Persistent Catechol-*O*-methyltransferase–dependent Pain Is Initiated by Peripheral β-Adrenergic Receptors

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ABSTRACT

Background: Patients with chronic pain disorders exhibit increased levels of catecholamines alongside diminished activity of catechol-*O*-methyltransferase (COMT), an enzyme that metabolizes catecholamines. The authors found that acute pharmacologic inhibition of COMT in rodents produces hypersensitivity to mechanical and thermal stimuli *via* β-adrenergic receptor (βAR) activation. The contribution of distinct βAR populations to the development of persistent pain linked to abnormalities in catecholamine signaling requires further investigation.

Methods: Here, the authors sought to determine the contribution of peripheral, spinal, and supraspinal βARs to persistent COMT-dependent pain. They implanted osmotic pumps to deliver the COMT inhibitor OR486 (Tocris, USA) for 2 weeks. Behavioral responses to mechanical and thermal stimuli were evaluated before and every other day after pump implantation. The site of action was evaluated in adrenalectomized rats receiving sustained OR486 or in intact rats receiving sustained βAR antagonists peripherally, spinally, or supraspinally alongside OR486.

Results: The authors found that male (N = 6) and female (N = 6) rats receiving sustained OR486 exhibited decreased paw withdrawal thresholds (control 5.74 ± 0.24 *vs.* OR486 1.54 ± 0.08, mean ± SEM) and increased paw withdrawal frequency to mechanical stimuli (control 4.80 ± 0.22 *vs.* OR486 8.10 ± 0.13) and decreased paw withdrawal latency to thermal heat (control 9.69 ± 0.23 *vs.* OR486 5.91 ± 0.11). In contrast, adrenalectomized rats (N = 12) failed to develop OR486-induced hypersensitivity. Furthermore, peripheral (N = 9), but not spinal (N = 4) or supraspinal (N = 4), administration of the nonselective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A blocked the development of OR486-induced hypersensitivity.

Conclusions: Peripheral adrenergic input is necessary for the development of persistent COMT-dependent pain, and peripherally-acting βAR antagonists may benefit chronic pain patients. **(Anesthesiology 2016; 124:1122-35)**

CHRONIC pain disorders, including fibromyalgia, headache, temporomandibular disorder (TMD), and vestibulodynia, constitute a significant healthcare problem, affecting more than 100 million Americans.[1–7] These disorders occur more frequently in women than in men[8] and are persistent in nature, characterized by pain that occurs daily and spans years. While the mechanisms underlying chronic pain are poorly understood, emerging evidence indicates a role for adrenergic pathways. Patients with chronic pain exhibit increased levels of catecholamines[9–11] alongside diminished activity of catechol-*O*-methyltransferase (COMT),[12,13] a ubiquitously expressed enzyme that metabolizes catecholamines to their inactive derivatives.[14] An increase in catecholamines is similarly observed in patients with inflammatory conditions such as arthritis and complex regional pain syndrome (CRPS).[15–17] Furthermore, functional variants in the *COMT* gene that reduce COMT activity[13,18,19] are associated with increased susceptibility to fibromyalgia,[20–24] TMD,[25] and experimental pain[25,26] as well as impaired response to treatment.[27,28] It is estimated, based on the frequency of allele variation, that nearly two thirds of patients with chronic pain disorders possess the low-activity COMT variants.[20,29]

What We Already Know about This Topic

- Decreased catecholamine-*O*-methyltransferase activity is associated with increased clinical and experimental pain in humans, and inhibition of catecholamine-*O*-methyltransferase in animals results in hypersensitivity
- Although β-adrenoceptors appear important to these observations, the sites of receptor activation are unknown

What This Article Tells Us That Is New

- In rats, sustained administration of a catecholamine-*O*-methyltransferase inhibitor produces hypersensitivity to mechanical and thermal stimuli, which is prevented by peripheral, but not spinal or supraspinal, administration of β-adrenoceptor antagonists, suggesting a peripheral site of action

Consistent with clinical disorders, we found in our laboratory that administration of the COMT inhibitor OR486 (Tocris, USA) in rodents produces increased hypersensitivity at multiple body sites and alters cognitive–affective

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behaviors linked to pain (*e.g.*, avoidance of painful heat and bright light).[30–32] Pharmacologic studies further revealed that OR486-induced hypersensitivity is blocked by administration of the nonselective β-adrenergic receptor (βAR) antagonist propranolol or by combined administration of selective β_2- and β_3AR antagonists.[30–32] These results are in line with those from clinical studies, showing that propranolol alleviates pain among fibromyalgia and TMD patients.[33,34] Collectively, these studies suggest that increased catecholamine levels, resulting from reduced COMT activity, drive pain *via* β_2- and β_3ARs.

β_2- and β_3ARs are G-protein–coupled receptors expressed in peripheral and central regions where they could drive pain. β_2ARs are located on peripheral terminals[35–39] and cell bodies[40–42] of primary afferent nociceptors; keratinocytes,[43–45] immune cells,[46–49] and adipocytes[50] in the periphery; and neurons[51,52] and glial cells[53] in the central nervous system. β_3ARs are located on primary afferent nociceptors,[54] adipocytes[50] and immune cells[47,48] in the periphery, and noradrenergic neurons in the brain.[55] Thus, we hypothesized that peripheral, spinal, and/or supraspinal β_2- and β_3ARs contribute to persistent COMT-dependent pain.

To test this hypothesis, we employed a clinically-relevant model of persistent COMT-dependent pain and evaluated responses to mechanical and thermal stimuli in adrenalectomized rats lacking peripheral epinephrine, and in intact rats receiving continuous delivery of βAR antagonists *via* intraplantar, intrathecal, or intracerebroventricular routes. Potential sexual dimorphism in the contribution of adrenergic systems to persistent COMT-dependent pain was also assessed.

Results demonstrated that male and female rats receiving sustained OR486 exhibited COMT-dependent mechanical and thermal hypersensitivity, persisting for 2 weeks. In contrast, adrenalectomized rats failed to develop OR486-induced hypersensitivity. Furthermore, intraplantar, but not intrathecal or intracerebroventricular, administration of the nonselective βAR antagonist propranolol, β_2AR antagonist ICI118,551, or β_3AR antagonist SR59230A blocked OR486-induced hypersensitivity. These findings demonstrate the importance of peripheral β_2- and β_3ARs in mediating persistent pain and suggest that peripherally-acting βAR antagonists may provide an effective treatment option for patients with chronic pain disorders.

