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Persistent COMT-dependent pain is initiated by peripheral, but not spinal or central, β_2- and β_3- adrenergic receptors

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Summary

Adrenalectomized rats or intact rats receiving peripheral administration of β-adrenergic receptor antagonists do not develop pain following sustained COMT inhibition, suggesting a peripheral adrenergic site of action for COMT-dependent pain.

Background—Patients with chronic pain disorders exhibit increased levels of catecholamines alongside diminished activity of catechol-O-methyltransferase (COMT), an enzyme that metabolizes catecholamines. Consistent with clinical observations, our lab found that acute pharmacologic inhibition of COMT in rodents produces pain. Furthermore, we found that the development of acute COMT-dependent pain is mediated by β_2-and β_3-adrenergic receptors (ARs). However, the contribution of distinct populations of β_2- and β_3ARs to the development of persistent pain linked to abnormalities in catecholamine signaling requires further investigation.

Methods—Here, we sought to determine the contribution of peripheral, spinal, and central β_2- and β_3ARs to persistent COMT-dependent pain. Specifically, we implanted osmotic pumps to achieve sustained systemic delivery of the COMT inhibitor OR486 over 2-weeks. Behavioral responses to mechanical and thermal stimuli were evaluated prior to and every other day following pump implantation. Site of action was evaluated in adrenalectomized rats receiving sustained OR486 or in intact rats receiving sustained βAR antagonists peripherally, spinally or centrally alongside sustained OR486.

Results—We found that male and female rats receiving sustained OR486 exhibited mechanical allodynia, mechanical hyperalgesia and thermal hyperalgesia that lasted throughout the 2-week period. In contrast, adrenalectomized rats failed to develop COMT-dependent pain. Furthermore, peripheral, but not spinal or central, administration of the non-selective βAR antagonist propranolol, β_2AR antagonist ICI-118,511 or β_3AR antagonist SR59230A blocked the development of COMT-dependent pain.

Conclusions—These results demonstrate that peripheral adrenergic input is necessary for the development of persistent COMT-dependent pain, and that peripherally-acting βAR antagonists may benefit patients with chronic pain disorders.

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Introduction

Chronic pain disorders including fibromyalgia (FM), headache, temporomandibular disorder (TMD) and vestibulodynia (VBD) constitute a significant healthcare problem, affecting over 100 million Americans.[1-7] These disorders occur more frequently in females than males[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 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] Furthermore, functional variants in the COMT gene that reduce COMT activity[13,15,16] are associated with increased susceptibility to FM,[17-21] TMD[22] and experimental pain[22,23] as well as impaired response to treatment.[24,25] 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.[26,27]

Consistent with clinical disorders, our lab found that administration of the COMT inhibitor OR486 in rodents produces increased pain at multiple body sites and altered cognitive-affective behaviors linked to pain (e.g. avoidance of painful heat and bright light).[28-30] Pharmacologic studies further revealed that COMT-dependent pain is blocked by administration of the non-selective βAR antagonist propranolol or by combined administration of selective β_2- and β_3AR antagonists.[28-30] These results are in line with those from clinical studies, showing that propranolol alleviates pain among FM and TMD patients.[31,32] 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 (GPCRs) expressed in peripheral, spinal and central regions where they could drive pain. β_2ARs are located on peripheral terminals[33-37] and cell bodies[38-40] of primary afferent nociceptors; keratinocytes;[41-43] immune cells;[44-47] and adipocytes[48] in the periphery and neurons[49,50] and glial cells[51] in the central nervous system. β_3ARs are located on primary afferent nociceptors,[52] adipocytes[48] and immune cells[45,46] in the periphery and noradrenergic neurons in brain.[53] Thus, we hypothesized that peripheral, spinal, and/or central 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 (i.pl.), intrathecal (i.t.), or intracerebroventricular (i.cv.) 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 pain, persisting for 2-weeks. In contrast, adrenalectomized rats failed to develop COMT-dependent pain. Furthermore, i.pl., but not i.t. or i.c.v., administration of the non-selective βAR antagonist propranolol, β_2AR

antagonist ICI118,551, or β_3AR antagonist SR59230A blocked COMT-dependent pain. 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, Raleigh, NC) 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 400g 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 (IACUC) at the University of North Carolina at Chapel Hill (UNC).

General Experimental Conditions

First, the effects of sustained COMT inhibition on pain were evaluated in intact rats receiving the COMT inhibitor OR486 or vehicle systemically for 14 days *via* a 2002 Alzet osmotic pump (Durect Corporation, Cupertino, CA). Next, the contribution of peripheral adrenergic systems to persistent COMT-dependent pain 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 central βARs to persistent COMT-dependent pain was evaluated in separate groups of intact rats receiving i.pl., i.t. or i.c.v.. 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 prior to testing. Responses to punctuate mechanical and thermal stimuli were assessed in intact and adrenalectomized animals 1 day prior to and on days 1, 3, 5, 7, 9, 11 and 13 following pump implantation. For βAR antagonist experiments, pain behaviors were assessed 1 day prior to and on days 2, 4, 6, 8, 10, 12 and 14 following 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 10-15 minutes.

