Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 350 (2006) 238–243
BBRC
www.elsevier.com/locate/ybbrc

Silencing of vanilloid receptor TRPV1 by RNAi reduces neuropathic and visceral pain *in vivo*

Thomas Christoph [a,1], Arnold Grünweller [b,c,1], Joanna Mika [d,e], Martin K.-H. Schäfer [d], Erik J. Wade [a], Eberhard Weihe [d], Volker A. Erdmann [b], Robert Frank [a], Clemens Gillen [a], Jens Kurreck [b,*]

[a] *Research and Development, Grünenthal GmbH, Aachen, Germany*
[b] *Institute for Chemistry and Biochemistry, Free University Berlin, Germany*
[c] *Institute for Pharmaceutical Chemistry, Philipps-University Marburg, Germany*
[d] *Institute of Anatomy and Cell Biology, Philipps-University Marburg, Germany*
[e] *Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

Received 8 September 2006
Available online 18 September 2006

Abstract

RNA interference (RNAi) has proven to be a powerful technique to study the function of genes by producing knock-down phenotypes. Here, we report that intrathecal injection of an siRNA against the transient receptor potential vanilloid receptor 1 (TRPV1) reduced cold allodynia of mononeuropathic rats by more than 50% over a time period of approximately 5 days. A second siRNA targeted to a different region of the TRPV1 gene was employed and confirmed the analgesic action of a TRPV1 knock-down. Furthermore, siRNA treatment diminished spontaneous visceral pain behavior induced by capsaicin application to the rectum of mice. The analgesic effect of siRNA-mediated knockdown of TRPV1 in the visceral pain model was comparable to that of the low-molecular weight receptor antagonist BCTC. Our data demonstrate that TRPV1 antagonists, including TRPV1 siRNAs, have potential in the treatment of both, neuropathic and visceral pain.

Keywords: BCTC; Capsaicin; Neuropathic pain; RNA interference; Small interfering RNA; Vanilloid receptor; Visceral pain

Chronic pain affects more than 320 million people worldwide [1], but current pharmaceutical treatment of pain sufferers is frequently unsatisfactory, demonstrating the obvious need to develop new strategies for the treatment of pain with acceptable adverse side effects [2]. One of the major new targets for novel pain medication is TRPV1, which is activated by heat, protons, and capsaicin, the hot component of chili peppers [3,4]. Its sensitivity is further modulated and increased by pathophysiological states such as inflammation [5]. TRPV1 has thus been considered to be a central integrator of numerous endogenous and exogenous stimuli [6,7].

As a result of the findings that demonstrated the important function of TRPV1 in pain perception, low molecular weight compounds have been developed that modulate the activity of the receptor and attenuate inflammatory pain conditions in rodents (summarized in [8]). The role of TRPV1 during neuropathic pain, however, is not fully clarified, although the potent TRPV1 antagonist BCTC has been shown to reduce mechanical hyperalgesia and allodynia in neuropathic pain models [9].

RNA interference (RNAi) has been established as a powerful technique to investigate gene functions. For this purpose, small interfering RNA (siRNA) molecules 21 nucleotides in length are usually employed to specifically

* Corresponding author. Fax: +49 30 83856413.
E-mail address: jkurreck@chemie.fu-berlin.de (J. Kurreck).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.09.037

inhibit the expression of genes (for reviews, see [10,11]). RNAi has been reported to be more efficient than traditional antisense approaches [12,13]. The method has been used widely in neuroscience and is suited to investigate the function of genes that are likely to be involved in pain processes (for reviews, see [1,14]). Since the blood–brain barrier prevents efficient delivery of large molecules into the CNS, siRNAs have been administered intrathecally, close to the spinal cord [15–17].

The aim of the present study was to evaluate the potential of intrathecally administered siRNAs as a rapid technique to validate targets for pain research by targeting TRPV1 as a receptor expressed on spinal endings of primary nociceptive somatic and visceral afferents. Our results obtained with intrathecally delivered siRNAs for TRPV1 in a neuropathic rat model and in an acute visceral pain model of the mouse as compared to the TRPV1 inhibitor BCTC support the view that TRPV1 plays an important role in neuropathic and visceral pain.

