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Involvement of the transient receptor potential vanilloid 1 (TRPV1) in the development of acute visceral hyperalgesia during colorectal distension in rats

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ABSTRACT

Transient receptor potential vanilloid 1 (TRPV1) channels have been implicated in pain mechanisms and, particularly, in the development of hyperalgesia. We used selective TRPV1 antagonists (NGV-1, SB-750364 and JYL 1421) to assess the role of TRPV1 channels in repetitive noxious colorectal distension (CRD)-induced visceral pain responses in rats. Isobaric CRD (80 mmHg) induced a viscerosomatic response, indicative of visceral pain associated to the distension procedure. Repetition (12 consecutive distensions) of the CRD resulted in an increase in the response over time (119±23% increase at distension 12, $P<0.05$ vs response during the 1st distension) indicative of acute mechanical sensitization. NGV-1 (0.1, 0.3, 1 or 3 μmol/kg, i.v.) prevented in a dose-related manner the development of sensitization, without inducing hypoalgesic responses. SB-750364 (30 μmol/kg, i.v.) had a transitory effect, partially reducing the sensitization response, while JYL 1421 (4.7 μmol/kg, i.v.) was without effect. In the same conditions, the cannabinoid receptor 1 (CB_1) agonist, WIN55,212-2 (0.1 μmol/kg) reduced pain responses leading to a hypoalgesic state. At 3 μmol/kg, NGV-1, did not affect the pressure–volume relationship during CRD, indicating that TRPV1 channels do not modulate colonic compliance. These observations suggest that TRPV1 channels are involved in the development of acute mechanical colonic hyperalgesia during repetitive noxious CRD in rats. Antagonism of TRPV1 channels might result in antihyperalgesic effects without hypoalgesic activity and might be beneficial in the treatment of visceral pain disorders, such as irritable bowel syndrome. These observations warrant the clinical assessment of TRPV1 antagonists for the treatment of visceral pain.

1. Introduction

Visceral hypersensitivity, with increased perception of balloon distension of the colorectal area, has been implicated in the pathophysiology of the functional gastrointestinal disorder, irritable bowel syndrome (Ritchie, 1973; Stacher and Christensen, 2006; Azpiroz et al., 2007). The exact cause of this hypersensitivity is unknown and several mechanisms, pathways and mediators have been implicated in the process (Azpiroz et al., 2007; Blackshaw et al., 2007). The transient receptor potential vanilloid 1 (TRPV1; also known as the capsaicin receptor) is a non-selective ligand-gated cation channel that seems to be essential for certain modalities of pain sensation (Jia et al., 2005; Holzer, 2008). Recent data suggest that TRPV1 channels might be involved in visceral hypersensitivity. TRPV1-positive nerve fibers are abundant in the gastrointestinal tract. For instance, the majority (>80%) of spinal afferents with nerve endings innervating the mouse distal colon, largely functioning as intestinal nociceptors, are TRPV1-immunoreactive (Robinson et al., 2004; Spencer et al., 2008). These morphological findings are supported by functional observations that indicate that TRPV1 channels participate in intestinal mechanosensation and are necessary for the induction and maintenance of chemically-induced visceral hypersensitivity in mice (Rong et al., 2004; Winchester et al., 2004; Jones et al., 2005; Winston et al., 2007). Moreover, an increased density of TRPV1-immunoreactive fibers within the gastrointestinal tract has been correlated with decreased sensory thresholds to rectal distension in patients with fecal urgency and incontinence (Chan et al., 2003) and with abdominal pain in irritable bowel syndrome patients (Akbar et al., 2008).

The objective of the present study was to assess the involvement of TRPV1 channels in the development of visceral hypersensitivity in a model of colorectal distension (CRD)-induced acute visceral hyperalgesia in conscious rats using the highly selective TRPV1 antagonists, with proven analgesic-like effects in several experimental models, NGV-1 (Hutchison et al., 2002; Cortright et al., 2003; Winchester et al.,

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2004), SB-750364 (Hutchison et al., 2002; Phillis et al., 2007; Biggs et al., 2008) and JYL 1421 (Wang et al., 2002).

2. Materials and methods

2.1. Animals

Adult female Sprague–Dawley rats (Harlan, The Netherlands; 250–300 g) were used. The rats were allowed to acclimatize to the animal facility for at least 1 week after arrival. Rats were housed in groups of five in an enriched environment with free access to food (Standard pellets, R3, Lactamin, Sweden) and water under controlled conditions of temperature (21 °C) and humidity (50%) on a 12:12 h light–dark cycle. The phase of the estrous cycle was not taken into consideration in the current study. All experiments were approved by the local animal ethics review committee in Göteborg, Sweden.