Drug Preparation

OR486 (Tocris, Ellisville, MO) was dissolved in a 5:3:2 ratio of dimethylsulfoxide (DMSO), 0.9% saline and ethanol.[30] For peripheral experiments βAR antagonists propranolol hydrochloride (Tocris, Ellisville, MO), ICI-118,511 (Tocris, Ellisville, MO) and SR59230A (Tocris, Ellisville, MO) were each dissolved in 5:3:2 ratios of DMSO, 0.9% saline and ethanol. For i.t. and i.c.v. experiments, βAR antagonists were dissolved in 0.9% saline. Drug

solutions were injected into pumps, which were placed in 15mL conical tubes containing sterile 0.9% saline and primed overnight in a dry heat bath (Lab Armor, Cornelius, OR) at 37°C. All pumps (other than those for i.t. delivery) were attached to corresponding catheters prior to priming. Subcutaneous delivery of OR486 was at a constant rate of 15mg/kg/day for a 2-week period. Peripheral delivery of propranolol hydrochloride was at 9mg/kg/day, ICI-118,511 was at 1.5mg/kg/day and SR59230A was at 1.67mg/kg/day. I.t. delivery of propranolol hydrochloride was at 50ug/day for the low dose experiments and 100ug/day for the high dose experiments; ICI-118,511 was at 30ug/day; and SR59230A was at 20ug/day. I.c.v. delivery of propranolol hydrochloride was at 50ug/day for the low dose experiments and 100ug/day for the high dose experiments; ICI-118,511 was at 30ug/day; and SR59230A was at 20ug/day.

Surgical Procedures

For all surgical procedures, rats were anesthetized by isoflurane inhalation (5% induction, 1.5-5% maintenance). Incision sites were shaved and disinfected with ethanol and betadine. Sterile technique was employed throughout the duration of all procedures according to IACUC requirements. Stainless steel wound clips (Braintree Scientific, Braintree, MA) 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 i.pl. delivery of βAR antagonists, a modified version of the protocol published by Haddad et al[54] was used. Pumps were attached to a Y-shaped, bifurcated 3F silicone catheter (SAI Infusion Technologies, Libertyville, IL). The pump was implanted subcutaneously over the right shoulder blade and a stainless steel 10G × 20cm semi-blunt tip trocar (SAI Infusion Technologies, Libertyville, IL) 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, Mettawa, IL).

For i.t. delivery[55] of βAR antagonists, a small incision was made on the nape of the neck, and scissors and hemostats were used in order to lift muscle and expose the atlanta-occipital membrane. The membrane was carefully incised using the tip of scissors, causing the escape of cerebral spinal fluid (CSF). A polyurethane Alzet Short Rat IT Catheter (Durect Corporation, Cupertino, CA) was inserted into the intathecal space, dorsal to the spinal cord. The other end of the catheter was sutured to surrounding tissue and attached to the osmotic pump, which was subcutaneously implanted over the right shoulder blade.

For i.c.v. delivery[56] 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, Cupertino, CA). The cannula was implanted into the right lateral ventricle (from the bregma: -0.8mm anteroposterior, -1.6mm mediolateral, -5mm 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.[57] 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 15.0g; Stoelting, Wood Dale, IL) were applied to the plantar hind paw. First, the middle filament (3.4g) was applied to the hind paw for 3 seconds. 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 6 total responses were recorded for each paw. Results were entered into the Paw Flick module within the National Instruments LabVIEW 2.0 software (LabVIEW, Austin, TX), which uses a logarithmic algorithm in order 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 normally noxious mechanical stimulus (15.0g) applied to the hind paw 10 times for a duration of 1 second, with an interstimulus interval of 1 second.[30] The number of paw withdrawals was recorded for each hind paw. 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.[58] 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 seconds of the first, a third measure was recorded. The 2 latencies closest in value were averaged in order to determine overall latency to withdrawal. Thermal behavioral data is 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

Mechanical allodynia, mechanical hyperalgesia and thermal hyperalgesia data were analyzed by 2-way analysis of variance (ANOVA). 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, La Jolla, CA).

