σ_1 Receptors Are Involved in the Visceral Pain Induced by Intracolonic Administration of Capsaicin in Mice

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ABSTRACT

Background: Visceral pain is an important and prevalent clinical condition whose treatment is challenging. Sigma-1 (σ_1) receptors modulate somatic pain, but their involvement in pure visceral pain is unexplored.

Methods: The authors evaluated the role of σ_1 receptors in intracolonic capsaicin-induced visceral pain (pain-related behaviors and referred mechanical hyperalgesia to the abdominal wall) using wild-type (WT) (n = 12 per group) and σ_1 receptor knockout (σ_1-KO) (n = 10 per group) mice, selective σ_1 receptor antagonists (BD-1063, S1RA, and NE-100), and control drugs (morphine and ketoprofen).

Results: The intracolonic administration of capsaicin (0.01–1%) induced concentration-dependent visceral pain-related behaviors and referred hyperalgesia in both WT and σ_1-KO mice. However, the maximum number of pain-related behaviors induced by 1% capsaicin in σ_1-KO mice (mean ± SEM, 22 ± 2.9) was 48% of that observed in WT animals (46 ± 4.2). Subcutaneous administration of the σ_1 receptor antagonists BD-1063 (16–64 mg/kg), S1RA (32–128 mg/kg), and NE-100 (8–64 mg/kg) dose-dependently reduced the number of behavioral responses (by 53, 62, and 58%, respectively) and reversed the referred hyperalgesia to mechanical control threshold (0.53 ± 0.05 g) in WT mice. In contrast, these drugs produced no change in σ_1-KO mice. Thus, the effects of these drugs are specifically mediated by σ_1 receptors. Morphine produced an inhibition of capsaicin-induced visceral pain in WT and σ_1-KO mice, whereas ketoprofen had no effect in either mouse type.

Conclusion: These results suggest that σ_1 receptors play a role in the mechanisms underlying capsaicin-induced visceral pain and raise novel perspectives for their potential therapeutic value.

What We Already Know about This Topic

- Visceral and somatic pain have distinct physiological mechanisms and responses to analgesics
- Although visceral pain is common clinically, it is poorly understood and difficult to treat compared with somatic pain

What This Article Tells Us That Is New

- σ_1 Receptors were shown to play an important role in a mouse model of pure visceral pain using a combined genetic and pharmacological approach
- Pharmacological blockade of σ_1 receptors might provide a therapeutic approach to visceral pain

VISCERAL pain is the most frequent type of pathological pain and one of the main reasons for patients to seek medical assistance.[1,2] However, most of our knowledge about pain mechanisms derives from experimental studies of somatic (principally cutaneous) pain rather than visceral pain.[3,4] The characteristics, pathophysiological mechanisms, and response to drug treatment of visceral and somatic pain are different; consequently, it is not valid to indiscriminately extrapolate findings from the somatic–cutaneous to visceral domain.[5–10]

Most existing animal models of visceral pain are either technically complex (*e.g.*, require surgery) or combine visceral and somatic mechanisms of peritoneal pain (*e.g.*, writhing test). However, a technically simple and purely visceral mouse model of chemical stimulation of the colon by capsaicin has been developed.[11] This model permits examination of both acute visceral pain and referred mechanical hyperalgesia to the abdominal wall, which is an important clinical

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feature of visceral pain.[11,12] The intracolonic capsaicin model in mice may represent an appropriate translational model of visceral pain, because application of capsaicin to the human gut evokes intense (acute) pain and referred mechanical hyperalgesia and is a well-validated human model of visceral pain.[13–15]

Sigma receptors have been classified into two distinct subtypes, sigma-1 (σ_1) and sigma-2 (σ_2), although only the σ_1 receptor has been cloned.[16,17] σ_1 Receptors are highly expressed in the central nervous system, including important areas for pain control such as the superficial layers of the spinal cord dorsal horn, periaqueductal gray matter, locus coeruleus, and rostroventral medulla.[18,19] The role of σ_1 receptors in somatic pain control is well documented. Thus, σ_1 receptor knockout (σ_1-KO) mice[20–22] and wild-type (WT) mice treated with σ_1 receptor antagonists[22–29] showed a marked reduction of pain-related behaviors in different models of somatic pain. However, it is not known whether σ_1 receptors also play a role in models of pure visceral pain.

To test this possibility, we performed studies using the intracolonic capsaicin model in mice with the following objectives: to compare the behavioral responses to chemical stimulation of the colon and the referred hyperalgesia to abdominal mechanical stimulation after the intracolonic instillation of capsaicin between naïve σ_1-KO and WT mice; and to evaluate the effects of the systemic administration of σ_1 receptor antagonists (BD-1063, NE-100, and S1RA) and control drugs (morphine and ketoprofen) on the spontaneous pain-related behaviors and referred mechanical hyperalgesia induced by intracolonic capsaicin in both mouse types.

Materials and Methods

Animals

Experiments were performed in female WT (Charles River, Barcelona, Spain) and σ_1-KO (Laboratorios Esteve, Barcelona, Spain) CD-1 mice weighing 25–30 g. The σ_1-KO mice were generated on a CD-1 background as described previously.[22] Animals were acclimated in our animal facilities for at least 1 week before testing, housed in colony cages in temperature- and light-controlled rooms (22 ± 1°C, lights on at 8:00 AM and off at 8:00 PM, air replacement every 20 min). A standard laboratory diet (Harlan Teklad Research Diets, Madison, WI) and tap water were available *ad libitum* until the beginning of the experiments. Testing took place during the light phase (from 9:00 AM to 3:00 PM). Mice were handled in accordance with the European Communities Council Directive of November 24, 1986 (86/609/ECC), and the experimental protocol was approved by the University of Granada Research Ethics Committee (Granada, Spain).

