Pharmacology, Biochemistry and Behavior xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Dissociated modulation of conditioned place-preference and mechanical hypersensitivity by a TRPA1 channel antagonist in peripheral neuropathy

Hong Wei [a], Hanna Viisanen [a], Diana Amorim [a,b], Ari Koivisto [c], Antti Pertovaara [a,*]

[a] Institute of Biomedicine/Physiology, University of Helsinki, Helsinki, Finland
[b] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal
[c] OrionPharma, Orion Corporation, Turku, Finland

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 11 December 2012
Accepted 19 December 2012
Available online xxxx

Keywords:
Chembridge-5861528
Clonidine
Conditioned place-preference
Diabetic neuropathy
Mechanical hypersensitivity
Ongoing pain
Peripheral nerve injury
Transient receptor potential ankyrin 1 channel

ABSTRACT

Transient receptor potential ankyrin 1 (TRPA1) channel antagonists have suppressed mechanical hypersensitivity in peripheral neuropathy, while their effect on ongoing neuropathic pain is not yet known. Here, we assessed whether blocking the TRPA1 channel induces place-preference, an index for the relief of ongoing pain, in two experimental rat models of peripheral neuropathy. Diabetic neuropathy was induced by streptozotocin and spared nerve injury (SNI) model of neuropathy by ligation of two sciatic nerve branches. Conditioned place-preference (CPP) paradigm involved pairing of the drug treatment with one of the chambers of a CPP device once or four times, and the time spent in each chamber was recorded after conditioning sessions to reveal place-preference. The mechanical antihypersensitivity effect was assessed by the monofilament test immediately after the conditioning sessions. Intraperitoneally (30 mg/kg; diabetic and SNI model) or intrathecally (10 μg; diabetic model) administered Chembridge-5861528 (CHEM) was used as a selective TRPA1 channel antagonist. In diabetic and SNI models of neuropathy, CHEM failed to induce CPP at a dose that significantly attenuated mechanical hypersensitivity, independent of the route of drug administration or number of successive conditioning sessions. Intrathecal clonidine (an α_2-adrenoceptor agonist; 10 μg), in contrast, induced CPP in SNI but not control animals. The results indicate that ongoing pain, as revealed by CPP, is less sensitive to treatment by the TRPA1 channel antagonist than mechanical hypersensitivity in peripheral neuropathy.

1. Introduction

Transient receptor potential ankyrin 1 (TRPA1) is a nonselective calcium-permeable ion channel that is expressed on a subpopulation of nociceptive primary afferent nerve fibers (Story et al., 2003; Jordt et al., 2004). In the periphery, TRPA1 channel contributes to transduction of harmful stimuli to neuronal discharge, whereas on central endings of nociceptive nerve fibers it mediates glutamatergic transmission to spinal dorsal horn interneurons (see for reviews, Patapoutian et al., 2009; Stucky et al., 2009; Moran et al., 2011; Pertovaara and Koivisto, 2011; Andrade et al., 2012).

Peripheral neuropathies are among pathophysiological conditions that are associated with chronic ongoing pain and hypersensitivity to cutaneous stimulation, symptoms which are significant clinical problems (Scadding and Koltzenburg, 2006). Interestingly, there is recent experimental evidence indicating that blocking the TRPA1 channel attenuates mechanical hypersensitivity induced by peripheral diabetic neuropathy (Wei et al., 2009, 2010a; Koivisto et al., 2012; Koivisto and Pertovaara, in press) or spinal nerve injury (Eid et al., 2008; Wei et al., 2011). While these findings indicate that the TRPA1 channel exerts an important role in the facilitation of mechanical stimulus-evoked pain in peripheral neuropathy, these findings still leave open whether the TRPA1 channel is involved in maintenance of ongoing neuropathic pain.

Assessment of ongoing neuropathic pain in experimental animals is notoriously difficult. One approach is to apply conditioned place-preference (CPP) paradigm. If animals have ongoing pain that is reduced by drug treatment in one of the test chambers, the animals are expected to prefer the test chamber paired with the analgesic treatment (Sufka, 1994). Unmasking the tonic-aversive state using the CPP paradigm has been successfully applied to study sustained pain in various models of peripheral neuropathy (King et al., 2009; De Felice et al., 2011; King et al., 2011; Qu et al., 2011; He et al., 2012; Leite-Almeida et al., 2012) as well as in some other experimental models of chronic pain (Davoody et al., 2011; He et al., 2012; Okun et al., 2012). Here, we administered a selective TRPA1 channel antagonist in the CPP paradigm that was modified from that of King et al. (2009) to study whether the TRPA1 channel is involved in maintenance of ongoing neuropathic pain. The experiments were performed in two models of experimental peripheral neuropathy, one induced by a metabolic disorder (diabetes mellitus) and one by nerve ligations (spared nerve injury, SNI).

