Neuropharmacology and analgesia

Mechanisms involved in abdominal nociception induced by either TRPV1 or TRPA1 stimulation of rat peritoneum

Gabriela Trevisana, Mateus F. Rossato, Carin Hoffmeister, Sara M. Oliveira, Cássia R. Silva, Filipe C. Matheus, Gláucia C. Mello, Edson Antunes, Rui D.S. Prediger, Juliano Ferreira


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ABSTRACT

Abdominal pain is a frequent symptom of peritoneal cavity irritation, but little is known about the role of the receptors for irritant substances, transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1), in this painful condition. Thus, we investigated the abdominal nociception caused by peritoneal stimulation with TRPV1 (capsaicin) and TRPA1 (allyl isothiocyanate, AITC) agonists and their mechanisms in rats. The intraperitoneal (i.p.) injection of either capsaicin or AITC (0.03–10 mg/kg) induced short-term (up to 20 min) and dose-dependent abdominal nociception, and also produced c-fos expression in spinal afferents of the dorsal horn. TRPV1 antagonism prevented (94 ± 4% inhibition) nociception induced by capsaicin but not by AITC. In contrast, the TRPA1 antagonism almost abolished AITC-induced nociception (95 ± 2% inhibition) without altering the capsaicin response. Moreover, nociception induced by either capsaicin or AITC was reduced by the desensitisation of TRPV1-positive sensory fibres with resiniferatoxin (73 ± 18 and 76 ± 15% inhibitions, respectively) and by the NK1 receptor antagonist aprepitant (56 ± 5 and 53 ± 8% inhibitions, respectively). Likewise, the i.p. injections of capsaicin or AITC increased the content of substance P in the peritoneal fluid. Nevertheless, neither the mast cell membrane stabiliser cromoglicate, nor the H3 antagonist promethazine, nor depletion of peritoneal macrophages affected abdominal nociception induced either by capsaicin or AITC. Accordingly, neither capsaicin nor AITC increased the histamine content in the peritoneal fluid or provoked peritoneal mast cell degranulation in vitro. Collectively, our findings suggest that TRPV1 and TRPA1 stimulation in the peritoneum produces abdominal nociception that is mediated by sensory fibres activation.

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1. Introduction

Transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1) are non-selective cation channels involved in the peripheral detection of several painful stimuli (Andrade et al., 2012; Jara-Oseguera et al., 2008; Moran et al., 2011). An important feature of both channels is that they are activated by irritant substances, such as capsaicin (the “hot” component of chilli peppers), which activates TRPV1, and allyl isothiocyanate (AITC, the major component of mustard oil), which stimulates TRPA1 (Moran et al., 2011).

TRPV1 and TRPA1 are usually co-expressed in a subset of small diameter sensory fibres together with the neuropeptide substance P (Bautista et al., 2005; Story et al., 2003). It has been well described that activation of TRPV1 and TRPA1 in the sensory neurons releases neuropeptides and transmits painful stimuli to the central nervous system, which causes pain (Corrigian and Szallasi, 2009; Geppetti et al., 2008). In fact, the receptors TRPV1 and TRPA1 have been implicated in painful processes observed in somatic (such as skin and joints) and visceral (such as intestine, pancreas and urinary bladder) organs (Akbar et al., 2008; Andrade et al., 2012; Lapointe and Altier, 2011; Moran et al., 2011; Schwartz et al., 2011). In addition to sensory neuron activation and neuropeptide release, mast cell stimulation and histamine secretion have been implicated in nociception caused by triggering TRPA1 and TRPV1 in the skin and viscera (Andrade et al., 2008; Futamura et al., 2009; Inoue et al., 1993; Massaad et al., 2004).
The peritoneum is the largest and most complex serous membrane of the body and consists of two layers: the parietal peritoneum lines the abdomino-pelvic cavity and the visceral peritoneum over the external surface of the viscera and consists of two layers: the parietal peritoneum (Elsayes et al., 2006). The irritation or inflammation of the peritoneum by chemical substances or microorganisms usually induces abdominal pain, which is a common complaint in all settings of medical practice (Flasar and Goldberg, 2006; Mactier et al., 1998). The peritoneal cavity contains both visceral and somatic sensory innervations and has a large number of resident cells, such as mast cells (Anaf et al., 2006; Flasar and Goldberg, 2006; Lantéri-Minet et al., 1993).

In addition, both TRPV1 and TRPA1 are expressed in somatic and visceral sensory neurons, as well as in mast cells (Biro et al., 1998; Malin et al., 2011; Prasad et al., 2008; Standier et al., 2004; Weller et al., 2011), and are important receptors for chemical irritants (Moran et al., 2011). However, the ability of such receptors to induce abdominal pain after peritoneum stimulation and the mechanisms involved are poorly understood. The goal of this study was to investigate whether abdominal nociception is induced by the activation of either TRPV1 or TRPA1 and some of the mechanisms involved in this response.

2. Material and methods

2.1. Animals

Adult male Wistar rats (200–250 g) bred in our animal house were used in all of the experiments. The animals were housed in groups of 5 to a cage in a controlled temperature environment maintained at 22 ± 1 °C with a 12-h light/dark cycle (lights on from 6:00 a.m. to 6 p.m.) and fed standard lab chow and tap water ad libitum. The animals were acclimated to the experimental room for at least 2 h before the experiments. Each animal was used only once. All of the experiments were carried out according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983). The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments. All of the protocols were approved by the Ethics Committee of the Federal University of Santa Maria (CIETEA, protocol number: 029/2012).

2.2. Drugs

Capsaicin, allyl isothiocyanate (AITC), 4′-chloro-3-methoxy-cinnamaldehyde (SB-366791), sodium cromoglicate, compound 48/80, α-phthaldialdehyde, resiniferatoxin (RTX), metronidazole, HC-030031, and histamine dihydrochloride were purchased from Sigma (Sigma, St Louis, MO, USA). Camphor was purchased from VETEC (Rio de Janeiro, Brazil). Promethazine was obtained from Cristália (São Paulo, Brazil). Aprepitant (MK-869) was extracted from commercially available capsules (Emend®, Merck, USA), and its identity and purity (greater than 98%) were confirmed by nuclear resonance methods. The stock solutions of capsaicin and AITC were prepared in 90% ethanol and 10% Tween 80. Resiniferatoxin was diluted in 10% ethanol and 10% Tween 80 in phosphate-buffered saline (PBS). Camphor and SB-366791 were suspended in 1% Tween 80 and 1% DMSO in PBS. Sodium cromoglicate, compound 48/80, promethazine and aprepitant were diluted in PBS for injection. The PBS had the following composition: 137 mM NaCl and 10 mM sodium phosphate buffer (pH 7.4). The final concentrations of ethanol and Tween 80 did not exceed 1% and also did not produce any effect on their own.

