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Potent analgesic effects of a store-operated calcium channel inhibitor

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Abstract

Chronic pain often accompanies immune responses and immune cells are known to be involved in chronic pain. Store-operated calcium (SOC) channels are calcium-selective cation channels and play an important role in the immune system. YM-58483, a potent SOC channel inhibitor, has been shown to inhibit cytokine production from immune cells and attenuate antigen-induced hypersensitivity reactions. Here, we report that YM-58483 has analgesic actions in chronic pain and produces antinociceptive effects in acute pain and prevents the development of chronic pain in mice. Oral administration of 10 mg/kg or 30 mg/kg YM-58483 dramatically attenuated Complete Freund's adjuvant (CFA)-induced thermal hyperalgesia and prevented the development of thermal and mechanical hypersensitivity in a dose-dependent manner. Analgesic effects were observed when YM-58483 administered systemically, intrathecally, and also intraplantarly. YM-58483 decreased spared nerve injury (SNI)-induced thermal and mechanical hypersensitivity and prevented the development of SNI-induced pain hypersensitivity. Pretreatment with YM-58483 strongly reduced both the first and second phases of formalin-induced spontaneous nocifensive behavior dose-dependently. YM-58483 produced antinociception in acute pain induced by heat or chemical or mechanical stimuli at the dose of 30 mg/kg. YM-58483 diminished CFA-induced paw edema, and reduced production of TNF- α , IL-1 β and PGE₂ in the CFA-injected paw. In vitro, SOC entry in nociceptors was more robust than in nonnociceptors, and the inhibition of SOC entry by YM-58483 in nociceptors was much greater than in non-nociceptors. Our findings indicate that YM-58483 is a potent analgesic and suggest that SOC channel inhibitors may represent a novel class of therapeutics for pain.

Keywords

Store-operated calcium channels; Pain; Inflammation; YM-58483; Dorsal root ganglia; Tumor necrosis factor-

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1. Introduction

Chronic pain continues to be a major public health concern and the underlying mechanisms remain to be determined. Chronic pain can result from tissue damage or injury and is often associated with inflammation and immune responses. Evidence suggests that immune cells play important roles in chronic pain [1; 5; 25; 26; 39]. For example, rats lacking mature T cells showed a significant reduction in mechanical allodynia and thermal hyperalgesia following chronic constriction injury (CCI) [39]. Immune cells have been shown to infiltrate into the injury site, the dorsal root ganglion (DRG) and the spinal cord after peripheral tissue or nerve injury [5; 10; 27]. These cells produce proinflammatory cytokines, which have been indicated as mediators of inflammation and pain hypersensitivity [10; 66; 67].

Store-operated calcium (SOC) channels are highly Ca^{2+} -selective cation channels that are activated by the release and depletion of calcium from endoplasmic reticulum (ER) stores [48]. Activation of SOC channels leads to sustained high levels of cytosolic Ca^{2+} , which is required for many calcium-dependent cellular processes. SOC entry is a major mechanism for triggering Ca^{2+} influx in immune cells [21]. SOC channels have been implicated as critical players in immune and inflammatory diseases [13; 43; 46; 53; 68]. The SOC channel proteins STIM1 and STIM2 (stromal interaction molecules) and Orai1/2/3 were identified recently [16; 35; 54; 65; 69]. Deficiency of STIMs significantly impairs the generation of antigen-specific T-cell responses and prevents the development of autoimmune encephalomyelitis [58]. A recent study suggested that SOC channels are expressed in DRG neurons, and their function is enhanced in rat DRG neurons in a neuropathic pain model [18]. Despite the important physiological and pathological role of SOC channels in the immune system, their role in the central nervous system is largely unknown.

The function of SOC channels in pain would be facilitated by the availability of specific inhibitors. Unfortunately, most of the commercially available SOC channel inhibitors, such as lanthanum, SKF 96365, and 2-aminoethyldiphenylborate, are nonspecific or have complex effects on SOC entry [24; 37; 38; 47; 60; 70]. Two groups identified YM-58483, a pyrazole derivative, as a potent inhibitor of store-operated Ca^{2+} entry [29; 71]. YM-58483 appeared selective because it does not affect Ca^{2+} handling by mitochondria or ER or other channel activities, such as K^{+} channels or voltage-operated Ca^{2+} channels [29; 71]. YM-58483 blocks SOC entry in T-lymphocytes and other immune cells and reduces cytokine production from these cells [33; 71]. Previous studies have also shown that YM-58483 inhibits the sheep red blood cell-induced delayed-type hypersensitivity response and reduces antigen-induced bronchial asthma [42; 43; 68]. However, the effects of YM-58483 on immune cells in pain models have not been examined.

