σ₁ 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 (σ₁) receptors modulate somatic pain, but their involvement in pure visceral pain is unexplored.

Methods: The authors evaluated the role of σ₁ 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 σ₁ receptor knockout (σ₁-KO) (n = 10 per group) mice, selective σ₁ 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 σ₁-KO mice. However, the maximum number of pain-related behaviors induced by 1% capsaicin in σ₁-KO mice (mean ± SEM, 22 ± 2.9) was 48% of that observed in WT animals (46 ± 4.2). Subcutaneous administration of the σ₁ 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 σ₁-KO mice. Thus, the effects of these drugs are specifically mediated by σ₁ receptors. Morphine produced an inhibition of capsaicin-induced visceral pain in WT and σ₁-KO mice, whereas ketoprofen had no effect in either mouse type.

Conclusion: These results suggest that σ₁ receptors play a role in the mechanisms underlying capsaicin-induced visceral pain and raise novel perspectives for their potential therapeutic value.

Visceral pain is the most frequent type of pathological pain and one of the main reasons for patients to seek medical assistance. However, most of our knowledge about pain mechanisms derives from experimental studies of somatic (principally cutaneous) pain rather than visceral pain. 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.

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. This model permits examination of both acute visceral pain and referred mechanical hyperalgesia to the abdominal wall, which is an important clinical

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

• σ₁ 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 σ₁ receptors might provide a therapeutic approach to visceral pain

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Anesthesiology, V 118 • No 3

691 March 2013
feature of visceral pain.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{18,19} The role of σ\(_1\) receptors in somatic pain control is well documented. Thus, σ\(_1\) receptor knockout (σ\(_1\)-KO) mice\textsuperscript{20–22} and wild-type (WT) mice treated with σ\(_1\) receptor antagonists\textsuperscript{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.

\section*{Materials and Methods}

\subsection*{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.\textsuperscript{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).

\subsection*{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\textsuperscript{19}; and S1RA (4-[2-[[5-methyl-1-(2-naphthalenyl)-1H-pyrazol-3-yl]oxy]ethyl] morpholine,\textsuperscript{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 Quimica 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 Quimica 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.

\subsection*{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,\textsuperscript{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. Perianal and external genitalia areas were avoided, concentrating the stimulation on the lower and mid abdomen, as reported previously. 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 \( \sigma \)-KO groups, solvent- or capsaicin-treated groups, and saline- or drug-treatment groups were run in parallel. Each animal (\( n = 12 \) WT or \( n = 10 \) \( \sigma \)-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 \( \sigma \)-KO Mice**

WT and \( \sigma \)-KO mice were administered different capsaicin concentrations (0.01–1%) intracolonomically, 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 \( x \), 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 \( \sigma \)-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 \( \sigma \) receptor antagonist on the responses induced by different concentrations of capsaicin. Second, we tested the effects of several doses of various \( \sigma \) 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 \( \sigma \)-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 \( \sigma \)-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 \( \sigma \) 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 \( \sigma \)-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: 50% mechanical threshold (g) = \([(10^{0.5\cdot x\pm \delta})/10,000]\), where \( x \) = value (in logarithmic units) of the final von Frey filament used, \( \kappa \) = tabular value for the pattern of positive/negative responses, and \( \delta \) = 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 allogene, or drug and allogene, 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
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 $\sigma_1$-KO Mice

In both WT and $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-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

Effects of $\sigma_1$ Receptor Antagonists on Visceral Pain Induced by Intracolonic Capsaicin

The subcutaneous administration of the selective $\sigma_1$ 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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-KO mice (fig. 3B).

To determine whether the effects of BD-1063 were dose-dependent and shared by other $\sigma_1$ antagonists, we administered various doses of the selective $\sigma_1$ antagonists BD-1063, S1RA, and NE-100 at 30 min before instillation of the capsaicin concentrations found to induce the

![Fig. 1](http://anesthesiology.pubs.asahq.org/10/30/2018) 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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-KO animals at the same concentration of capsaicin: **$P < 0.01$ (two-way ANOVA followed by Bonferroni test). WT, wild type; $\sigma_1$-KO, $\sigma_1$ 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 σ1-KO mice (fig. 4). None of the σ1 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 σ1-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...
The effect of morphine (1–4 mg/kg) was significantly greater in WT and $\sigma_1$-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 $\sigma_1$-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; $\sigma_1$-KO, $\sigma_1$ receptor knockout.