2.3. Evaluation of capsaicin- and AITC-induced abdominal nociception in rats

Animals were placed individually in chambers (transparent glass boxes) and were allowed to adapt for 20 min before the algogen injection. Abdominal nociception was elicited by the intraperitoneal (i.p.) administration of either capsaicin or AITC. The control animals received the same volume of the vehicle (10 mL/kg, 0.95% ethanol and 0.05% Tween 80 in PBS). Abdominal nociception was qualitatively evaluated using a scale from 0 to 3 points for each 10-min interval, as previously described with some modifications (Schmauss and Yaksh, 1984). The abdominal nociceptive score was assigned as follows: 0 = normal body position of the rat and normal exploratory behaviour, 1 = leaning posture favouring the left or right body side, 2 = stretching of the hindlimbs, dorsoflexion of the hind paws, and body stretched and flat on the bottom, frequently with the pelvis rotated sideward, 3 = contraction of the abdominal muscles followed by a stretching of the body and extension of the hind limbs (writhing response). Abdominal nociception was also quantitatively measured by the amount of time an animal presented a nociceptive score ≥1 timed in 10-min blocks (Schmauss and Yaksh, 1984).

Initially, we observed the abdominal nociception elicited by the i.p. administration of capsaicin (0.1 mg/kg) or AITC (3 mg/kg) at 10-min intervals over a total time of 30 min. Afterwards, a dose-response curve for the abdominal nociception induced by the i.p. administration of capsaicin (0.03–0.3 mg/kg) or AITC (1–10 mg/kg) was carried out. For this experiment, the abdominal nociception time and score were observed for 10 min after the algogen injection.

To observe a possible co-participation of the TRPV1 and TRPA1 receptors in the abdominal nociception induced by capsaicin or AITC, we have co-injected these substances. Then, we have co-administered the capsaicin (0.01 mg/kg, i.p.) and AITC (1 mg/kg, i.p.) in doses that did not induce nociception previously. In addition, we have also co-injected the TRPV1 (SB-366791, 0.25 mg/kg, i.p.) and TRPA1 (camphor, 0.25 mg/kg, s.c.) antagonists 30 min before the administration of capsaicin (0.01 mg/kg, i.p.) plus AITC (3 mg/kg, i.p.) or its vehicles.

2.4. Fos immunohistochemistry and quantification

Fos immunohistochemistry was performed as previously described (Bonaz et al., 1994, 2000). Rats were sacrificed 10 min after i.p. injection of vehicle, capsaicin (0.1 mg/kg, i.p.) or AITC (3 mg/kg, i.p.). Animals were transcardially perfused with 0.1 M phosphate buffer (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.1 M PBS. Spinal cords segments were rapidly removed, postfixed for 24 h at the same fixative, and subsequently cryoprotected overnight in 30% sucrose in 0.1 M PBS. Frozen coronal sections (40 μm) of the spinal cord (segments thoracic or cervical) were cut on a cryostat (Leica 1850, Germany) and processed for Fos-IR. Free-floating sections were incubated for 16–18 h at 4 °C with the primary antibody (Fos AB-5 rabbit polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:1000 in PBS 0.02 M, containing 0.5% Triton X-100 and 10% normal goat serum) and then with a biotinylated secondary antibody (goat anti-rabbit, Dako, USA; dilution 1: 250) for 2 h at room temperature. Sections were finally processed for avidin-biotin-peroxidase using diaminobenzidine as a chromogen (Sigma, St Louis, MO, USA), then mounted on gelatin-coated slides, dehydrated, cleared in xylene, and cover-slipped.

The presence of Fos immunoreactivity (Fos-IR) was detected by optic microscopy as a brown-black reaction product in cell nuclei. Fos positive cells were counted with the software Image J at the thoraco-lumbar (T2-L2) and cervical (C1-C5) levels of the spinal cord on 5 consecutive sections, in the dorsal horn of the gray
mater. Results were expressed by the mean number of Fos-IR-cells per dorsal horn.

2.5. Role of sensory fibres, TRPV1, TRPA1 and NK1 in abdominal noiception evoked by capsaicin or AITC in rats

To assess the possible contribution of the TRPV1, TRPA1 and NK1 in the nociceptive responses induced by capsaicin (0.1 mg/kg) and AITC (3 mg/kg), the animals were treated with SB-366791 (a selective TRPV1 antagonist, 0.25 mg/kg, i.p. or s.c.), camphor (a non-selective TRPA1 antagonist, 0.25 mg/kg, s.c.), HC-030031 (a selective TRPA1 antagonist, 30 mg/kg, i.p.), aprepitant (a tachykinin NK1 receptor antagonist, 2.5 mg/kg, i.p.) or the respective vehicles 30 min before the algogen injection. Then, abdominal noiception was observed individually for 10 min after the i.p. administration of capsaicin (0.1 mg/kg), AITC (3 mg/kg) or the vehicle, as described above. The dose of the antagonists and their treatment time were based on previous data described in the literature as well as those determined in pilot experiments (Kopczyńska, 2008; Millan et al., 2010).

In order to investigate the effect of TRPV1-positive sensory fibres on noiception induced by capsaicin or AITC, we used a desensitisation protocol employing resiniferatoxin, as described previously (Steiner et al., 2007). The animals were pre-treated with a single i.p. injection of resiniferatoxin (200 μg/kg) or the vehicle (10% ethanol and 10% Tween 80 in PBS, 1 mL/kg) under anaesthesia induced by a mixture of ketamine and xylazine (90 mg/kg and 3 mg/kg, respectively, i.p.). The animals were tested 7 days after treatment with resiniferatoxin or the vehicle.

To assess whether a complete desensitisation of the TRPV1-positive fibres had occurred after treatment with RTX, the animals were submitted to an eye-wiping test, as previously described (Ikeda et al., 2001; Jakab et al., 2005). For this experiment, 20 μL of 10 μg/mL capsaicin solution was administered into the eye, and the number of wiping movements that occurred in a 1-min time interval was counted. The animals that wiped their eyes no more than 5 times were considered to be desensitised by the RTX treatment. Subsequently, the animals received an i.p. injection of capsaicin (0.1 mg/kg), AITC (3 mg/kg) or the vehicle, and their nociceptive responses were observed individually for 10 min, as described above.