2.2. Colorectal distension (CRD)

Rats were habituated to Bollmann cages (Plexi-glass tubes, length 18 cm, diameter 6 cm, AstraZeneca, Mölndal, Sweden) 30 min per day for three consecutive days prior to experiments, to reduce motion artifacts and confounding effects due to stress-related responses.

A 3 cm polyethylene balloon (made in-house) with connecting catheter (PE-50) was inserted in the distal colon, 2 cm from the base of the balloon to the anus, during light isoflurane anesthesia (Forene®, Abbott Scandinavia AB, Solna, Sweden) and the catheter fixed to the tail with tape. Rats were allowed to recover from sedation in the Bollmann cages for at least 15 min before the start of experiments.

A customized barostat (AstraZeneca, Mölndal, Sweden) was used to manage air inflation and balloon pressure control. A customized computer software (PharmLab on-line 5.0) was used to control the barostat and to perform data collection.

Colorectal distension-induced contractions of the abdominal musculature (the so called visceromotor response) were assessed in conscious animals as a surrogate marker of visceral pain (Ness and Gebhart, 1988). For CRD, repeated phasic distensions, 12 times at 80 mmHg, with a pulse duration of 30 s at 5 min intervals, were used. Such a protocol induces acute mechanical sensitization leading to the development of acute visceral hyperalgesia, as previously described (Tammpere et al., 2005; Martínez et al., 2007). For the assessment of compliance, increasing phasic distensions from 2 to 20 mmHg, at 2 mmHg increasing steps, with a pulse duration of 1 min at 5 min intervals were used. In this case, low distension pressures (within a range considered non-noxious) were chosen in order to minimize pain-related visceromotor responses that might interfere with the measurements of the intraballoon volume. Similar protocols have been used before to assess responses to colorectal distension in rats (Tammpere et al., 2005; Käll et al., 2007; Martínez et al., 2007; Lindström et al., 2008; Brusberg et al., 2009).

2.3. Data collection and analysis

The analog input channels were sampled with individual sampling rates, and digital filtering was performed on the signals. The balloon pressure signals (average rectified value of the pressure changes inside the balloon) were sampled at 50 samples/s. A highpass filter at 1 Hz was used to separate the contraction-induced pressure changes from the slow varying pressure generated by the barostat. A resistance in the airflow between the pressure generator and the pressure transducer further enhanced the pressure variations induced by abdominal contractions of the animal. In addition, a band-stop filter at 49–51 Hz was used to remove line frequency interference. A customized computer software (PharmLab off-line 5.0) was used to quantify the magnitude of the highpass-filtered balloon pressure signals. The highpass-filtered balloon pressure signals were calculated for 30 s before the pulse (i.e. baseline response) and for the duration of the pulse. When calculating the magnitude of the highpass-filtered balloon pressure signals, the first and last seconds of each pulse were excluded since these reflect artifact signals produced by the barostat during inflation and deflation and do not originate from the animal.

For the determination of compliance, the maximal intracolonic volume achieved during each distension (2–20 mmHg) was determined and pressure–volume curves were constructed.

2.4. Drugs

NGV-1 [(R)(−)-4-(3-Cl-pyridine-2-yl)-2-me-piperazine-1-carboxilic acid (CF3-phenyl)-amide; AstraZeneca R&D] (Hutchison et al., 2002; Cortright et al., 2003) was dissolved in 28% cyclodextrin. SB-750364 [N-(2-bromophenyl)-N′-[((R)-1-(5-CF3-2pyridyl)pyrrolidin-3-yl)]urea; AstraZeneca R&D] (Hutchison et al., 2002) was dissolved in 28% cyclodextrin. JYL 1421 [N-(4-tert-butylbenzyl)-N′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea; AstraZeneca R&D] was dissolved in 28% cyclodextrin. WIN55,212-2 [(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone, mesylate form; Tocris Cookson, Bristol, England] was dissolved in 5% ethanol:5% Solutol® HS 15:90% saline (v:v:v). Doses were selected based on preliminary experiments and previous reports showing efficacy in different pain models (Cortright et al., 2003; Jakab et al., 2005; Jia et al., 2005; Biggs et al., 2008). The corresponding vehicles were used as controls.

2.5. Experimental protocols

Each rat received both vehicle and a dose of compound on different occasions, with at least 4 days between consecutive experiments. Hence, each rat served as its own vehicle control. Experiments were performed in a counterbalanced cross-over fashion in which vehicle and different doses of compounds were tested during the same experiment, and repeated at several occasions.