Materials and methods

Oligonucleotides and chemicals. Purified 19mer siRNA duplexes were purchased from IBA GmbH (Göttingen, Germany). Sequences of the siRNAs used in the present study are summarized in Table 1. The siRNAs were dissolved and annealed in DEPC-treated water for in vivo administrations. BCTC (*N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2*H*)-carbox-amide; Grünenthal GmbH, Germany) was dissolved in 10% DMSO, 5% Cremophor EL, in 5% glucose solution and administered in a volume of 5 μl/animal intrathecally. Capsaicin (Tocris, UK) was dissolved in vehicle solution (10% EtOH, 10% Tween 80 in 0.9% NaCl solution) and administered intrarectally (50 μl of a 10% solution per animal).

Cell culture experiments. Cos-7 cells (African green monkey kidney fibroblasts) were cultivated under standard conditions as described previously [13]. Co-transfection experiments were carried out with a pcDNA3.1/TRPV1-GFP plasmid and increasing concentrations of siRNA as indicated with Lipofectamine 2000 (Invitrogen, Germany). Twenty-four hours after transfection, cells were harvested and the protein expression was analyzed by Western blotting. The TRPV1-GFP fusion protein was detected with anti-GFP serum (Invitrogen, Germany). For further details, see [13].

Animal models. Adult male Sprague–Dawley rats (170–310 g, obtained from Janvier, France) and male NMRI mice (28–38 g, obtained from Iffa Credo, France) were used. Animals were kept under standard laboratory conditions with free access to standard laboratory food and tap water. Spinal administrations under short ether narcosis were performed according to [18]. Data were analyzed by means of two-way analysis of variance (ANOVA) with repeated measures. In case of a significant treatment effect, pairwise comparison was performed by post hoc analysis with Bonferroni adjustment. Results were considered statistically significant if $P < 0.05$. Group sizes were $n = 5$–10 (chronic constriction injury model) and $n = 7$ (capsaicin-induced visceral pain model in mice). The experiments were performed in accordance with ECC guidelines (86/609/EEC). All efforts were made to minimize animal suffering and the number of animals used.

Table 1
siRNA sequences used in the present study

siRNA	Sequence
VsiRl	5′-GCGCAUCUUCUACUUCAACTT-3′ 3′-TTCGCGUAGAAGAUGAAGUUG-5′
VsiRl inv	5′-CAACUUCAUCUUCUACGCGTT-3′ 3′-TTGUUGAAGUAGAAGAUGCGC-5′
VsiR2	5′-GUUCGUGACAAGCAUGUACTT-3′ 3′-TTCAAGCACUGUUCGUACAUG-5′
VsiR2 inv	5′-CAUGUACGAACAGUGCUUGTT-3′ 3′-TTGUACAUGCUUGUCACGAAC-5′

Animal model of neuropathic pain. Rats underwent surgery involving four loose ligations of the sciatic nerve according to the Bennett model [19]. Under pentobarbital anesthesia (Narcoren®, 60 mg/kg i.p.), the right common sciatic nerve was exposed by blunt dissection at the level of mid-thigh and four loose ligatures (softcat®chrom USP 4/0, metric2; Braun Melsungen, Germany) were placed around the nerve, taking care not to interrupt the epineural circulation. After operation, animals were allowed to recover for one week. Cold allodynia was stable for several weeks and was tested on a metal plate cooled by a water bath to a constant temperature of 4 °C by means of counting the number of brisk paw withdrawals during 2 min. Animals were observed for periods of 2 min before and several days after intrathecal administration of TRPV1-specific siRNAs, inverted control siRNAs or a vehicle control (0.9% NaCl).

Animal model of acute visceral pain. Analgesic effects of siRNAs or BCTC in capsaicin-induced visceral pain were investigated according to a mouse model described in [20]. Initially, the animals received an intrathecal bolus application (5 μl) of 1 ng of the TRPV1-specific siRNA 'VsiR1', the control siRNA 'VsiR1 inv' or a vehicle control (0.9% NaCl). Four days after pre-treatment, 50 μl of a 10% capsaicin solution was administered rectally. The resulting spontaneous pain behavior was scored (1 = licking of abdominal wall, 2 = stretching, squashing, mounting, backward movement or contraction of the flank muscles; number of reactions recorded 2–12 min after capsaicin). One group of animals received rectal administration of capsaicin vehicle only. Other groups of animals received BCTC at different doses 5 min before rectal administration of capsaicin. Each group consisted of 7 animals.