* Corresponding author at: Institute of Biomedicine/Physiology, P.O. Box 63, University of Helsinki, 00014 Helsinki, Finland. Tel.: +358 9 191 25280; fax: +358 9 191 25302.
E-mail address: antti.pertovaara@helsinki.fi (A. Pertovaara).

0091-3057/$ – see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pbb.2012.12.014

2. Material and methods

2.1. Experimental animals

The experiments were performed with male Hannover–Wistar rats (220–260 g; Harlan, Horst, The Netherlands) in Biomedicum Helsinki. All experiments were approved by the ethical committee for experimental animals studies of the State Provincial Office of Southern Finland (Hämeenlinna, Finland) and the experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. The animals were housed in polycarbonate cages with a deep layer of saw dust, one to three animals in each cage, in a thermostatically controlled room at 24.0 ± 0.5 °C. The room was artificially illuminated from 8.30 AM to 8.30 PM. The animals received commercial pelleted rat feed (CRM-P pellets, Special Diets Services, Witham, Essex, England) and tap water ad libitum.

2.2. Induction of diabetes mellitus

Diabetes mellitus was induced under pentobarbitone anesthesia by tail vein injection of streptozotocin (60 mg/kg; Sigma-Aldrich, St.Louis, MO, USA) in citrate buffer (pH 4.5). Streptozotocin-induced diabetes mellitus is known to cause a marked hypersensitivity to various types of stimuli (Courteix et al., 1993). While peripheral diabetic neuropathy is a complex disorder with multiple underlying mechanisms (Obrosova, 2007), the TRPA1 channel was recently shown to exert an important role in its pathophysiology (Koivisto et al., 2012). The development of diabetes mellitus was confirmed 3 and 10 days later by measurements of blood glucose concentration (One Touch Ultra, Life Scan Inc., Milpitas, CA, USA). All streptozotocin-treated animals developed diabetes and had a blood glucose level of >20 mmol/l. Weight of the animals was assessed every other day. If the animal had a weight decrease of >20% or it showed signs of suffering, then the animal was immediately sacrificed by administering a lethal dose of pentobarbitone.

2.3. Techniques for producing spared nerve injury model of peripheral neuropathy

There are a number of surgically induced models of peripheral neuropathy (Honoré et al., 2011), of which we chose for this study the spared nerve injury (SNI) model (Decosterd and Woolf, 2000). For SNI, the unilateral axotomy and ligation of the tibial and common peroneal nerves on the left side was performed under pentobarbitone anesthesia (60 mg/kg i.p.) as described in detail earlier (Decosterd and Woolf, 2000). Briefly, the skin of the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle exposing the sciatic nerve and its three terminal branches. Following ligation and removing 2–4 mm of the distal nerve stumps of the tibial and common peroneal nerves, muscle and skin were closed in two layers. In sham-operated animals, the surgical procedure was identical, except that the tibial and common peroneal nerves were not ligated or sectioned. After the surgery, the animals were allowed to recover before the actual testing that was performed one week after the operation.

2.4. Surgical procedures for the installation of intrathecal catheter

In one group of animals, drug was administered intrathecally (i.t.). For i.t. drug injections, a catheter (Intramedic PE-10, Becton Dickinson and Company, Sparks, MD) was administered into the lumbar level of the spinal cord under pentobarbital anesthesia (60 mg/kg intraperitoneally) as described in detail elsewhere (Størkson et al., 1996). Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4%, 7–10 μl followed by a 20 μl of saline for flushing) with a 50-μl Hamilton syringe (Hamilton Company, Bonaduz, Switzerland). Only those rats that had no motor impairment before lidocaine injection but had a bilateral paralysis of hind limbs following intrathecal administration of lidocaine were studied further. The installation of the intrathecal catheter was performed about one week before the start of the actual experiments. In the actual experiments, the drugs were microinjected i.t. with a 50-μl Hamilton microsyringe in a volume of 5 μl followed by a saline flush in a volume of 20 μl.

2.5. Conditioned place preference (CPP)

For analysis of ongoing pain, conditioned place-preference (CPP) paradigm modified from that of King et al. (2009) was used. When using a single-drug exposure paradigm, rats initially went a 3 day habituation, in which they were placed in automated CPP boxes (Place Preference System, San Diego Instruments, Inc., San Diego, CA) with access to all 3 chambers for 30 min per day during the first two days. The device records time spent in each chamber using a computer-controlled 4×16 array of photobeams. Among differences between the test chambers was the roughness of the floor (rough versus smooth) and the painting of the walls (black triangles versus bars on white surface). Time spent in each of the boxes was recorded for 15 min on day 3 (D3). Rats that spent more than 720 s in one of the conditioning chambers were eliminated from the study. The following day (D4), all rats received a morning injection of vehicle and were immediately (or in two groups, 15 min after vehicle) placed in one of the pairing chambers for 30 min. Five hours later, all rats received drug (clonidine, Chembridge-5861528, their combination, or in one control condition the second dose of vehicle) and were immediately (or in two groups, 15 min after Chembridge-5861528) placed in the opposite chamber for 30 min.