2.6. Assessment of the participation of resident peritoneal mast cells and macrophages in capsaicin- and AITC-elicted abdominal noiception in rats

The role of resident peritoneal mast cells on capsaicin- and AITC-induced abdominal noiception in rats was investigated by using the mast cell membrane stabiliser cromoglycate and the histaminergic H1 receptor antagonist promethazine. Cromoglycate (80 mg/kg, i.p.), promethazine (20 mg/kg, i.p.) were administered 1 or 0.5 h, respectively, before the i.p. injection of capsaicin (0.1 mg/kg), AITC (3 mg/kg), the mast cell degranulator compound 48/80 (0.75 mg/kg, used as a positive control) or the vehicle (10 mL/kg, i.p.). The nociceptive response was observed individually for 10 min, as described above. The dose of the drugs and their treatment time were based on previous data described in the literature as well as those determined in pilot experiments of our group using positive controls (Ohita et al., 2003).

To observe the effect of resident peritoneal macrophages in the nociceptive effect of capsaicin and AITC, we have used the protocol of peritoneal macrophage depletion as the method described before (Ribeiro et al., 2000; Kamei et al., 2010). In order to deplete the macrophage content in the peritoneal cavity, rats were i.p. injected with PBS (10 mL) under isoflurane anesthesia and the peritoneal cavity was washed three times. Immediately after the procedure, animals were injected with capsaicin (0.1 mg/kg, i.p.), AITC (3 mg/kg, i.p.), or vehicle (10 mL/kg, i.p.) and their nociceptive responses were observed individually for 10 min, as described above. Control animals were not submitted to peritoneal macrophage depletion, but were anesthetized as described above. We detected that peritoneal lavage decreased (about 90%) the number of resident macrophages (data not shown), in accordance with previous studies (Ribeiro et al., 2000).

2.7. Determination of the histamine and substance P contents in the peritoneal cavity

To observe if the i.p. injections of either capsaicin or AITC were capable of inducing the degranulation of mast cells, the histamine content in the peritoneal fluid was determined, as previously described (Oliveira et al., 2011). The animals received an i.p. administration of capsaicin (0.1 mg/kg, i.p.), AITC (3 mg/kg, i.p.) or the vehicle (10 mL/kg, i.p., 0.95% ethanol and 0.05% Tween 80 in PBS). After 10 min, the animals were euthanised using a carbon dioxide chamber, and their peritoneal cavities were washed with 3 mL of PBS containing 1 mM metronidazole (a histamine methyl transferase inhibitor used to reduce histamine degradation). Aliquots of the peritoneal fluids were centrifuged at 12,000 × g and 4 °C for 10 min, and the resulting supernatants were used to evaluate the histamine content. Next, 150 μL of NaOH (1 M) was added to 400 μL of the supernatant and incubated with 40 μL of 1% o-phthalaldehyde for 4 min. Next, 75 μL of HCl (3 M) was added to stop the reaction and allow for the development of fluorescence. The histamine contents were determined spectrophotometrically with an excitation wavelength of 360 nm and an emission wavelength of 450 nm using a fluorescence photometer. The histamine release was expressed as the content of histamine (in μg) per mL of peritoneal fluid, as compared to a standard curve of histamine.

The substance P levels were also measured from an aliquot of the peritoneal fluid supernatant obtained 10 min after the i.p. injection of capsaicin, AITC, or the vehicle using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Cayman Chemicals Company, Ann Arbor, USA). For this experiment, the animals received an i.p. administration of capsaicin (0.1 mg/kg, i.p.), AITC (3 mg/kg, i.p.) or the vehicle (10 mL/kg, i.p., 0.95% ethanol and 0.05% Tween 80 in PBS). After 10 min, the animals were euthanised using a carbon dioxide chamber, and their peritoneal cavities were washed with 3 mL of PBS. Aliquots of the peritoneal fluids were centrifuged at 12,000 × g and 4 °C for 10 min, and the resulting supernatants were used to evaluate the content of substance P, which was expressed as the SP (in pg) content per mL of peritoneal fluid, as compared to a standard curve of substance P.

2.8. Mast cell degranulation in vitro

Mast cells from the peritoneal cavities of the rats were purified on a Percoll gradient, as described previously (Desouza et al., 2009). Briefly, the rats were exsanguinated under halothane anesthesia, and 10 mL of Krebs-Ringer phosphate solution (pH 7.4) was injected into their intraperitoneal cavities. The Krebs-Ringer solution had the following composition (mM): NaCl (150), KCl (6.1), Na2HPO4 (10), MgSO4 (1.5), CaCl2 (42.9), and glucose (5.6). The abdomen was carefully massaged, and the peritoneal fluid was withdrawn, placed in polypropylene centrifuge tubes and centrifuged at 300 × g for 5 min at 4 °C. The resulting cell pellet (of which mast cells comprise 10%) was gently resuspended in a small volume of Krebs-Ringer phosphate solution, layered over the isotonic Percoll gradient and left at room temperature for 10 min prior to centrifugation (150 × g for 25 min at 4 °C). The gradient zone containing the mast cells was removed and washed twice in Krebs-Ringer phosphate solution. The purity of the cells in the final preparation was 90–95%, and their viability (as assessed by 0.1% Trypan blue dye exclusion) was
approximately 95%. Aliquots of the mast cell suspension (0.5 mL containing $4 \times 10^6$ cells/mL) were warmed to $37^\circ C$ for 10 min. Capsaicin (0.001–10 μM), AITC (1–1000 μM), the vehicle (0.01% ethanol in Krebs-Ringer phosphate solution), or compound 48/80 (1 μg/mL, used as a positive control) was added to the suspension (final volume of 1 mL). After 15 min of incubation, the reaction was quenched by placing the test tubes in ice-cold water. The cells were then centrifuged (3000 $\times g$ for 10 min at 4 $^\circ C$), and the supernatant was removed for histamine determination. Then, Krebs-Ringer phosphate solution (1 mL) was added to the pellet, which was boiled at 100 $^\circ C$ for 10 min to release the residual histamine. Then, 150 μL of NaOH (1 M) was added to 400 μL of the supernatant or the resuspended pellet and incubated with 40 μL of 1% o-phthaldialdehyde for 4 min. Next, 75 μL of HCl (3 M) was added to stop the reaction and allow for the development of fluorescence. The histamine contents were determined spectrophotometrically with an excitation wavelength of 360 nm and an emission wavelength of 450 nm using a fluorescence photometer, as previously described (Oliveira et al., 2011). The histamine release was expressed as a percentage of the total cellular content of the amine.