Results

Silencing of TRPV1 in cell culture

In a previous study, we screened six siRNAs to obtain an efficient inhibitor of TRPV1 expression [13]. Here, we performed a more detailed analysis of the concentration dependency of the two most efficient siRNAs in co-transfection experiments with a plasmid encoding the cDNA of a TRPV1-GFP fusion protein. As can be seen in Fig. 1, we observed the expected concentration-dependent silencing of target gene expression. Both siRNAs tested almost completely inhibited expression of TRPV1-GFP even at the very low concentration of 1 nM. In contrast, the inverted control siRNA, VsiR2 inv, did not influence gene expression in the concentration range investigated. Thus, we have identified two siRNAs against separate sequences of the TRPV1 mRNA that can be used as tools to efficiently and specifically inhibit the expression of the TRPV1 gene.

Analgesic effect of siRNAs against TRPV1 in a neuropathic pain model

To examine the impact of siRNA treatment *in vivo*, modulation of pain perception in the Bennett model of neuropathic pain was investigated [19] since previous

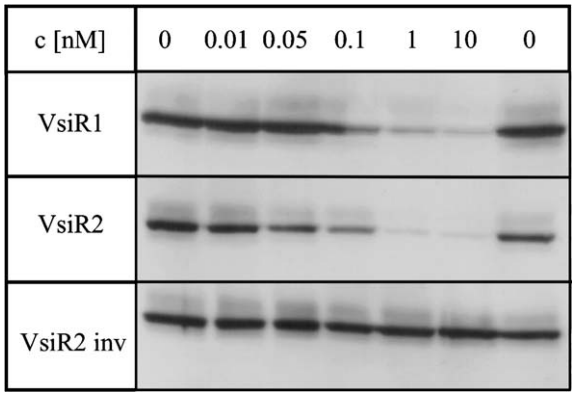

Fig. 1. Concentration-dependent inhibition of TRPV1-GFP expression in cell culture. Cos-7 cells were co-transfected with a plasmid encoding the TRPV1-GFP fusion protein and increasing concentrations of the siRNAs VsiR1, VsiR2 and the inverted control VsiR2 inv, respectively. Twenty-four hours after transfection, cells were harvested and TRPV1-GFP expression was analyzed by Western blotting.

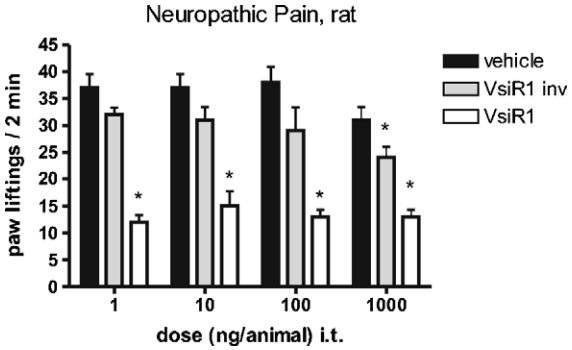

Fig. 3. Effects of different doses of siRNA treatment. Neuropathic rats received a single bolus injection of physiological saline solution (black) or of 1–1000 ng TRPV1-specific siRNA (white) and control siRNA (grey), respectively. Cold-evoked paw withdrawals were measured 24–48 h after siRNA treatment. $n = 10$ per treatment group, $^{*}P < 0.05$ versus saline control.

experiments revealed that this model produces reliable and robust responses to modulation of TRPV1. Mononeuropathic rats received intrathecal bolus administration of 1 μg VsiR1, its inverted control VsiR1 inv or vehicle. The TRPV1-specific siRNA VsiR1 diminishes the number of cold-induced paw liftings by approximately 50%, indicating a reduction of cold allodynia (Fig. 2A). Seven days after RNAi treatment pain sensitivity returned to normal level. The inverted control siRNA resulted in an initial slight unspecific modulation of pain. Lower doses down to 1 ng VsiR1 still showed analgesic activity, whereas the unspecific effects of the inverted control RNA were diminished (Fig. 3).