Given that chronic pain is associated with immune responses and SOC channels play important roles in these responses, we hypothesized that SOC channel inhibitors have analgesic effects on pain hypersensitivity. Here, we demonstrated that YM-58483 relieved complete Freund's adjuvant (CFA)- and spared nerve injury (SNI)- induced pain hypersensitivity, attenuated formalin-induced spontaneous nociceptive behavior, and reduced mechanical, heat and chemical stimuli-induced acute pain. These findings indicate that the SOC channel inhibitor produces antinociception and anti-inflammation and may represent a novel class of analgesics.

2. Methods

2.1. Behavioral tests

All behavioral tests were performed using 7- to 10-week-old CD-1 or C57BL/6 male mice purchased from Charles River (Wilmington, MA). CD1 mice were used for thermal sensitivity, capsaicin tests, and paw edema measurements. C57BL/6 mice were used for mechanical sensitivity and formalin tests. Experiments were done in accordance with the guidelines of the National Institutes of Health and with the guidelines of the Committee for Research and Ethical Issues of IASP and were approved by the Animal Care and Use Committee of Drexel University College of Medicine. Mice were housed in 12 h/12 h light/dark cycles, and habituated in the testing room 2-3 days before experiments. All experiments were performed with the experimenter blind to the drug treatments.

2.1.1. Complete Freund's adjuvant model—CFA-induced inflammatory pain was measured as previously described [44]. Twenty μL of 50% CFA (Sigma) was injected subcutaneously into the plantar surface of the animal's right hind paw. Mechanical sensitivity was measured using a series of von Frey filaments (North Coast Medical, Inc., Gilroy, CA) as previously described [23]. The smallest monofilament that evoked paw withdrawal responses on three out of five trials was taken as the mechanical threshold. Thermal sensitivity was measured using the Hargreaves' method as previously described [23]. The baseline latencies were set to around 10 seconds with a maximum of 20 seconds as the cutoff to prevent potential injury. The latencies were averaged over three trials, separated by 30-minute intervals.

Paw volume was measured before and 24 hr after injection of CFA by plethysmometry using a modified plethysmometer as described previously [8]. Briefly, the mouse hind paw was dipped into a water column up to the junction of hairy and glabrous skin (labeled with a waterproof color marker), which was connected by tubing to a 1 ml syringe with a resolution of 10 μL . Paw volume was measured in triplicate before and after CFA injection. Paw edema was determined by subtraction of preinjection from postinjection volume.

2.1.2. Formalin model—The formalin test was performed as previously described [44]. C57BL/6 mice were habituated for 1-2 h in a transparent Plexiglas test box ($5 \times 5 \times 10$ inches) before any injections. Twenty μL of 2% formalin solution (Sigma) was injected subcutaneously into the plantar surface of the right hind paw, and the mouse was returned to the test box immediately. The total time that the animal spent in spontaneous nociceptive behavior (licking and lifting of the formalin-injected paw) was recorded in 5-minute intervals for 1 hour.

2.1.3. Spared nerve injury model—SNI was performed as described previously [11]. Briefly, mice were anesthetized under isoflurane; the common peroneal and tibial nerves of the left paw were ligated and sectioned distal to the ligation. The distal nerve stump (2 to 4 mm) was removed. The sham surgical procedure was identical to that performed on the SNI group, but no ligature and section were made. Mechanical and thermal sensitivity were measured before and after SNI using von Frey filaments and the Hargreaves' method at different time points.

2.1.4. Acute pain—The tail-flick test was performed by applying focused, radiant heat to the tail as previously described [50]. Mice were loosely held with soft tissues for 1 minute each time (more than three times) before testing. The withdrawal response was detected using the tail-flick test analgesia meter (model 33, IITC Life Science, Woodland Hills, CA). Noxious heat was applied to the caudal end (2.5 cm from the tip) of the tail. The intensity of

heat was adjusted to evoke a tail flick baseline latency of approximately 5 to 6 seconds. A cutoff time of 10 seconds was set to avoid tissue damage. The baseline latencies were averaged over three trials, separated by 30-minute intervals before drug administration.

The paw pressure test (the Randall–Selitto test) was conducted using the Basile Analgesy-Meter (model 37215, Ugo Basile, Comerio, Italy). C57BL/6 mice were held with soft tissues for 2 minutes each time (six times) to get used to manipulation before testing. Animal paws were probed with a round probe to the medial portion of the dorsal surfaces of hind paws until a withdrawal response resulted. The force at which the animal withdrew the paw or struggled was recorded as a threshold. A cutoff of 200 g was set to avoid tissue damage or injury. The baseline thresholds were averaged over three trials, separated by 60-minute intervals before drug administration.