On the next day (D5), 20 h following drug pairing, animals were placed drug-free in the CPP boxes with access to all chambers. The amount of time spent in each of the two chambers (saline- and drug-paired) was automatically registered and used to quantify the conditioning effect by drug treatment. In one test group, a multiple drug exposure-paradigm was used (for details, see Section 2.7). It was expected that if the animal had ongoing pain that was reduced by drug treatment, the animal preferred the drug-paired chamber.

2.6. Assessment of pain-related behavior evoked by peripheral test stimulation

All animals were habituated to pain testing procedures at least 1–2 h per day for two days before assessing drug effects on pain behavior. Since mechanical rather than heat hypersensitivity is a frequent problem in patients with peripheral neuropathy (Scadding and Koltzenburg, 2006), the focus in testing of stimulus-evoked pain behavior was on mechanically evoked responses. Hypersensitivity to cold is also common in peripheral neuropathies (Scadding and Koltzenburg, 2006) and the spinal nerve injury-induced cold hypersensitivity has also been attenuated by a TRPA1 channel antagonist (Chen et al., 2011). The testing schedule in the present study, however, did not allow assessing cold hypersensitivity.

To assess mechanically evoked pain behavior, the frequency of withdrawal responses to the application of monofilaments (von Frey hairs) to the hind paw was examined. A series of monofilaments that produced forces varying from 1 g to 26 g (North Coast Medical, Inc., Morgan Hill, CA) was applied in ascending order five times to the plantar skin at a frequency of 0.5 Hz. A visible lifting of the stimulated hind limb was considered a withdrawal response. If the rat failed to withdraw to any of the five presentations of a monofilament, the response rate for the studied force level was 0%. If the rat withdrew every time the monofilament was applied to the paw, the response rate for the studied force level was 100%. Thus, an increase in the

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response rate represents facilitation of mechanical stimulus-evoked pain behavior (hypersensitivity). When assessing treatment effects on mechanical hypersensitivity, the treatment effect on the cumulative response rate to a series of monofilaments was calculated in the following way: the cumulative response rate after treatment − the cumulative response rate before treatment. Treatment-induced changes in cumulative response rates that were less than 0 represent treatment-induced antihypersensitivity effects. Pain behavior was assessed on day 4 of the conditioning. Testing of pain behavior was performed before placing the animal into the CPP device and immediately after its removal from the CPP device, both in the vehicle (morning) and drug (afternoon) treatment conditions.

2.7. Course of the study

In general, animals were tested 1–2 weeks after induction of diabetes or SNI/sham surgery. Each animal participated only in one testing condition, each of which lasted five days (see Section 2.5 for details). When single-exposure CPP paradigm was used, vehicle (morning) and drug (afternoon) were administered only on D4, whereas in the multiple-exposure CPP paradigm, vehicle and drug were administered on four consecutive days (days 1–4); in the multiple-exposure CPP paradigm the experimental procedure on days 1–4 was identical to that of day 4 in the single-exposure CPP condition. Place-preference (time spent in the vehicle- versus drug-paired chamber) was assessed on D5. Mechanical pain behavior (see Section 2.6) was assessed on D4. After completion of the study, the animals were sacrificed with a lethal dose of pentobarbitone.

2.8. Drugs

Chembridge-5861528, (CHEM; a derivative of HC-030031) that was synthesized by ChemBridge Corporation (San Diego, CA) was used as a TRPA1 channel antagonist. Its chemical structure is illustrated in our previous publication (Fig. 1 in Wei et al., 2009). Our calcium imaging results in human TRPA1 and TRPV1 transfected HEK cells showed that when mustard oil or 4-hydroxynonenal (4-HNE) was used as a TRPA1 channel agonist, IC_{50} value of CHEM was 14.3 ± 0.7 μM or 18.7 ± 0.3 μM, respectively (Wei et al., 2009). Moreover, CHEM showed no TRPA1 or TRPV1 channel agonism and no TRPV1 channel antagonism up to a dose of 100 μM (Wei et al., 2009). CHEM was administered i.p. or i.t. at doses (30 mg/kg or 10 μg, respectively) that have proved to have a significant mechanical antihypersensitivity effect, without motor or other side-effects (e.g., Wei et al., 2009, 2010a, 2011, 2012). With i.p. administration of CHEM, the onset of action is within 15 min, the peak effect is reached at 30 min, and the duration of effect is less than two h (Wei et al., 2009). With i.t. administration of CHEM, the onset of action is within 5 min, the peak effect is reached at 15 min, and the duration of action is less than 2 h (Wei et al., 2010a). It should be noted that due to dissolving problems it was not possible to administer a higher i.t. dose of CHEM than the currently used 10 μg. Moreover, the currently used i.p. dose of CHEM (30 mg/kg) was the highest i.p. dose that was expected to reduce selectively pathophysiological pain hypersensitivity, without a significant suppression of (physiological) nociception that is needed for protecting the tissues from damage. Clonidine, an α_2-adrenoceptor agonist (Sigma-Aldrich, St.Louis, MO) was used in control experiments at an antinociceptive dose of 10 μg i.t. as in the study of King et al. (2009). In general, drugs were administered immediately before placing the animal in the test chamber. However, since it may take up to 15 min before CHEM has a significant effect following i.p. administration (Wei et al., 2009) and since the lack of significant drug effect during the first 10–15 min of the pairing period of 30 min duration might prevent making the association between the i.p. CHEM treatment and the test chamber (Bardo and Bevins, 2000), in two experimental groups i.p. administration of CHEM (or vehicle) was performed 15 min before placing the animal in the test chamber.