2.5.1. Effects of the TRPV1 antagonists NGV-1, SB-750364 and JYL 1421 on repetitive noxious CRD-evoked visceral pain

NGV-1 (0.1. 0.3, 1 or 3 μmol/kg; equivalent to 0.04, 0.12, 0.4 and 1.2 mg/kg, respectively), SB-750364 (30 μmol/kg; equivalent to 7 mg/kg), JYL 1421 (4.7 μmol/kg; equivalent to 2 mg/kg), or the appropriate vehicle (1 ml/kg) was administered intravenously (i.v.) between distensions 3 and 4. Because of the relative large time interval needed to complete the NGV-1 experiments, 2 control (vehicle treatment) CRDs were performed, one at the start and one at the end of the experimental period, as to account for time-related effects in the responses observed.

In a separate experiment, the effects of intraperitoneal (i.p.) JYL 1421 were also tested. In this case, JYL 1421 (4.7 μmol/kg) or vehicle (1 ml/kg) was administered i.p. 10 min before the start of the repetitive noxious CRD procedure.

2.5.2. Effects of the cannabinoid 1 (CB_1) receptor agonist WIN55,212-2 on repetitive noxious CRD-evoked visceral pain

In order to make a comparison to a compound with a different mechanisms of action we tested the effect of the CB_1 receptor agonist WIN55,212-2 on CRD. WIN55,212-2 (0.1 μmol/kg, equivalent to 0.05 mg/kg) or vehicle 1 (ml/kg) was administered i.v. between distensions 3 and 4, during repetitive CRD at 80 mmHg.

2.5.3. Effects of NGV-1 on colonic compliance

NGV-1 (3 μmol/kg) or vehicle (1 ml/kg) was administered i.v. at the start of the CRD protocol (2–20 mmHg). The same group of animals received both treatments, in a random manner, in two separate experiments, with an interval of at least 4 days.

2.6. Statistical analysis

When assessing pain responses during repetitive noxious CRD (12×80 mmHg), the first 3 distensions were considered a measure of a normal pain response to distension. Sensitization responses and the effects of compounds were determined in the following 9 distensions. Data are expressed as mean ± S.E.M. Differences between two groups were assessed by paired Student's *t* test. Differences between multiple groups were determined by a repeated or non-repeated measures one-way analysis of variance (ANOVA), as appropriate, followed, when necessary, by a Student–Newman–Keuls multiple comparisons test. Data were considered statistically significant when P was <0.05.

3. Results

3.1. Viscerosomatic responses during repetitive noxious CRD

CRD at 80 mmHg induced a viscerosomatic response that resulted in a 3-fold increase in the mechanical activity of the abdominal musculature compared to the basal activity, as determined during the first 3 consecutive distensions before treatments (basal: 0.056 ± 0.003; distension: 0.167 ± 0.018; $P<0.05$; $n=70$, pooled data corresponding to all CRD experiments). Thereafter, in vehicle-treated animals, the response to CRD increased over time, indicating the presence of acute mechanical sensitization. Responses were similar regardless the vehicle used. From distensions 4 to 12, the response to CRD increased by 119 ± 23% over the mean response observed before treatment [$F(11,31)=38.42$; $P<0.0001$; $P<0.05$ for distensions 7–12 vs distensions 1–3, $n=32$ pooled data from all vehicle groups]. Basal mechanical activity of the abdominal musculature between distensions was not modified during the CRD protocol.

Initial responses to CRD were similar in consecutive experiments indicating that the sensitization induced by the CRD protocol used was transitory and did not induce long-term changes in sensitivity.

3.2. Effects of NGV-1, SB-750364 and JYL 1421 on CRD-induced pain responses

Responses in the two CRD experiments performed with the vehicle for NGV-1 were similar in magnitude. For the sake of clarity, the individual means of these two experiments have been used as a vehicle group ($n=8$). From distensions 4 to 12, the response to CRD in vehicle-treated rats increased by 114 ± 31% over the main response observed before treatment [$F(11,7)=7.055$; $P<0.0001$; $P<0.05$ for distensions 7–12 vs distensions 1–3; Fig. 1A]. NGV-1, administered i.v. between distensions 3 and 4, inhibited in a dose-related manner the acute mechanical sensitization elicited by repetitive CRD (Fig. 1). At 1 μmol/kg, NGV-1 ($n=7$) reduced the hyperalgesic response to CRD by 89 ± 16%, compared with the response in the vehicle group ($P<0.05$) (Fig. 1B). Responses to CRD were of similar magnitude to those observed before treatment with NGV-1 and the expected sensitization observed with repeated distensions was prevented (Fig. 1A). A further increase in the dose to 3 μmol/kg ($n=8$), resulted in a similar effect, with an 85 ± 15% reduction of the hyperalgesic response to CRD compared with the response in the vehicle group ($P<0.05$), with responses to distension of similar magnitude to those observed before treatment with NGV-1 (Fig. 1). Lower doses of NGV-1 (0.1 or 0.3 μmol/kg, i.v.) reduced the hyperalgesic response to CRD by 51 ± 14% and 57 ± 14%, respectively ($n=7$–8; both $P>0.05$ vs the sensitization response in the vehicle-treated group). However, these doses did not completely prevent the progressive sensitization in response to CRD from distensions 4 to 12 [0.1 μmol/kg: $F(11,6)=2.234$, $P=0.0225$; 0.3 μmol/kg: $F(11,7)=2.055$, $P=0.034$] (Fig. 1).