2.9. Gastrointestinal transit

We have also observed if capsaicin (0.1 mg/kg) or AITC (3 mg/kg) i.p. administration alters the normal gastrointestinal transit it was analysed, as described previously (Holtman et al., 2010). Rats were housed in cages without food for 18 h. Then, animals were treated with capsaicin (0.1 mg/kg), AITC (3 mg/kg), or vehicle (10 mL/kg, 0.95% ethanol and 0.05% Tween 80 in PBS). Consecutively, rats were given a standard charcoal meal (5% charcoal and 20% Arabic gum, 2 mL) by gavage. Ten minutes after administration of the charcoal meal, the animals were euthanized and their stomachs and small intestines were removed. The length of the intestine (from the pyloric sphincter to the ileo-caecal junctions) and the distance travelled by the charcoal meal were measured. The propulsive activity of the gut was evaluated by determining the percentage of gastrointestinal travelled charcoal, using the following equation: travelled (%) = $100 \times (\text{distance travelled/total gut length})$.

2.10. Myeloperoxidase activity assessment

Myeloperoxidase (MPO) activity was determined as described before (Sauzem et al., 2009). Animals were i.p. injected with capsaicin (0.1 mg/kg), AITC (3 mg/kg), or vehicle (10 mL/kg, 0.95% ethanol and 0.05% Tween 80 in PBS). Ten minutes after samples of intestine were used to analyze the MPO activity. Samples were homogenized in 0.3 mL of sodium acetate buffer (80 mM, pH 5.5) plus 0.5% hexadecyltrimethylammonium bromide (HTAB), and centrifuged (10,000 $\times g$) at 4 $^\circ C$ for 20 min; supernatants were used for the assay. Then, 10 μL of supernatant was added to 200 μL of sodium acetate buffer (80 mM, pH 5.5) and 10 μL of 5-(N,N-diethylamino)-pentyl-3,4,5-trimethoxybenzoate. After an incubation period of 3 min at 37 $^\circ C$, the reaction was stopped on ice by the addition of 30 μL acetic acid. The visible absorbance was analyzed on a spectrophotometer at 610 nm, a Fisher Biotech.

Fig. 1. Intrapertoneal injection of the TRPV1 agonist capsaicin induced a short-term abdominal nociceptive response. (A) and (B) Time-course of the abdominal nociception elicited by the i.p. injection of capsaicin in rats. The animals received an i.p. injection of capsaicin (CPS, 0.1 mg/kg) or the vehicle (Veh, 0.95% ethanol and 0.05% Tween 80 in PBS, 10 mL/kg), and then the nociception time (A) and nociception score (B) were observed for 30 min. Then, the nociception time (C) and nociception score (D) were observed for 10 min. Each column represents the mean ± S.E.M. (A and C) or the median ± interquartile range (B and C) of five to six rats. The asterisks denote the significance levels: *P < 0.05, **P < 0.01, and ***P < 0.001 in comparison to the vehicle-treated group. Student’s “t” test (B); Student’s “t” test followed by the Mann-Whitney test (B); one-way ANOVA followed by Bonferroni’s post-hoc test (C) or the Kruskal Wallis test followed by Dunn’s test (D).
Microkinetics BT 2000 (Fisher Scientific, Pittsburgh, PA, USA) microplate reader. Values were expressed as optical density corrected by mg of tissue.

2.11. Measurement of plasma protein extravasation

The vascular permeability induced by the i.p. injection of capsaicin or AITC was determined according to previously described with some modifications (An et al., 2011). The plasma protein extravasation was observed after 10 min of the injection of capsaicin (0.1 mg/kg), AITC (3 mg/kg), or vehicle (10 mL/kg, 0.95% ethanol and 0.05% Tween 80 in PBS). Animals were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and the Evans blue (50 mg/mL in saline solution, NaCl 0.9%) was injected through the tail vein (1 mL/kg, i.v.). Then, ear or meningeal samples were removed and immersed in N,N-dimethylformamide at 55 °C for 24 h. Afterwards, the extract of

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**Fig. 2.** Intraperitoneal injection of the TRPA1 agonist allyl isothiocyanate caused short-term abdominal nociception. ((A) and (B)) Time-course of the abdominal nociception provoked by i.p. injection of AITC in rats. The animals received an i.p. injection of AITC (3 mg/kg) or the vehicle (Veh, 0.95% ethanol and 0.05% Tween in PBS, 10 mL/kg), and then the nociception time (A) and nociception score (B) were observed for 30 min. ((C) and (D)) Dose–response curve of AITC-elicited abdominal nociception in rats. Different doses of AITC (1–10 mg/kg) or the vehicle (0.95% ethanol and 0.05% Tween in PBS, 10 mL/kg) were administered by i.p. injection and the nociception time (C) and nociception score (D) were observed for 10 min. The co-injection of sub-effective doses of capsaicin plus AITC induced nociception, and these responses were reduced by the co-administration of TRPV1 and TRPA1 antagonists. The selective TRPV1 antagonist SB-366791 (0.25 mg/kg, s.c.) plus the non-selective TRPA1 antagonist camphor (0.25 mg/kg, s.c.) administered systemically 30 min before the i.p. injection of capsaicin (CPS) plus AITC (1 mg/kg, i.p.) diminished the nociceptive time (E) and score (F). Each column represents the mean ± S.E.M. ((A), (C), and (E)) or the median ± interquartile range ((B), (C), and (F)) of five to six rats. The asterisks denote the significance levels: *P < 0.05, **P < 0.01, and ***P < 0.001 in comparison to the vehicle-treated group, and ##P < 0.01 in comparison to the capsaicin plus AITC-treated groups (pre-treated with vehicle); Student “t” test (A); Student “t” test followed by the Mann–Whitney test (B); one-way ANOVA followed by Bonferroni’s post-hoc test ((C) and (E)) or the Kruskal Wallis test followed by Dunn’s test ((D) and (F)).
each sample was centrifuged at (5000 × g, 10 min at 20°C). The Evans blue content in supernatants was quantified by reading the optical density at 620 nm (measured in a Plate reader, Biotech, USA). The content of Evans blue was compared with a standard curve, and plasma extravasation was expressed as µmol of Evans blue extracted per mg of tissue.

2.12. Statistical analysis

The results were expressed as the mean ± S.E.M for the nociception time and mediator concentration and the median followed by the interquartile range for the nociception scores. The ED_{50} values were reported as the geometric means accompanied by their respective 95% confidence limits. Parametric data were analysed by one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test or by the Student's "t" test when appropriate. Non-parametric data were analysed either by the Student's "t" test followed by the Mann–Whitney test or the Kruskal–Wallis test followed by Dunn's test. The ED_{50} values were determined by non-linear regression analyses with a sigmoid dose–response equation using GraphPad Software 5.0 (GraphPad Software, San Diego, CA, USA). The E_{max} (maximum effect for the TRPV1 and TRPA1 agonists) was described in seconds for the nociception time response or in a percentage for the maximal nociceptive score (3). The percentages of inhibition are reported as the mean ± S.E.M. obtained in each individual experiment in relation to the control values. P values less than 0.05 (P<0.05) were considered significant.