2.9. Statistical analysis

When assessing CPP during the 30 min observation period on D5, the absolute time each animal spent in the drug-paired chamber was compared with that spent in the vehicle-paired chamber. When assessing mechanical antihypersensitivity effects on D4, the CHEM-induced change in the cumulative response rate to repetitive stimulation with a series of monofilaments was compared with that induced by vehicle treatment. These comparisons were performed using a paired *t*-test. $P<0.05$ was considered to represent a significant difference.

3. Results

3.1. CHEM- and clonidine-induced CPP in control experiments

In healthy controls, CHEM failed to produce CPP as revealed by equal amounts of time spent in the chamber paired with CHEM (30 mg/kg i.p.) versus vehicle ($t_{11}=0.11$; Fig. 1A). Also, when both chambers were paired with vehicle in control animals, the animals spent equal amounts of time in the chamber paired with the first administration of vehicle as in the chamber paired with the second administration of vehicle ($t_5=0.75$; Fig. 1B).

In order to have a positive control, we replicated the single-exposure CPP experiment described by King et al. (2009) in the SNI model of peripheral neuropathy. I.t. treatment with clonidine (10 μg; a prototype α_2-adrenoceptor agonist) on D4 produced a significant CPP effect on D5 as revealed by a significantly longer time spent in the chamber paired with clonidine than vehicle ($t_9=3.9$, $P=0.0037$; Fig. 1C). In sham-operated animals, i.t. treatment with clonidine on D4 failed to produce a CPP effect on D5 ($t_5=0.24$; Fig. 1D).

3.2. CHEM-induced CPP and mechanical antihypersensitivity effect in diabetic animals

In diabetic animals, i.p. treatment with CHEM (30 mg/kg, immediately before placing the animal in the test chamber) on D4 failed to produce a CPP effect on D5, as revealed by the lack of significant difference in times spent in the vehicle- versus CHEM-paired chamber ($t_7=2.0$; Fig. 2A). Nor did i.t. treatment of diabetic animals with CHEM (10 μg) produce a significant CPP effect ($t_4=0.22$; Fig. 2B).

Mechanical hypersensitivity was measured in diabetic animals by assessing cumulative withdrawal response rates to repetitive stimulation of the hind paw with a calibrated series of monofilaments before and after treatments. Before CHEM treatment, diabetic animals were hypersensitive to mechanical stimulation as shown by an increased mean withdrawal response rate to monofilament stimulation (e.g., at the stimulus force of 8 g: 23 ± 3% in diabetes versus 10 ± 4% in controls; $t_{11}=2.6$, $P=0.023$). In contrast to the failure to induce a significant CPP effect, CHEM produced a significant antihypersensitivity effect in diabetic animals both in the i.p. ($t_6=4.9$, $P=0.0026$; Fig. 2C) and i.t. ($t_4=3.9$, $P=0.017$; Fig. 2D) treatment conditions.

3.3. CHEM-induced CPP and mechanical antihypersensitivity effect in the SNI model of neuropathy

In SNI animals, i.p. treatment with CHEM (30 mg/kg, immediately before placing the animal in the test chamber) on D4 failed to produce a CPP effect on D5, as revealed by the lack of significant difference in times spent in the vehicle- versus CHEM-paired chamber ($t_{10}=0.52$; Fig. 3A). Since pairing of the test chamber only once (on D4) with CHEM failed to induce significant CPP in SNI animals, we tested whether pairing the test chamber on four consecutive days (D1–D4) induced a significant CPP effect on D5. However, pairing of the test chamber on four consecutive days with CHEM failed to induce a significant CPP effect ($t_5=0.47$; Fig. 3B).

