Neuropharmacology 58 (2010) 578–584

Contents lists available at ScienceDirect

Neuropharmacology

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

Roles of cutaneous versus spinal TRPA1 channels in mechanical hypersensitivity in the diabetic or mustard oil-treated non-diabetic rat

Hong Wei [a], Hugh Chapman [b], Marja Saarnilehto [b], Katja Kuokkanen [b], Ari Koivisto [b], Antti Pertovaara [a,*]

[a] Institute of Biomedicine/Physiology, University of Helsinki, Biomedicum Helsinki, POB 63, 00014 Helsinki, Finland
[b] In Vitro Pharmacology, Orion Corporation, OrionPharma, Turku, Finland

ARTICLE INFO

Article history:
Received 15 September 2009
Received in revised form
11 November 2009
Accepted 1 December 2009

Keywords:
Diabetic neuropathy
Mechanical hypersensitivity
Rat
Transient receptor potential
ankyrin 1 channel

ABSTRACT

Previous results indicate that intaperitoneal administration of a TRPA1 channel antagonist attenuates diabetic hypersensitivity. We studied whether the antihypersensitivity effect induced by a TRPA1 channel antagonist in diabetic animals is explained by action on the TRPA1 channel in the skin, the spinal cord, or both. For comparison, we determined the contribution of cutaneous and spinal TRPA1 channels to development of hypersensitivity induced by topical administration of mustard oil in healthy controls. Diabetes mellitus was induced by streptozotocin in the rat. Hypersensitivity was assessed by the monofilament- and paw pressure-induced limb withdrawal response. Intrathecal (i.t.) administration of Chembridge-5861528 (CHEM, a TRPA1 channel antagonist) at doses 2.5–5.0 μg/rat markedly attenuated diabetic hypersensitivity, whereas 20 μg of CHEM was needed to produce a weak attenuation of diabetic hypersensitivity with intraplantar (i.pl.) administrations. In controls, i.pl. administration of CHEM (20 μg) produced a weak antihypersensitivity effect at the mustard oil-treated site. I.t. administration of CHEM (10 μg) in controls produced a strong antihypersensitivity effect adjacent to the mustard oil-treated area (site of secondary hyperalgesia), while it failed to influence hypersensitivity at the mustard oil-treated area (site of primary hyperalgesia). A reversible antagonism of the rat TRPA1 channel by CHEM was verified using *in vitro* patch clamp recordings. The results suggest that while cutaneous TRPA1 channels contribute to mechanical hypersensitivity induced by diabetes or topical mustard oil, spinal TRPA1 channels, probably on central terminals of primary afferent nerve fibers, play an important role in maintenance of mechanical hypersensitivity in these conditions.

1. Introduction

Peripheral neuropathy is a frequent complication of diabetes mellitus (Wheeler et al., 2007). Our recent study indicated that a TRPA1 channel antagonist attenuated mechanical hypersensitivity in diabetic animals at a low dose that did not influence pain-related responses in control animals (Wei et al., 2009). Moreover, diabetic animals receiving prolonged treatment with a TRPA1 receptor antagonist did not show mechanical hypersensitivity (Wei et al., 2009) suggesting that the TRPA1 channel is involved in development as well as maintenance of diabetic hypersensitivity.

The TRPA1 channel is expressed particularly by nociceptive primary afferent neurons. On peripheral terminals, they can contribute to transduction of potentially harmful stimuli to electric signals (Bandell et al., 2004; Jordt et al., 2004), and on central terminals in the spinal dorsal horn they can modulate neurotransmitter release (Kosugi et al., 2007; Wrigley et al., 2009). While our recent results indicated that systemic administration of a TRPA1 channel antagonist effectively attenuates diabetic hypersensitivity, this finding still left open whether the antihypersensitivity effect was due to block of TRPA1 channels in the skin, the spinal cord, or both.

Here we assessed pain-related behavior of diabetic animals following intraplantar (i.pl.) versus intrathecal (i.t.) administration of a TRPA1 channel antagonist to determine the roles of cutaneous versus spinal TRPA1 channels in maintenance of diabetic hypersensitivity. For comparison, we assessed the roles of cutaneous and spinal TRPA1 channels in topical mustard oil-induced primary and secondary hypersensitivity/hyperalgesia in healthy control animals. This was done by determining the influence of i.pl. versus i.t. administration of a TRPA1 channel antagonist on development of hypersensitivity at the mustard oil-treated area (primary hyperalgesia) or adjacent to it (secondary hyperalgesia). Furthermore, since the properties of the currently used TRPA1 channel

* Corresponding author. Tel.: +358 9 191 25280; fax: +358 9 191 25302.
E-mail address: Antti.Pertovaara@helsinki.fi (A. Pertovaara).

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.12.001

antagonist were previously characterized in human TRPA1-inducible cells (Wei et al., 2009), we verified with patch clamp recordings that the antagonist blocks the rat TRPA1 channel.

2. Materials and methods

2.1. Experimental animals

The experiments were performed with male Hannover-Wistar rats (220–260 g; Harlan, Horst, Netherland). 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.

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 (Calcutt, 2004; Courteix et al., 1993). 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 > 20 mmol/l. Weight of the animals was assessed every other day. If the animal had a weight decrease >20% or it showed signs of suffering or spontaneous pain, then the animal was immediately sacrificed by administering a lethal dose of pentobarbitone. Mustard oil-induced hypersensitivity was studied in healthy control rats.