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 pain in rats.[30] To evaluate the effects of sustained COMT inhibition on pain, responses to

mechanical and thermal stimuli were measured in separate groups of rats receiving systemic OR486 (15mg/kg/day) or vehicle over a 2-week period. Compared to rats receiving vehicle, those receiving OR486 exhibited mechanical allodynia ($F_{7,368}$=3.020, p=0.0043; Fig 1A), mechanical hyperalgesia ($F_{7,368}$=2.653, p=0.0109; Fig 1B) and thermal hyperalgesia ($F_{7,272}$=4.891, 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 $F_{1,80}$=101.6, p<0.0001 and Female $F_{1,80}$=135.4, p<0.0001; Fig 1A, Supplemental Digital Content), mechanical hyperalgesia (Male $F_{1,80}$=76.01, p<0.0001 and Female $F_{1,80}$=78.18, p<0.0001; Fig 1B, Supplemental Digital Content) and thermal hyperalgesia (Male $F_{1,56}$=88.98, p<0.0001 and Female $F_{1,56}$=97.66, p<0.0001; Fig 1C, Supplemental Digital Content).

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 central 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 (15mg/kg/day) 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 ($F_{3,720}$=114.5, p<0.0001; Fig 2A), mechanical hyperalgesia ($F_{3,736}$=36.52, p<0.0001; Fig 2B) and thermal hyperalgesia ($F_{3,720}$=73.75, 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 $F_{1,160}$=72.50, p<0.0001 and Female $F_{1,176}$=223.70, p<0.0001; Fig 2A, Supplemental Digital Content), mechanical hyperalgesia (Male $F_{1,160}$=8.009, p=0.0053 and Female $F_{1,176}$=118.7, p<0.0001; Fig 2B, Supplemental Digital Content) and thermal hyperalgesia (Male $F_{1,160}$=87.99, p<0.0001 and Female $F_{1,176}$=134.20, p<0.0001; Fig 2C, Supplemental Digital Content). Both male and female adrenalectomized rats failed to develop mechanical allodynia (Fig 2D, Supplemental Digital Content), mechanical hyperalgesia (Fig 2E, Supplemental Digital Content) and thermal hyperalgesia (Fig 2F, Supplemental Digital Content).

Peripheral βAR antagonist administration prevents the development of persistent COMT-dependent pain

Adrenalectomized rats fail to develop persistent COMT-dependent pain, 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 central βARs to persistent COMT-dependent pain. First, the contribution of peripheral βARs to mechanical and thermal pain was evaluated in separate groups of rats receiving sustained i.pl. administration of propranolol (9mg/kg/day), ICI-118,551 (1.5mg/kg/day), SR59230A (1.67mg/kg/day), or vehicle alongside sustained systemic administration of OR486 (15mg/kg/day) 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.

Compared to rats receiving vehicle, those receiving sustained i.pl. administration of the non-selective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A alongside systemic OR486 did not develop mechanical allodynia (Fig 3A, $F_{2,164}$=72.87, p<0.0001; Fig. 3D, $F_{2,164}$=65.43, p<0.0001; Fig 3G, $F_{2,164}$=90.51, p<0.0001) or mechanical hyperalgesia (Fig 3B, $F_{2,162}$=41.76, p<0.0001; Fig 3E, $F_{2,162}$=43.15, p<0.0001; Fig 3H, $F_{2,162}$=61.97, p<0.0001). Rats receiving sustained i.pl. administration of the β_3AR antagonist SR59230A also did not develop OR486-induced thermal hyperalgesia (Fig 3I, $F_{2,163}$=46.24, P<0.0001). In contrast, rats receiving propranolol (Fig 3C) or ICI-118,551 (Fig 3F) alongside OR486 exhibited a 15% decrease in paw withdrawal latency from baseline, similar to rats receiving vehicle.

Intrathecal βAR antagonist administration does not alter persistent COMT-dependent pain

Next, the contribution of spinal βARs to mechanical and thermal pain was evaluated in separate groups of rats receiving sustained i.t. administration of propranolol (50ug/day), ICI-118,551 (30ug/day), SR59230A (20ug/day), or vehicle alongside sustained systemic administration of OR486 (15mg/kg/day) or vehicle over a 2-week period. Intrathecal delivered antagonist doses were selected based on their ability to block pain or related behaviors in other rat models when administered i.t..[59-61] Similar to animals receiving vehicle, those receiving sustained i.t. administration of the non-selective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A alongside systemic OR486 exhibited mechanical allodynia (Fig 4A, $F_{2,159}$=16.63, p<0.0001; Fig 4D, $F_{2,158}$=16.60, p<0.0001; Fig 4G, $F_{2,173}$=16.13, p<0.0001), mechanical hyperalgesia (Fig 4B, $F_{2,164}$=9.149, p=0.0002; Fig 4E, $F_{2,165}$=13.33, p<0.0001; Fig 4H, $F_{2,181}$=6.544, p=0.0018) and thermal hyperalgesia (Fig 4C, $F_{2,164}$=85.26, p<0.0001; Fig 4F, $F_{2,164}$=86.01, p<0.0001; Fig 4I, $F_{2,178}$=81.55, p<0.0001).