Immunohistochemical analysis of dissected spinal cords did not reveal a clear change of TRPV1 protein staining after siRNA treatment (data not shown). Technical hurdles of the immunohistochemical analysis limit the measurements to determine global expression levels within recognizable tissue structures.

One way to enhance confidence in the outcome of RNAi experiments is the use of multiple siRNAs with independent sequences. As has been shown in cell culture (Fig. 1), the second siRNA, VsiR2, was at least as potent at silencing TRPV1 as the initially used siRNA VsiR1. To minimize unspecific side-effects, a dose of 1 ng siRNA was administered intrathecally to mononeuropathic rats. Assessment of cold allodynia revealed a pattern comparable to the one observed before (Figs. 2A and B): pain sensitivity is reduced initially by approximately 50% and returns to a normal level after several days. The inverted siRNA did not modulate pain behavior as compared to vehicle.

Analgesic effect of BCTC in capsaicin-induced colitis

To investigate the modulation of pain perception induced by the TRPV1 agonist capsaicin, we used a model to study visceral pain in the mouse [20]. Rectal administration of 50 μl of a 10% capsaicin solution induced spontaneous visceral pain, resulting in a pain score between 25 and 30 (Fig. 4A). Pre-treatment of the animals with an intrathecally administered TRPV1 antagonist, BCTC, resulted in significant and dose-dependent inhibition of capsaicin-

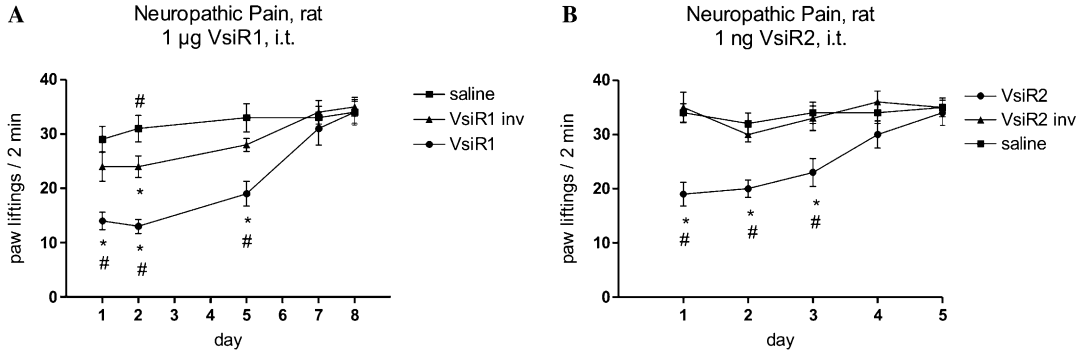

Fig. 2. Analgesic effects of intrathecally administered siRNAs VsiR1 (A) or VsiR2 (B) against TRPV1 in rats with chronic constriction injury as measured by cold evoked paw withdrawals during 2 min intervals (means ± SEM). Bolus injection of 1 μg (VsiR1) or 1 ng (VsiR2) of either TRPV1-specific siRNA (circle) or the corresponding inverted control (triangle) as well as a saline control injection (square). $n = 10$ for VsiR1 and $n = 5$ for VsiR2 per treatment group, $^{*}P < 0.05$ versus saline control, $^{\#}P < 0.05$ versus inverted RNA control.