The chemically-induced acute pain was tested in naive mice by intraplantar injection of capsaicin. Mice were placed individually in a Plexiglas cage for at least 1 h before injection of capsaicin (10 μ L, 1 nmol). The intensity of nociceptive behavior was quantified by recording the total time that the animal spent licking or lifting the injected paw with a stopwatch for 5 minutes immediately after the injection of capsaicin.

2.2. Cell culture

Primary cultures of DRG neurons were prepared as described previously [22]. Briefly, DRGs were removed from 6- to 8-week-old CD-1 mice and collected in cold (4°C) Hank's balanced saline solution (HBSS; Life Technologies, Grand Island, NY). Ganglia were incubated in 15 U/mL papain/L-cysteine and 1.5 mg/mL collagenase in HBSS for 30 minutes at 37°C. After they were washed with neurobasal medium (Life Technologies), ganglia were gently triturated with a flame-polished Pasteur pipette until the solution turned cloudy. The dispersed cells were plated at a density of 3000 cells per well on 12-mm glass coverslips coated with poly-D-lysine and collagen (Sigma, St. Louis, MO). Cultures were maintained for 24 to 48 h in growth medium containing neurobasal medium supplemented with 2% B-27, 2% fetal bovine serum, 2% horse serum and 2 mM L-alanyl-L-glutamine dipeptide (Glutamax) (all from Life Technologies) at 37°C in humidified air with 5% CO₂.

2.3. Calcium imaging

The changes in intracellular Ca²⁺ concentration were examined using fura-2 based microfluorimetry and imaging analysis as previously described [30]. Cells were loaded with 4 μ M fura-2AM (Invitrogen, Burlington, ON, Canada) for 30 minutes at room temperature in HBSS, washed and further incubated in HBSS for an additional 20 minutes. Coverslips were mounted in a small laminar-flow perfusion chamber (Model RC-25; Warner Instruments, Hamden, CT) and continuously perfused at 4 to 5 mL/minute with a normal Tyrode's solution containing (mM) 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 5.6 glucose. Images were acquired at 3-second intervals at room temperature using an Olympus inverted microscope equipped with a CCD camera (ORCA-03G; Hamamatsu, Japan). The 340/380 ratios were recorded and analyzed using the software Metafluor 7.7.9 (Molecular Devices, Sunnyvale, CA). The excitation shutter was closed between acquisitions to prevent photobleaching.

2.4. Measurement of Tumor necrosis factor- α (TNF- α) and interleukin-1b (IL-1 β)

Mice were anesthetized with isoflurane after behavioral testing 24 h following CFA injection. Blood samples (about 1 mL) were collected from the orbital venous sinus and the injected paws were removed. Blood samples were centrifuged at 2000 rpm for 20 minutes. The entire paw was weighed and homogenized using a tissuemiser (Thermo Fisher Scientific, Pittsburgh, PA) with three volumes of buffer containing (mM) 50 NaCl, 10 Tris,

2.5 MgCl₂, 0.1 phenylmethanesulfonyl fluoride, and protease inhibitor cocktails (Thermo Fisher Scientific, Rockford, IL), pH adjusted to 7.4. The tissue samples were centrifuged at 10,000 rpm for 10 minutes. Serum samples and tissue supernatants were collected for the assay. TNF- α and IL-1 β concentrations were measured by ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, MN). The concentrations of total protein were determined using the Pierce bicinchoninic acid protein assay kit (Thermo Fisher Scientific) following the manufacturer's instructions. The TNF- α and IL-1 β concentrations were normalized to the total protein.

2.5 Measurement of prostaglandin E₂ (PGE₂)

Mice were euthanized after 24 h post CFA or saline injection. The entire injected paws were cut at the ankle joint and four incisions were made on both of plantar and dorsal surfaces of the hind paw with a #11 scalpel blade. The exudate was immediately collected by adding 1 ml PBS containing 20 μ g/ml indomethacin to each paw to avoid further activation of COX and centrifuged at 10,000 rpm for 10 minutes [17]. The PGE₂ level in the supernatant was measured by ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, MN).