Drugs and Drug Administration

We used the selective σ_1 receptor antagonists BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride), supplied by Tocris Cookson Ltd. (Bristol, UK); NE-100 (*N,N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]ethylamine hydrochloride), synthesized as reported previously[30]; and S1RA (4-[2-[[5-methyl-1-(2-naphthalenyl)-1H-pyrazol-3-yl]oxy]ethyl] morpholine),[27] supplied by Laboratorios Esteve. Morphine hydrochloride (General Directorate of Pharmacy and Drugs, Spanish Ministry of Health, Madrid, Spain) was used as a control drug against visceral pain, and ketoprofen (Sigma-Aldrich Química S.A., Madrid, Spain) served as a negative control. All of these drugs were dissolved in sterile physiologic saline, with the exception of ketoprofen, which was dissolved in 10% absolute ethanol and 90% saline. Drug solutions were prepared immediately before the start of the experiments, and 5 ml/kg of the drug or its solvent was injected subcutaneously into the interscapular area.

Capsaicin (Sigma-Aldrich), which was used to induce visceral pain, was prepared to make up a 1% (weight/volume) stock solution in a solvent comprising 10% absolute ethanol (Panreac Química SA, Barcelona, Spain), 10% Tween 80 (Sigma-Aldrich), and 80% sterile saline. The capsaicin solution was prepared once per week and stored at −20°C in aliquots, which were thawed and diluted at the appropriate concentrations on the day of the experiment. The capsaicin solution (50 μl) was instilled into the colon by introducing through the anus a fine round-tip cannula (external diameter, 0.61 mm; length, 4 cm) connected to a 1710 TLL Hamilton microsyringe (Teknokroma, Barcelona, Spain). Control animals were intracolonically instilled with the same volume of capsaicin solvent.

General Procedures for Evaluating Intracolonic Capsaicin-evoked Visceral Pain and Referred Hyperalgesia

Spontaneous pain-related behaviors and referred mechanical hyperalgesia induced by intracolonic capsaicin were tested following a previously described protocol,[11] with small modifications. Mice were housed in individual transparent plastic boxes (7 × 7 × 13 cm) on an elevated platform with a wire mesh floor (small mirrors behind and below the chambers enhanced observation of the animals). After a 40-min habituation period, animals were removed from the compartments, and a capsaicin solution (or its solvent) was instilled intracolonically after application of petroleum jelly on the perianal area to avoid stimulation of somatic areas through contact with the algogen. The animal was immediately returned to the compartment, where the number of pain-related behaviors (licking of the abdomen, stretching of the abdomen, and abdominal retractions) were counted by direct observation for 20 min.

The presence of capsaicin-induced referred hyperalgesia was determined by measuring the withdrawal response to a punctate mechanical stimulation of the abdomen at 20 min after the instillation of capsaicin (or its solvent). Forces ranging from 0.02 to 2 g (0.19–19.6 mN) were applied to the abdomen with a series of calibrated von Frey filaments (Touch-Test Sensory Evaluators; North Coast Medical Inc.,

Gilroy, CA) using the up–down paradigm.[31] Perianal and external genitalia areas were avoided, concentrating the stimulation on the lower and mid abdomen, as reported previously.[11] Filaments were applied three times for 2–3 s, with interapplication intervals of 5 s. Testing was initiated with the 0.4-g (3.92-mN) von Frey filament (*i.e.*, the middle of the range). In each consecutive test, if there was no response to the filament, a stronger stimulus was then selected; if there was a positive response, a weaker one was then used. The response to the filament was considered positive if immediate licking/scratching of the application site, sharp retraction of the abdomen, or jumping was observed.

The experimenter who evaluated the behavioral responses was blinded to the treatment and genotype of experimental subjects. In all cases, experiments in WT or σ_1-KO groups, solvent- or capsaicin-treated groups, and saline- or drug-treatment groups were run in parallel. Each animal (n = 12 WT or n = 10 σ_1-KO mice per group were tested in all experiments) was used only once and received a single concentration of capsaicin (or its solvent) and a single dose of one drug (or its solvent).

Comparison of the Effect of Different Concentrations of Intracolonic Capsaicin in Naïve WT and σ_1-KO Mice

WT and σ_1-KO mice were administered different capsaicin concentrations (0.01–1%) intracolonically, and the pain-related behaviors and referred hyperalgesia to abdominal mechanical stimulation induced by each concentration were recorded consecutively in the same animal following the procedure described earlier in the first and second paragraphs of the General Procedures for Evaluating Intracolonic Capsaicin-evoked Visceral Pain and Referred Hyperalgesia section. This allowed the construction of concentration–response curves (concentration *vs.* number of behaviors or mechanical threshold) and identification of the optimal concentrations of capsaicin for the pharmacologic studies (see the next section for details).

Comparison of Drug Effects on Visceral Pain Induced by Intracolonic Administration of Capsaicin in WT and σ_1-KO Mice

Two experimental approaches were used to evaluate the effect of the drugs on capsaicin-induced visceral pain and referred hyperalgesia. First, we tested the effect of a fixed dose of a σ_1 receptor antagonist on the responses induced by different concentrations of capsaicin. Second, we tested the effects of several doses of various σ_1 receptor antagonists and control drugs on the pain-related behavior and referred hyperalgesia induced by a fixed concentration of capsaicin.