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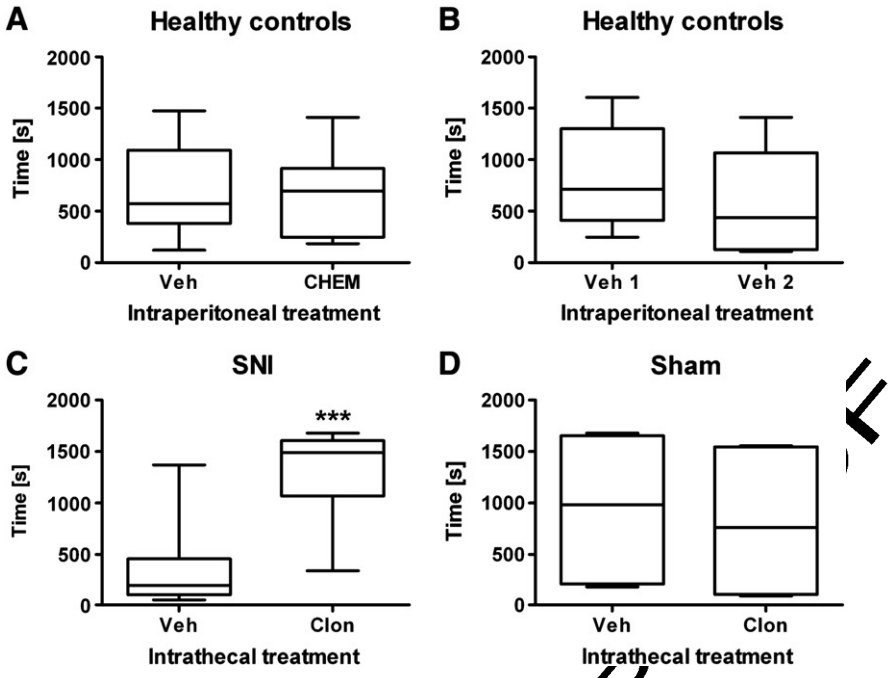

Fig. 1. Assessment of conditioned place-preference (CPP) in control experiments. (A) CPP in healthy control animals (n = 12) following single intraperitoneal (i.p.) treatment with Chembridge-5861528 (CHEM, a TRPA1 channel antagonist; 30 mg/kg). (B) CPP control experiment in which both chambers of the CPP device were paired with vehicle in healthy controls (n = 6). (C) CPP in the spared nerve injury (SNI) model of peripheral neuropathy (n = 10) following single intrathecal (i.t.) treatment with clonidine (Clon, an α_2-adrenoceptor agonist; 10 μg). (D) CPP in sham-operated control animals (n = 6) following single i.t. treatment with clonidine (10 μg). Pairing of each test chamber with drug/vehicle was performed only once on day 4 and CPP was assessed as time spent in each chamber (shown by the Y-axis) on the following day. The boxes represent median and its interquartile values, while whiskers represent the range.

Before CHEM treatments, SNI animals were hypersensitive to mechanical stimulation as shown by an increased mean withdrawal response rate to monofilament stimulation (e.g., at the stimulus force of 8 g: 50 ± 4 in SNI % versus 10 ± 4% in controls; $t_{10} = 7.1$, $P < 0.0001$). CHEM treatment (30 mg/kg i.p.) had a significant mechanical antihypersensitivity effect assessed in SNI animals on D4