To confirm that i.t. βAR antagonists were unable to block OR486-induced pain, we performed a duplicate set of experiments using a higher dose of the non-selective βAR antagonist propranolol (100ug/day). Similar to the original dose, i.t. administration of the higher dose did not block OR486-induced mechanical allodynia ($F_{3,182}$=17.42, p<0.0001; Fig 3A, Supplemental Digital Content), mechanical hyperalgesia ($F_{3,188}$=5.552, p=0.0011; Fig 3B, Supplemental Digital Content) or thermal hyperalgesia ($F_{3,187}$=50.96, p<0.0001; Fig 3C, Supplemental Digital Content).

Intracerebroventricular βAR antagonist administration does not alter persistent COMT-dependent pain

Finally, the contribution of central βARs to mechanical and thermal pain was evaluated in separate groups of rats receiving sustained i.c.v. administration of propranolol (50ug/day), ICI-118,551 (30ug/day), SR59230A (20ug/day), or vehicle alongside sustained systemic administration of OR486 (15mg/kg/day) or vehicle over a 2-week period. I.c.v. antagonist doses were selected based on their ability to block pain or related behaviors in other rat models.[59-61] Similar to animals receiving vehicle, those receiving sustained i.c.v.

administration of the non-selective βAR antagonist propranolol, the β_2AR antagonist ICI-118,511, or the β_3AR antagonist SR59230A alongside systemic OR486 exhibited mechanical allodynia (Fig 5A, $F_{2,155}$=42.51, p<0.0001; Fig 5D, $F_{2,157}$=57.15, p<0.0001; Fig 5G, $F_{2,157}$=48.66, p<0.0001), mechanical hyperalgesia (Fig 5B, $F_{2,165}$=41.6, p<0.0001; Fig 5E, $F_{2,164}$=51.30, p<0.0001; Fig 5H, $F_{2,164}$=55.35, p<0.0001) and thermal hyperalgesia (Fig 5C, $F_{2,164}$=31.36, p<0.0001; Fig 5F, $F_{2,163}$=43.52, p<0.0001; Fig 5I, $F_{2,165}$=22.98, p<0.0001).

To confirm that i.c.v. βAR antagonists are unable to block OR486-induced pain, we performed a duplicate set of experiments using a higher dose of the non-selective βAR antagonist propranolol (100ug/day). Similar to the original dose, i.c.v. administration of the higher dose did not block OR486-induced mechanical allodynia ($F_{3,193}$=43.70, p<0.0001; Fig 4A, Supplemental Digital Content), mechanical hyperalgesia ($F_{3,205}$=28.26, p<0.0001; Fig 4B, Supplemental Digital Content) or thermal hyperalgesia ($F_{3,203}$=61.76, p<0.0001; Fig 4C, Supplemental Digital Content).

Discussion

Though 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 behaviors are mediated *via* peripherally, but not spinally or centrally, located β_2- and β_3ARs.

In previous studies, we established a causal link between low COMT and pain. Specifically, we demonstrated that a single injection of the COMT inhibitor OR486 produces pronounced increases in behavioral responses to mechanical and thermal stimuli, similar to that produced by intraplantar carrageenan. Subsequent pharmacological studies further demonstrated that the development of acute COMT-dependent pain requires the activation of β_2- and β_3ARs.[28,30] Within hours, administration of OR486 results in increased circulating levels of nitric oxide (NO) and the pro-inflammatory cytokines tumor necrosis factor-α (TNFα), interleukin 1β (IL-1β), interleukin 6 (IL-6), and chemokine (C-C motif) ligand 2 (CCL2),[28] which are nociceptive transmitters implicated in chronic pain disorders. Individuals with FM, headache and TMD exhibit increased levels of these molecules,[62-65] which elicit pain by reducing nociceptor firing thresholds.[66-76] NO and pro-inflammatory cytokines also elicit pain by working synergistically to potentiate one another's biosynthesis, as we observed in the OR486 model.[28]

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 that lack peripheral epinephrine fail to develop OR486-induced mechanical and thermal pain. This finding is in line with those from previous studies showing that adrenalectomized rats have blunted pain responses following formalin

administration[77] or chronic constriction injury.[78] Together, these results suggest that peripherally circulating catecholamines contribute to the transmission of pain in models of inflammatory and neuropathic pain as well as chronic pain disorders. This conclusion is further supported by studies that have demonstrated increased levels of urinary catecholamines in patients with myofacial pain[10] and increased circulating plasma catecholamines in women with FM.[9]

As previous preclinical and clinical studies have reported sex-specific differences in COMT-related phenotypes[79-83] and as males and females exhibit different COMT expression patterns,[84,85] we examined the contribution of peripheral adrenergic systems to COMT-dependent pain in both males and females. Counter to our expectation, male and female rats exhibited a comparable increase in mechanical and thermal pain following sustained systemic OR486 administration, which was blocked by suppressing peripheral adrenergic tone. Our findings emphasize the importance of examining chronic pain disorders in the context of both males and females, while continuing to consider possible gender-specific effects of COMT-dependent pain in future studies and clinical applications.