In the first experimental approach, BD-1063 (32 mg/kg) or its solvent was administered subcutaneously 30 min before the intracolonic instillation of different concentrations (0.01–1%) of capsaicin, and the number of pain-related behaviors was counted for 20 min after the capsaicin instillation. Immediately afterward (*i.e.*, 50 min after injection of the drug), its effect on the capsaicin-induced referred hyperalgesia was assessed in the same animal by stimulation of the abdomen with von Frey filaments as described earlier in the General Procedures for Evaluating Intracolonic Capsaicin-evoked Visceral Pain and Referred Hyperalgesia section. Experiments were performed in both WT and σ_1-KO mice.

In the second experimental approach, different doses of BD-1063 (16–64 mg/kg), S1RA (32–128 mg/kg), NE-100 (8–64 mg/kg), morphine (1–16 mg/kg), ketoprofen (32–128 mg/kg), or their solvents were administered subcutaneously at 30 min before the intracolonic instillation of 1% capsaicin, and the number of pain-related behaviors was counted for 20 min after administration of capsaicin. This concentration of capsaicin was selected because it produces the maximum number of pain-related behaviors in WT and σ_1-KO mice (fig. 1A) and therefore offers the maximum window for observing any reductions in this response. In separate experiments, we tested the effect of the same doses of the σ_1 receptor antagonists and the control drugs on the referred hyperalgesia induced by a fixed concentration of capsaicin. In these experiments, the drug under study or its solvent was injected subcutaneously at 30 min before the intracolonic instillation of 0.1% capsaicin and, 20 min after the instillation, the response of the animal to abdominal stimulation with von Frey filaments was tested using the up–down method, as described earlier under General Procedures for Evaluating Intracolonic Capsaicin-evoked Visceral Pain and Referred Hyperalgesia. A capsaicin concentration of 0.1% was selected for these experiments because it reaches the maximum reduction in the mechanical threshold for referred hyperalgesia in WT and σ_1-KO mice (fig. 1B).

Statistical Analysis

The degree of referred pain, expressed as the mechanical threshold that produces 50% of responses, was calculated using the formula of Dixon[32]: 50% mechanical threshold (g) = $[(10^{(Xf + \kappa\delta)})/10{,}000]$, where Xf = value (in logarithmic units) of the final von Frey filament used, κ = tabular value for the pattern of positive/negative responses, and δ = mean difference (in log units) between stimuli.

The number of behaviors and mechanical thresholds were compared across experimental groups with one-way ANOVA or two-way non–repeated measures ANOVA (factors were genotype and algogen, or drug and algogen, or genotype and drug, depending on the experiment performed) followed by the Bonferroni test, using the SigmaPlot 12.0 program (Systat Software Inc., San Jose, CA). ED_{50} values (dose producing half of the maximal response) and their standard errors were calculated using nonlinear regression analysis of the equation for a sigmoid plot and were compared by means of Snedecor's F test using the GraphPad Prism 5.00 program (GraphPad Software Inc., San Diego, CA). No data were

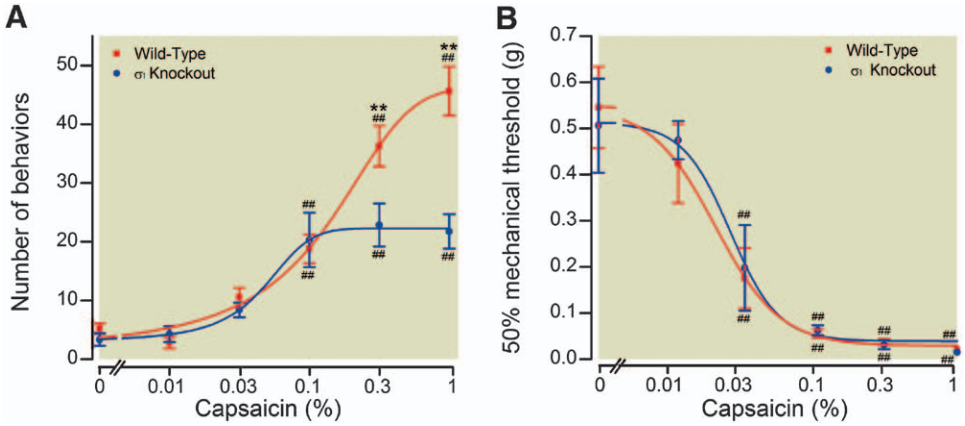

Fig. 1. Pain-related behaviors (*A*) and referred mechanical hyperalgesia (*B*) induced by intracolonic administration of different concentrations of capsaicin (0.01–1%) or its solvent (0) in WT and σ_1-KO mice. The behavioral pain responses (licking of abdomen, stretching, abdominal retractions) were recorded during the first 20 min after instillation of capsaicin, and referred mechanical hyperalgesia (evaluated by stimulation of the abdomen with von Frey filaments) was measured at 20 min after instillation of capsaicin. Each *point* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. Statistically significant differences between the values obtained in capsaicin- and solvent-treated animals: ##$P < 0.01$. Statistically significant differences between the values obtained in WT and σ_1-KO animals at the same concentration of capsaicin: **$P < 0.01$ (two-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

missing for any of the variables. A value of $P < 0.05$ was considered statistically significant.

Results

Comparison of Capsaicin-induced Visceral Pain between WT and σ_1-KO Mice

In both WT and σ_1-KO mice, intracolonic administration of the capsaicin solvent elicited a small number of abdominal licking behaviors that could be clearly differentiated from normal grooming activity (fig. 1A), whereas intracolonic instillation of capsaicin-induced multiple types of pain-related behavior (*e.g.*, licking, stretching, and contraction of the abdomen). The number of pain-related behaviors induced by capsaicin (0.01–1%) increased in a concentration-dependent manner in both WT and σ_1-KO mice (fig. 1A), but the pattern of this response differed markedly between them ($P < 0.001$, two-way ANOVA). In WT mice, the maximum number of pain responses (46 ± 4.2) was evoked at the highest concentration tested (1%); in σ_1-KO mice, the maximum number of pain responses, which was half that in WT mice, reached a plateau from a concentration of 0.1% (fig. 1A). The difference in number of pain responses between WT and σ_1-KO animals was statistically significant at concentrations of 0.3% and 1% ($P < 0.01$) (fig. 1A).