3. Results

3.1. Characterisation of abdominal nociception induced by i.p. injections of capsaicin and AITC

The i.p. injection of the TRPV1 agonist capsaicin (0.1 mg/kg) induced a short-term nociceptive response, observed by the nociception time and score, that peaked during the first 10 min and disappeared within 20–30 min after administration (Fig. 1A and B). The capsaicin-induced nociceptive response was dose-dependent with calculated values of ED_{50} and E_{max} of 0.083 (0.067–0.102) mg/kg and 563 ± 20 s and 0.124 (0.094–0.163) mg/kg and 100% for the nociception time and score, respectively (Fig. 1C and D).

Similar to the results obtained using capsaicin, i.p. injection of the TRPA1 agonist AITC (3 mg/kg) was also capable of inducing short-term nociception, peaking at 10 and lasting for a maximum of 20 min (Fig. 2A and B). The calculated ED_{50} and E_{max} values were 2.3 (1.4–3.9) mg/kg and 514 ± 54 s or 3.1 (2.1–4.7) mg/kg and 100% for nociception time and score, respectively (Fig. 2C and D).

Moreover, we have observed that the co-injection of sub-effective doses of capsaicin (0.03 mg/kg, i.p.) plus AITC (1 mg/kg, i.p.) induced nociception (indicated as nociceptive time and nociceptive score).

Fig. 3. Capsaicin or AITC i.p. injection induced c-fos expression in thoraco-lumbar level spinal cord lumbar sections. (A) Photomicrographs of transverse sections of the thoraco-lumbar spinal cord and (B) pooled data representative of the action of i.p. injection of capsaicin (CPS, 0.1 mg/kg) or AITC (3 mg/mg) on the expression of c-fos-like immunoreactivity (LI) in rats 10 min after administration (5 animals for each treatment). The arrows indicate the c-fos immunoreactive cells. The representative photomicrograph of each group is an example one section (T2-L2) of the dorsal horn. Each column represents the mean ± S.E.M. of of Fos-IR-cells per dorsal horn; n≥5 slices per condition in T2-L2 segments; **p<0.001 in comparison to the vehicle (Veh)-treated group one-way ANOVA followed by Bonferroni's post-hoc test.
These responses were reduced by the administration of the combination of the TRPV1 (SB-366791, 0.25 mg/kg, s.c.) plus the TRPA1 (camphor, 0.25 mg/kg, s.c.) antagonists (97 ± 8 or 100% inhibition, for nociceptive time and score, respectively) (Fig. 2E and F).

Confirming the behavioral findings and indicating neuronal activation, capsaicin or AITC i.p. injection caused an increase in the c-Fos immunoreactivity (Fos-IR) of the dorsal horn cells in the thoraco-lumbar level of the spinal cord, when compared with vehicle i.p. injection (Fig. 3A and B). On the other hand, capsaicin, AITC, or vehicle i.p. treatment did not induce Fos-IR of cells in the cervical level of the spinal cord (Suppl. Fig. 1A and B).

To avoid supra-maximal stimulation and unnecessary animal discomfort, we selected the doses of 0.1 mg/kg for capsaicin and 3 mg/kg for AITC with an observational time of 10 min for the next experiments.

### 3.2. Role of TRPV1, TRPA1 and positive sensory fibres in abdominal nociception elicited by capsaicin and AITC

The nociception induced by capsaicin (0.1 mg/kg) was almost abolished by i.p. pre-treatment with the selective TRPV1 receptor antagonist SB-366791 (0.25 mg/kg i.p.; 94 ± 4 and 100% inhibition,

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**Fig. 4.** The effect of TRPA1 and TRPV1 antagonists on agonist-induced nociception. The selective TRPV1 antagonist SB-366791 administered systemically diminished the nociceptive time (A) and score (B) elicited by capsaicin (CPS) i.p. injection. SB-366791 (0.25 mg/kg, i.p.) or the vehicle (Veh, 1% Tween 80 and 1% DMSO in PBS, 1 mL/kg) was injected 30 min before the i.p. administration of capsaicin (0.1 mg/kg), AITC (3 mg/kg) or the vehicle (0.95% ethanol and 0.05% Tween in PBS, 10 mL/kg). The non-selective (camphor) or the selective (HC-030031) TRPA1 antagonists were able to reduce the nociceptive time ((C) and (E)) and score ((D) and (F)) provoked by AITC i.p. administration. Camphor (0.25 mg/kg, s.c.), HC-030031 (30 mg/kg, i.p.), or the vehicle (1% Tween 80, 1% DMSO in PBS, 1 mL/kg) was injected 30 min before the i.p. injection of capsaicin (0.1 mg/kg), AITC (3 mg/kg) or the vehicle (0.95% ethanol and 0.05% Tween in PBS, 10 mL/kg). Afterwards, the nociception time ((A), (C), and (E)) and nociception score ((B), (D), and (F)) were observed for 10 min. Each column represents the mean ± S.E.M. (A), (C), and (E) or the median ± interquartile range (B), (D), and (F) of five rats. The asterisks denote the significance levels: *p < 0.05 and ***p < 0.001 in comparison to the vehicle-treated group (pre-treated with vehicle), and #p < 0.05 and ###p < 0.001 in comparison to the capsaicin- or AITC-treated groups (pre-treated with vehicle); one-way ANOVA followed by Bonferroni’s post-hoc test ((A), (C), and (E)) or the Kruskal Wallis test followed by Dunn’s test ((B), (D), and (F)).
for the nociception time and score, respectively; Fig. 4A and B). However, the response induced by AITC (3 mg/kg) was not prevented by pre-treatment with SB-366791 (Fig. 4A and B). The antagonism effect of SB-366791 was not related to a neutralizing effect because we have also injected SB-366791 by subcutaneous route (0.25 mg/kg, s.c.) and it was also able to reduce capsaicin in a similar way that was observed to the i.p. injection of this TRPV1 antagonist (98 ± 3 and 100% inhibition, for the nociception time and score, respectively) (data not shown).