SB-750364 (30 μmol/kg, i.v., $n=4$) induced a transitory inhibition of the CRD response, when compared with the vehicle-treated group, that lasted from the 4th to the 6th distension (all $P<0.05$ vs vehicle),

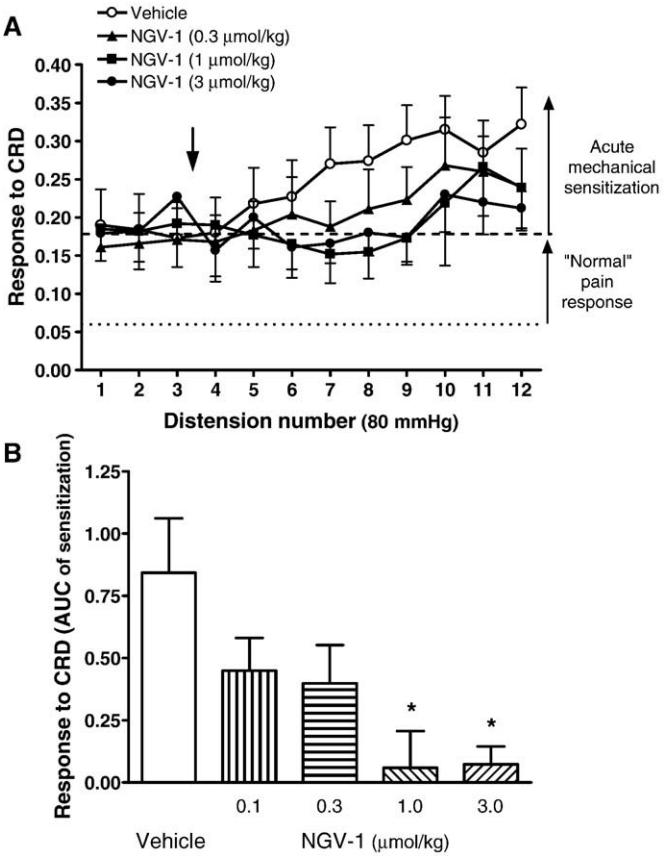

Fig. 1. Effects of the selective TRPV1 antagonist, NGV-1, on visceral pain responses during repetitive phasic noxious CRD (12 distensions at 80 mmHg) in conscious rats. A: Changes in the visceromotor response during repetitive noxious CRD (12 consecutive distension at 80 mmHg). The dotted line corresponds to the mean basal mechanical activity of the abdominal musculature between distensions. The broken line corresponds to the mean visceromotor response during distensions 1 to 3. As indicated in the figure, the margin between the basal mechanical activity and the mean response to the first 3 distensions represents the "normal" pain response elicited by the mechanical distension of the colorectal area. Visceromotor responses above the mean response for distensions 1 to 3 are indicative of hyperalgesia due to acute mechanical sensitization. Vehicle or NGV-1 was administered i.v. between distensions 3 and 4, as indicated by the arrow. Data are mean ± S.E.M. of 7–8 animals per group. For the sake of clarity, data for NGV-1 at 0.1 mg/kg have not been included in the graph. B: Quantification of the effect of NGV-1 on the sensitization response induced by repetitive noxious CRD. Data correspond to the area under the curve (AUC) of the sensitization component observed between distensions 4 and 12. Data are mean ± S.E.M. of 7–8 animals per group. *: $P<0.05$ vs vehicle [$F(4,34)=2.837$, $P=0.0393$].

while the 7th distension showed a tendency towards recovery ($P=0.0542$ vs vehicle) and distensions 9 to 12 were of similar magnitude in SB-750364- and vehicle-treated animals (Fig. 2A). The overall sensitization response during distensions 4 to 12 was reduced by 50 ± 21% in SB-750364-treated animals ($P=0.0834$ vs vehicle; Fig. 2B).

JYL 1421 (4.7 μmol/kg, $n=8$) did not affect the responses to CRD compared with the vehicle-treated group (Fig. 2C–D). A similar lack of effect was also observed when JYL 1421 was administered i.p. (overall response to CRD, distensions 1 to 12: vehicle, 1.91 ± 0.26; JYL 1421, 1.52 ± 0.26; $n=4$ for each, $P=0.3379$).