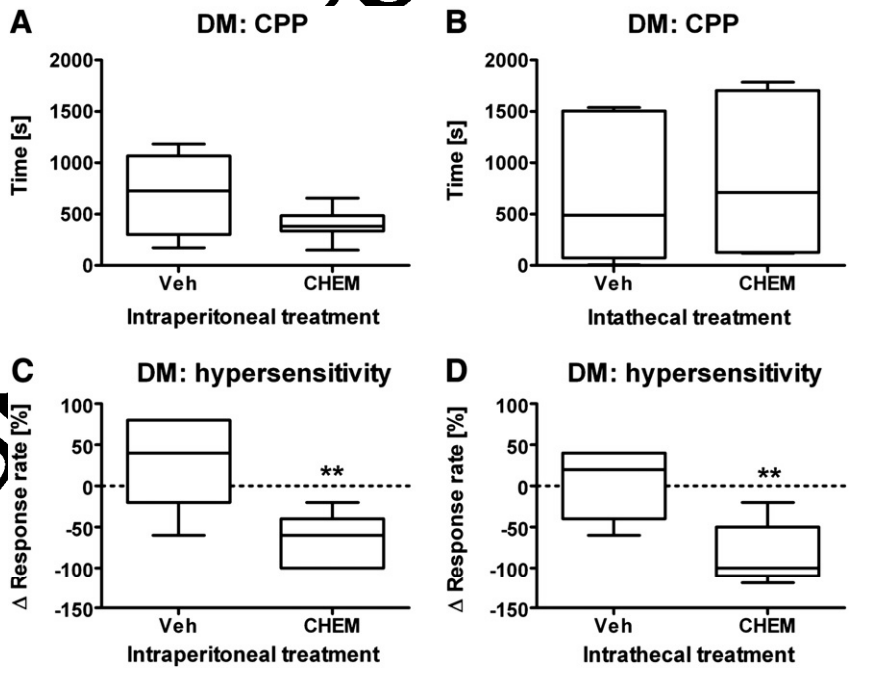

Fig. 2. Assessment of conditioned place-preference (CPP) and mechanical hypersensitivity in diabetic (DM) animals. (A) CPP and (C) the attenuation of mechanical hypersensitivity (n = 7) following single intraperitoneal (i.p.) treatment with Chembridge-5861528 (CHEM, a TRPA1 channel antagonist; 30 mg/kg). (B) CPP and (D) the attenuation of mechanical hypersensitivity (n = 5) following single intrathecal (i.t.) treatment with CHEM (10 μg). Pairing of each test chamber with drug/vehicle was performed only once on day 4. CPP was assessed as time spent in each chamber (shown by the Y-axis) on the following day. Mechanical hypersensitivity was assessed as the cumulative response rate to a series of monofilaments. Mechanical hypersensitivity was assessed on day 4 (before and immediately after pairing one of the test chambers for 30 min with vehicle/drug administration). In graphs C and D, 0% (shown by the dotted horizontal line) represents the mean pre-drug response. Values<0% represent a drug-induced suppression of hypersensitivity. The boxes represent median and its interquartile values, while whiskers represent the range. **P<0.01 (paired *t*-test).

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both following a single-exposure to CHEM ($t_5 = 4.8$, P = 0.0047; Fig. 3C) and four exposures to CHEM ($t_5 = 10.7$, P = 0.0001; Fig. 3D).

Animals in the above mentioned experiments were placed in the test chamber immediately after intraperitoneal injection of (vehicle or) CHEM, while it may take up to 15 min before i.p. administration of CHEM produces a significant antihypersensitivity effect (Wei et al., 2009). Therefore, it might be argued that the failure to induce CPP by i.p. treatment with CHEM was due to the slow onset of the significant drug effect (15 min), due to which the animals placed immediately after drug injection in the test chamber failed to associate the test chamber with the (rewarding) pain relief induced by CHEM. To exclude this possibility, a group of SNI animals were placed in the test chamber 15 min after i.p. administration of (vehicle or) CHEM. I.p. treatment with 30 mg/kg of CHEM failed to induce CPP in SNI animals ($t_5 = 0.3$; Fig. 4A), although the animals were placed in the test chamber 15 min after i.p. drug administration (i.e., at or after the onset of the significant antihypersensitivity effect).

In case CHEM treatment abolished ongoing pain in SNI animals, it might be expected that pretreatment with CHEM prevents observing a relief of ongoing pain induced by i.t. treatment with clonidine. To address this question, we determined CPP induced by i.t. clonidine (10 μg) in SNI animals that were pretreated with CHEM (30 mg/kg i.p., 15 min prior to i.t. treatment with clonidine). In spite of i.p. pretreatment with CHEM, i.t. treatment with clonidine produced CPP in SNI animals ($t_9 = 2.4$, P = 0.037; Fig. 4B).

4. Discussion

The main finding of this study was that the selective TRPA1 channel antagonist CHEM administered at a high systemic or intrathecal dose produced a marked mechanical antihypersensitivity effect that was not associated with CPP (an index for the drug-induced relief of ongoing pain) in experimental models of peripheral neuropathy. This finding suggests that the TRPA1 channel-mediated facilitation of stimulus-evoked pain dissociates from mechanisms contributing to maintenance of sustained pain in peripheral neuropathy. The result allows concluding that ongoing pain is less sensitive to blocking the TRPA1 channel than mechanical hypersensitivity in peripheral neuropathy. It should be noted that the present results don't exclude the possibility that a further increase in the dose of the TRPA1 channel antagonist might induce CPP in neuropathic animals. However, higher doses may not be clinically feasible, due to suppression of physiological nociception that helps in protecting tissues from harmful stimuli. In healthy controls, the TRPA1 channel antagonist CHEM failed to induce CPP indicating that the antagonist alone had neither rewarding nor aversive properties.