2.6. Drug application

YM-58483 (4-methy-4'-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide) was purchased from Tocris (Minneapolis, MN). Cyclopiazonic acid (CPA), thapsigargin, indomethacin and capsaicin were purchased from Sigma. Compounds were dissolved in dimethyl sulfoxide (DMSO) as stock solutions and further diluted to final concentrations in 0.1% DMSO for in vitro application; in 1% DMSO saline solution for intraplantar injection of capsaicin; and in 2% DMSO/cremophor EL saline solution for intraplantar injection of YM-58483; in 3% DMSO/cremophor EL with 2% polysorbate 80/0.5% methylcellulose sterile water for oral administration of YM-58483 or indomethacin.

2.7. Data analysis

Data are presented as mean $\pm$ SEM. Treatment effects were statistically analyzed with a one- or two-way analysis of variance. Pair wise comparisons between means were tested using the post hoc Bonferroni method. Paired or two-sample t tests were used when comparisons were restricted to two means. Error probabilities of $P < .05$ were considered statistically significant. The statistical software Origin 8.1 (OriginLab Corp., Northampton, MA) was used to perform all statistical analyses.

3. Results

3.1. YM-58483 relieves CFA-induced thermal and mechanical hypersensitivity

To explore potential analgesic actions of YM-58483, the SOC channel inhibitor, we first tested the effect of YM-58483 on CFA-induced inflammatory pain with the same route of administration and dosage range used in previous studies [33; 68]. Injection of 20 μ L of 50% CFA into the hind paw of a CD-1 mouse produced thermal hyperalgesia in the ipsilateral paw, but not in the contralateral paw, which was consistent with our previous study [44]. A single dose of YM-58483 (10 and 30 mg/kg) relieved thermal hyperalgesia when orally administered on day 3 post-CFA injection; the effect was dose-dependent with the maximal effect occurring at 1 h and lasting for 2 h (Fig. 1A). CFA-treated mice developed robust mechanical allodynia after CFA injection. Similarly, YM-58483 dose-dependently diminished mechanical allodynia in C57BL/6 mice with the maximal effect occurring at 2 h and last for 3 h (Fig. 1B). These results demonstrate that YM-58483 has an

analgesic action on both thermal hypersensitivity and mechanical hypersensitivity in the CFA inflammatory pain model.

3.2. YM-58483 prevents the development of pain hypersensitivity

We also tested whether YM-58483 prevents the development of pain hypersensitivity. Thermal and mechanical sensitivities were measured before and at 2 and 24 hours after CFA injection. CD1 mice pretreated orally with YM-58483 or indomethacin (a commercial non-steroidal anti-inflammatory drug) 1 hour prior to CFA injection showed a reduction or absence of thermal hypersensitivity (Fig. 2A). The anti-hyperalgesia of YM-58483 was dose-dependent, lasted for 24 hours and was comparable to that of indomethacin. YM-58483-induced analgesic effect on mechanical allodynia was also observed in C57BL/6 mice and had greater efficacy than indomethacin (Fig. 2B). These results demonstrate that YM-58483 prevents the development of inflammatory pain.

3.3 YM-58483 attenuates CFA-induced inflammation

YM-58483 has been implicated as an immunomodulator [14; 63], and has been shown to inhibit the sheep red blood cell-induced delayed-type hypersensitivity response [42]. We sought to test whether YM-58483 has an anti-inflammatory effect. A single dose of YM-58483 or indomethacin (10 and 30 mg/kg) were orally administered 30 minutes prior to CFA injection in CD-1 mice. CFA-induced paw edema was measured 3 and 24 hours after CFA injection. YM-58483 significantly decreased CFA-induced paw edema (Fig. 3A). However, an indomethacin-induced anti-inflammatory effect was observed only at the high dose at both the 3 and 24 hour testing observations (Fig. 3A).

To investigate whether long-lasting analgesic and anti-inflammatory actions of YM-58483 are associated with proinflammatory cytokine and inflammatory mediator levels, we measured the TNF- α , IL-1 β and PGE₂ 24 hours after CFA injection in CD-1 mice. CFA dramatically increased TNF- α , IL-1 β and PGE-2 levels in the inflamed paw (Fig. 3B, C and D), but did not alter TNF- α level in the serum (data not shown). Oral administration of YM-58483 30 minutes prior to CFA injection significantly reduced TNF- α , IL-1 β and PGE₂ production in the inflamed paw (Fig. 3B, C and D). Indomethacin completely inhibited PGE₂ production, decreased IL-1 β level, however, increased TNF- α level in the inflamed paw. These results suggest that YM-58483-induced anti-inflammation and protection from the development of pain hypersensitivity are associated with reduction of proinflammatory cytokine and inflammatory mediator production.