Abdominal nociception caused by AITC (3 mg/kg) was markedly reduced by the non-selective TRPA1 antagonist camphor (0.25 mg/kg, s.c.), with inhibitions of 95 ± 2 and 67% for the nociception time and score, respectively (Fig. 4C and D). A similar reduction was observed for the selective TRPA1 antagonist (HC-030031, 30 mg/kg, i.p.), with inhibitions of 96 ± 5 and 100% for the nociception time and score, respectively (Fig. 4E and F). Moreover, pre-treatment with camphor (0.25 mg/kg) or HC-030031 (30 mg/kg, i.p.) did not affect capsaicin-induced abdominal nociception (Fig. 4C–F).

To confirm the ablation of TRPV1- and TRPA1-positive fibres, wiping movements induced by the administration of capsaicin (10 μg/ml, 20 μl) into the eye were counted. The rats pre-treated with RTX (200 μg/kg, i.p., 7 days prior) showed an almost complete reduction in nociception induced by capsaicin when compared with the vehicle-treated animals (12.0 ± 0.8 and 3.0 ± 0.3 wiping movements, for the vehicle- and RTX-treated animals, respectively; P < 0.001, Student’s t test).

In addition, the desensitisation of TRPV1-positive sensory fibres caused by pre-treatment with RTX largely reduced capsaicin-(0.1 mg/kg) and AITC-elicited (3 mg/kg) abdominal nociception with inhibitions of 73 ± 18 and 76 ± 15% for the nociception time, respectively, and 100% for the nociceptive score for both treatments (Fig. 5A and B).

### 3.3. Participation of substance P and the tachykinin NK1 receptor in abdominal nociception induced by TRPV1 and TRPA1 agonists

To determine the participation of substance P and its NK1 receptor in nociceptive behaviours induced by either capsaicin or AITC, we first used the selective tachykinin NK1 receptor antagonist aprepitant (MK-869). Pre-treatment with aprepitant (2.5 mg/kg, i.p.) partially, but significantly, reduced the nociception time and score induced by capsaicin (56 ± 5 and 50% inhibition, respectively) and AITC (53 ± 8 and 50% inhibition, respectively; Fig. 6A and B).

Further, we measured the substance P content in the peritoneal fluid 10 min after i.p. injection of capsaicin (0.1 mg/kg), AITC (3 mg/kg) or the vehicle (10 mL/kg, i.p.; 0.95% ethanol and 0.05% Tween 80 in PBS; Fig. 5C). The concentration of substance P in the peritoneal fluid of the vehicle-treated animals was below the detection limit of the high-sensitivity EIA kit (3.9 pg/mL). However, treatment with capsaicin and AITC significantly elevated the concentration of substance P in the peritoneal fluid (12 ± 3 and 12 ± 2 pg/mL, respectively; Fig. 6C).

### 3.4. Role of resident peritoneal mast cells and macrophages in abdominal nociception induced by TRPV1 and TRPA1 agonists

Afterwards, we evaluated the possible participation of mast cells in nociception elicited by capsaicin and AITC. Pre-treatment with the mast cell membrane stabiliser cromoglycate (80 mg/kg, i.p.) did not affect the nociceptive responses elicited by capsaicin (0.1 mg/kg) or AITC (3 mg/kg) (Fig. 7A and B). Nevertheless, abdominal nociception trigged by the mast cell degranulator compound 48/80 was almost abolished by cromoglycate (inhibitions of 90 ± 5 and 100%, for the nociception time and score, respectively). Similarly, pre-treatment with the H1 receptor antagonist promethazine did not affect nociception induced by capsaicin or AITC, but markedly reduced nociception induced by compound 48/80 (inhibitions of 91 ± 6 and 100% for the nociception time and score, respectively; Fig. 7C and D).

In addition, neither the i.p. administration of capsaicin (0.1 mg/kg) nor AITC (3 mg/kg) was capable of altering the histamine concentration in the peritoneal fluid when compared with the vehicle-treated animals, while the i.p. injection of compound 48/80 (0.75 mg/kg) significantly increased the histamine content of the peritoneal fluid (2.6-fold, when compared to vehicle; Fig. 8A). Accordingly, incubation of the isolated peritoneal mast cells with capsaicin (0.001–10 μM) and AITC (1–1000 μM) in vitro did not induce the release of histamine (Fig. 8B and C). In contrast,
compound 48/80 (positive control) caused a release of 85 ± 3% of the total histamine content of the mast cells in vitro.

Moreover, we observed that peritoneal macrophage depletion by repeated peritoneal lavage was not able to decrease the nociceptive time (452 ± 54 and 456 ± 23 s, for depleted or control animals, respectively) and score (2 (2–3) and 2 (2–2.75) score medians (25–75 percentiles), for depleted or control animals, respectively) induced by capsaicin administration (0.1 mg/kg, i.p.). In addition, AITC (3 mg/kg, i.p.) induced nociceptive time (423 ± 63 and 372 ± 35 s, for depleted or control animals, respectively) or score (2 (1–3) and 2 (1.25–2.75) score medians (25–75 percentiles) for depleted or control animals, respectively) were unaltered by the macrophage depletion protocol. Vehicle i.p. injection in control or depleted animals did not induce nociception (data not shown).

3.5. Effect of capsaicin or AITC i.p. injection on gastrointestinal transit, gut inflammation or plasma extravasation

Demonstrating that TRPV1 and TRPA1 agonists did not alter gastrointestinal function or produced gastrointestinal inflammation, capsaicin (0.1 mg/kg, i.p.) or AITC (3 mg/kg, i.p.) i.p. injection did not alter gastrointestinal transit or induced MPO activity when observed 10 min after administration (Suppl. Fig. 2A and B). TRPA1 and TRPV1 agonists did not produce systemic effects thus the i.p. injection of capsaicin (0.1 mg/kg, i.p.) or AITC (3 mg/kg, i.p.) was not able to induce plasma extravasation in peripheral and central tissues, such as ear and meningeal samples, after 10 min of i.p. administration (Suppl. Fig. 3A and B).

4. Discussion

Irritation of the peritoneum by chemical substances induces abdominal pain, which is a common complaint in many areas of medical practice. However, little is known about the role of TRPV1 and TRPA1 in this painful condition. In the present study, we focused on the mechanisms involved in abdominal pain induced by TRPV1 and TRPA1 agonists in rats. We observed that an injection of either capsaicin or AITC into the rat peritoneum induces nociception mediated by TRPV1 and TRPA1 receptor activation. Furthermore, stimulation of TRPV1-positive sensory fibres triggers the release of
Bonferroni were equieffective and short-lived (Mactier et al., 1998). Although capsaicin and AITC induced by peritoneal irritation in humans is very severe and present nociception over nearly the entire observation period (600 s). Similarly, it has been demonstrated that abdominal pain by pre-treatment with either TRPV1 or TRPA1 antagonists, respectively. A recent study reported that some aversive and nociceptive actions resulting from high concentrations of AITC (Everaerts et al., 2011). In our study, AITC seems to be selective for TRPA1 because the selective TRPV1 antagonist SB366791 was not capable of altering AITC-induced nociception in a dose where it fully prevented capsaicin-induced abdominal nociception. One may also argue that camphor is a TRPV1 agonist at high concentrations (Xu et al., 2005). However, this activity was not observed in our experimental setting because camphor did not induce nociception alone or potentiate capsaicin- or AITC i.p. injection was caused by a local action of agonists on peritoneum sensory fibers.