The independent contribution of peripheral, spinal and central βARs to persistent COMT-dependent pain were next examined in separate groups of intact rats receiving targeted delivery of the non-selective βAR antagonist propranolol, the selective β_2AR antagonist ICI-118,551, or the selective β_3AR antagonist SR59230A alongside systemic OR486. We found that peripheral, but not spinal or central, administration of propranolol, ICI-118,511 or SR59230A blocked the development of COMT-dependent pain throughout the duration of the 2-week testing period. While all three antagonists blocked the development of mechanical pain, only SR59230A blocked the development of thermal heat pain. These findings significantly extend those from earlier acute COMT inhibition studies,[28,30] demonstrating that peripheral β_2- and β_3ARs both contribute to the development of persistent mechanical pain, while peripheral β_3ARs independently contribute to the development of persistent thermal pain following 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.[33-37] Also, elevations in plasma levels of norepinephrine activate β_2ARs to promote visceral hypersensitivity.[36] In humans, variants of the β_2AR gene known to influence receptor expression are associated with increased risk of TMD.[86]

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.[87] 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 behavior.[88] Recently, β_3ARs have also been shown to mediate formalin-induced TMJ pain.[89] In contrast to acute COMT-dependent thermal pain, which requires coincident activation of both β_2- and β_3ARs,[30] persistent COMT-dependent thermal pain requires independent activation of peripheral β_3ARs. Unlike most GPCRs, including

β_2ARs, β_3ARs do not undergo desensitization after agonist stimulation.[90,91] 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 on numerous peripheral cell types where 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, following their activation by epinephrine or norepinephrine, orchestrate inflammatory responses. Increased catecholamine levels following stress or pharmacologic manipulation lead to activation of T-cells, increased expression of β_2- and β_3ARs,[47] and production of IL-1, IL-6, and CCL2.[92] T-cell infiltration in the spinal dorsal horn of adult rats has been shown to contribute to pain following nerve injury.[93,94] In line with these findings, patients with FM have more activated T-cells circulating in blood compared to healthy controls.[95] Epinephrine activates mast cells and stimulates release of IL-1β, IL-6 and other pro-inflammatory cytokines in a β_2AR-dependent manner.[44] Increased activation of mast cells has been observed in numerous chronic pain disorders, including FM, headache, vestibulodynia, and irritable bowel syndrome.[96-101] Agonist activation of β_2ARs expressed on macrophages *in vitro* results in activation of intracellular kinases and subsequent release of IL-6. In line with these findings, sustained systemic administration of epinephrine in mice results in β_2AR-mediated increases in the activation of macrophages and production of IL-6.[45,46]

Adipocytes are cells in the periphery that express both β_2- and β_3ARs and specialize in storing energy as fat.[48] In addition, they interface with immune cells to regulate inflammatory responses.[102] Notably, adipocytes produce 30% of the IL-6 circulating in the body[103] and studies have shown that activation of β_3ARs on adipocytes produces a robust increase in IL-6 levels in plasma[104] as well as in TNFα,[105] CCL2,[106] and NO[107] 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 β_2ARs expressed on keratinocytes results in increased activation of intracellular kinases and release of IL-6.[41-43] Similarly, activation of satellite glia by catecholamines results in βAR-mediated increases in intracellular cyclic nucleotides that facilitate neuronal-glial communication.[108]

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 FM and TMD.[31,32,109] 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_2DARs in the spinal dorsal horn.[110,111] Thus, catecholamines can exert divergent influences on nociception as a function of localization and net influence on neuronal excitability. 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 pain. 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 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. complex regional pain syndrome[112]).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What we already know about this topic

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

What this article tells us that is new

- In rats, sustained administration of a COMT 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