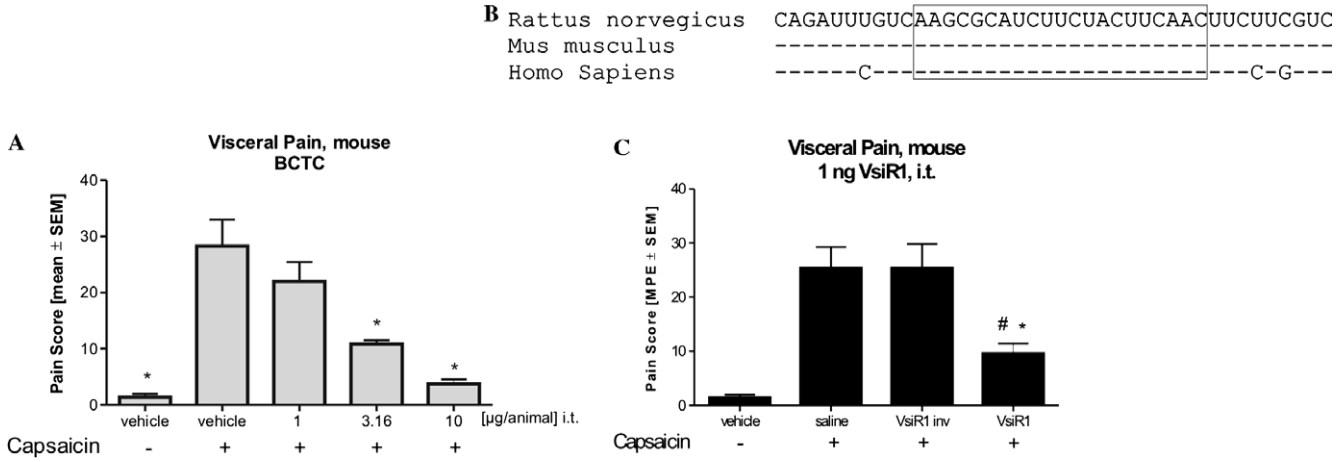

Fig. 4. Analgesic effect of intrathecally administered BCTC or siRNA against TRPV1 in capsaicin-induced visceral pain. (A) Animals received an intrathecal bolus application (5 µl) of 1, 3, or 10 µg BCTC or vehicle solution. One group received rectal injection of capsaicin-vehicle only. Pain reactions were scored within 2–12 min after capsaicin injection (means ± SEM). $n = 7$ per treatment group, $^{*}P < 0.05$ versus saline control. (B) Sequence homology of the siRNA target region in TRPV1 from rat, mouse, and human. The box indicates the 21 nucleotide long binding site of VsiR1. (C) Four days prior to capsaicin treatment, the animal received an intrathecal bolus application (5 µl) of 1 ng siRNA against TRPV1 (VsiR1), the inverted control siRNA (VsiR1 inv) or a physiological saline solution. One group received rectal injection of capsaicin-vehicle only. Pain reactions were scored within 2–12 min after capsaicin injection (means ± SEM). $n = 7$ per treatment group, $^{*}P < 0.05$ versus saline control, $^{\#}P < 0.05$ versus inverted RNA control.

induced spontaneous pain behavior with an ED_{50} (95% confidence interval) of 2.5 (1.7–3.4) µg/animal.

Analgesic effect of siRNAs against TRPV1 in capsaicin-induced colitis

We finally wanted to analyze, whether treatment with siRNAs against TRPV1 changes the response to the receptor agonist capsaicin in a visceral pain model. Since VsiR1 is directed against a target site on the TRPV1 mRNA that is conserved in rats, mice, and humans (Fig. 4B), the experiments to study capsaicin-induced colitis in mice were carried out with the same siRNA that has been employed to investigate the role of TRPV1 in the rat.

One group of animals received intrathecal injection of a vehicle control followed by rectal administration of the capsaicin solution on day four. As expected, this treatment induced spontaneous visceral pain, resulting in a pain score of approximately 25 (Fig. 4C). Intrathecal injection of 1 ng TRPV1-specific siRNA four days prior to capsaicin administration was found to have a significant analgesic effect similar to the reduction of pain sensitivity observed after administration of BCTC. In contrast, the inverted control siRNA did not influence pain perception as reflected in the animals' observed behavior. These results demonstrate that spinal administration of the siRNA against TRPV1 leads to a loss of response to the TRPV1 agonist capsaicin. It is therefore reasonable to assume that the intended specific silencing of TRPV1 was achieved by the RNAi treatment.

Discussion

A crucial point in the development of new analgesic drugs is the validation of new targets for low molecular weight compounds. Here, we evaluate the potential of intrathecally injected siRNAs as tools for rapid functional investigation of pain targets. We chose the vanilloid receptor, TRPV1, as a target, which is considered a central molecular integrator of nociceptive signaling [6].

In vivo delivery of siRNAs into the central nervous system is complicated by the fact that oligonucleotides do not efficiently cross the blood–brain barrier. Previous studies applied siRNAs locally into the spinal cord to investigate the functional role of pain-related receptors by RNAi-mediated knockdown: siRNAs have either been continuously infused via a minipump [15] or have been mixed with delivery agents like the transfection reagent i-Fect [17] or a polyethyleneimine-based gene-delivery system [16].