In contrast, there were no significant differences between WT and σ_1-KO mice in the intracolonic capsaicin-induced referred mechanical hyperalgesia as evaluated with von Frey filaments (fig. 1B). Instillation of capsaicin (0.01–1%) reduced the mechanical threshold in a concentration-dependent manner in both groups, which showed almost identical stimulus–response curves (fig. 1B). The difference between these two groups and solvent-treated mice reached statistical significance at a capsaicin concentration of 0.03% (fig. 1B). No significant difference was found between the 50% mechanical thresholds in animals treated with capsaicin solvent (0.54 ± 0.08 and 0.52 ± 0.05 g in WT and σ_1-KO mice, respectively) and those observed in naïve animals without any instillation (0.53 ± 0.09 and 0.51 ± 0.10 g in WT and σ_1-KO mice, respectively).

In summary, the intracolonic administration of capsaicin evoked concentration-dependent visceral pain-related behaviors and referred mechanical hyperalgesia in both WT and σ_1-KO mice. The number of pain-related behaviors was lower in the σ_1-KO mice, but the mechanical hyperalgesia did not differ between the mouse types.

Effects of σ_1 Receptor Antagonists on Visceral Pain Induced by Intracolonic Capsaicin

The subcutaneous administration of the selective σ_1 antagonist BD-1063 (32 mg/kg) at 30 min before the intracolonic instillation of 0.1–1% capsaicin reduced the number of pain-related behaviors in WT mice ($P < 0.001$, two-way ANOVA) (fig. 2A) but not in σ_1-KO mice (fig. 2B). The maximum number of 1% capsaicin–induced pain-related behaviors was highly similar between WT mice treated with BD-1063 (22 ± 3.9) and σ_1-KO mice treated with saline (22 ± 2.9) or with BD-1063 (20 ± 2.8). BD-1063 (32 mg/kg) also inhibited the referred mechanical hyperalgesia in WT mice, promoting a shift to the right of the concentration–response curve of capsaicin ($P < 0.05$, two-way ANOVA) (fig. 3A), but it had no effect on the referred hyperalgesia in σ_1-KO mice (fig. 3B).

To determine whether the effects of BD-1063 were dose-dependent and shared by other σ_1 antagonists, we administered various doses of the selective σ_1 antagonists BD-1063, S1RA, and NE-100 at 30 min before instillation of the capsaicin concentrations found to induce the

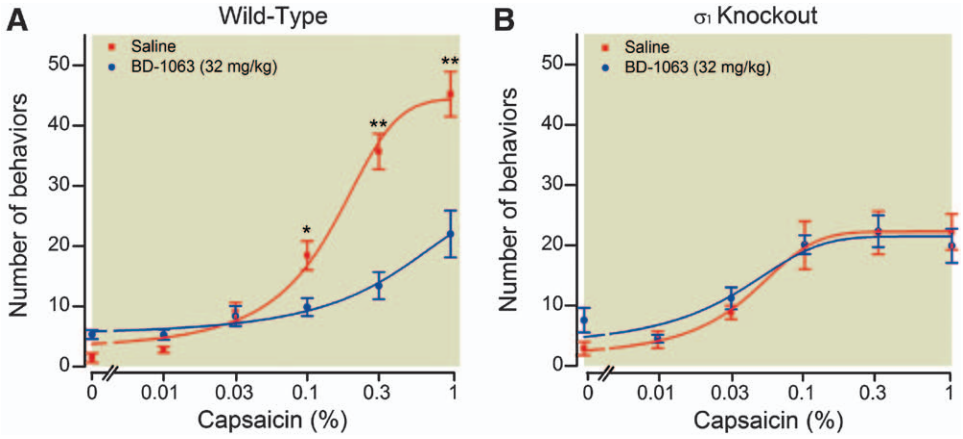

Fig. 2. Effects of the subcutaneous administration of BD-1063 (32 mg/kg) or saline on the pain-related behaviors evoked by intracolonic administration of different concentrations of capsaicin (0.01–1%) or its solvent (0) in WT (*A*) and σ_1-KO (*B*) mice. BD-1063 or saline was injected at 30 min before the administration of capsaicin or its solvent. The behavioral pain responses (licking of abdomen, stretching, abdominal retractions) were recorded during the first 20 min after instillation of capsaicin or its solvent. Each *point* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. Statistically significant differences compared to BD-1063–injected mice: $^*P < 0.05$; $^{**}P < 0.01$ (two-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

maximum number of pain-related behaviors and maximum referred hyperalgesia (1% and 0.1% capsaicin, respectively). The subcutaneous administration of BD-1063 (16–64 mg/kg), S1RA (32–128 mg/kg), and NE-100 (8–64 mg/kg) induced a dose-dependent inhibition of capsaicin-induced pain responses in WT mice but produced no change in σ_1-KO mice (fig. 4). None of the σ_1 antagonists abolished pain-related behaviors in WT mice; however, at the highest doses tested, all of them reduced the number of behaviors in WT mice to the same number observed in capsaicin-treated σ_1-KO mice (fig. 4).