3.4. YM-58483 has direct analgesic action at the spinal cord level

Based on the anti-inflammatory effect of YM-58483, we wanted to determine whether YM-58483 produces an analgesic effect at the peripheral level. Ten μ L of YM-58483 was administered subcutaneously at concentrations of 0.5 mM (5 nmol), 1 mM (10 nmol) or 2 mM (20 nmol) 10 minutes prior to CFA injection. Thermal hypersensitivity and paw edema were measured following 10 μ L CFA injection in CD-1 mice. Interestingly, peripheral treatment with YM-58483 did not prevent CFA injection-induced acute pain at 1 h time point, however, YM-58483 significantly reduced CFA-induced thermal hypersensitivity at the 2 and 3 hour time points and also decreased paw edema (Fig. 4A and B). We then examined the analgesic effect of YM-58483 at the spinal level. YM-58483 5 μ L was administered intrathecally at concentrations of 100 μ M (0.5 nmol) and 300 μ M (1.5 nmol). YM-58483 markedly diminished thermal hypersensitivity in a dose-dependent manner (Fig. 4C). These results suggest that YM-58483 produces the analgesic effect at both the spinal cord level and the peripheral levels.

3.5. YM-58483 reduces formalin-induced spontaneous nociception

The formalin test is a widely used model of nociception. It allows for an assessment of analgesic action and provides the ability to distinguish the site of action of analgesics [28; 59]. Formalin induces spontaneous biphasic nociceptive behavior. The first phase of nociception results from acute stimulation of nociceptors. The second phase is thought to be involved in central sensitization of dorsal horn neurons [9]. Injection of 20 μ L of formalin (2%) subcutaneously in the C57 BL/6 mouse hind paw resulted in intensive spontaneous licking or lifting of the injected paw with a classic biphasic response. Pretreatment with oral administration of YM-58483 strongly decreased both the first and second phases of formalin-induced nociception in a dose-dependent manner (Fig. 5A and B). The higher doses of YM-58483 completely diminished the second phase. This feature of blocking both phases is similar to that observed with centrally acting drugs such as narcotics.

3.6. YM-58483 attenuates SNI-induced thermal and mechanical hypersensitivity

Immune responses have been implicated in neuropathic pain [2; 34]. SNI is a well-established neuropathic pain model developed by Decosterd and Woolf [11]. To examine whether YM-58483 has an analgesic action on neuropathic pain, we tested the effect of YM-58483 on SNI-induced pain hypersensitivity. We performed SNI surgeries on mice and observed robust mechanical allodynia 24 hours after surgery and significant thermal hyperalgesia 3 days after surgery, which lasted for more than 1 month (data not shown). A single dose of YM-58483 10 and 30 mg/kg dramatically and dose-dependently reduced thermal hyperalgesia in CD-1 mice and mechanical allodynia in C57BL/6 mice when orally administered on day 7 post-surgery. The maximal antihyperalgesia effect occurred at 1 hour and lasted for 2 hours (Fig. 6A and B); however, the maximal antiallodynia effect occurred at 2 hours and lasted for 3 hours (Fig. 6B). In addition, mice receiving daily administration of 10 mg/kg YM-58483 for 7 days completely or partially prevented the development of thermal and mechanical hypersensitivity (Fig. 6C and D). These results demonstrate that YM-58483 not only attenuates established neuropathic pain, but also prevents the development of pain hypersensitivity.

3.7. YM-58483 reduces acute nociception

As demonstrated above, YM-58483 has analgesic effects in multiple chronic pain models. We sought to test whether YM-58483 changes pain sensitivity in naive mice. We first tested the effects of 10 mg/kg or 30 mg/kg YM-58483 in non-noxious mechanical sensation in C57BL/6 mice. Tactile threshold was measured with von Frey filaments. YM-58483 had a significant effect on nonnoxious mechanical threshold at the higher dose (30 mg/kg) (Fig. 7A), but not at 10 mg/kg (data not shown). We then tested the effect of YM-58483 on noxious mechanical stimulus-induced pain in C57BL/6 mice. Application of noxious pressure to the paw elicited a paw withdrawal response or struggling movement. Pretreatment of YM-58483 30 mg/kg increased the threshold of noxious mechanical stimulus (Fig. 7B). We also tested the effect of YM-58483 in the tail-flick test in CD-1 mice. Noxious heat induced a reliable and reproducible withdrawal reflex. Oral administration of YM-58483 30 mg/kg significantly increased tail flick latency (Fig. 7C). In addition, we tested the effect of YM-58483 on chemically-induced acute pain in CD-1 mice. Intraplantar injection of capsaicin (1 nmol) induced robust nocifensive (licking/lifting) responses. Oral administration of YM-58483 30 mg/kg significantly reduced this response (Fig. 7D). Taken together, these results suggest that YM-58483 at the high dose has antinociceptive effects on acute pain induced by heat, mechanical or chemical stimuli.