We decided to use a simple intrathecal injection of unmodified siRNAs, since it has previously been shown that naked oligonucleotides are efficiently taken up by neuronal cells (e.g. [21]). This method is unlikely to result in sustained silencing of the targeted gene, but even a transient knockdown can be sufficient to obtain a cellular response that allows the performance of functional studies. In fact, we observed an analgesic effect of siRNA treatment that lasted for four to five days before pain sensitivity returned to a normal level.

The function of TRPV1 has previously been investigated by analyzing loss-of-function phenotypes of knockout animals [22–24]. These studies demonstrated a lack of vanilloid-evoked pain behavior, diminished heat response, and perturbed micturition. The role of spinal TRPV1 in pain transduction and its involvement in neuropathic pain, however, is less clear. Recent findings suggest that expression of TRPV1 is regulated after chronic constriction injury and is thus involved in the development and maintenance of mechanical allodynia in this model [25]. We have

previously used antisense oligonucleotides to investigate the functional relevance of TRPV1 for tactile allodynia in a rat model of spinal nerve ligation [26].

In the present study, we analyzed cold allodynia in neuropathic rats. A single bolus injection of an siRNA against TRPV1 was found to be sufficient to reduce pain sensitivity of the animals on a cold plate by ~50%. TRPV1 is connected to a complex network of pain-related mechanisms [27], which suggests a possible link to cold allodynia in chronic neuropathic pain conditions. We are currently unable to distinguish whether the remaining pain perception is due to incomplete knockdown of target gene expression or whether additional pathways mediate signaling of the cold stimulus.

Beside neuropathy-induced down regulation an increased TRPV1 expression has been demonstrated in uninjured nerve cells after peripheral nerve injury [28]. It is well known that knockdown of gene expression is extremely difficult to analyze in the spinal cord. The immunohistochemical analysis performed after siRNA treatment did not reveal significant reduction in TRPV1 expression (data not shown). Apparently, it was impossible to discriminate the siRNA-induced reduction of functionally relevant TRPV1 protein against the large TRPV1 pool existing prior to siRNA application. We might have been unable to detect a partial reduction of TRPV1 expression, since decreased protein expression in a critical subset of cells of a tissue or within subcellular structures of cells could easily pass undetected. Furthermore, the extremely low dose that was sufficient to evoke an analgesic response suggests that only a small number of neuronal cells had to be reached by the siRNA treatment. Similarly, knockdown of TRPV1 by antisense oligonucleotides was shown to be too small to be detected in spite of proven efficacy to reduce neuropathic pain behavior [26]. It has been discussed previously that the biological effect observed after antisense treatment can be much more pronounced than the detected reduction of target protein level [29].

In order to confirm the results of our first RNAi experiment we used a second siRNA with an unrelated sequence against the same target mRNA. These experiments confirmed the initial experiments that RNAi-mediated knockdown of TRPV1 reduces cold allodynia. Sequence-dependent off-target effects are thus highly unlikely to be responsible for the observed analgesic effect. Since siRNAs with inverted control sequences did not exert pronounced effects, we can also rule out that the observed phenotypes are a general consequence of the RNAi procedure.

TRPV1 has a high degree of sequence homology and functional similarity between different species [30]. One of the siRNAs used in the present study targets a region that is conserved between rat, mouse, and humans. This siRNA could therefore be employed in a mouse model for visceral pain to demonstrate that knockdown reduces spontaneous pain behavior induced by the TRPV1 agonist capsaicin. These results are similar to those obtained with the receptor antagonist BCTC (Fig. 4A) as well as to those of capsaicin-evoked nociception in TRPV1 knockout animals [22]. Insensitivity to capsaicin further supports the assumption that siRNA-treatment results in silencing of TRPV1.