Materials and Methods

Subjects

Adult male and female Sprague–Dawley rats (N = 24 intact, N = 24 adrenalectomized, and N = 23 sham) were purchased (Charles River Laboratories, USA) for the first set of experiments. For subsequent βAR antagonist experiments, adult male Sprague–Dawley rats (N = 111) were bred in-house. Rats weighed between 200 and 400 g for all experimental studies. Rats had *ad libitum* access to standard laboratory chow and water. Adrenalectomized rats were provided with saline water (0.9%) to compensate for the loss of sodium in urine due to the absence of aldosterone. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. Although rodent models of pain only partially correlate with human conditions, rats were chosen for these experiments because an extensive body of literature exists regarding nociceptive pathways and behavior in this species and because rat pain behavior assays are readily available and well characterized.[56–58]

General Experimental Conditions

First, the effects of sustained COMT inhibition on hypersensitivity were evaluated in intact rats receiving the COMT inhibitor OR486 or vehicle systemically for 14 days *via* a 2002 Alzet Osmotic Pump (Durect Corporation, USA). Next, the contribution of peripheral adrenergic systems to persistent OR486-induced hypersensitivity was evaluated in adrenalectomized rats lacking peripheral epinephrine or sham rats receiving OR486 or vehicle systemically for 14 days *via* an osmotic pump. Finally, the contribution of peripheral, spinal, and supraspinal βARs to persistent OR486-induced hypersensitivity was evaluated in separate groups of intact rats receiving intraplantar, intrathecal, or intracerebroventricular βAR antagonists alongside systemic delivery of OR486 or vehicle for 14 days *via* an osmotic pump. The βAR antagonists were delivered *via* a catheter attached to a separate 2002 Alzet Osmotic Pump.

Animals were handled and habituated to the experimenter and environment for 4 days before testing. Responses to punctuate mechanical and thermal stimuli were assessed in intact and adrenalectomized animals 1 day before and on days 1, 3, 5, 7, 9, 11, and 13 after pump implantation. For βAR antagonist experiments, pain behaviors were assessed 1 day before and on days 2, 4, 6, 8, 10, 12, and 14 after pump implantation. The rest day between surgery and testing allowed animals to fully recover from catheter implantation. On baseline and testing days, rats were habituated to the mechanical and thermal testing environments for 10 to 15 min. Although we were unable to eliminate all environmental factors (*e.g.*, season, humidity, and noise) from this study, we minimized others (*e.g.*, experimenter consistency, testing time of day, and cage density) that were in our control.[59,60] Animals were randomly assigned to groups; were tested by a single, blinded experimenter at a consistent time of day (morning); and were housed with one to two other rats. The primary outcome reported in this study is behavioral changes, in the form of mechanical allodynia, mechanical hyperalgesia, and thermal hyperalgesia, which are described in detail below under their respective subtitles.

Drug Preparation

OR486 (Tocris) was dissolved in a 5:3:2 ratio of dimethylsulfoxide, 0.9% saline, and ethanol.[32] For peripheral

experiments, βAR antagonists propranolol hydrochloride (Tocris), ICI-118,511 (Tocris), and SR59230A (Tocris) were each dissolved in 5:3:2 ratios of dimethylsulfoxide, 0.9% saline, and ethanol. For intrathecal and intracerebroventricular experiments, βAR antagonists were dissolved in 0.9% saline. Drug solutions were injected into pumps, which were placed in 15-ml conical tubes containing sterile 0.9% saline and primed overnight in a dry heat bath (Lab Armor, USA) at 37°C. All pumps (other than those for intrathecal delivery) were attached to corresponding catheters before priming. Subcutaneous delivery of OR486 was at a constant rate of 15 mg · kg^{-1} · day^{-1} for a 2-week period. Peripheral delivery of propranolol hydrochloride was at 9 mg · kg^{-1} · day^{-1}, ICI-118,511 was at 1.5 mg · kg^{-1} · day^{-1}, and SR59230A was at 1.67 mg · kg^{-1} · day^{-1}. Intrathecal delivery of propranolol hydrochloride was at 50 μg/day for the low-dose experiments and 100 μg/day for the high-dose experiments, ICI-118,511 was at 30 μg/day, and SR59230A was at 20 μg/day. Intracerebroventricular delivery of propranolol hydrochloride was at 50 μg/day for the low-dose experiments and 100 μg/day for the high-dose experiments; ICI-118,511 delivery was at 30 μg/day, and SR59230A delivery was at 20 μg/day.

Surgical Procedures

For all surgical procedures, rats were anesthetized by isoflurane inhalation (5% induction, 1.5 to 5% maintenance). Incision sites were shaved and disinfected with ethanol and betadine. Sterile technique was employed throughout the duration of all procedures according to Institutional Animal Care and Use Committee requirements. Stainless steel wound clips (Braintree Scientific, USA) were used to close the wounds.

For systemic delivery of OR486, a small incision was made over the left shoulder blade of the rat. Hemostats were used to create a small subcutaneous pocket in which the pump was placed.

For intraplantar delivery of βAR antagonists, a modified version of the protocol published by Haddad and Adams[61] was used. Pumps were attached to a 15-cm, Y-shaped, bifurcated 3-French silicone catheter (SAI Infusion Technologies, USA). The pump was implanted subcutaneously over the right shoulder blade, and a stainless steel 10-gauge × 20-cm semiblunt tip trocar (SAI Infusion Technologies) was used to subcutaneously route the catheter ends to incisions made at either hind paw. The catheter ends were attached to the plantar fascia using 4-0 silk sutures (Oasis Medical, USA).

For intrathecal delivery[62] of βAR antagonists, a small incision was made on the nape of the neck, and scissors and hemostats were used to lift muscle and expose the atlantooccipital membrane. The membrane was carefully incised using the tip of scissors, causing the escape of cerebrospinal fluid. A 27.3-cm, polyurethane Alzet Short Rat IT Catheter (Durect Corporation) was inserted into the intrathecal space, dorsal to the spinal cord. The other end of the catheter was sutured to the surrounding tissue and attached to the osmotic pump, which was subcutaneously implanted over the right shoulder blade. Four animals did not wake up after intrathecal surgery. These animals were replaced in future intrathecal groups to account for the decrease in sample size.

For intracerebroventricular delivery[63] of βAR antagonists, pumps were attached to a 38-gauge stainless steel cannula *via* a short vinyl catheter (Alzet Brain Infusion Kit 2; Durect Corporation). The cannula was implanted into the right lateral ventricle (from the bregma: −0.8 mm anteroposterior, −1.6 mm mediolateral, −5 mm dorsoventral) and was cemented to two anchoring screws on the skull. The attached pump was subcutaneously implanted over the right shoulder blade.