In the present study, capsaicin and AITC were selective in inducing pain through TRPV1 and TRPA1 stimulation because the abdominal nociception induced by these agonists was fully abolished by pre-treatment with either TRPV1 or TRPA1 antagonists, respectively. A recent study reported that some aversive and nociceptive actions resulting from high concentrations of AITC (> 1 mM) in mice are mediated by TRPV1 (Everaerts et al., 2011). In our study, AITC seems to be selective for TRPA1 because the selective TRPV1 antagonist SB366791 was not capable of altering AITC-induced nociception in a dose where it fully prevented capsaicin-induced abdominal nociception. One may also argue that camphor is a TRPV1 agonist at high concentrations (Xu et al., 2005). However, this activity was not observed in our experimental setting because camphor did not induce nociception alone or potentiate capsaicin- or AITC-induced nociception, as expected for a TRPV1 agonist. Moreover, the selective TRPA1 antagonist, HC-030031, was also able to reduce the nociception induced by AITC and not capsaicin effects. These results indicated that the nociceptive effect induced by capsaicin or AITC i.p. injection was caused by a local action of agonists on peritoneum sensory fibers.

In the present study, capsaicin and AITC were able to induce short-term abdominal nociception in a dose-dependent manner and with similar efficacy. Analysis of the $E_{\text{max}}$ values showed that both agonists were very effective, reaching the maximal score on the abdominal nociception scale (3) and presenting nociception over nearly the entire observation period (600 s). Similarly, it has been demonstrated that abdominal pain induced by peritoneal irritation in humans is very severe and short-lived (Mactier et al., 1998). Although capsaicin and AITC were equieffective, the TRPV1 agonist was approximately 25-fold more potent than the TRPA1 agonist. This result is consistent with previous studies showing that capsaicin is more potent than allyl isothiocyanate in inducing depolarisation of somatic and visceral nociceptors and pain/nociception in the skin and viscera (Andrade et al., 2006, 2008; Brozmanova et al., 2012; Weller et al., 2011). Confirming our behavioral findings, we observed that i.p. capsaicin and AITC were able to increase the Fos-IR in dorsal horn cells of the spinal cord at the thoraco-lumbar level, but not at cervical level. These findings are in accordance with the literature that demonstrates that i.p. injection of the irritant substance acetic acid caused nociception and Fos-IR mainly at the thoraco-lumbar levels of the spinal cord (where there are the spinal terminations of afferent fibers that innervates the peritoneum), but not at cervical level (indicating a selective stimulation of the peritoneal afferent fibers) (Lantéri-Minet et al., 1993; Sinniger et al., 2005). In addition, we have observed that capsaicin or AITC i.p. injection did not alter gut function or induce gut inflammation, however, no systemic inflammatory effects were observed. Together, these results indicated that the nociceptive effect induced by capsaicin or AITC i.p. injection was caused by a local action of agonists on peritoneum sensory fibers.

It was previously demonstrated that TRPV1 activation may cross-sensitise TRPA1 or vice versa (Akopian, 2011). However, it is unlikely that cross-sensitisation occurred in the abdominal pain induced by either the TRPV1 or TRPA1 agonists because selective antagonism of substance P and, in turn, the activation of the tachykinin NK1 receptor but not by mast cell degranulation.

We first observed that intraperitoneal injections of capsaicin or AITC are able to induce short-term abdominal nociception in a dose-dependent manner and with similar efficacy. Analysis of the $E_{\text{max}}$ values showed that both agonists were very effective, reaching the maximal score on the abdominal nociception scale (3) and presenting nociception over nearly the entire observation period (600 s). Similarly, it has been demonstrated that abdominal pain induced by peritoneal irritation in humans is very severe and short-lived (Mactier et al., 1998). Although capsaicin and AITC were equieffective, the TRPV1 agonist was approximately 25-fold more potent than the TRPA1 agonist. This result is consistent with previous studies showing that capsaicin is more potent than allyl isothiocyanate in inducing depolarisation of somatic and visceral nociceptors and pain/nociception in the skin and viscera (Andrade et al., 2006, 2008; Brozmanova et al., 2012; Weller et al., 2011). Confirming our behavioral findings, we observed that i.p. capsaicin and AITC were able to increase the Fos-IR in dorsal horn cells of the spinal cord at the thoraco-lumbar level, but not at cervical level. These findings are in accordance with the literature that demonstrates that i.p. injection of the irritant substance acetic acid caused nociception and Fos-IR mainly at the thoraco-lumbar levels of the spinal cord (where there are the spinal terminations of afferent fibers that innervates the peritoneum), but not at cervical level (indicating a selective stimulation of the peritoneal afferent fibers) (Lantéri-Minet et al., 1993; Sinniger et al., 2005). In addition, we have observed that capsaicin or AITC i.p. injection did not alter gut function or induce gut inflammation, however, no systemic inflammatory effects were observed. Together, these results indicated that the nociceptive effect induced by capsaicin or AITC i.p. injection was caused by a local action of agonists on peritoneum sensory fibers.
both of the receptors did not alter the nociceptive response of either AITC or capsaicin. However, cross-sensitisation may occur with non-selective irritants. The intraperitoneal injection of acetic acid is one of the oldest and most studied models of abdominal pain in rodents (Porreca et al., 1987). Nociception caused by acetic acid is reduced by both TRPV1 and TRPA1 antagonists and also by ablation of TRPV1-positive fibres in rodents (Ikeda et al., 2001; Pereira et al., 2012). Moreover, acetic-acid-induced abdominal pain is mediated by TRPV1-positive fibres, mast cells, and macrophages in rodents (Ikeda et al., 2001; Ribeiro et al., 2000). Because TRPV1 and TRPA1 are expressed in somatic and visceral sensory neurons as well as in mast cells (Biro et al., 1998; Malin et al., 2011; Prasad et al., 2008; Stander et al., 2004; Weller et al., 2011), we further investigated the role of mast cells and sensory fibres in the abdominal pain caused by capsaicin and AITC.