In WT mice, the referred mechanical hyperalgesia induced by 0.1% capsaicin was almost completely reversed by pretreatment with the highest doses of BD-1063 or S1RA (fig. 5), yielding a 50% mechanical threshold (0.48 ± 0.1 g and 0.47 ± 0.07 g in BD-1063 32 mg/kg plus capsaicin and S1RA 128 mg/kg plus capsaicin groups, respectively) that was close to the threshold obtained in capsaicin solvent-treated animals (0.53 ± 0.05 g). The subcutaneous administration of NE-100 also dose-dependently reversed the capsaicin-induced mechanical hyperalgesia in WT mice (fig. 5). However, NE-100 was less effective than BD-1063 and S1RA, and the 50% maximal mechanical threshold was lower in the capsaicin-treated mice preadministered with the highest dose (64 mg/kg) of NE-100 (0.33 ± 0.06 g) than in the mice treated with capsaicin solvent (0.53 ± 0.05 g) (fig. 5). NE-100 doses

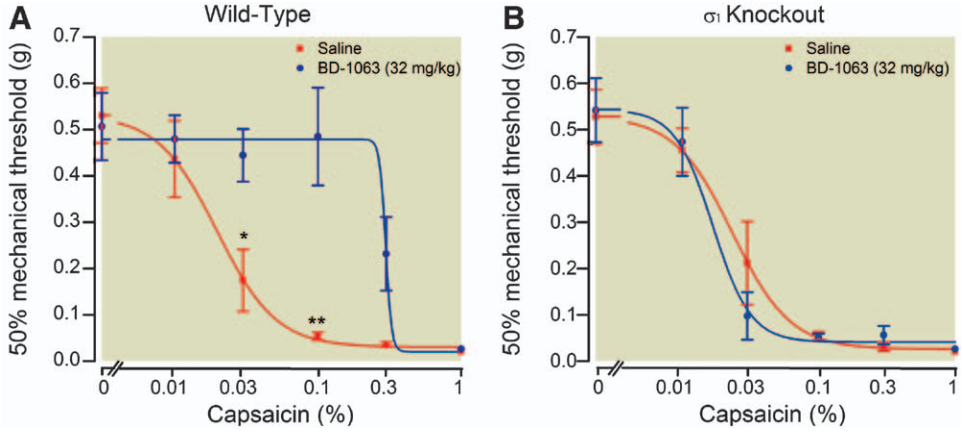

Fig. 3. Effects of the subcutaneous administration of BD-1063 (32 mg/kg) or saline on the referred mechanical hyperalgesia induced by intracolonic administration of different concentrations of capsaicin (0.01–1%) or its solvent (0) in WT (*A*) and σ_1-KO (*B*) mice. BD-1063 or saline was injected at 30 min before the administration of capsaicin or its solvent. The referred mechanical hyperalgesia (evaluated by stimulation of the abdomen with von Frey filaments) was measured at 20 min after instillation of capsaicin or its solvent. Each *point* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. Statistically significant differences compared to BD-1063–injected mice: $^*P < 0.05$; $^{**}P < 0.01$ (two-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

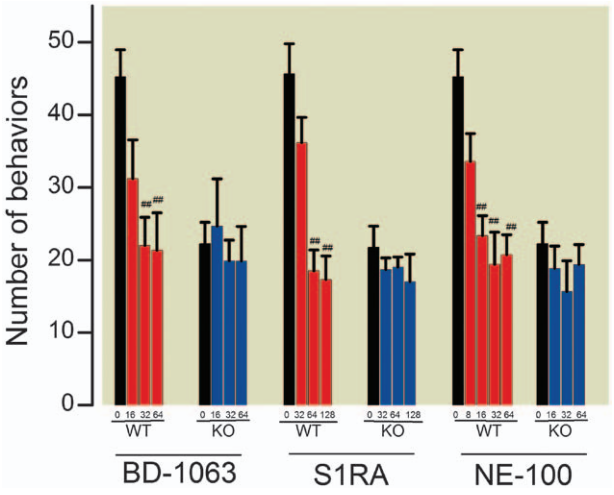

Fig. 4. Effects of the subcutaneous administration of BD-1063 (16–64 mg/kg), S1RA (32–128 mg/kg), NE-100 (8–64 mg/kg), and saline (0) on the pain-related behaviors evoked by intracolonic administration of 1% capsaicin in WT and σ_1-KO mice. The drug or saline was injected at 30 min before instillation of capsaicin. Behavioral pain responses (licking of abdomen, stretching, abdominal retractions) were recorded during the first 20 min after instillation of capsaicin. Each *bar* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. Statistically significant differences between the values obtained in drug- and saline-injected mice: ##P < 0.01 (one-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

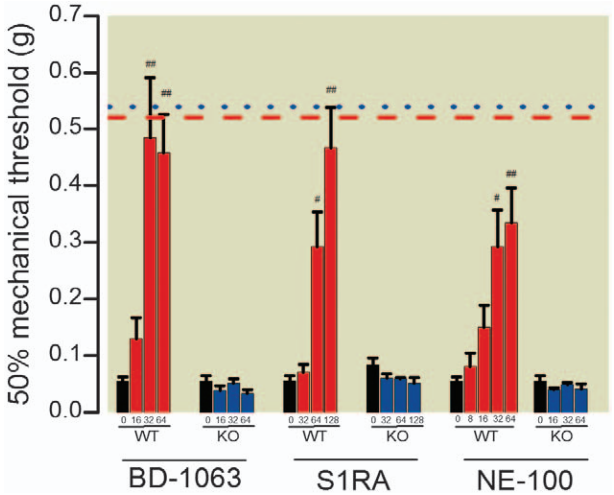

Fig. 5. Effects of the subcutaneous administration of BD-1063 (16–64 mg/kg), S1RA (32–128 mg/kg), NE-100 (8–64 mg/kg), and saline (0) on the referred mechanical hyperalgesia induced by intracolonic administration of capsaicin 0.1% in WT and σ_1-KO mice. The drug or saline was injected at 30 min before the capsaicin instillation. The referred mechanical hyperalgesia (evaluated by stimulation of the abdomen with von Frey filaments) was measured at 20 min after the capsaicin instillation. Each *bar* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. The *dashed* and *dotted lines* indicate the 50% threshold in capsaicin solvent-treated WT and σ_1-KO mice, respectively. Statistically significant differences between the values obtained in drug- and saline-injected mice: #P < 0.05; ##P < 0.01 (one-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