3.8. YM-58483 blocks SOC entry in nociceptive neurons

A previous study has shown that SOC entry is present in DRG neurons [18]. We wondered whether YM-58483 blocks SOC entry in DRG neurons. We performed calcium imaging recordings in cultured adult CD-1 mouse DRG neurons. In the absence of extracellular calcium, neurons were treated with thapsigargin 1 μ M or CPA 10 μ M, specific inhibitors of ER Ca^{2+} -ATPase [12; 31; 62] for 5 minutes to deplete ER calcium. Subsequent addition of 2 mM of calcium evoked calcium influx (SOC entry) with varied magnitudes. CPA evoked a more stable and sustained calcium response than did thapsigargin (data not shown). Interestingly, the calcium responses in small-diameter (<34 μ m) or capsaicin positive neurons were more robust than that in large-diameter (>34 μ m) neurons (Fig. 8A and B). Bath application of YM-58483 decreased SOC entry in a concentration-dependent manner (Fig. 8C and D). Inhibition of SOC entry by YM-58483 in small or capsaicin positive neurons was much stronger than that in large neurons. These results suggest that the functional expression of SOC channels is different in subtypes of DRG neurons and YM-58483-induced inhibition of SOC entry is more effective in nociceptors.

4. Discussion

Results from the present study reveal that the SOC channel inhibitor YM-58483 reduces acute pain, neuropathic pain, and inflammatory pain, indicating that YM-58483 has the characteristics of a broad-spectrum analgesic. YM 58483 prevents the development of chronic pain when it is administered before induction of chronic pain and significantly suppresses chronic pain when it is administered after pain is already established. These findings demonstrate that YM-58483 not only protects against chronic pain but also is effective against established chronic pain. Our study provides the first evidence that the SOC channel inhibitor has potent analgesic actions, suggesting that SOC channels may contribute significantly to the modulation of pain transmission.

Our results also demonstrated that YM-58483 reduces CFA-induced inflammation. It is well known that proinflammatory cytokines and inflammatory mediators contribute to the generation of inflammatory pain [45; 67]. Analysis of TNF- α , IL-1 and PGE₂ levels revealed that production of IL-1, TNF- α and PGE₂ was dramatically increased in the CFA-injected paw. These results are consistent with previous reports that CFA induces elevation of TNF- α , IL-1 and PGE₂ levels in the inflamed rat paw [36; 67]. YM-58483 significantly reduced production of TNF- α , IL-1 and PGE₂ in the inflamed paw, which is consistent with a previous report that YM-58483 inhibits activation-induced secretion of TNF- α [33]. We also confirmed a previous finding that indomethacin, a potent nonselective inhibitor of cyclooxygenase (COX), reduced IL-1 production in the CFA injected paw [55]. Indomethacin had a greater effect on PGE₂ production and less effect on IL-1 production than YM-58483. Intriguingly, both indomethacin and YM-58483 have anti-inflammatory and analgesic effects, however, YM-58483 markedly decreased TNF- α production while indomethacin dramatically increased TNF- α production in the inflamed paw, which is consistent with previous reports that indomethacin increases TNF- α level in the rat subcutaneous air pouch model and intestinal ulcerations [3; 41]. These results suggest that YM-58483 and indomethacin produce analgesic actions through different mechanisms. It is well known that cyclooxygenase-2 and prostaglandin E synthases are inducible enzymes that can be induced in response to IL-1 and TNF- α [64]. YM-58483-induced decrease in the PGE₂ level may be mediated by reduction of TNF- α and IL-1 production. We could not rule out the possibility that YM-58483 partially inhibits COX-2 without direct evidence. It is conceivable that reduction of TNF- α , IL-1 and PGE₂ contributes to the mechanisms by which pretreatment with YM-58483 reduces CFA-induced inflammation and pain hypersensitivity.

