response to colorectal distension in unoperated rats (●) or those after spinal nerve ligation (○). Each symbol represents the mean ± SEM of seven to nine animals. * $P < 0.05$ by one-way analysis of variance followed by Dunnett test compared with no stimulus control.

tion of the paw, with no effect from 0.1 μg, a modest effect from 0.3 μg, and complete return to presurgical withdrawal threshold from 1.0 μg (fig. 2). Intrathecal milnacipran had no effect on grooming behavior or on ambulation.

Effect of Intrathecal Milnacipran on Visceral Stimulation

Neither intrathecal saline nor intrathecal milnacipran altered the response to a 75-mmHg colorectal distension stimulus in normal animals (fig. 3). In nerve-ligated animals, there was a tendency for intrathecal milnacipran to actually increase the visceromotor response to colorectal distension, although this was not significant ($P > 0.2$; fig. 3).

Discussion

Chronic visceral and somatic pain often coexist. For example, there is a high incidence of comorbidity of
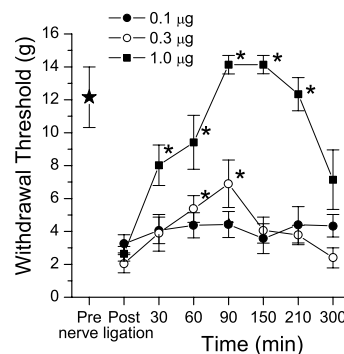

Fig. 2. Withdrawal threshold to mechanical stimulation with von Frey filaments to the left hind paw in animals receiving intrathecal injection of 0.1 μg (●), 0.3 μg (○), or 1.0 μg (■) milnacipran at time 0. Withdrawal threshold before spinal nerve ligation is shown as a *star*. Each symbol represents the mean ± SEM of seven animals. *$P < 0.05$ compared with post–nerve ligation baseline.
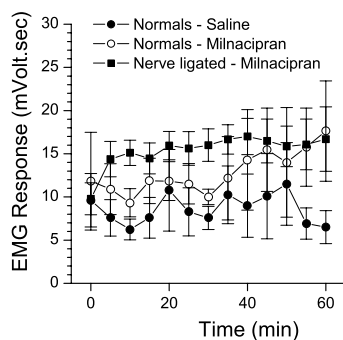
Fig. 3. Electromyographic (EMG) response to colorectal distension at a fixed stimulus of 75 g after intrathecal injection, at time 0 of saline (●) or 1.0 μg milnacipran (○) in normal animals and of 1.0 μg milnacipran (■) in animals with spinal nerve ligation. Each symbol represents the mean ± SEM of four to seven animals. There were no significant differences among groups.

irritable bowel syndrome with fibromyalgia and temporomandibular joint disorder.[24] Previous research has focused on whether chronic visceral pain leads to hypersensitivity to stimulation of somatic structures, and this has been demonstrated in animals and humans.[7,25] Therefore, it may be that the coexistence of chronic visceral and somatic pain reflects cross-sensitization to somatic structures from chronic visceral nociceptive input. The current study suggests that the converse may also be true: peripheral nerve injury not only sensitizes the response to somatic structures in nearby areas, but also to visceral input from organs with innervation to the same dermatomes of the spinal cord.

Although there is extensive innervation of the gut by peptidergic C fibers, the majority of these carry information probably related to the local environment, and this information does not reach consciousness. Inflammation results in sensitization of peripheral afferents, such that the threshold for nerve firing from distension decreases,[26] peptides are released from C fibers,[27] and sensitization of spinal dorsal horn neurons occurs.[1] Patients with irritable bowel syndrome also exhibit a reduced pain threshold to controlled distension of the rectum,[25] suggesting similar peripheral or central processes may occur in humans in the absence of acute inflammation. Although we did not assess the possibility of inflammation of the colon or rectum in the current study, there is no reason to suspect that injury to the spinal nerves should produce such inflammation.

We speculate that the reduced threshold to colorectal distension–evoked contraction of the abdominal muscles after spinal nerve ligation reflects sensitization of dorsal horn neurons receiving convergent input from somatic and visceral structures. Although others have shown that this viscerosomatic inhibition is reduced in rats with spinal nerve ligation,[28] their study did not test whether spinal nerve ligation altered the response to colorectal distension itself. Because we did not include a sham surgery control with nerve exposure but without

overt nerve injury, we cannot entirely distinguish the sensitization that might have occurred from the somatic injury of the surgery itself rather than the nerve injury. However, although sham surgery does produce hypersensitivity, this is not uniformly observed and does not occur to the degree that is seen after nerve injury in this model.

Intrathecal injection of the monoamine reuptake inhibitor, milnacipran, increased withdrawal threshold to somatic stimulation in this animal model of neuropathic pain, as anticipated from previous studies with other agents of this class.[29,30] Lack of efficacy of intrathecal milnacipran against colorectal distension could reflect pharmacokinetic factors, such as less penetration deep in the spinal cord to the termination of visceral afferents, pharmacodynamic factors, such as less spontaneous release of norepinephrine in spinal circuits of visceral nociception, or stimulus factors, such as the different modality tested or that of a threshold response with von Frey filaments *versus* that of a suprathreshold stimulus with colorectal distension. Although we only studied the effect of milnacipran for 60 min after colorectal distension, this time was adequate to demonstrate an effect to somatic stimulation. As noted in figure 3, the probe stimulus used for colorectal distension to test milnacipran resulted in a near maximum response, a level at which there was minimal difference between normal and nerve-injured animals. This intense stimulus level may have reduced our ability to observe an antinociceptive effect to visceral stimulation, in contrast with somatic stimulation, where a very low intensity (threshold) stimulation was studied. Although definitive statements regarding reasons for this discrepancy cannot be stated from the current experiments, these results raise the possibility that hypersensitivity to somatic stimuli after nerve injury may be inhibited by different drugs than hypersensitivity to visceral stimuli.

In summary, ligation of the L5 and L6 spinal nerves reduces the threshold to withdrawal to mechanical stimulation of the ipsilateral paw in the L4 dermatome and also reduces the threshold to response to mechanical stimulation of the colon in lower lumbar and sacral dermatomes. This hypersensitivity across stimulus modalities may underlie the coexistence of chronic visceral and somatic pain in some individuals. Intrathecal injection of 1.0 μg milnacipran removed the hypersensitivity to somatic stimulation but had no effect on hypersensitivity to visceral stimulation, reinforcing the emerging literature indicating that the physiology and pharmacology of visceral afferents differ significantly from those of somatic afferents.

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