3.3. Effects of the cannabinoid 1 (CB_1) receptor agonist WIN55,212-2 on repetitive noxious CRD-evoked visceral pain

In vehicle-treated animals, the response to CRD during distensions 4 to 12 increased by 94 ± 32% over the response before treatment [$F(11,3)=2.364$; $P=0.0277$; Fig. 3A]. WIN55,212-2, administered between distensions 3 and 4, induced a transitory inhibition of CRD

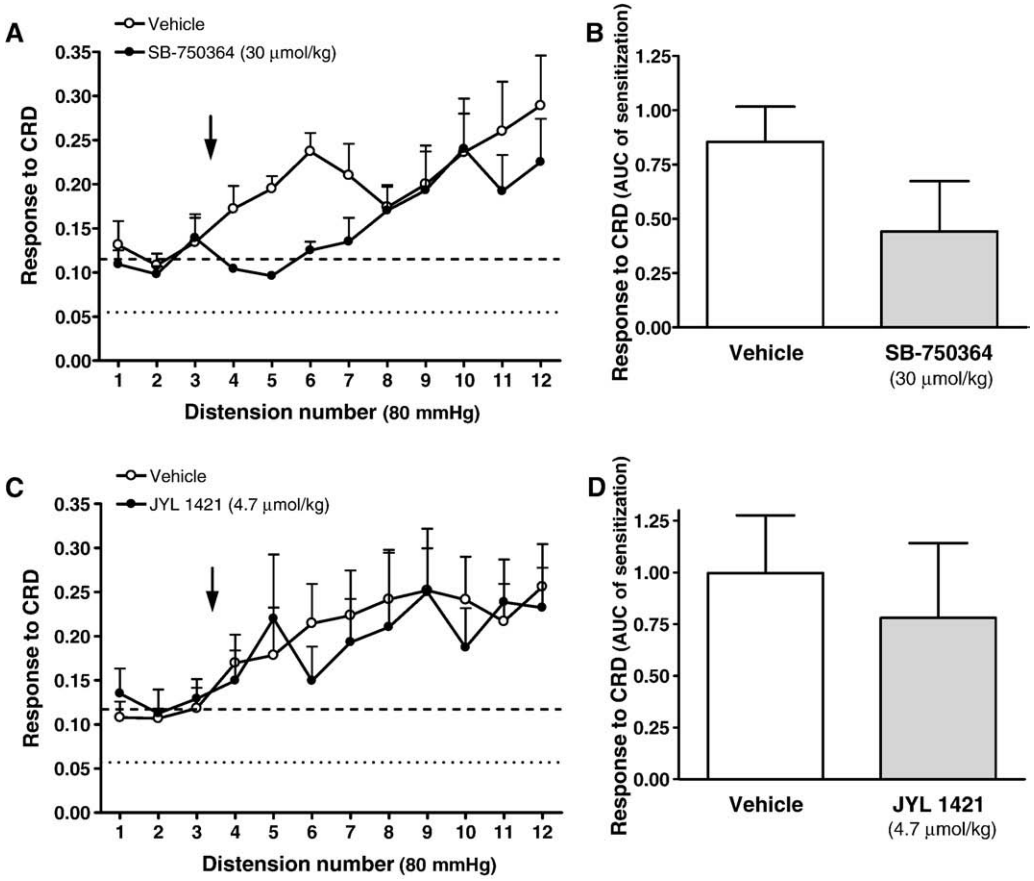

Fig. 2. Effects of the TRPV1 antagonists, SB-750364 and JYL 1421, on visceral pain responses during repetitive phasic noxious CRD (12 distensions at 80 mmHg) in conscious rats. A, C: Changes in the visceromotor response during repetitive noxious CRD. In both graphs, the dotted line corresponds to the mean basal mechanical activity of the abdominal musculature between distensions and the broken line to the mean visceromotor response during distensions 1 to 3 (see legend to Fig. 1 for details). Compounds were administered i.v. between distensions 3 and 4, as indicated by the arrow. Data are mean ± S.E.M. of 4–8 animals per group. B, D: Quantification of the effect of SB-750364 and JYL 1421 on the sensitization response induced by repetitive noxious CRD, as shown in panels A and C, respectively. Data correspond to the area under the curve (AUC) of the sensitization component observed between distensions 4 and 12. Data are mean ± S.E.M. of 4–8 animals per group.

responses. Responses to distensions 4 to 6 were completely inhibited, reaching values similar to those in basal (non-distension) conditions ($P<0.05$ vs corresponding responses in vehicle-treated animals); while distension 7 showed a clear tendency ($P=0.0562$ vs vehicle). Thereafter, responses from distensions 8 to 12 were similar to those observed in the vehicle-treated group. The overall sensitization response during distensions 4 to 12 was reduced by 135 ± 18% in WIN55,212-2-treated animals ($P=0.0296$ vs vehicle; Fig. 3B), indicating hypoalgesic responses below the level of normal pain-related response to distension.