A single oral administration of YM-58483 reduced CFA- and SNI- induced pain hypersensitivity. Intrathecal application of YM-58483 had a similar effect on thermal hypersensitivity, suggesting an action of YM-58483 at the spinal level. In addition, the results from the formalin tests demonstrated that YM-58483 strongly decreased the first and second phases of formalin-induced spontaneous nociceptive responses. It has been suggested that centrally acting drugs inhibit both phases [44]; peripherally acting drugs such as non-steroidal anti-inflammatory drugs inhibit only the second phase [28; 59]. The reduction of both phases by YM-58483 suggests that YM-58483 has a central action. The central mechanism of YM-58483-induced analgesic actions remains to be determined. It has been shown that cortical and hippocampal neurons express SOC channels, which are involved in the modulation of neuronal functions [4; 15; 19]. Spinal cord dorsal horn neurons may also express SOC channels and YM-58483-induced analgesic effect might be mediated by blocking SOC channels in dorsal horn neurons. We also examined the peripheral analgesic action of YM-58483 in the present study. The subcutaneous application of YM-58483 directly into the plantar region of CFA-treated mice had significant analgesic and anti-inflammatory effects. This observation strongly suggests that YM-58483 has a peripheral analgesic effect. Intriguingly, local injection of YM-58483 had no effect at 1 h time point post CFA injection when inflammation had not significantly developed yet, indicating that the analgesic effect of YM-58483 is mediated by reduction of inflammation.

Under normal conditions, YM-58483 at the high dose (30 mg/kg) attenuated pain sensation induced by heat, mechanical, or chemical stimuli, but had no effect on innocuous or noxious stimulus-induced nociception at the low dose (10 mg/kg). This result demonstrates that the high dose of YM-58483 nonselectively modulates nociceptive pain as is the case with opioids and non-steroidal anti-inflammatories (NSAIDs), suggesting that SOC channels may be involved in the process of nociception. The antinociceptive effects of YM-58483 in naive mice could not be explained by reduction of proinflammatory and inflammatory mediators because these mediators are induced only under inflammatory conditions. A previous study has suggested that SOC channels are expressed in rat DRG neurons [18]. To understand the peripheral mechanism underlying YM-58483-induced antinociception in DRG neurons, we performed calcium imaging recordings in cultured adult mouse DRG neurons. Consistent with the findings from the previous study [18], SOC entry can be activated by 1 μ M thapsigargin. However, 10 μ M of CPA induced more sustained SOC entry, which is important for pharmacological analysis of blocking SOC entry. Interestingly, SOC entry was more robust in small DRG neurons than in large DRG neurons. These findings support the notion that SOC channels may be mainly functionally expressed in nociceptors. Previous studies demonstrated that painful nerve injury induces depletion of intracellular Ca^{2+} stores and enhances SOC channel function in sensory neuron [18; 52]. These reports further support the view that SOC channels are involved in chronic pain. Moreover, YM-58483 inhibited SOC entry in DRG neurons in a concentration-dependent manner. Inhibition potency of YM-58483 in small DRG neurons was greater than in large DRG neurons. This result suggests that YM-58483-induced antihyperalgesia and antinociception may be mediated by inhibition of these channels.

Interestingly, we observed the differential effect of the SOC channel inhibitor in CD1 and C57BL/6 mice. YM-58483 produced a strong analgesic effect in CFA- and SNI-induced mechanical hypersensitivity in C57BL/6 mice, but had no such effect in CD1 mice at the same dose. It has been reported that there are strain differences in morphine- and gabapentin-induced antinociception in some nociceptive tests [7; 40]. The strain difference may contribute to different efficacy of YM-58483 in CD1 and C57BL/6. SOC channels have been proposed since 1986 [49], however, their molecular identities were discovered only recently [16; 35; 54; 65; 69]. It has been demonstrated that TRPC (transient receptor potential canonical) family is also involved in SOC entry [6; 56; 61]. A previous study has

reported that YM-58483 inhibits TRPC channels [20; 51]. Most TRPC channel proteins (except TRPC2) are expressed in DRG neurons [32]. YM-58483 blocks both Orai- and TRPC- mediated SOC entry [57]. The molecular mechanism underlying SOC entry and YM-58483-induced analgesic effects needs to be explored.

In summary, our results indicate that the SOC channel inhibitor YM-58483 produces strong analgesic and anti-inflammatory actions. The peripheral mechanisms of these actions appear to be mainly mediated by the reduction of TNF- α , IL-1 and PGE2 production, but may also occur via inhibition of SOC channels in DRG neurons. Although the central mechanisms mediating the effects of YM-58483 remain to be explored, it is clear from the data presented here that YM-58483 acts as an analgesic and anti-inflammatory agent, which suggests a potential therapeutically beneficial and novel approach to the treatment of chronic pain, especially chronic pain associated with inflammation.

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Synopsis

The SOC channel inhibitor YM-58483 has analgesic actions in chronic pain, demonstrates antinociceptive effects in acute pain and prevents the development of chronic pain in mice.

Fig. 1.