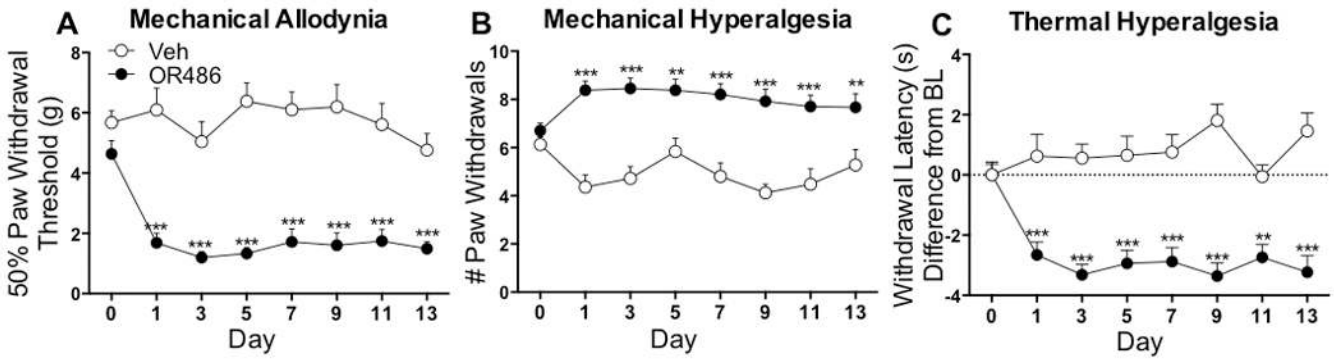

Figure 1.

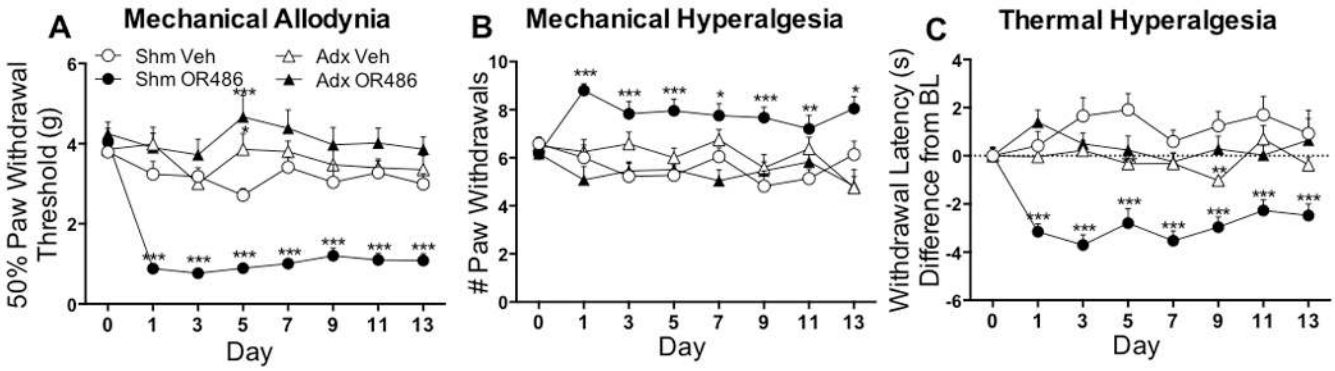

Figure 2.

