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

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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 cromoglycate, nor the H_1 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.

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 (Cortright 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).

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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 reflects over the external surface of the viscera (Tanaka et al., 2002). Diseases involving the peritoneum are frequently encountered in medical practices (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; Stander 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-methoxycinnamanilide (SB-366791), sodium cromoglycate, compound 48/80, *o*-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 cromoglycate, 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