3.4. Effects of NGV-1 on colonic compliance

When assessing colorectal compliance, a positive pressure–volume relationship was observed during increasing phasic colorectal distension (2 to 20 mm Hg × 1 min) in vehicle-treated animals ($n=7$). In the same animals, NGV-1 (3 μmol/kg, $n=7$) did not affect the pressure–volume relationship during phasic (2–20 mmHg) CRD compared to vehicle, indicating that NGV-1 does not affect colorectal compliance in rats (Fig. 4).

4. Discussion

Growing evidence suggest that TRPV1 channels, expressed on primary sensory afferents, are implicated in the processing of visceral pain signals arising from the gastrointestinal tract. In particular, several studies have shown that TRPV1 channels are important for the development of chemically-induced visceral hyperalgesia and might participate in mechanotransduction. Here we show, for the first time, that TRPV1 channels are implicated in the development of mechanically-induced visceral hypersensitivity during repetitive CRD in rats. Interestingly, selective antagonism of TRPV1 channels reduced acute mechanical hyperalgesia but did not affect normal pain responses during noxious CRD, suggesting that TRPV1 antagonism could restore normal pain sensitivity without hypoalgesic effects.

Previous reports showed that the TRPV1 agonist, capsaicin, administered into the colon, induced visceral pain-related responses in mice and rats (Laird et al., 2001; Christoph et al., 2006). Likewise, intracolonic capsaicin increased CRD responses in rats (Miranda et al., 2007; unpublished observations). All together, these observations implicate TRPV1 channels, expressed in sensory afferents, in visceral pain arising from the gut. Supporting these observations, recent data showed that TRPV1 channels are necessary to develop and maintain colonic hypersensitivity (Jones et al., 2005; Winston et al., 2007) and that the TRPV1 antagonist JYL 1421 reduced inflammation-induced hyperalgesic responses during CRD (Miranda et al., 2007). Here we show that highly selective TRPV1 antagonists, namely NGV-1 and SB-750364, block the acute hyperalgesic responses associated with repetitive noxious colorectal distension in conscious rats. Interestingly, the effects observed seem to affect only the part of the response to CRD associated to acute hyperalgesia, but not that due to the manifestation of “normal” pain-related responses. This agrees with

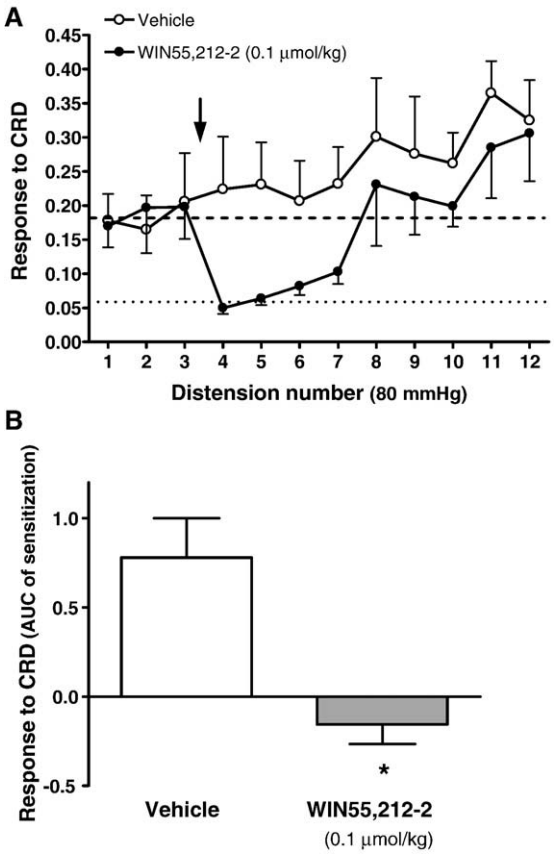

Fig. 3. Effects of the cannabinoid 1 (CB_1) receptor agonist, WIN55,2121-2, on visceral pain responses during repetitive phasic noxious CRD (12 distensions at 80 mm Hg) in conscious rats. A: Changes in the visceromotor response during repetitive noxious CRD. The dotted line corresponds to the mean basal mechanical activity of the abdominal musculature between distensions and the broken line to the mean visceromotor response during distensions 1 to 3 (see legend to Fig. 1 for details). Treatments were administered i.v. between distensions 3 and 4, as indicated by the arrow. Data are mean ± S.E.M. of 4 animals per group. B: Quantification of the effect of WIN55,212-2 on the sensitization response induced by repetitive noxious CRD, as shown in panels A. Data correspond to the area under the curve (AUC) of the sensitization component observed between distensions 4 and 12. Data are mean ± S.E.M. of 4 animals per group. *: $P = 0.0296$ vs vehicle group.