higher than 64 mg/kg produced locomotor alterations (ataxia and motor incoordination) that interfered with the mechanical threshold measurement (data not shown).

Comparison of the Effects of Control Drugs on Visceral Pain Induced by Intracolonic Capsaicin

As expected, the subcutaneous administration of morphine (1–16 mg/kg) produced a dose-dependent inhibition of capsaicin-induced pain-related behavioral responses and mechanical referred hyperalgesia in WT and σ_1-KO mice (figs. 6 and 7). In both mouse types, the pain-related behaviors induced by intracolonic 1% capsaicin were significantly reduced by morphine at doses of greater than or equal to 3 mg/kg and completely abolished by a dose of 8 mg/kg (fig. 6A). Morphine administration fully reversed the referred mechanical hyperalgesia and exerted a clear and robust analgesic action in both mouse types (fig. 7A), yielding a higher mechanical threshold than that observed in mice treated with capsaicin solvent (0.53 ± 0.05 g and 0.52 ± 0.06 g in WT and σ_1-KO mice, respectively). In fact, at the highest morphine doses tested (8 and 16 mg/kg), most of the mice made no response to the strongest stimulus (2 g). The overall effect of morphine on the referred mechanical hyperalgesia evoked by 0.1% capsaicin was statistically different in σ_1-KO compared with WT mice (P < 0.001, two-way ANOVA) (fig. 7A). In particular, the effect of morphine (1–4 mg/kg) was significantly greater in σ_1-KO mice than in WT mice (P < 0.05) (fig. 7A). The subcutaneous administration of ketoprofen (32–128 mg/kg) had no effect on the pain-related responses or referred mechanical hyperalgesia at any dose tested in either σ_1-KO or WT mice (figs. 6B and 7B).

Discussion

The main findings of this study were that the pain-related behaviors induced by intracolonic capsaicin were reduced markedly in σ_1-KO mice and that the pharmacological blockade of σ_1 receptors inhibited the capsaicin-induced pain-related behaviors and mechanical referred hyperalgesia in WT mice but not in σ_1-KO animals. This is the first report to present evidence of a role for the σ_1 receptor in a pure visceral pain model.

In agreement with previous studies,[11,12,33] we found that intracolonic capsaicin evoked concentration-dependent visceral pain-related behaviors and referred mechanical hyperalgesia. The number of capsaicin-induced pain-related behaviors was significantly lower in σ_1-KO mice than in WT mice. It has been postulated that the acute visceral pain evoked by intracolonic capsaicin is attributable to the direct activation of nociceptors in the colon.[34] In this regard, our group previously reported that the response in the first phase

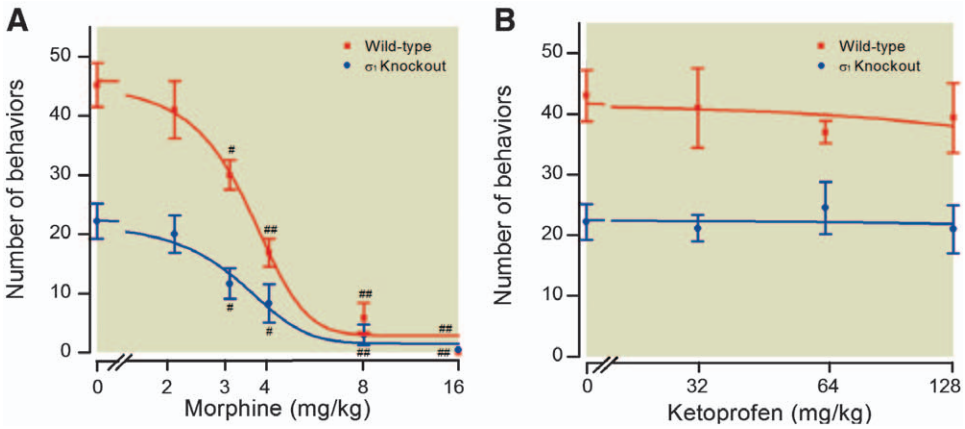

Fig. 6. Effects of the subcutaneous administration of morphine (2–16 mg/kg) (*A*) and ketoprofen (32–128 mg/kg) (*B*) on the pain-related behaviors evoked by intracolonic administration of capsaicin 1% in WT and σ_1-KO mice. The drug or its vehicle was injected at 30 min before the administration of capsaicin. Behavioral pain responses (licking of abdomen, stretching, abdominal retractions) were recorded during the first 20 min after the capsaicin instillation. Each *point* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. Statistically significant differences between the values obtained in drug- and vehicle-injected mice: #$P < 0.05$; ##$P < 0.01$ (one-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

of intraplantar formalin-induced pain, also attributed to the direct activation of nociceptors, was attenuated by 61% in σ_1-KO mice,[20] similar to the reduction in the number of pain-related behaviors (52%) observed in the present σ_1-KO mice.