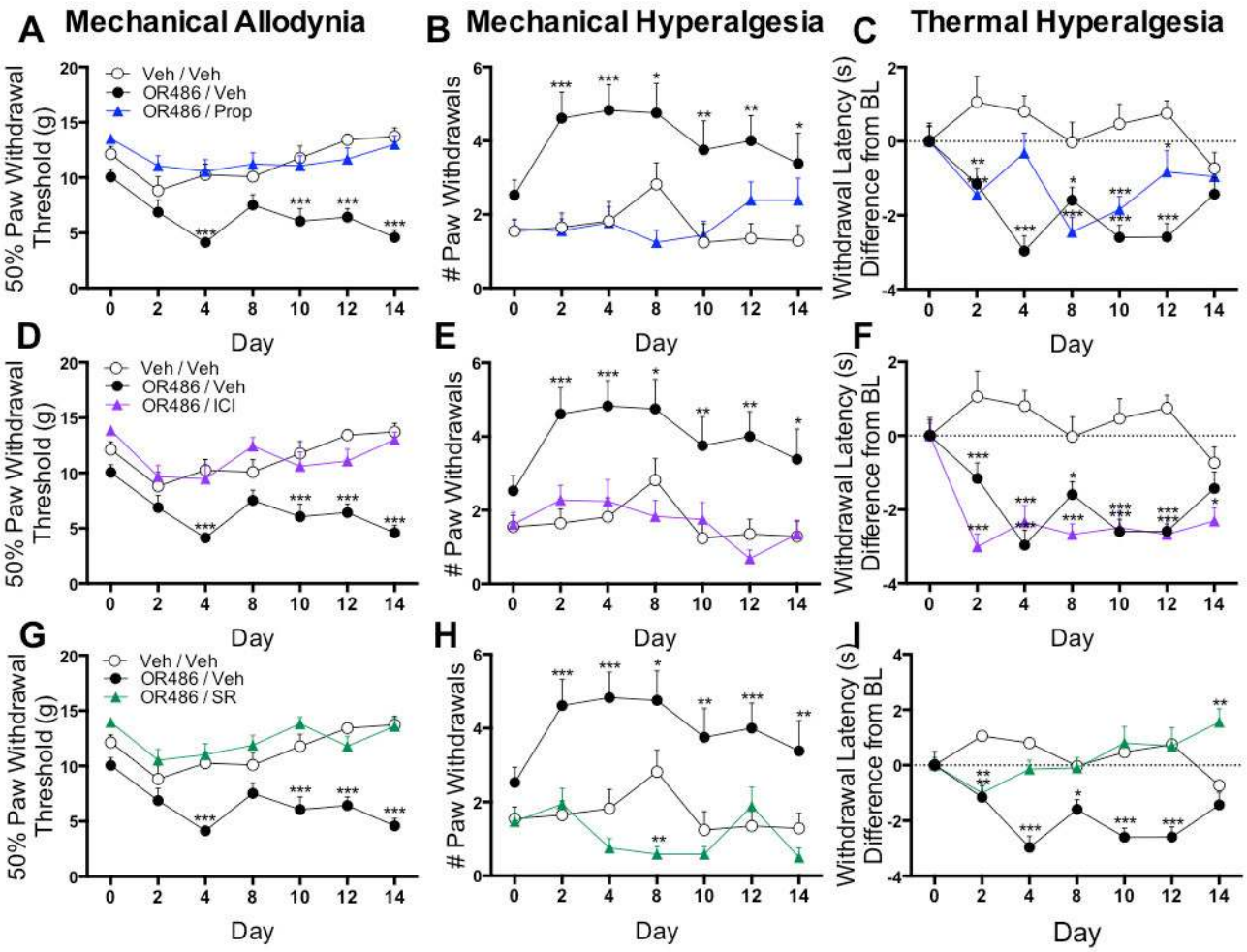

Figure 3.