preliminary data indicating that NGV-1, given orally at a dose of 10 mg/kg, attenuated visceromotor responses to CRD in rats (Winchester et al., 2004). On the other hand, the effects observed contrast with those of the CB_1 agonist, WIN55,212-2, used as a comparator in the present study. In the same experimental conditions, WIN55,212-2 completely inhibited the response to distension leading to a state of transitory hypoalgesia, without affecting colonic compliance (Brusberg et al., 2009), while TRPV1 blockade with NGV-1 inhibited hyperalgesia but did not induce hypoalgesia. This might suggest that TRPV1 channels are recruited during the noxious stimulation process and are necessary for the development of hyperalgesia, but might not be essential for normal pain responses. On the other hand, CB_1 receptors seem to modulate both "normal" pain and hyperalgesic responses (Sanson et al., 2006; Brusberg et al., 2009; present observations). This agrees with recent data showing that TRPV1 channels were necessary for the induction and maintenance of colonic hyperalgesia during CRD in a model of neonatally-induced, long-lasting, visceral hypersensitivity in rats (Winston et al., 2007). Interestingly, in the same report, a TRPV1 antagonist did not affect CRD-evoked pain responses in animals without visceral hyperalgesia, and therefore, characterized as normosensitive. This finding coincides with our observations and reinforces the concept that TRPV1-dependent mechanisms are only involved in hyperalgesic responses but not in "normal" pain responses. Interestingly, TRPV1 knockout mice showed reduced visceromotor responses during CRD compared with wild-type controls (Jones et al., 2005), although this apparent discrepancy might be related to the constitutive deletion of TRPV1-dependent pathways in these animals.

The mechanisms mediating TRPV1-dependent hyperalgesia are currently unknown and warrant further studies. Although TRPV1 channels seem to function mainly as thermo- and chemosensory transducers (Tominaga et al., 1998; Jia et al., 2005; Sugiura et al., 2007), recent data suggest that colonic TRPV1 channels might also respond to mechanical stimuli and function, therefore, as mechanoreceptors (Jones et al., 2005; Spencer et al., 2008). Nevertheless, from the present study, the implication of chemical mechanisms in the activation and/or recruitment of TRPV1 channels during CRD cannot be excluded. CRD at noxious pressures (such as 80 mmHg) is likely to induce a relative ischemic state in the tissue leading to the induction of local acidification (Steen et al., 1995), which is a recognized activator of TRPV1 channels (Tominaga et al., 2004; Sugiura et al., 2007). In addition, TRPV1 channels might be activated/sensitized by several mediators likely to be released during colon distension (such as ATP) (Moriyama et al., 2003; Wynn et al., 2003; Lakshmi and Joshi, 2005) and the activation of pain modulatory mechanisms, such as the release of endogenous cannabinoid-related mediators (anandamide) (Smart et al., 2000) or the activation of protease-activated receptors (Amadesi et al., 2004). Therefore, integrity of TRPV1-dependent pathways might be necessary to ensure the development of hyperalgesia mediated by a variety of chemical, mechanical, and probably thermal, stimuli.

Although the relationship between colonic tone/accommodation and pain is not clear (Martínez et al., 2007; Brusberg et al., 2008, 2009; Ravnefjord et al., 2008), altered colonic tone and accommodation to distension have been suggested as contributing mechanisms to the altered colonic sensitivity observed in functional gastrointestinal disorders (Delgado-Aros and Camilleri, 2005). Results obtained here show that the pressure–volume relationship during colon distension was not affected by NGV-1 at doses having maximal antihyperalgesic effects during noxious CRD. Thus, indicating that TRPV1 channels are not involved in regulation of smooth muscle tone and that, therefore, the analgesic effect of NGV-1 is not due to the modulation of colonic compliance.