2.2. Chembridge-5861528, a TRPA1 channel antagonist

Chembridge-5861528, (CHEM; a derivative of HC-030031) that was synthesized by ChemBridge Corporation (San Diego, CA) was used as a TRPA1 channel antagonist in this study. 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). In each experiment, CHEM was dissolved in physiological saline immediately before its i.pl. or i.t. administration.

2.3. Assessment of pain-related behavior in diabetic animals

The rats were habituated to the experimental conditions by allowing them to spend 1–2 h daily in the laboratory during two to three days preceding any testing. Animals were tested 10–20 days after induction of diabetes. This study focused on assessing mechanical hypersensitivity, since previous results indicate that streptozotocin-treated animals develop a marked and reproducible hypersensitivity to mechanical stimulation, whereas hypersensitivity to thermal stimulation may be more variable or non-existent (e.g., Malcangio and Tomlinson, 1998; Pertovaara et al., 2001). For assessment of mechanical hyperalgesia, the hind limb withdrawal threshold to noxious mechanical stimulation (the paw pressure test) was determined with a Basile Analgesy-meter (Ugo Basile, Varese, Italy). With this device a mechanical force was applied at a rate of 32 g/s to the hind paw until limb withdrawal or the cut-off force of 250 g. At each time point, two threshold determinations were performed at 1 min intervals. The mean of the two threshold values at each time point was used in further calculations.

To assess tactile allodynia-like behavior elicited by mechanical stimulation of the skin in diabetic animals, the frequency of withdrawal responses to the application of monofilaments (von Frey hairs) to the hind paw was examined. Nine hairs with forces varying from 1.0 g to 60 g (North Coast Medical, Inc., Morgan Hill, CA, USA) were applied five times at a frequency of approximately of 0.5 Hz. Hairs were tested in ascending order of force. A visible lifting of the stimulated hind limb was considered a withdrawal response.

2.4. Assessment of mustard oil-induced hypersensitivity in non-diabetic animals

In healthy control animals, mustard oil-induced mechanical hypersensitivity/hyperalgesia was induced as described earlier (Mansikka and Pertovaara, 1995). Mustard oil (50% in ethanol, Merck, Darmstadt, Germany) was applied for 2 min on

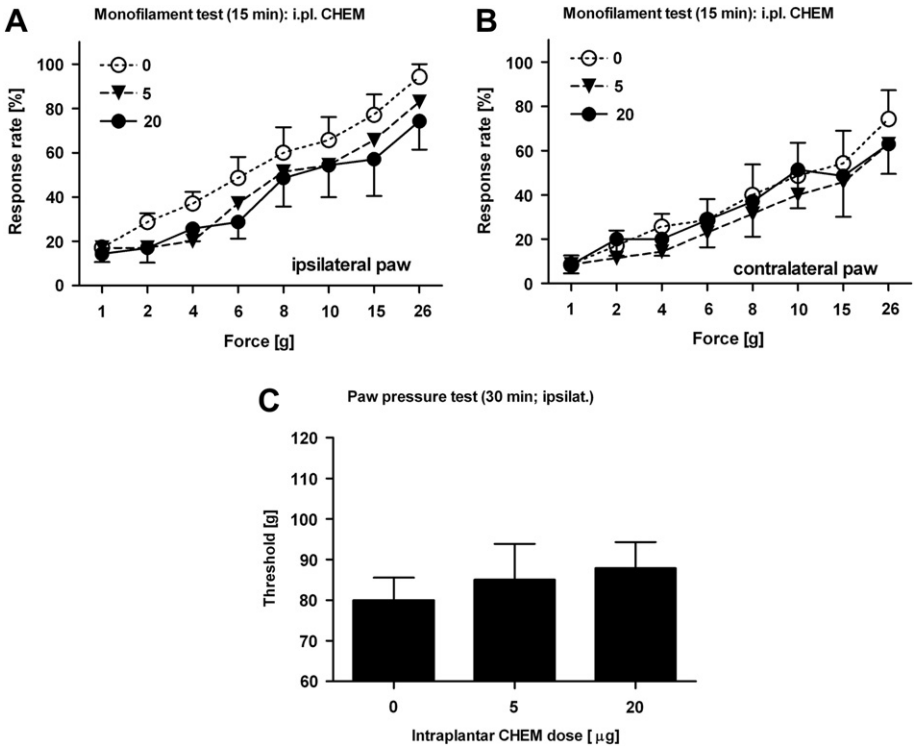

Fig. 1. Influence by intraplantar (i.pl.) treatment with Chembridge-5861528 (CHEM, a TRPA1 channel antagonist) on pain-related behavior in diabetic animals. A. Withdrawal response rates evoked by monofilaments 15 min following i.pl. administration of CHEM in the test site. B. Withdrawal response rates evoked by monofilaments 15 min following i.pl. administration of CHEM in contralateral to the test site. C. Paw pressure threshold 30 min following i.pl. administration of CHEM in the test site. Decrease in the monofilament-induced response rate and increase in the paw pressure threshold are considered to represent antihypersensitivity effect. CHEM doses in μg are indicated in graphs. Error bars represent S.E.M. (in each group, $n = 7$).