Assessment of Behavioral Responses to Mechanical and Thermal Stimuli

Paw withdrawal threshold was assessed using the von Frey up–down method.[64] Nine calibrated and logarithmically spaced von Frey monofilaments (bending forces: 0.40, 0.68, 1.1, 2.1, 3.4, 5.7, 8.4, 13.2, and 15.0 g; Stoelting, USA) were applied to the plantar hind paw. First, the middle filament (3.4 g) was applied to the hind paw for 3 s. If the rat responded with a withdrawal, an incrementally lower filament was applied. In the absence of a withdrawal, an incrementally higher filament was applied. A series of six total responses were recorded for each paw. Results were entered into the Paw Flick module within the National Instruments LabVIEW 2.0 software (LabVIEW, USA), which uses a logarithmic algorithm to determine the gram-force value that would elicit paw withdrawal in 50% of trials ($10^{(Xf + k\delta)}$/10,000, where X_f = value [in log units] of the final von Frey hair used; k = tabular value of positive and negative responses, and δ = mean difference [in log units] between stimuli). Mechanical allodynia was defined as a heightened response to a normally innocuous stimulus, as determined by a decrease in paw withdrawal threshold.

Mechanical hyperalgesia was assessed using a 15.0-g von Frey filament. This filament was chosen as a normally noxious stimulus, as it has a gram-force value well over the 50% withdraw threshold for animals tested in this study. The filament was applied to the hind paw 10 times for a duration of 1 s, with an interstimulus interval of 1 s.[32] The number of paw withdrawals (which could range from 0 to 10) was recorded for each hind paw at each time point. Mechanical hyperalgesia was defined as an increase in the number of paw withdrawals in response to a normally noxious mechanical stimulus.

Thermal hyperalgesia was assessed using the Hargreaves method.[65] Animals were placed in plexiglass chambers, and a radiant beam of light was applied to the hind paw through a glass floor heated to 30°C. Paw withdrawal latencies were recorded in duplicate per paw. If the second latency recorded was not within ±4 s of the first, a third measure was recorded. The two latencies closest in value were averaged to determine overall latency to withdrawal. Thermal behavioral data are

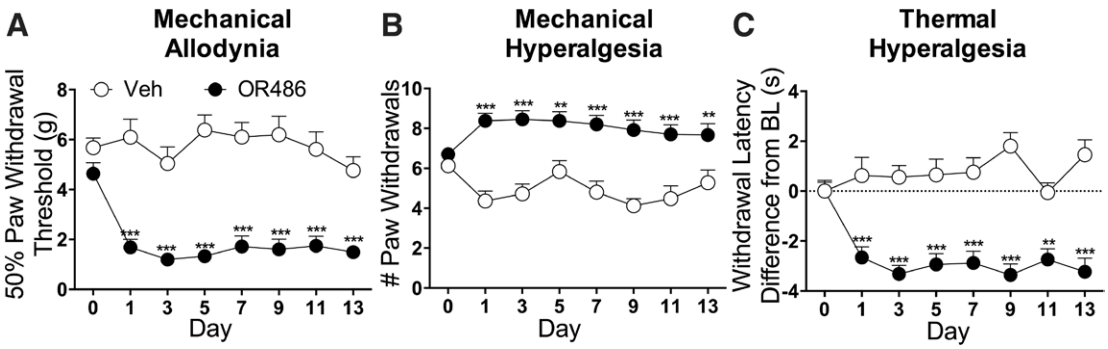

Fig. 1. Sustained administration of the catecholamine-*O*-methyltransferase inhibitor OR486 leads to mechanical and thermal hypersensitivity. Compared to vehicle, sustained systemic OR486 administration produces (*A*) mechanical allodynia, (*B*) mechanical hyperalgesia, and (*C*) thermal hyperalgesia. N = 12 (6 males and 6 females) per group. Data are expressed as mean ± SEM. ***P < 0.001, **P < 0.01 different from vehicle. BL = baseline; Veh = vehicle.

reported in text and figures as the difference in paw withdrawal latency from baseline (day 0). Thermal hyperalgesia was defined as a decrease in paw withdrawal latency in response to a noxious thermal stimulus.

Statistical Analyses

Sample sizes were selected based on their ability in previous, similarly structured rat studies to accurately demonstrate behavioral differences between groups.[30–32] Mechanical allodynia, mechanical hyperalgesia, and thermal hyperalgesia data were analyzed by 2-way ANOVA (for group × time). In ANOVA analyses, groups correspond to the separate groups on the graph of interest, as denoted by different symbols and names (*e.g.*, groups in fig. 1 = vehicle and OR486). *Post hoc* comparisons were performed using the Bonferroni test, which corrected for multiple comparisons. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using GraphPad Prism (GraphPad Software, USA).

Results

Sustained COMT Inhibition Produces Persistent Pain

Genetic and pharmacologic alterations resulting in reduced COMT activity are associated with increased experimental pain and likelihood of developing chronic pain disorders. Acute administration of the COMT inhibitor OR486 results in enhanced mechanical and thermal hypersensitivity in rats.[32] To evaluate the effects of sustained COMT inhibition on hypersensitivity, responses to mechanical and thermal stimuli were measured in separate groups of rats receiving systemic OR486 ($15\,mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle over a 2-week period. Compared to rats receiving vehicle, those receiving OR486 exhibited mechanical allodynia (group: $P < 0.0001$; group × day: $P = 0.0043$; fig. 1A), mechanical hyperalgesia (group: $P < 0.0001$; group × day: $P = 0.0109$; fig. 1B), and thermal hyperalgesia (group: $P < 0.0001$; group × day: $P < 0.0001$; fig. 1C) beginning on day 1 and lasting throughout the duration of the experiment. Sexual dimorphism was not observed, as both male and female rats developed mechanical allodynia (male group: $P < 0.0001$; female group: $P < 0.0001$; fig. 1A, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (male group, $P < 0.0053$; female group: $P < 0.0001$; fig. 1B, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), and thermal hyperalgesia (male group: $P < 0.0001$; female group: $P < 0.0001$; fig. 1C, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). See figure 1, Supplemental Digital Content 1 (http://links.lww.com/ALN/B262), for all sexual dimorphism data in intact rats.

Adrenalectomized Rats Fail to Develop Persistent COMT-dependent Pain

Previous work has demonstrated that acute COMT-dependent pain is mediated *via* β_2- and β_3ARs, which are located in peripheral, spinal, and supraspinal regions where they could potentially drive pain transmission. To evaluate the potential contribution of peripheral adrenergic systems to COMT-dependent pain, separate groups of adrenalectomized rats (lacking peripheral epinephrine) or sham surgery rats received systemic OR486 ($15\,mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle over a 2-week period, and responses to mechanical and thermal stimuli were measured. Compared to sham rats receiving vehicle, those receiving OR486 developed mechanical allodynia (group: $P < 0.0001$; group × day: $P < 0.0001$; fig. 2A), mechanical hyperalgesia (group: $P < 0.0001$; group × day: $P = 0.0044$; fig. 2B), and thermal hyperalgesia (group: $P = 0.0005$; group × day: $P < 0.0001$; fig. 2C). In contrast, adrenalectomized rats did not develop mechanical allodynia, mechanical hyperalgesia, or thermal hyperalgesia.