The mechanical threshold was similar between naïve WT and σ_1-KO animals (0.53 ± 0.09 and 0.51 ± 0.1 g, respectively), indicating that σ_1-KO animals perceive and respond normally to a punctate mechanical stimulus applied to the abdomen. This finding is in agreement with various reports showing that the absence of σ_1 receptors in naïve σ_1-KO animals does not interfere with the perception of mechanical and thermal stimuli applied to the hind paw or with the motor response to these stimuli.[21–23,27,29]

Capsaicin-induced referred mechanical hyperalgesia did not differ between WT and σ_1-KO mice, in strong contrast to a previous finding by our group that intraplantar capsaicin was completely unable to induce mechanical hypersensitivity to a punctate stimulus in σ_1-KO mice.[22] Although somatic- and visceral-induced hyperalgesic states are both mediated by

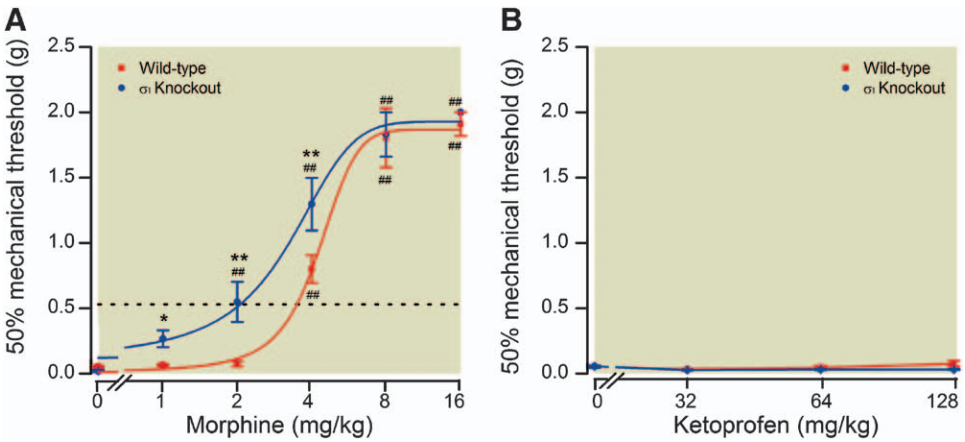

Fig. 7. Effects of the subcutaneous administration of morphine (1–16 mg/kg) (*A*) and ketoprofen (32–128 mg/kg) (*B*) on the referred mechanical hyperalgesia induced by intracolonic administration of capsaicin 0.1% in WT and σ_1-KO mice. The drug or its vehicle was injected at 30 min before instillation of capsaicin. The referred mechanical hyperalgesia (evaluated by stimulation of the abdomen with von Frey filaments) was measured at 20 min after the capsaicin instillation. Each *point* and *vertical line* represents the mean ± SEM of values obtained in 12 WT or 10 σ_1-KO mice per group. The *dashed line* indicates the 50% threshold in capsaicin solvent-treated WT and σ_1-KO mice. Note that the higher doses of morphine increase the mechanical threshold to above the control value (i.e., exert analgesic effects). Statistically significant differences between the values obtained in morphine- and vehicle-injected mice: ##$P < 0.01$. Statistically significant differences between the values obtained in WT and σ_1-KO animals at the same dose of morphine: *$P < 0.05$; **$P < 0.01$ (two-way ANOVA followed by Bonferroni test). WT, wild type; σ_1-KO, σ_1 receptor knockout.

an enhanced sensitivity of nociceptive neurons in the central nervous system (central sensitization), they differ widely in the neurobiological mechanisms that mediate the sensory process.[4,34] Thus, mouse colonic spinal primary afferent neurons are located in T8–L1 and L6–S1 dorsal root ganglia, and are mostly peptidergic calcitonin gene-related peptide– and transient receptor potential vanilloid 1 (TRPV1)–positive neurons,[4,35–37] whereas neurons innervating the hind paw are found at L3–L5 dorsal root ganglia, and a higher percentage of them are nonpeptidergic IB4-positive than are peptidergic calcitonin gene-related peptide–positive or TRPV1-positive.[8,36] Thus, this apparent discrepancy may be related to the distinct neurochemistries of the primary afferents activated after the intraplantar (somatic) and intracolonic (visceral) administration of capsaicin. In this context, a greater abdominal referred hyperalgesia was reported in α_{2A}-adrenoceptor knockout mice than in WT mice after intracolonic capsaicin, whereas paw mechanical hyperalgesia after intraplantar capsaicin was similar in the two mouse types.[38] Furthermore, the differences between somatic and visceral mechanical hypersensitivity induced by capsaicin are confirmed by the fact that, in our laboratory, the maximum mechanical pain hypersensitivity in WT mice was obtained after the intraplantar administration of 1 μg of capsaicin (20 μl of a 0.005% capsaicin solution),[22] whereas 50 μg of intracolonic capsaicin (50 μl of a 0.1% capsaicin solution) was required to reach the maximum referred mechanical hyperalgesia in the present study. Moreover, the duration of acute pain induced by intraplantar capsaicin (5 min) is much shorter than that of intracolonic capsaicin (20 min). Therefore, it could be possible that after intracolonic administration of capsaicin, the painful stimulus duration and intensity may be too strong to permit a suppression of referred mechanical hyperalgesia in σ_1-KO mice in comparison with σ_1-KO mice treated with capsaicin intraplantarly.