One might argue that the failure to induce CPP by administering a TRPA1 channel antagonist was due to lack of ongoing pain in the currently used models of peripheral neuropathy. This argument is not supported by the finding that intrathecal clonidine produced CPP in the SNI model of peripheral neuropathy in the present as in the earlier study by King et al. (2009). Importantly, intrathecal clonidine produced CPP also in SNI animals that were pretreated intraperitoneally with CHEM, which finding indicates that SNI animals had ongoing pain that was not abolished by CHEM treatment at a dose producing a marked antihypersensitivity effect. Earlier neurophysiological studies in the streptozotocin-induced model of diabetic neuropathy have reported increased discharge rates in nociceptive primary afferent nociceptive nerve fibers (Khan et al., 2002) and spinal dorsal horn neurons (Pertovaara et al., 2001; Chen and Pan, 2002). Moreover, SNI has increased the ongoing discharge rate of pronociceptive medullary neurons and decreased the discharge rate of antinociceptive medullary neurons (Gonçalves et al., 2007). These neurophysiological findings are in line with the hypothesis that diabetes or SNI induces ongoing pain. On the other hand, one needs to be cautious with interpretations from correlative neurophysiological evidence to ongoing pain and CPP. This is indicated by the recent finding that systemically administered morphine and pregabalin reduced mechanical hyperalgesia and the spontaneous discharge rate of the presumed pain-relay neurons of diabetic animals, without inducing CPP (Rutten et al., 2011). Furthermore, it has been pointed out that what is often considered spontaneous pain

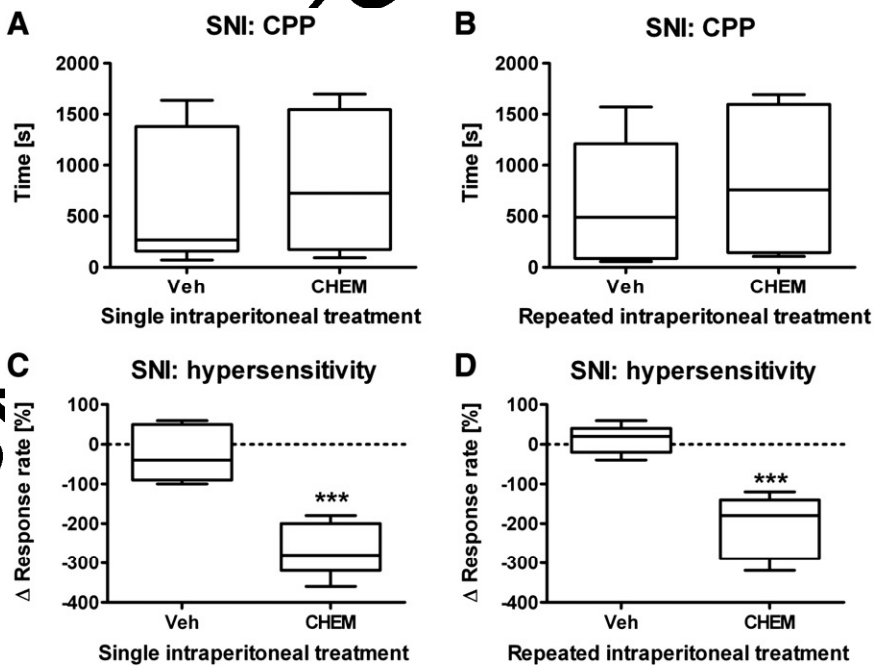

Fig. 3. Assessment of conditioned place-preference (CPP) and mechanical hypersensitivity in animals with the spared nerve injury (SNI) model of peripheral neuropathy. (A and B) CPP and (C and D) the attenuation of mechanical hypersensitivity following intraperitoneal (i.p.) treatment with Chembridge-5861528 (CHEM, a TRPA1 channel antagonist; 30 mg/kg). Pairing of each test chamber with drug/vehicle was performed only once on day 4 (A and C; n = 11) or on four consecutive days (B and D; n = 6). CPP was assessed as time spent in each chamber (shown by the Y-axis) on the fifth day. Mechanical hypersensitivity was assessed as the cumulative response rate to a series of monofilaments. In both groups, mechanical hypersensitivity was assessed on day 4 (before and immediately after pairing one of the test chambers for 30 min with vehicle/drug administration). In graphs C and D, 0% (shown by the dotted horizontal line) represents the mean pre-drug response. Values<0% represent a drug-induced suppression of hypersensitivity. The boxes represent median and its interquartile values, while whiskers represent the range. ***P<0.005 (paired *t*-test).