Sexual dimorphism was not observed, as both male and female sham rats developed mechanical allodynia (male group: $P < 0.0001$; female group: $P < 0.0001$; fig. 2A, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (male group: $P = 0.0053$; female group: $P < 0.0001$; fig. 2B, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), and

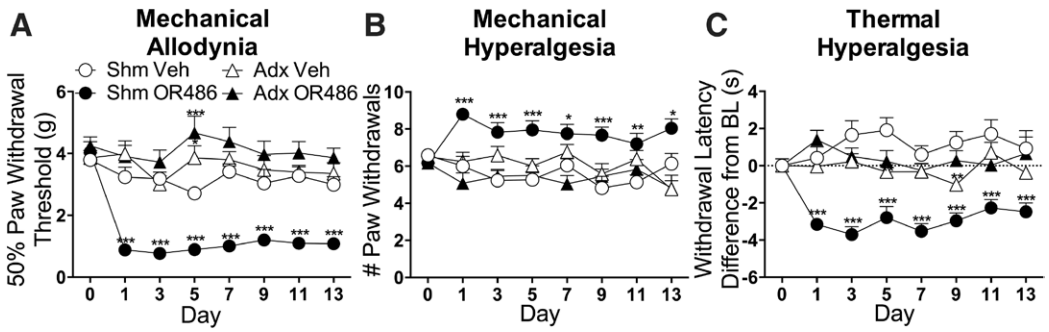

Fig. 2. Adrenalectomized (Adx) rats fail to develop OR486-induced hypersensitivity. In Sham (Shm), but not Adx, animals, sustained systemic OR486 administration produces (*A*) mechanical allodynia, (*B*) mechanical hyperalgesia, and (*C*) thermal hyperalgesia. N = 11 (5 males and 6 females) for Shm/vehicle (Veh) and N = 12 (6 males and 6 females) for all other groups. Data are expressed as mean ± SEM. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ different from Shm/Veh. BL = Baseline.

thermal hyperalgesia (male group: $P < 0.0001$; female group: $P < 0.0001$; fig. 2C, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). Both male and female adrenalectomized rats failed to develop mechanical allodynia (fig. 2D, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (fig. 2E, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), and thermal hyperalgesia (fig. 2F, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). See figure 2, Supplemental Digital Content 1 (http://links.lww.com/ALN/B262), for all sexual dimorphism data in sham and adrenalectomized rats.

Peripheral βAR Antagonist Administration Prevents the Development of Persistent COMT-dependent Pain

Adrenalectomized rats failed to develop persistent hypersensitivity after COMT inhibition, suggesting a peripheral adrenergic site of action. In order to further investigate this hypothesis, pharmacological methods were used to determine the contribution of peripheral, spinal, and supraspinal βARs to persistent COMT-dependent pain. First, the contribution of peripheral βARs to mechanical and thermal hypersensitivity was evaluated in separate groups of rats receiving sustained intraplantar administration of propranolol (9 mg · kg^{-1} · day^{-1}), ICI-118,551 (1.5 mg · kg^{-1} · day^{-1}), SR59230A (1.67 mg · kg^{-1} · day^{-1}), or vehicle alongside sustained systemic administration of OR486 (15 mg · kg^{-1} · day^{-1}) or vehicle over a 2-week period. Peripheral antagonist doses were selected based on the results from a preliminary study that evaluated the ability of three different doses per antagonist to reduce or block COMT-dependent pain (fig. 3).

Compared to rats receiving vehicle, those receiving sustained intraplantar administration of the nonselective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A alongside systemic OR486 did not develop mechanical allodynia (group: fig. 3A, $P < 0.0001$; fig. 3B, $P < 0.0001$; fig. 3C, $P < 0.0001$) or mechanical hyperalgesia (group: fig. 3D, $P < 0.0001$; fig. 3E, $P < 0.0001$; fig. 3F, $P < 0.0001$). Rats receiving sustained intraplantar administration of the β_3AR antagonist SR59230A also did not develop OR486-induced thermal hyperalgesia (group: fig. 3I, $P < 0.0001$). In contrast, rats receiving propranolol (fig. 3G) or ICI-118,551 (fig. 3H) alongside OR486 exhibited a 15% decrease in paw withdrawal latency from baseline, similar to rats receiving vehicle. Animals receiving sustained intraplantar administration of βAR antagonists alongside systemic vehicle failed to develop mechanical allodynia (fig. 3A, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (fig. 3D, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), or thermal hyperalgesia (fig. 3G, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). See figure 3, Supplemental Digital Content 1 (http://links.lww.com/ALN/B262), for control data demonstrating no effect of antagonists on hypersensitivity irrespective of administration route.

Intrathecal βAR Antagonist Administration Does Not Alter Persistent COMT-dependent Pain

Next, the contribution of spinal βARs to mechanical and thermal hypersensitivity was evaluated in separate groups of rats receiving sustained intrathecal administration of propranolol (50 μg/day), ICI-118,551 (30 μg/day), SR59230A (20 μg/day), or vehicle alongside sustained systemic administration of OR486 (15 mg · kg^{-1} · day^{-1}) or vehicle over a 2-week period (fig. 4). Intrathecal delivered antagonist doses were selected based on their ability to block hypersensitivity or pain-relevant behaviors in other rat models when administered intrathecally.[66–68] Similar to animals receiving vehicle, those receiving sustained intrathecal administration of the nonselective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A alongside systemic OR486 exhibited mechanical allodynia (group: fig. 4A, $P < 0.0001$; fig. 4B, $P < 0.0001$; fig. 4C, $P < 0.0001$), mechanical hyperalgesia (group: fig. 4D, $P = 0.0002$; fig. 4E, $P < 0.0001$; fig. 4F, $P = 0.0018$), and thermal hyperalgesia (group: fig. 4G, $P < 0.0001$; fig. 4H, $P < 0.0001$; fig. 4I, $P < 0.0001$). Animals receiving sustained intrathecal administration of βAR antagonists alongside systemic vehicle failed