Finally, the partial effects observed for SB-750364 and the lack of activity of JYL 1421, compared with NGV-1, are likely to be related to differences in the relative potency of these compounds blocking

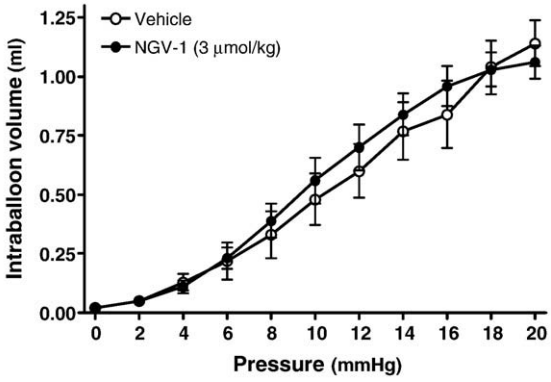

Fig. 4. Effects of the selective TRPV1 antagonist, NGV-1, on colorectal compliance during colorectal distension in rats. The figure shows the pressure–volume curves during phasic colorectal distension (2–20 mmHg) in animals receiving i.v. vehicle or NGV-1 at the start of the distension protocol. Data are mean ± S.E.M. of 7 animals per group and represent the maximal intracolonic volume (ml) for each pressure level.

TRPV1 channels. This agrees with the higher potency of NGV-1 blocking capsaicin-induced effects in cellular systems *in vitro* compared with SB-750364 or JYL 1421 (Wang et al., 2002; Cortright et al., 2003; Jakab et al., 2005; unpublished observations). Nevertheless, at the dose tested in the present study, intraperitoneal JYL 1421 inhibited several capsaicin-evoked pain-related responses in rats (Jakab et al., 2005). However, as part of this study, we also tested the effect of intraperitoneal JYL 1421 on CRD-evoked pain responses with similar negative results as those obtained after intravenous administration. A recent report showed that repeated administration of JYL 1421, at a 5-fold higher dose than that used in the current study, blocked inflammation-induced visceral hyperalgesia in rats (Miranda et al., 2007), further indicating that the lack of effect observed in the present study might be related to the relative low potency of the compound blocking TRPV1 channels compared with NGV-1. As it relates to SB-750364, preliminary reports indicated that this antagonist was more potent than capsazepine at blocking capsaicin effects in a model of neurogenic colonic inflammation (Kaur et al., 2005). Furthermore, SB-750364 at doses of 0.6 and 2 mg/kg, i.v., inhibited neural activity in a model of injury-induced discharge of the lingual nerve in ferrets; with only a minor increase in effect when the dose was raised to 6 mg/kg (Yates et al., 2006; Biggs et al., 2008). Thus, suggesting that the dose used in the current study is likely to provide an effective blockade of TRPV1 channels in *in vivo* conditions. Moreover, preliminary data also suggested that SB-750364 was effective modulating the activity of colonic splanchnic afferent nerves during colitis in rats (Phillis et al., 2007). In any case, differences in the pharmacokinetic properties of these compounds cannot be excluded as a contributing factor for the differences in efficacy observed here. In addition, differences between antagonists in the interaction with TRPV1 channels at a molecular level might result in the selective blockade of some responses but not others, leading to specific *in vivo* activity profiles, as recently described for several TRPV1 blockers (Lehto et al., 2008). Hence, the pharmacokinetic/pharmacodynamic relationship for TRPV1 antagonists with respect to visceral pain warrants further investigation.

Recently, a lot of interest has risen in relation to transient receptor potential (TRP) channels as targets for the treatment of multiple pain-related disorders in several systems (Jia et al., 2005; Hicks, 2006; Okuhara et al., 2007;Gunthorpe and Szallasi, 2008; Holzer, 2008). The effects observed here suggest that therapeutic targeting of TRPV1 channels in visceral pain might function as a normalizer of pain and, therefore, TRPV1 antagonists should be regarded as antihyperalgesics. Interestingly, this implies that, compared to other compounds that might impair normal pain mechanisms (such as opioids or cannabinoids) (Kamp et al., 2003; Sanson et al., 2006; Brusberg et al., 2009; current observations), TRPV1 antagonist might present the advantage of not inducing hyposensitivity, acting selectively preventing hyperalgesic responses. Nevertheless, clinical studies in patients with colonic hypersensitivity are necessary to confirm the clinical significance of these observations.

In summary, these results demonstrate for the first time that TRPV1 channels are involved in the development of acute mechanical colonic hyperalgesia. Interestingly, blockade of the channels prevented hyperalgesic responses without inducing hyposensitivity. These observations warrant further studies designed to address in more detail if similar effects are present in other models of visceral hyperalgesia and if other channels of the TRPV family contribute to these mechanisms, particularly modulating long-term effects. In addition, studies characterizing the blocking activity of the antagonists tested in this study against the activation of TRPV1 channels with exogenous agonists (such as capsaicin or resineferatoxin) are also necessary to fully characterize these compounds. Independently of these studies, the present observations have significant value per se, suggesting that TRPV1 channels might represent a potential target for the normalization of visceral hypersensitivity in functional gastrointestinal disorders, such as irritable bowel syndrome.

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