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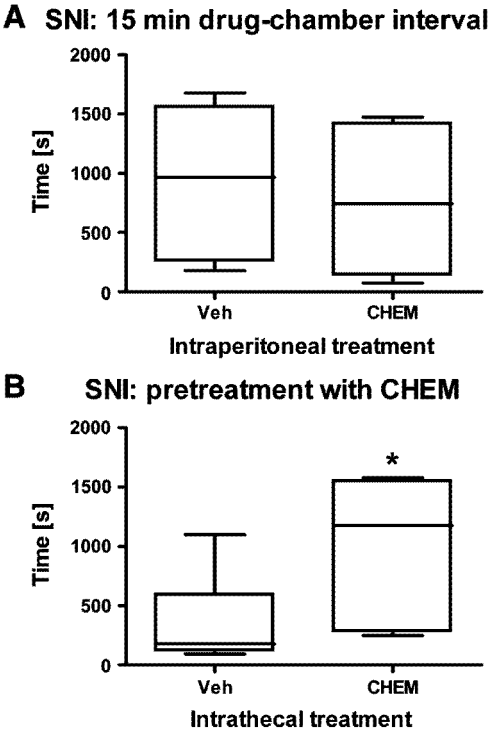

Fig. 4. Assessment of conditioned place-preference (CPP) in animals with the spared nerve injury (SNI) model of peripheral neuropathy. (A) CPP following single intraperitoneal treatment with vehicle (Veh) or Chembridge-5861528 (CHEM, a TRPA1 channel antagonist; 30 mg/kg 15 min prior to placing the animal in the test chamber). (B) CPP following single intrathecal treatment with vehicle or clonidine (Clon, an α_2-adrenoceptor agonist; 10 μg). Animals were pretreated 15 min before intrathecal vehicle treatment with intraperitoneally administered vehicle, and 15 min before intrathecal clonidine treatment with intraperitoneally administered CHEM (30 mg/kg). Pairing of each test chamber with drug(s)/vehicle was performed only once on day 4. CPP was assessed as time spent in each chamber (shown by the Y-axis) on the fifth day. The boxes represent median and its interquartile values, while whiskers represent the range. In graph A, n = 6 and in graph B, n = 10. *$P < 0.05$ (paired *t*-test).

may actually represent summated pains caused by the stimuli of daily life (Bennett, 2012).

The present CPP results failed to give evidence that supports a role for the TRPA1 channel in maintenance of ongoing pain in peripheral neuropathy. Previous results, however, indicate that in a number of other conditions the peripheral TRPA1 channel may induce afferent barrage driving ongoing pain. For example, cutaneous administration of a selective TRPA1 channel agonist in healthy control animals (e.g., Andrade et al., 2008; Tsagareli et al., 2010) induced sustained pain behavior. In human subjects, cutaneous administrations of a TRPA1 channel agonist (mustard oil or cinnamaldehyde) also produced sustained pain (Koltzenburg et al., 1992; Namer et al., 2005). Conversely, a TRPA1 channel antagonist adjacent to a wound attenuated guarding, an index of ongoing postoperative pain behavior in the rat (Wei et al., 2012).

The spinal TRPA1 channel on central terminals of nociceptive nerve fibers, in contrast, has so far been associated only with modulation of stimulus-evoked pain responses, such as secondary or central hypersensitivity (Da Costa et al., 2010; Kremeyer et al., 2010; Wei et al., 2010a, 2011; Sisignano et al., 2012; Klafke et al., 2012), or a dorsal root reflex-mediated aggravation of cutaneous neurogenic inflammation (Wei et al., 2010b), but not yet with spontaneous pain (Pertovaara and Koivisto, 2011; Wei et al., 2012). In stimulus-evoked neuropathic hypersensitivity the spinal TRPA1 channel has proved to play an important role as shown by the mechanical antihypersensitivity effect induced by spinal administration of a TRPA1 channel antagonist in nerve-injured or diabetic animals (Wei et al., 2010a, 2011).

5. Conclusions

The results of this study indicate that the TRPA1 channel-mediated mechanical hypersensitivity may not reflect ongoing pain in peripheral neuropathy. The significant TRPA1 channel antagonist-induced mechanical antihypersensitivity effect in SNI and diabetic animals of the present study adds to the accumulating evidence indicating that selective TRPA1 channel antagonists are promising candidates for treating pain hypersensitivity associated with peripheral neuropathy, while the CPP paradigm of the present study failed to confirm their efficacy against ongoing neuropathic pain.

Conflict of interest

One of the authors (A.K.) is an employee of the pharmaceutical company (OrionPharma, Finland) that has supported this study.

Acknowledgments

The study was supported by the Sigrid Jusélius Foundation, Helsinki, the Academy of Finland, Helsinki, and OrionPharma, Orion Corporation, Turku, Finland. Diana Norim was supported by the Portuguese Science Foundation (FCT) grant SFRH/BD/71219/2010.

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Please cite this article as: Wei H, et al, Dissociated modulation of conditioned place-preference and mechanical hypersensitivity by a TRPA1 channel antagonist in peripheral neuropathy, Pharmacol Biochem Behav (2013), http://dx.doi.org/10.1016/j.pbb.2012.12.014

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Please cite this article as: Wei H, et al, Dissociated modulation of conditioned place-preference and mechanical hypersensitivity by a TRPA1 channel antagonist in peripheral neuropathy, Pharmacol Biochem Behav (2013), http://dx.doi.org/10.1016/j.pbb.2012.12.014