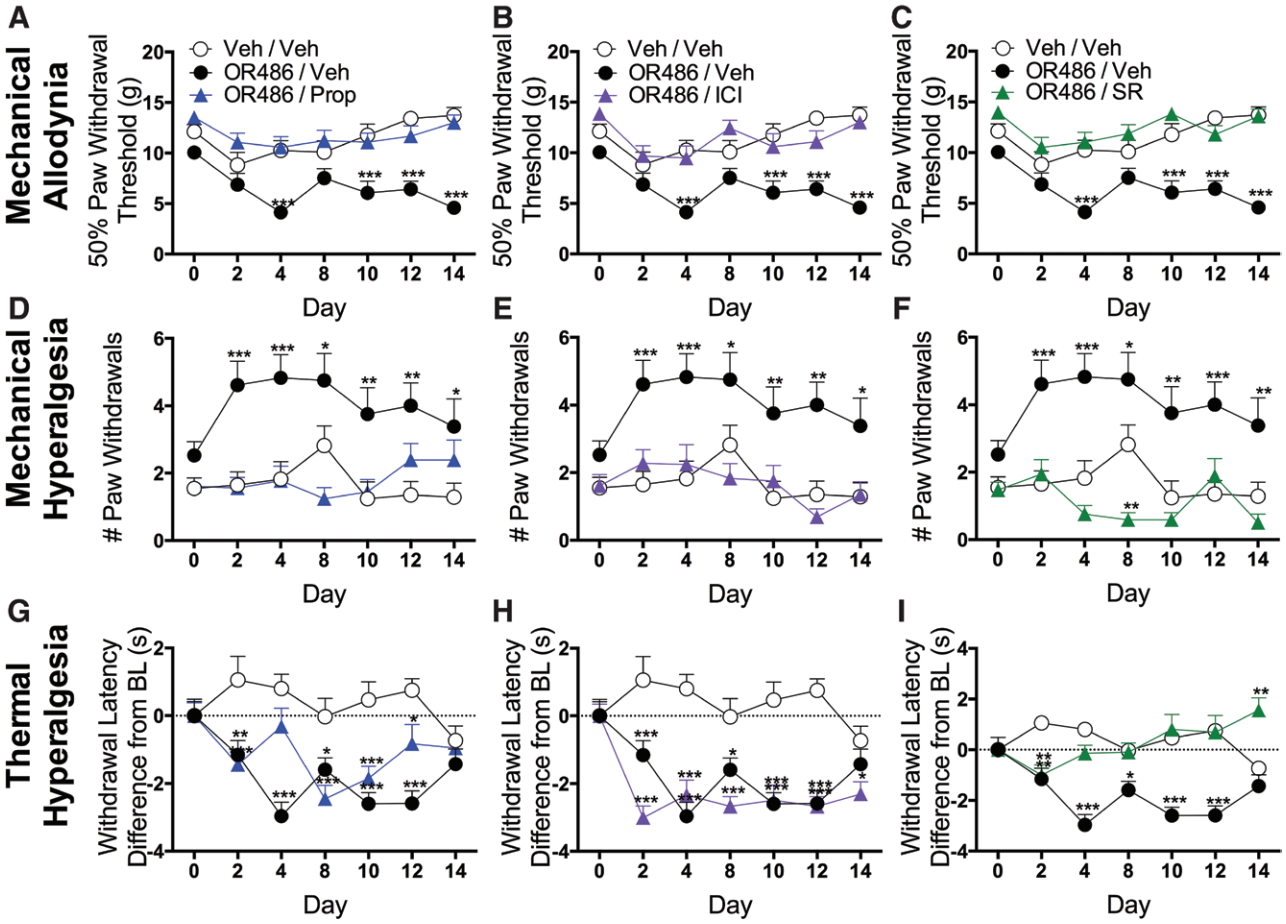

Fig. 3. Peripheral administration of β-adrenergic receptor (βAR) antagonists blocks OR486-induced hypersensitivity. Peripheral delivery of the nonselective βAR antagonist propranolol (Prop) alongside sustained systemic OR486 administration prevents (*A*) mechanical allodynia and (*D*) mechanical hyperalgesia but does not alter (*G*) thermal hyperalgesia. Similarly, peripheral delivery of the β_2AR antagonist ICI-118,551 (ICI) alongside sustained systemic OR486 administration prevents (*B*) mechanical allodynia and (*E*) mechanical hyperalgesia but does not alter (*H*) thermal hyperalgesia. Finally, peripheral delivery of the β_3AR antagonist SR59230A (SR) alongside sustained systemic OR486 administration prevents (*C*) mechanical allodynia, (*F*) mechanical hyperalgesia, and (*I*) thermal hyperalgesia. N = 9 per group. Data are expressed as mean ± SEM. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ different from vehicle (Veh)/Veh. BL = Baseline.

to develop mechanical allodynia (fig. 3B, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (fig. 3E, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), or thermal hyperalgesia (fig. 3H, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). Animals receiving SR59230A alongside vehicle did exhibit transient elevations in paw withdrawal threshold on days 2 (vehicle/vehicle 4.47 ± 0.63 *vs.* vehicle/SR59230A 10.80 ± 3.26, mean ± SEM) and 10 (vehicle/vehicle 3.50 ± 0.73 *vs.* vehicle/SR59230A 10.97 ± 3.13) likely due to higher baseline values (vehicle/vehicle 4.76 ± 0.55 *vs.* vehicle/SR59230A 8.54 ± 2.59) and increased intergroup variability as compared to control animals (fig. 3B, Supplemental Digital Content 1, http://links.lww.com/ALN/B262).

To confirm that intrathecal βAR antagonists were unable to block OR486-induced hypersensitivity, we performed a duplicate set of experiments using a higher dose of the nonselective βAR antagonist propranolol (100 μg/day). Similar to the original dose, intrathecal administration of the higher dose did not block OR486-induced mechanical allodynia (group: $P < 0.0001$; fig. 4A, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (group: $P = 0.0011$; fig. 4D, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), or thermal hyperalgesia (group: $P < 0.0001$; fig. 4G, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). See figure 4, Supplemental Digital Content 1 (http://links.lww.com/ALN/B262), for all intrathecal high-dose propranolol data.

Intracerebroventricular βAR Antagonist Administration Does Not Alter Persistent COMT-dependent Pain

Finally, the contribution of supraspinal βARs to mechanical and thermal hypersensitivity was evaluated in separate