In the present study, subcutaneous administration of the σ_1 receptor antagonists BD-1063, S1RA, and NE-100 dose-dependently inhibited the number of pain-related behaviors and the referred mechanical hyperalgesia induced by intracolonic capsaicin in WT mice but not in σ_1-KO mice. According to these results, the effects of the σ_1 antagonists are specifically mediated by their interaction with σ_1 receptors. The highest doses of all of the σ_1 receptor antagonists tested reduced the number of capsaicin-induced pain-related behaviors in WT mice to the same number observed in σ_1-KO animals, indicating that these doses of BD-1063, S1RA, and NE-100 are sufficient to totally block the fraction of σ_1 receptors required to fully express the pain-related behaviors. The remaining response to capsaicin in σ_1-KO and WT animals treated with the highest doses of σ_1 receptor antagonists indicates that mechanisms other than σ_1 receptor activation are also implicated in the acute visceral pain induced by capsaicin in both mouse types. It was previously reported that σ_1 receptor antagonists had no effect on acute pain induced by thermal or mechanical stimuli to the paw skin[22–24,39] but almost completely abolished the first phase of formalin-induced pain[23,27] and, in the present study, partially reduced capsaicin-induced visceral pain-related behaviors. These data indicate that the analgesic actions of σ_1 receptor antagonists may depend on the type of pain (cutaneous, somatic, or visceral) and on the nociceptive stimulus applied (thermal, mechanical, or chemical).

The referred mechanical hyperalgesia induced by intracolonic capsaicin in WT mice was almost totally reversed by pretreatment with BD-1063 and S1RA but only partially reversed by NE-100. The lesser effect of NE-100 was not attributable to a pharmacokinetic difference (shorter duration of action), because the same reversion was obtained when NE-100 (64 mg/kg) was administered at 10 or 30 min before intracolonic capsaicin (50% mechanical thresholds: 0.32 ± 0.02 and 0.33 ± 0.06 g, respectively). However, we cannot rule out a pharmacodynamic difference between NE-100 and the other two σ_1 antagonists. At any rate, the antihyperalgesic effect of BD-1063, S1RA, and NE-100 in WT mice was attributable to their interaction with σ_1 receptors, given their complete lack of effect in σ_1-KO mice. The role of σ_1 receptors in pain hypersensitivity in somatic pain models in WT mice has been documented previously. It has been reported that σ_1 receptor antagonists reduce the pain responses in the second phase of the formalin test,[23,25,27] the mechanical hypersensitivity induced by capsaicin,[22,24,27] and the neuropathic pain responses in various models.[27–29]

There is an apparent discrepancy between the normal occurrence of capsaicin-induced referred hyperalgesia in σ_1-KO mice and the inhibition of this hyperalgesia in WT mice treated with σ_1 receptor antagonists. This mismatch may be attributable to the development of compensatory mechanisms in σ_1-KO mice *versus* WT animals, a general fact in genetic knockout experiments.[40] Thus, there have been reports of compensatory effects and conflicting results between knockout animals and pharmacological experiments in different areas such as GABAergic modulation of seizure activity,[41] endocannabinoid signaling,[42] the role of 5-HT_7 receptors in depression,[43] and the role of δ-subunit–containing γ-aminobutyric acid subtype A receptors in nociception,[44] among others.

Ketoprofen proved ineffective against the visceral pain induced by intracolonic capsaicin, despite administration of doses that were up to 90-fold and 2.5-fold higher than those shown to be effective in the writhing test (ED_{50} = 1.41 mg/kg) and in the second inflammatory phase of the formalin test (ED_{50} = 49.56 mg/kg) in mice.[45] Thus, the lack of effect of ketoprofen in our study cannot be attributed to the use of low doses of the drug and appears to indicate that prostaglandins are not implicated in capsaicin-induced visceral pain. By contrast, morphine totally abolished the pain-related behaviors and the mechanical referred hyperalgesia induced by intracolonic capsaicin in both WT and σ_1-KO mice. These results agree with previous reports

that morphine significantly inhibited intracolonic capsaicin-evoked c-Fos activation in the spinal cord[46] and the visceral pain induced by intracolonic mustard oil.[11,47] Interestingly, we found that morphine produced a similar reduction in the number of pain-related behaviors in both types of mouse (ED_{50} = 3.12 ± 0.35 and 3.47 ± 0.22 mg/kg in σ_1-KO and WT mice, respectively; $P > 0.05$) but was significantly ($P < 0.01$, Snedecor's F test) more potent against pain induced by mechanical stimulation of the abdominal wall in σ_1-KO mice (ED_{50} = 3.25 ± 0.40 mg/kg) than in WT mice (ED_{50} = 4.34 ± 0.21 mg/kg). Studies in models of cutaneous pain induced by thermal stimuli previously demonstrated an enhancement of morphine analgesia by σ_1 receptor antagonists or by antisense oligodeoxynucleotides against σ_1 receptors.[48–50] However, the present study reports the first evidence that σ_1 receptors modulate morphine-induced analgesia in a visceral pain model.

An increased number of capsaicin receptor TRPV1-expressing nerve fibers were reported in rectosigmoid mucosa biopsy specimens from patients with irritable bowel syndrome, one of the most prevalent human gastroenterological pain disorders, and the level of TRPV1 expression correlated with the intensity of abdominal pain.[51] Our data may therefore have clinical relevance, because the visceral pain induced by intracolonic instillation of capsaicin to mice can mimic this painful condition through the activation of TRPV1 receptors, suggesting a possible role for σ_1 receptor antagonists in irritable bowel syndrome treatment.

In conclusion, these findings demonstrate that σ_1 receptors play a key role in enteric visceral pain, an important and prevalent pain condition, suggesting that σ_1 receptor blockade may possibly represent a novel therapeutic strategy for treating this pain condition.

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