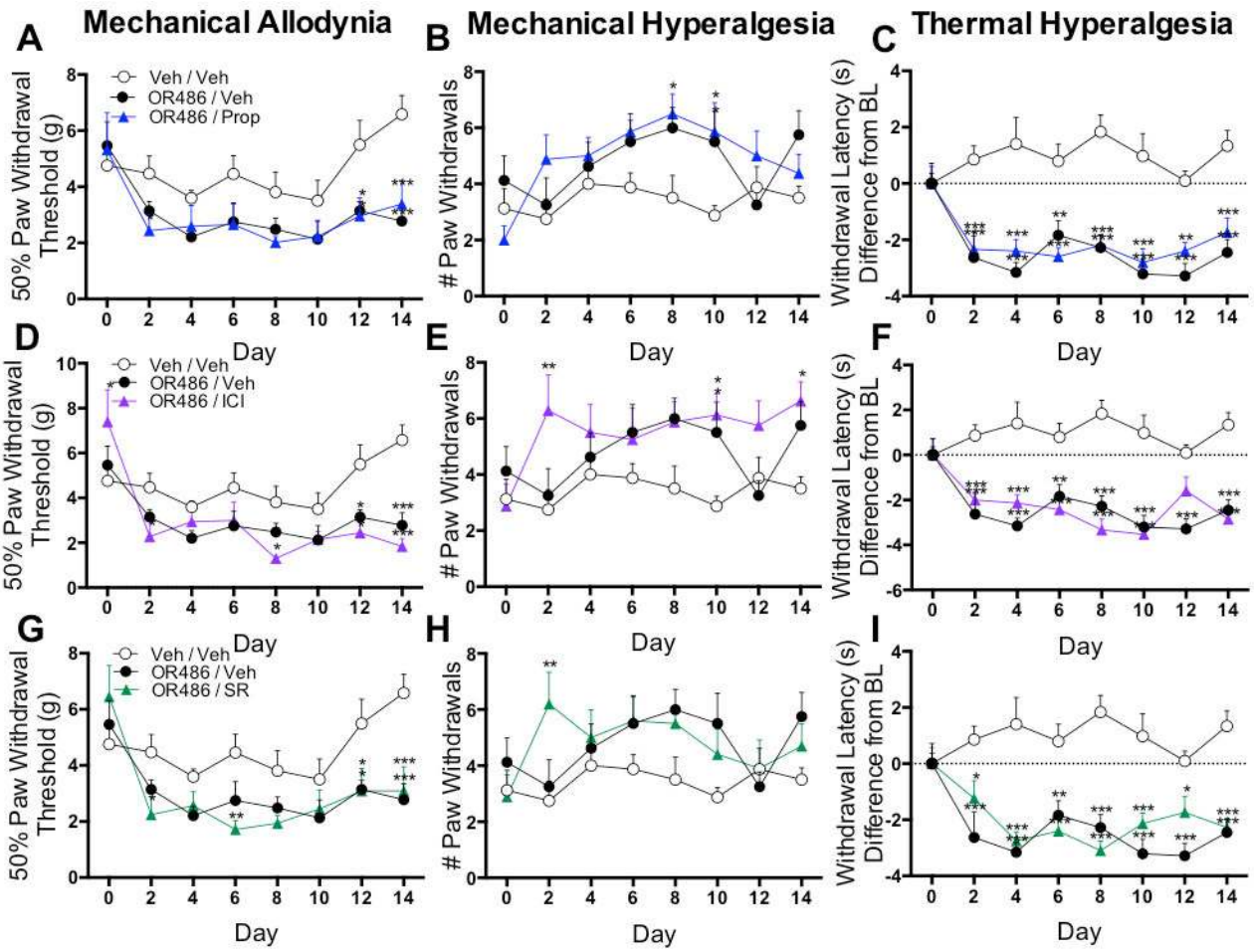

Figure 4.

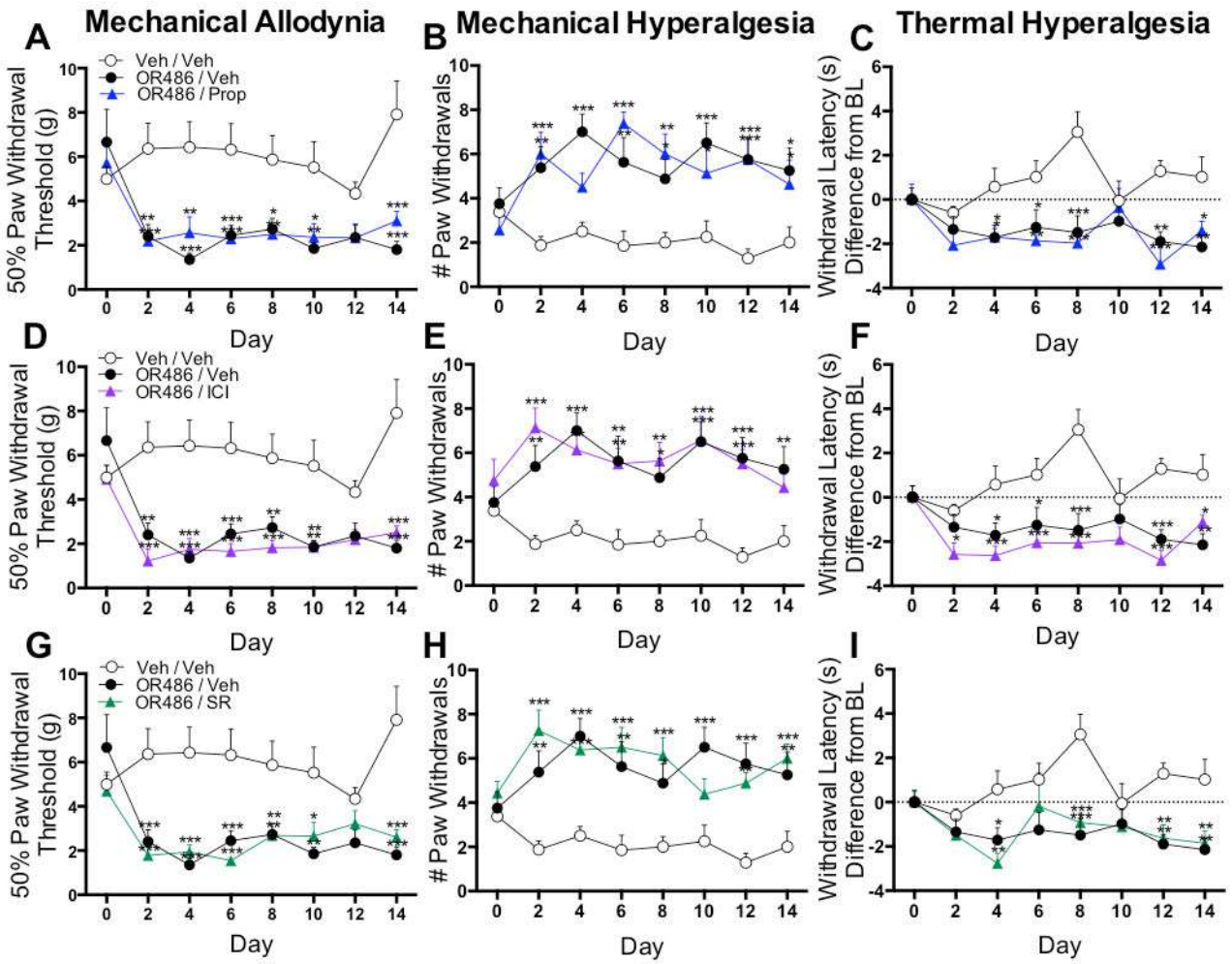

Figure 5.