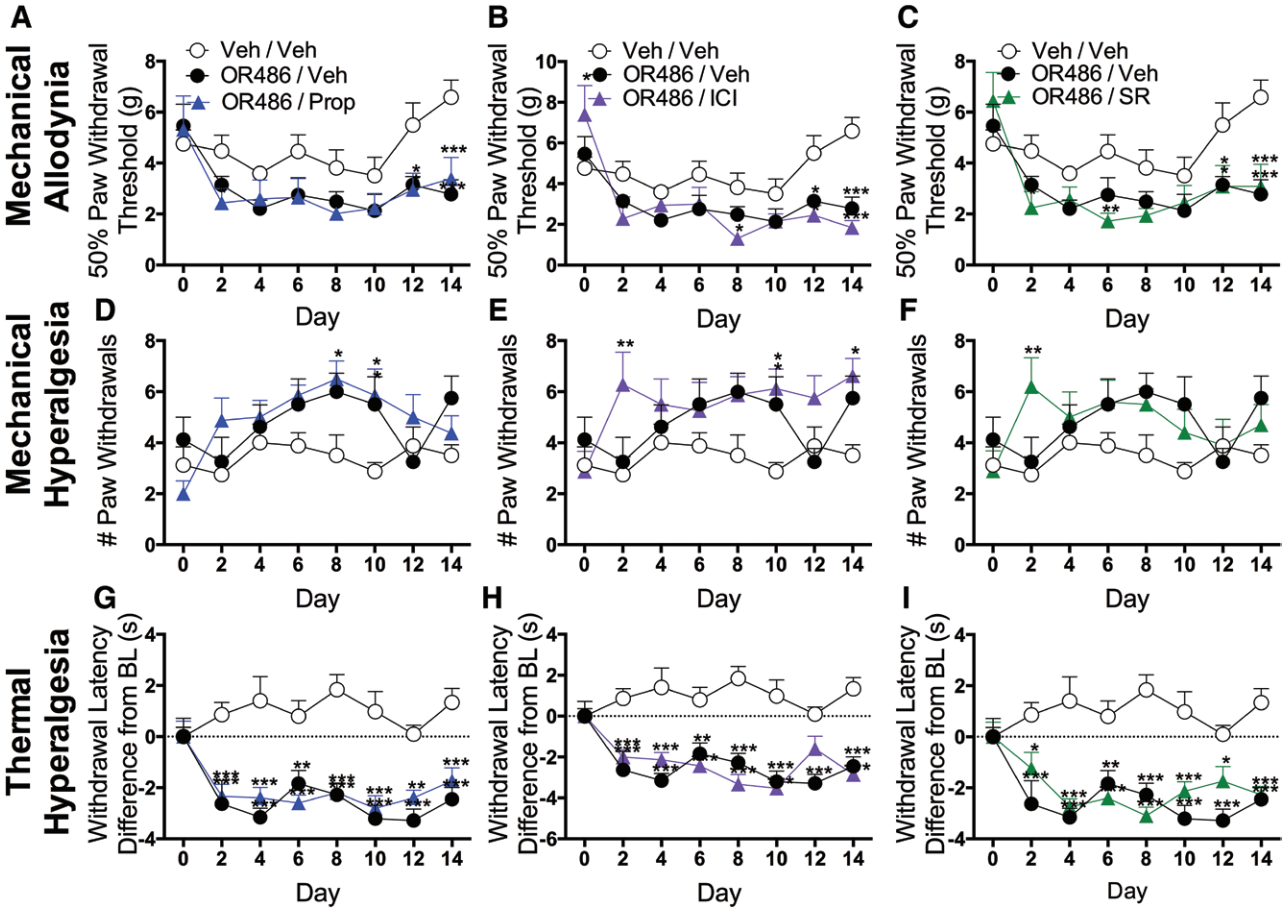

Fig. 4. Intrathecal administration of β-adrenergic receptor (βAR) antagonists does not alter OR486-induced hypersensitivity. Intrathecal delivery of the nonselective βAR antagonist propranolol (prop) (*A, D, G*), the β_2AR antagonist ICI-118,551 (ICI) (*B, E, H*), or the β_3AR antagonist SR59230A (SR) (*C, F, I*) alongside sustained systemic OR486 administration does not alter mechanical or thermal sensitivity. N = 4 per group. Data are expressed as mean ± SEM. ***P < 0.001, **P < 0.01, *P < 0.05 different from vehicle (Veh)/Veh. BL = Baseline.

groups of rats receiving sustained intracerebroventricular administration of propranolol (50 μg/day), ICI-118,551 (30 μg/day), SR59230A (20 μg/day), or vehicle alongside sustained systemic administration of OR486 (15 mg · kg^{-1} · day^{-1}) or vehicle over a 2-week period (fig. 5). Intracerebroventricular antagonist doses were selected based on their ability to block hypersensitivity or related behaviors in other rat models.[66–68] Similar to animals receiving vehicle, those receiving sustained intracerebroventricular administration of the nonselective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A alongside systemic OR486 exhibited mechanical allodynia (group: fig. 5A, P < 0.0001; fig. 5B, P < 0.0001; fig. 5C, P < 0.0001), mechanical hyperalgesia (group: fig. 5D, P < 0.0001; fig. 5E, P < 0.0001; fig. 5F, P < 0.0001), and thermal hyperalgesia (group: fig. 5G, P < 0.0001; fig. 5H, P < 0.0001; fig. 5I, P < 0.0001). Animals receiving sustained intracerebroventricular administration of βAR antagonists alongside systemic vehicle failed to develop mechanical allodynia (fig. 3C, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (fig. 3F, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), or thermal hyperalgesia (fig. 3I, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). Animals receiving SR59230A alongside vehicle did exhibit transient elevations in paw withdrawal frequency on days 2 (vehicle/vehicle 1.88 ± 0.40 *vs.* vehicle/SR59230A 4.62 ± 0.86) and 8 (vehicle/vehicle 2.00 ± 0.46 *vs.* vehicle/SR59230A 5.00 ± 1.20) likely due to increased intergroup variability as compared to control animals (fig. 3F, Supplemental Digital Content 1, http://links.lww.com/ALN/B262).

To confirm that intracerebroventricular βAR antagonists are unable to block OR486-induced hypersensitivity, we performed a duplicate set of experiments using a higher dose of the nonselective βAR antagonist propranolol (100 μg/day). Similar to the original dose, intracerebroventricular administration of the higher dose did not block OR486-induced mechanical allodynia (group: P < 0.0001; fig. 5A, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), mechanical hyperalgesia (group:

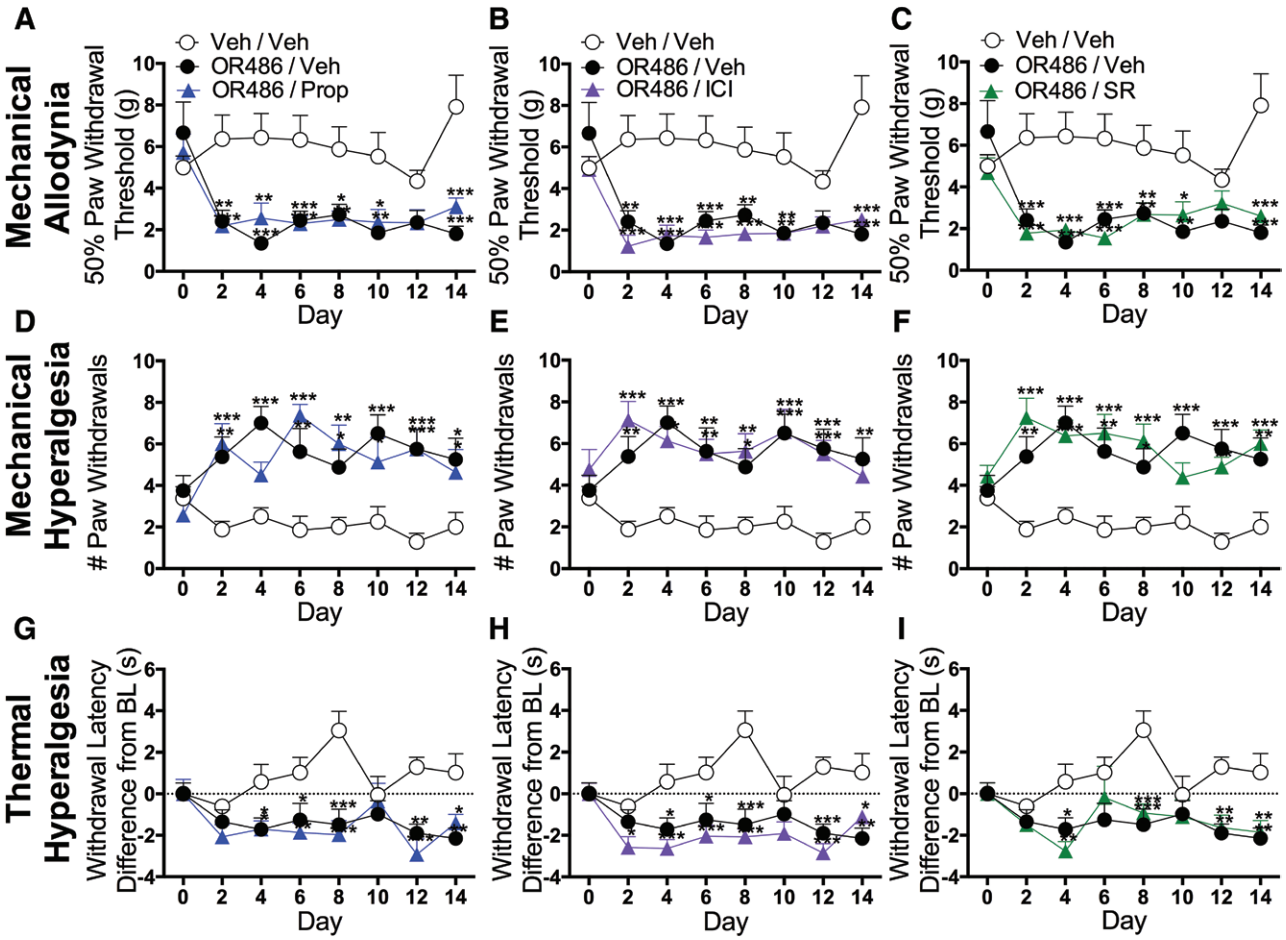

Fig. 5. Intracerebroventricular administration of β-adrenergic receptor (βAR) antagonists does not alter OR486-induced hypersensitivity. Supraspinal delivery of the nonselective βAR antagonist propranolol (prop) (*A, D, G*), β_2AR antagonist ICI-118,551 (ICI) (*B, E, H*), or the β_3AR antagonist SR59230A (SR) (*C, F, I*) alongside sustained systemic OR486 administration does not alter mechanical or thermal sensitivity. N = 4–5 per group. Data are expressed as mean ± SEM. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ different from vehicle (Veh)/Veh. BL = Baseline.

$P < 0.0001$; fig. 5D, Supplemental Digital Content 1, http://links.lww.com/ALN/B262), or thermal hyperalgesia (group: $P < 0.0001$; fig. 5G, Supplemental Digital Content 1, http://links.lww.com/ALN/B262). See figure 5, Supplemental Digital Content 1 (http://links.lww.com/ALN/B262), for all intracerebroventricular high-dose propranolol data.

Discussion

Although the mechanisms underlying chronic pain disorders are not well described, emerging evidence suggests a role for adrenergic pathways. Employing a rodent model of sustained COMT inhibition that mimics abnormalities in catecholamine signaling observed in patients with these disorders, we demonstrate that COMT-dependent pain is mediated *via* peripherally, but not spinally or supraspinally, located β_2- and β_3ARs.

In previous studies, we established a causal link between low COMT and pain. We demonstrated that a single injection of the COMT inhibitor OR486 produces mechanical and thermal hypersensitivity, similar to that produced by intraplantar carrageenan. Subsequent pharmacological studies further demonstrated that the development of acute OR486-induced hypersensitivity requires activation of β_2- and β_3ARs.[30,32] Within hours, administration of OR486 results in increased circulating levels of nitric oxide and the proinflammatory cytokines tumor necrosis factor-α, interleukin-1β, interleukin-6, and chemokine (C-C motif) ligand 2 (CCL2),[30] which are nociceptive transmitters implicated in chronic pain. Individuals with fibromyalgia, headache, and TMD exhibit increased levels of these molecules,[69–72] which elicit pain by reducing nociceptor firing thresholds.[73–83] Nitric oxide and proinflammatory cytokines also elicit pain by working synergistically to potentiate one another's biosynthesis, as observed in the OR486 model.[30]

Here, we utilized a more clinically-relevant model of sustained COMT inhibition, characterized by enhanced sensitivity to noxious stimuli and altered pain-relevant cognitive–affective behaviors that persist over a 2-week period, to

determine the site-of -action whereby βARs mediate persistent COMT-dependent pain. The contribution of peripheral adrenergic systems was first examined in adrenalectomized rats. We found that, compared to sham surgery rats, adrenalectomized rats lacking peripheral epinephrine fail to develop OR486-induced mechanical and thermal hypersensitivity. This finding is in line with those from previous studies showing that adrenalectomized rats have blunted hypersensitivity after formalin administration[84] or chronic constriction injury.[85] Together, these results suggest that peripherally circulating catecholamines contribute to the transmission of hypersensitivity in models of inflammatory and neuropathic pain, as well as chronic pain disorders. This conclusion is further supported by studies that have demonstrated increased urinary catecholamines in patients with myofascial pain[10] and increased circulating plasma catecholamines in women with fibromyalgia.[9] Of note, adrenalectomy also results in a reduction of circulating corticosterone levels.[86,87] Increased corticosterone levels after stress[88] or nerve injury[89,90] have been implicated in analgesia and pronociception. Thus, future experiments examining peripheral catecholamines should utilize adrenal medullectomized animals or should provide supplemental corticosterone to adrenalectomized animals to rule out corticosterone-mediated effects.

As previous preclinical and clinical studies have reported sex-specific differences in COMT-related phenotypes,[91–95] and as males and female rats exhibit different COMT expression patterns,[96,97] we examined the contribution of peripheral adrenergic systems to COMT-dependent pain in both sexes. Counter to our expectation, male and female rats exhibited a comparable increase in mechanical and thermal hypersensitivity after sustained systemic OR486 administration, which was blocked by suppressing peripheral adrenergic tone. Despite these findings, future studies and clinical applications related to COMT-dependent pain should continue to consider possible sex-specific effects.