The peritoneal cavity contains both visceral and somatic sensory innervations (Flasar and Goldberg, 2006; Lantéri-Minet et al., 1993). It has been well recognised that systemic treatment with TRPV1 agonists in rodents cause a discerning degeneration of TRPV1-expressing primary sensory fibres, mainly peptidergic C fibres (Ferreira et al., 2004; Hsieh et al., 2008; Kopczynska, 2008). Because TRPA1 and TRPV1 are co-expressed in sensory neurons, treatment with RTX reduces the expression of both TRPV1 and TRPA1 (Pecze et al., 2009). Accordingly, we observed that systemic treatment with RTX significantly reduced both capsaicin- and AITC-elicited abdominal nociception. Similar nociception results were found with the subcutaneous injection of either capsaicin or AITC into the paw skin of mice and rats (Andrade et al., 2008; Massaad et al., 2004). Collectively, our data clearly showed the essential role of primary sensory afferent fibres expressing TRPV1 and TRPA1 in the capsaicin- and AITC-induced abdominal nociceptive responses in rats.

An important feature of TRPV1- and TRPA1-positive fibres is the presence of neuropeptide substance P (Bautista et al., 2005; Story et al., 2003). Furthermore, the release of substance P is a critical mechanism that is associated with nociception provoked by the s.c. administrations of capsaicin and AITC of experimental animals and humans (Anand and Bley, 2011; Andrade et al., 2008; Banvolgyi et al., 2004; Massaad et al., 2004; Santos and Calixto, 1997). Similarly to the skin, pre-treatment with a selective NK1 antagonist largely reduced abdominal nociception induced by intraperitoneally injected capsaicin and AITC. Our results also indicate that the NK1 receptor is activated by substance P released from sensory neurons, because we also observed that the administration of either capsaicin or AITC

![Graph A](image)

**Fig. 8.** Evaluation of the histamine release induced by capsaicin and AITC in vivo (A) and in vitro ((B) and (C)). (A) The histamine levels were evaluated in the peritoneal fluid 10 min after the i.p. administration of capsaicin (CPS, 0.1 mg/kg), AITC (3 mg/kg), compound 48/80 (0.75 mg/kg, positive control), or the vehicle (Veh, 0.95% ethanol and 0.05% Tween in PBS, 10 mL/kg). ((B) and (C)) The histamine released from the isolated rat pleural mast cells was also measured in the presence of capsaicin (0.001–10 μM), AITC (1–1000 μM), compound 48/80 (1 μg/mL, positive control) or the vehicle (0.01% ethanol in Krebs-Ringer phosphate solution). Each column represents the mean ± S.E.M. of four rats or four samples. The asterisks denote the significance levels: *P < 0.05 and **P < 0.001 in comparison to the vehicle-treated group; one-way ANOVA followed by Bonferroni’s post-hoc test.
induced an increase of substance P in the peritoneal fluid at the dose and time that caused nociception.

In addition to pain, substance P may also cause neurogenic inflammation by inducing plasma extravasation, as well as activating resident immune cells such as mast cells (Geppetti et al., 2008; Richardson and Vasko, 2002). Of note, we detected that the intraperitoneal injection of capsaicin or AITC produced plasma extravasation in the rat peritoneum (data not shown). Moreover, it has been described that substance P is capable of inducing mast cell degranulation, provoking the release of histamine (Ansel et al., 1993; Ogawa et al., 1999; Okada et al., 1999; Suzuki et al., 1995, 1999). However, neither the mast cell membrane stabiliser cromoglicate nor the H3 receptor antagonist promethazine were able to reduce the abdominal nociceptive responses induced by either capsaicin or AITC. In addition, we failed in detecting an increase in the histamine content in the peritoneal fluid of rats after intraperitoneal injections of capsaicin and AITC. Accordingly, the concentration of substance P was markedly elevated in the peritoneal fluid after the capsaicin and AITC injections, but the levels were several orders of magnitude lower than that necessary to cause histamine release in rat peritoneal mast cells in vitro (Ogawa et al., 1999; Suzuki et al., 1995).

Because TRPA1 and TRPV1 are expressed in mast cells (Biro et al., 1998; Prasad et al., 2008; Stander et al., 2004), we further confirmed the potential ability of their agonists in directly inducing mast cell degranulation. Moreover, the ability of capsaicin in inducing mast cell degranulation in vitro is still controversial and was investigated only in cell lines and in bone-marrow-derived mast cells (Biro et al., 1998; Giudice et al., 2007). Currently, the direct activation of TRPV1 and TRPA1 in the mature peritoneal mast cells of rats has not been documented. In accordance with our in vivo results, capsaicin and AITC failed to provoke histamine release by the peritoneal mast cell of rats in vitro in concentrations that were efficacious in the activation sensory neurons (Caterina et al., 2000; Jodott et al., 2004). Thus, TRPA1 and TRPV1 agonists do not seem to induce a direct degranulation of mature mast cells. We next asked whether peritoneal mast cell degranulation is able to cause pain and histamine release. The intraperitoneal injection of a mast cell degranulator (compound 48/80) caused abdominal nociception and increased the peritoneal histamine content, which was fully prevented by cromoglicate and promethazine. This finding demonstrates clearly that peritoneal mast cell activation is able to cause abdominal nociception through histamine release, but this mechanism is not implicated in the nociceptive responses to selective TRPV1 and TRPA1 agonists.

We have also observed that resident peritoneal macrophages were not involved in the abdominal nociceptive responses induced by capsaicin or AITC i.p. injection. The TRPV1 and TRPA1 receptors have been highlighted as important sensors of noxious stimulus in different pain models, including visceral and pancreatic pain (Schwartz et al., 2011; Moran et al., 2011). However, in this study we have proposed to observe the participation of these channels in abdominal pain, which is an important feature in clinical settings (Flasar and Goldberg, 2006; Mactier et al., 1998). Until now, TRPV1 and TRPA1 antagonists are still not used in the clinical practice, but different evidences have pointed to a possible use of these substances for pain treatment (Moran et al., 2011). Collectively, our findings suggest that TRPV1 and TRPA1 stimulation in the peritoneum produces abdominal nociception that is mediated by sensory fibres and substance P but not by mast cells and histamine or macrophages. Because irritant substances, such as some peritoneal dialysis solutions and chemotherapy drugs, may produce pain when intraperitoneally injected and lead to the activation of TRPV1 and TRPA1 receptors, our results may benefit the understanding and treatment of abdominal pain.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2013.07.029.

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