**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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-KO mice and that the pharmacological blockade of $\sigma_1$ receptors inhibited the capsaicin-induced pain-related behaviors and mechanical referred hyperalgesia in WT mice but not in $\sigma_1$-KO animals. This is the first report to present evidence of a role for the $\sigma_1$ 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 $\sigma_1$-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...
of intraplantar formalin-induced pain, also attributed to the direct activation of nociceptors, was attenuated by 61% in $\sigma_1$-KO mice,20 similar to the reduction in the number of pain-related behaviors (52%) observed in the present $\sigma_1$-KO mice.

The mechanical threshold was similar between naïve WT and $\sigma_1$-KO animals (0.53 ± 0.09 and 0.51 ± 0.1 g, respectively), indicating that $\sigma_1$-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 $\sigma_1$ receptors in naïve $\sigma_1$-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 $\sigma_1$-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 $\sigma_1$-KO mice.22 Although somatic- and visceral-induced hyperalgesic states are both mediated by

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 $\sigma_1$-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 $\sigma_1$-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; $\sigma_1$-KO, $\sigma_1$ receptor knockout.

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 $\sigma_1$-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 $\sigma_1$-KO mice per group. The dashed line indicates the 50% threshold in capsaicin solvent-treated WT and $\sigma_1$-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 $\sigma_1$-KO animals at the same dose of morphine: *P < 0.05; **P < 0.01 (two-way ANOVA followed by Bonferroni test). WT, wild type; $\sigma_1$-KO, $\sigma_1$ 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,3,35–37 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.3,36 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,β-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 α,β-KO mice in comparison with α,β-KO mice treated with capsaicin intraplantarly.

In the present study, subcutaneous administration of the α,β 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 α,β-KO mice. According to these results, the effects of the α,β antagonists are specifically mediated by their interaction with α,β receptors. The highest doses of all of the α,β receptor antagonists tested reduced the number of capsaicin-induced pain-related behaviors in WT mice to the same number observed in α,β-KO animals, indicating that these doses of BD-1063, S1RA, and NE-100 are sufficient to totally block the fraction of α,β receptors required to fully express the pain-related behaviors. The remaining response to capsaicin in α,β-KO and WT animals treated with the highest doses of α,β receptor antagonists indicates that mechanisms other than α,β receptor activation are also implicated in the acute visceral pain induced by capsaicin in both mouse types. It was previously reported that α,β receptor antagonists had no effect on acute pain induced by thermal or mechanical stimuli to the paw skin22–24,39 but almost completely abolished the first phase of formalin-induced pain23,27 and, in the present study, partially reduced capsaicin-induced visceral pain-related behaviors. These data indicate that the analgesic actions of α,β 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 µg/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 α,β antagonists. At any rate, the antihyperalgesic effect of BD-1063, S1RA, and NE-100 in WT mice was attributable to their interaction with α,β receptors, given their complete lack of effect in α,β-KO mice. The role of α,β receptors in pain hypersensitivity in somatic pain models in WT mice has been documented previously. It has been reported that α,β 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 α,β-KO mice and the inhibition of this hyperalgesia in WT mice treated with α,β receptor antagonists. This mismatch may be attributable to the development of compensatory mechanisms in α,β-KO mice versus WT animals, a general fact in genetic knockout experiments.60 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,61 endocannabinoid signaling,62 the role of 5-HT7 receptors in depression,63 and the role of δ-subunit–containing γ-aminobutyric acid subtype A receptors in nociception,64 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 (ED50 = 1.41 mg/kg) and in the second inflammatory phase of the formalin test (ED50 = 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 α,β-KO mice. These results agree with previous reports.
that morphine significantly inhibited intracolonic capsaicin-evoked c-Fos activation in the spinal cord\cite{6} and the visceral pain induced by intracolonic mustard oil.\cite{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.\cite{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 gastrointestinal pain disorders, and the level of TRPV1 expression correlated with the intensity of abdominal pain.\cite{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.

The authors thank Richard Davies, M.A. (Granada, Spain), for editorial revision of the article.

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