The independent contribution of peripheral, spinal, and supraspinal βARs to persistent COMT-dependent pain was next examined in separate groups of intact rats receiving targeted delivery of the nonselective βAR antagonist propranolol, the β_2AR antagonist ICI-118,551, or the β_3AR antagonist SR59230A alongside systemic OR486. We found that peripheral, but not spinal or supraspinal, administration of propranolol, ICI-118,511, or SR59230A blocked the development of OR486-induced hypersensitivity throughout the duration of the testing period. While all three antagonists blocked the development of mechanical hypersensitivity, only SR59230A blocked the development of thermal hypersensitivity. These findings significantly extend those from acute COMT inhibition studies,[30,32] demonstrating that peripheral β_2- and β_3ARs both contribute to the development of persistent mechanical hypersensitivity, while peripheral β_3ARs independently contribute to the development of persistent thermal hypersensitivity after sustained COMT inhibition.

The peripheral contribution of β_2ARs to pain is in line with results from previous studies demonstrating that epinephrine activates β_2ARs located on the peripheral terminals of primary afferent nociceptors, increasing their excitability and producing a hyperalgesic state.[35–39] Also, elevated plasma norepinephrine activates β_2ARs to promote visceral hypersensitivity.[38] In humans, variants of the β_2AR gene known to influence receptor expression are associated with increased risk of TMD.[98]

The contribution of peripheral β_3ARs to persistent pain is more novel. Peripherally expressed β_3ARs are known for their ability to regulate norepinephrine-induced changes in metabolism and thermoregulation.[99] In 2010, it was discovered that β_3ARs are expressed on primary afferent nociceptors, where they drive norepinephrine-induced ATP release and contribute to neuropathic pain.[54] Recently, β_3ARs have also been shown to mediate formalin-induced temporomandibular joint pain.[100] In contrast to acute COMT-dependent thermal hypersensitivity, which requires coincident activation of both β_2- and β_3ARs,[32] persistent COMT-dependent thermal hypersensitivity requires independent activation of peripheral β_3ARs. Unlike most G-protein–coupled receptors, including β_2ARs, β_3ARs do not undergo desensitization after agonist stimulation.[101,102] Thus, β_3ARs are uniquely positioned to stimulate downstream effectors for prolonged periods of time.

In addition to their location on primary afferent nociceptors, β_2- and β_3ARs are expressed in numerous peripheral cell types, in which they could potentially mediate pain, including immune cells involved in adaptive responses (T cells, mast cells, and macrophages), adipocytes, keratinocytes, and satellite glia. T cells, mast cells, and macrophages are immune cells in the periphery that express βARs and, after their activation by epinephrine or norepinephrine, orchestrate inflammatory responses. Increased catecholamine levels after stress or pharmacologic manipulation led to activation of T cells, increased expression of β_2- and β_3ARs,[49] and production of interleukin-1, interleukin-6, and CCL2.[103] T-cell infiltration in the spinal dorsal horn of adult rats has been shown to contribute to hypersensitivity after nerve injury.[104,105] In line with these findings, patients with fibromyalgia have more activated T cells circulating in blood compared to healthy controls.[106] Epinephrine activates mast cells and stimulates the release of interleukin-1β, interleukin-6, and other proinflammatory cytokines in a β_2AR-dependent manner.[46] Increased activation of mast cells has been observed in numerous chronic pain disorders, including fibromyalgia, headache, vestibulodynia, and irritable bowel syndrome.[107–112] Agonist activation of β_2ARs expressed on macrophages *in vitro* results in activation of intracellular kinases and release of interleukin-6. Further, sustained systemic administration of epinephrine in mice results in β_2AR-mediated increases in macrophage activation and interleukin-6 production.[47,48]

Adipocytes are cells in the periphery that express both β_2- and β_3ARs and specialize in storing energy as fat.[50] They also interface with immune cells to regulate inflammatory responses.[113] Notably, adipocytes produce 30% of the interleukin-6 circulating in the body,[114] and studies have shown that activation of β_3ARs on adipocytes produces a robust increase in interleukin-6 levels in plasma,[115] as well as in tumor necrosis factor-α,[116] CCL2,[117] and NO[118] levels *in vitro*.

Keratinocytes and satellite glial cells reside near the peripheral terminals and cell bodies, respectively, of primary afferent nociceptors. While a direct link between βAR activation on these cell types and pain has yet to be established, catecholamine-induced activation of keratinocyte β_2ARs results in increased intracellular kinase activation and interleukin-6 release.[43–45] Similarly, activation of satellite glia by catecholamines results in βAR-mediated increases in intracellular cyclic nucleotides that facilitate neuronal–glial communication.[119]

Collectively, these findings demonstrate the importance of β_2- and β_3ARs located on immunoregulatory cells in the periphery to persistent COMT-dependent pain, accounting for clinical observations that βAR antagonists provide pain relief for patients with functional pain disorders, such as fibromyalgia and TMD,[33,34,120] as well as inflammatory conditions, such as arthritis, rosacea, and CRPS.[121–124] While these findings seem inconsistent with the ability of antidepressants to alleviate persistent pain by increasing synaptic levels of catecholamines, it is important to note that the analgesic effect of antidepressants is associated with descending inhibition of pain *via* actions at α_2ARs or D_2 dopamine receptors in the spinal dorsal horn.[125,126] Thus, catecholamines can exert divergent influences on nociception as a function of localization and net influence on neuronal excitability. Future studies are required to identify the specific cell type(s) in the periphery that express βARs and, upon activation, release proinflammatory molecules that initiate persistent hypersensitivity. By determining where, when, and how β_2- and β_3ARs and their downstream effectors mediate COMT-dependent pain, the field will better understand the diverse nature of catecholamine signaling so that patients suffering from disorders resulting from reduced COMT and/or elevated catecholamines receive the most relevant treatments.

While the studies herein utilized a clinically-relevant rodent model of sustained COMT inhibition, additional mechanistic studies will implement a COMT−/− mouse model in order to more accurately represent the endogenously low levels of COMT activity observed in pain patients. Future studies are also necessary to elucidate the specific cell signaling pathways responsible for the initiation and maintenance of β_2- and β_3AR-mediated hypersensitivity. Finally, clinical studies are required to evaluate the efficacy of peripheral β_2- and β_3AR antagonist therapy in patients with chronic pain disorders and related conditions.

In conclusion, we utilized a clinically-relevant animal model that portrays the characteristics of patients with chronic pain disorders to demonstrate that both male and female rats are susceptible to the development of persistent COMT-dependent pain, which is mediated *via* peripherally located β_2- and β_3ARs. These findings suggest that peripheral β_2- and β_3AR antagonist therapy may be an effective option for the treatment of chronic pain disorders, as well as those with overlapping peripheral β-adrenergic mechanisms (*e.g.*, CRPS[127]).

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Competing Interests

The authors declare no competing interests.

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