YM-58483 reverses complete Freund's adjuvant (CFA)-induced pain hypersensitivity. (A) Time-dependent effects of YM-58483 (YM) on CFA-induced thermal hyperalgesia measured 72 hrs after CFA injection in CD-1 mice ($n = 6-7$). (B) Time-dependent effects of YM-58483 on CFA-induced mechanical allodynia measured 72 hrs after CFA injection in C57BL/6 mice ($n=6-7$). * $P < .05$ compared with vehicle control

Fig. 2.

YM-58483 prevents the development of pain hypersensitivity. (A) Effects of YM-58483 and indomethacin (ind) on CFA-induced thermal hyperalgesia measured 2 and 24 hrs after CFA injection in CD-1 mice ($n = 4-7$). (B) Effects of YM-58483 and indomethacin on CFA-induced mechanical allodynia measured 2 and 24 hrs after CFA injection in C57BL/6 mice ($n = 4-7$). * $P < .05$ compared with vehicle control.

Fig. 3.

YM-58483 decreases complete Freund's adjuvant (CFA)-induced inflammation and TNF- α , IL-1 β and PGE₂ production in CD-1 mice. (A) Effects of YM-58483 (YM) and indomethacin (Ind) on CFA-induced paw edema measured at 3 and 24 hrs after CFA injection (n=4-7, * P < .05 compared with vehicle control). (B-D) The effect of YM-58483 on CFA-induced increase in TNF- α (B), IL-1 β (C) and PGE₂ (D). Control animals were intraplantarly injected with saline. The measurements were performed 24 hrs after CFA injection (n=4-6). * P < .05 compared with saline; # P < .05 compared with CFA.

Fig. 4. YM-58483 has both peripheral and central analgesic effects on CFA-induced thermal hyperalgesia in CD-1 mice. (A) Time-dependent effects of intraplantar injection of YM-58483 on CFA-induced thermal hyperalgesia measured 72 hrs after CFA injection (n = 6-7). (B) Effects of intraplantar administration of YM-58483 (YM) on CFA-induced paw edema measured at 3 hrs after CFA injection (n = 6-7). (C) Time-dependent effects of YM-58483 by intrathecal injection on CFA-induced thermal hyperalgesia measured 72 hrs after CFA injection (n = 6-7). * $P < .05$ compared with vehicle control).

Fig. 5.

YM-58483 attenuates formalin-induced nociceptive behavior in C57BL/6 mice. (A) The time course of nociceptive behavior after subcutaneous injection of formalin (2%, 20 μ L) into the hind paw following pretreatment with vehicle or YM-58483 ($n = 4-6$). (B) Total time spent in nociceptive behavior during the first (0-10 min) and second (15-60 min) phases of the formalin-induced spontaneous behavior with vehicle or YM-58483 ($n=4-6$). * $P < .05$ compared with vehicle control.

Fig. 6. YM-58483 reduces spared nerve injury (SNI)-induced pain hypersensitivity. (A) Time-dependent effects of YM-58483 on SNI-induced thermal hyperalgesia measured 7 days after surgery in CD-1 mice (n = 6-7). (B) Time-dependent effects of YM-58483 on SNI-induced mechanical allodynia measured 7 days after surgery in C57BL/6 mice (n=6-7). (C, D) Effects of YM-58483 on the development of SNI-induced thermal and mechanical hypersensitivity (n = 6-7). * $P < .05$ compared with vehicle control.

Fig. 7. YM-58483 diminishes acute pain induced by mechanical, heat and chemical stimuli. (A) Effects of YM-58483 on basal mechanical sensitivity in C57BL/6 mice (n=6-7). (B) Effects of YM-58483 on paw pressure-induced pain in C57BL/6 mice (n = 10). (C) Effects of YM-58483 on radiant heat-induced pain in CD-1 mice (n = 5). (D) Effects of YM-58483 on capsaicin-induced spontaneous nociceptive behavior in CD-1 mice (n=10-11). * $P < .05$ compared with vehicle control.

Fig. 8. YM-58483 inhibits store-operated calcium (SOC) entry in cultured adult CD-1 mouse dorsal root ganglion (DRG) neurons. (A) Representative traces of 10 μM cyclopiazonic acid-induced SOC entry in large, small and capsaicin positive (capsaicin+) DRG neurons. (B) Summary of SOC entry in large, small and capsaicin positive DRG neurons ($n = 34$ -66 neurons each), * $P < .05$ compared with large neurons. (C) Representative traces of SOC entry blocked by YM-58483. (D) Summary of YM-58483-induced inhibition of SOC entry in large, small and capsaicin-positive DRG neurons ($n = 27$ -35 neurons each). * $P < .05$ compared to control; # $P < .05$ compared with large neurons.