Taken together, we demonstrate that intrathecal injection of siRNAs is a suitable approach to investigate the relevance of potential pain targets. For TRPV1, we provide evidence for specific RNAi-mediated knockdown of the target: (1) two independent siRNAs induce the same phenotype; (2) inverted control siRNAs did not exert pronounced effects; (3) transient knockdown lasts for approximately 5 days; (4) siRNA-treated animals were insensitive to capsaicin. Together with the effects of the TRPV1 antagonist BCTC, our findings support an important role of TRPV1 in perception of a cold stimulus in a neuropathic pain condition and in the transmission of chemically evoked visceral pain stimuli. These results demonstrate that RNAi is a rapid and straightforward alternative to the generation of knockout animals for target validation.

Acknowledgments

The authors thank Birgit Bieber, Johanna Korioth, Simone Pfennings, Elke Schumacher, Elke Frank-Rodenberg, and Denise Werk for excellent technical assistance. Financial support by the German Ministry for Research and Technology (BMBF, Grant No. 01GG9819/0), the RiNA network for RNA technologies, and the Fonds der Chemischen Industrie is gratefully acknowledged.

References

[1] P. Ganju, J. Hall, Potential applications of siRNA for pain therapy, Exp. Opin. Biol. Ther. 4 (2004) 531–542.
[2] J. Scholz, C.J. Woolf, Can we conquer pain? Nat. Neurosci. 5 (2002) 1062–1067.
[3] M.J. Caterina, M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, D. Julius, The capsaicin receptor: a heat-activated ion channel in the pain pathway, Nature 389 (1997) 816–824.
[4] M. Tominaga, M.J. Caterina, A.B. Malmberg, T.A. Rosen, H. Gilbert, K. Skinner, B.E. Raumann, A.I. Basbaum, D. Julius, The cloned capsaicin receptor integrates multiple pain-producing stimuli, Neuron 21 (1998) 531–543.
[5] N. Zhang, S. Inan, A. Cowan, R. Sun, J.M. Wang, T.J. Rogers, M. Caterina, J.J. Oppenheim, A proinflammatory chemokine, CCL3, sensitizes the heat- and capsaicin-gated ion channel TRPV1, Proc. Natl. Acad. Sci. USA 102 (2005) 4536–4541.
[6] D.N. Cortright, A. Szallasi, Biochemical pharmacology of the vanilloid receptor TRPV1, Eur. J. Biochem. 271 (2004) 1814–1819.
[7] M. Tominaga, T. Tominaga, Structure and function of TRPV1, Pflugers Arch. 451 (2005) 143–150.
[8] R. González-Muñiz, New TRPV1-targeting agents for pain management, Drug Discov. Today 10 (2005) 1704–1705.
[9] J.D. Pomonis, J.E. Harrison, L. Mark, D.R. Bristol, K.J. Valenzano, K. Walker, *N*-(4-Tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2*H*)-carbox-amide (BCTC), a novel, orally-effective vanilloid receptor 1 antagonist with analgesic properties: II. In vivo characterization in rat models of inflammatory and neuropathic pain, J. Pharmacol. Exp. Ther. 306 (2003) 387–393.
[10] A. Grünweller, R.K. Hartmann, RNA interference as a gene-specific approach for molecular medicine, Curr. Med. Chem. 12 (2005) 3143–3161.

[11] R.K. Leung, P.A. Whittaker, RNA interference: from gene silencing to gene-specific therapeutics, Pharmacol. Ther. 107 (2005) 222–239.
[12] J.R. Bertrand, M. Pottier, A. Vekris, P. Opolon, A. Maksimenko, C. Malvy, Comparison of antisense oligonucleotides and siRNAs in cell culture and in vivo, Biochem. Biophys. Res. Commun. 296 (2002) 1000–1004.
[13] A. Grünweller, E. Wyszko, B. Bieber, R. Jahnel, V.A. Erdmann, J. Kurreck, Comparison of different antisense strategies in mammalian cells using locked nucleic acids, 2′*O*-methyl RNA, phosphorothioates and siRNA, Nucleic Acids Res. 31 (2003) 3185–3193.
[14] T. Röhl, J. Kurreck, RNA interference in pain research, J. Neurochem. (2006) in press, doi:10.1111/j.1471-4159.2006.04082.x.
[15] G. Dorn, S. Patel, G. Wotherspoon, M. Hemmings-Mieszczak, J. Barclay, F.J. Natt, P. Martin, S. Bevan, A. Fox, P. Ganju, W. Wishart, J. Hall, siRNA relieves chronic neuropathic pain, Nucleic Acids Res. 32 (2004) e49.
[16] P.-H. Tan, L.-C. Yang, H.-C. Shih, K.-C. Lan, J.-T. Cheng, Gene knockdown with intrathecal siRNA of NMDA receptor NR2B subunit reduces formalin-induced nociception in the rat, Gene Ther. 12 (2005) 59–66.
[17] M.-C. Luo, D.-Q. Zhang, S.-W. Ma, Y.-Y. Huang, S.J. Shuster, F. Porreca, J. Lai, An efficient intrathecal delivery of small interfering RNA to the spinal cord and peripheral neurons, Mol. Pain 1 (2005) 29.
[18] C. Mestre, T. Pélissier, J. Fialip, G. Wilcox, A. Eschalier, A method to perform direct transcutaneous injection in rats, J. Pharmacol. Toxicol. Meth. 32 (1994) 197–200.
[19] G.J. Bennett, Y.-K. Xie, A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man, Pain 33 (1988) 87–107.
[20] J.M. Laird, L. Martinez-Caro, E. Garcia-Nicas, F. Cernero, A new model of visceral pain and referred hyperalgesia in the mouse, Pain 92 (2001) 335–342.
[21] J. Lai, M.S. Gold, C.-S. Kim, D. Bian, M.H. Ossipov, J.C. Hunter, F. Porreca, Inhibition of neuropathic pain by decreased expression of the tetrodotoxin-resistant sodium channel, NaV1.8, Pain 95 (2002) 143–152.
[22] M.J. Caterina, A. Leffler, A.B. Malmberg, W.J. Martin, J. Trafton, K.R. Petersen-Zeitz, M. Koltzenburg, A.I. Basbaum, D. Julius, Impaired nociception and pain sensation in mice lacking the capsaicin receptor, Science 288 (2000) 306–313.
[23] J.B. Davis, J. Gray, M.J. Gunthorpe, J.P. Hatcher, P.T. Davey, P. Overend, M.H. Harries, J. Latcham, C. Clapham, K. Atkinson, S.A. Hughes, K. Rance, E. Grau, A.J. Harper, P.L. Pugh, D.C. Rogers, S. Bingham, A. Randall, S.A. Sheardown, Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia, Nature 405 (2000) 183–187.
[24] L.A. Birder, Y. Nakamura, S. Kiss, M.L. Nealen, S. Barrick, A.J. Kanai, E. Wang, G. Ruiz, W.C. De Groat, G. Apodaca, S. Watkins, M.J. Caterina, Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1, Nat. Neurosci. 5 (2002) 856–860.
[25] Y. Kanai, E. Nakazato, A. Fujiuchi, T. Hara, A. Imai, Involvement of an increased spinal TRPV1 sensitization through its up-regulation in mechanical allodynia of CCI rats, Neuropharmacology 49 (2005) 977–984.
[26] T. Christoph, C. Gillen, J. Mika, A. Grünweller, M. Schäfer, K Schiene, R. Jostock, G. Bahrenberg, E. Weihe, V.A. Erdmann, J. Kurreck, Antinoceptive effect of antisense oligonucleotides against VR1/TRPV1, Neurochem. Int., in press.
[27] L. De Petrocellis, V. Di Marzo, Lipids as regulators of the activity of transient receptor potential type V1 (TRPV1) channels, Life Sci. 77 (2005) 1651–1666.
[28] L.J. Hudson, S. Bevan, G. Wotherspoon, C. Gentry, A. Fox, J. Winter, VR1 protein expression increases in undamaged DRG neurons after partial nerve injury, Eur. J. Neurosci. 13 (2001) 2105–2114.
[29] L.S. Stone, L. Vulchanova, The pain of antisense: in vivo application of antisense oligonucleotides for functional genomics in pain and analgesia, Adv. Drug Deliv. Rev. 55 (2003) 1081–1112.
[30] D.N. Cortright, M. Crandall, J.F. Sanchez, T. Zou, J.E. Krause, G. White, The tissue distribution and functional characterization of human VR1, Biochem. Biophys. Res. Commun. 281 (2001) 1183–1189.