Neurochem Res (2008) 33:2151–2158
DOI 10.1007/s11064-008-9751-4

ORIGINAL PAPER

Behavioral and Electrophysiological Evidence for the Differential Functions of TRPV1 at Early and Late Stages of Chronic Inflammatory Nociception in Rats

Hao Luo · Isabella Shi Xu · Yi Chen · Fei Yang · Lu Yu · Guang-Xin Li · Feng-Yu Liu · Guo-Gang Xing · Yu-Shun Shi · Tan Li · Ji-Sheng Han · You Wan

Accepted: 14 May 2008 / Published online: 30 May 2008

Abstract We previously reported that vanilloid receptor type 1 (VR1, or TRPV1) was up-regulated in dorsal root ganglion (DRG) and the spinal dorsal horn after chronic inflammatory pain produced by complete Freund's adjuvant (CFA) injection into the plantar of rat hind paw. In the present study, we found that subcutaneous or intrathecal application of capsazepine (CPZ), a TRPV1 competitive antagonist, could inhibit thermal hyperalgesia on day 1 and on day 14 but not on day 28 after CFA injection. With extracellular electrophysiological recording, the effect of CPZ on noxious electrical or heat stimulation evoked responses of wide dynamic range (WDR) neurons in the deep layers of the spinal dorsal horn was evaluated. Under noxious electrical stimulation to sciatic nerve, CPZ applied to the spinal cord produced an inhibition on Aδ- and C-fiber evoked responses of WDR neurons on day 1 and 14, but not on day 28. Under radiant heat stimulation to the receptive field skin, subcutaneous application of CPZ significantly inhibited the background activity and extended the response latency of WDR neurons on day 14. These results provide new evidence for the functional significance of TRPV1 at the early stage, but not the late stage, in the rat model of CFA-induced inflammatory pain.

Keywords TRPV1 · Chronic pain · Dorsal root ganglion · Wide dynamic range neuron · Spinal dorsal horn

Special issue article in honor of Dr. Ji-Sheng Han.

Hao Luo, Isabella Shi Xu, Yi Chen are Co-first authors.

H. Luo · I. S. Xu · Y. Chen · F. Yang · L. Yu · G.-X. Li · F.-Y. Liu · G.-G. Xing · Y.-S. Shi · T. Li · J.-S. Han · Y. Wan (✉)
Neuroscience Research Institute, Peking University, 38 Xueyuan Road, Beijing 100083, China
e-mail: ywan@hsc.pku.edu.cn

Y. Wan
Department of Neurobiology, School of Basic Medical Science, Peking University, 38 Xueyuan Road, Beijing 100083, China

Y. Wan
The Key Laboratory for Neuroscience, The Ministry of Education, The Ministry of Public Health, Peking University, 38 Xueyuan Road, Beijing 100083, China

Introduction

Vanilloid receptor 1 (also known as TRPV1) has been cloned successfully by using an expression cloning strategy based on calcium influx from sensory neurons [1]. This receptor is a non-selective cation channel that is structurally related to members of the TRP family ion channel [1]. High expression level of TRPV1-immunoreactivity (TRPV1-*ir*) can be found in the small and medium-sized DRG neurons and trigeminal ganglion neurons primarily with unmyelinated axons [2, 3], whereas in a variety of brain regions, there is much lower level of TRPV1-immunoreactivity (*ir*) [1, 4]. The presence of TRPV1-*ir* appears to be prominent also in nerve fibers in lamina I and lamina II of the spinal dorsal horn both pre- and post-synaptically [2, 3, 5, 6].

Several lines of evidence support the role for TRPV1 in nociception after inflammatory pain. Blockade of the TRPV1 in electrophysiological and behavioral studies found that spinal TRPV1 participates in the transmission and modulation of nociceptive input [7–9]. A previous study showed that there was an up-regulation of TRPV1 mRNA in the DRG neurons and its central and peripheral terminals under carrageenan-induced inflammatory pain [10]. Capsaicin-evoked release of glutamate from the

dorsal horn was increased after peripheral inflammation and could be suppressed by intrathecal (*i.t.*) injection of TRPV1 anti-sense oligonucleotides [10]. Mutant mice lacking TRPV1 failed to develop heat hyperalgesia after inflammation [11], showed less thermal hypersensitivity under the state of inflammatory pain [12] and their sensory neurons exhibited impaired responses to noxious heat stimulation.

Chronic inflammatory pain is a severe problem in clinic. The behavioral thermal hyperalgesia is highly associated with the increased sensitivity of both peripheral and central nervous system in chronic inflammatory state and the role of TRPV1 in this process began to arouse more interest. We previously reported that in CFA-induced inflammatory pain the expression of TRPV1 increased in the rat DRG and spinal cord in a time-dependent manner [13]. Such an increase started on day 1 after CFA injection, peaked on day 14 and returned to control level on day 28.

Capsazepine (CPZ) is a specific, competitive antagonist of TRPV1 which could significantly inhibit capsaicin-induced responses in both isolated DRG neurons [14] and tissues [15, 16] from rats. In the present study, we investigated the role of TRPV1 at the early and the late stages of the CFA-induced inflammatory pain in rats. We also determined the role of spinal and peripheral TRPV1 in the responses of wide dynamic range (WDR) neurons to noxious electrical and thermal stimulation.

Experimental Procedures

Male Sprague–Dawley rats weighing 250–350 g were provided by the Department of Animal Science of Peking University Health Science Center. Animals were habituated to the behavioral testing paradigms for 3–5 days before experiment. All protocols are in concurrence with guidelines of the International Association for the Study of Pain (IASP).

CFA-induced Inflammatory Pain Model

Inflammatory pain model was induced by subcutaneous (*s.c.*) injection of 100 μl (1.0 mg/kg) complete Freund's adjuvant (CFA, Sigma–Aldrich Co. Ltd., St. Louis) into the lateral side of the plantar skin under the anesthesia with intraperitonous (*i.p.*) injection of chloral hydrate (300 mg/kg). Inflammation was verified by the presence of edema and erythema. It reached peak level 1–3 days after the injection and gradually recovered thereafter. Hind paw thickness was measured by a gauge to an accuracy of 1 mm. The inflammatory sign lasted over 4 weeks as described in our previous report [13].

Implantation of Intrathecal (*i.t.*) Catheterization

i.t. catheterization was implanted according to the technique by Storkson et al. [17] with a modification. After anesthetizing the rats with chloral hydrate (300 mg/kg; *i.p.*), a PE 10 catheter (o.d. 0.61 mm) was inserted into the subarachnoid space through a guide cannula connected to a 20 gauge needle which punctured the dura at the level of the cauda equina. The catheter was then carefully implanted rostrally, aiming its tip at the lumbar enlargement. The proper location of the catheter was tested 24 h before the pharmacological experiment by assessing sensory and motor blockade after *i.t.* injection of 7 μl lidocaine (50 mg/ml).

Hot Plate Test

Thermal hyperalgesia was assessed with hot plate test as described in our previous report [13]. After being habituated to the experimental environment in cage for at least 30 min, the rat was placed on the hot plate (52 ± 0.5°C). The time was recorded as hot plate latency (HPL) until the rat jumped or licked either of its hind paws. The rat was removed immediately from the hot plate once showing the response. A 60-s cut-off time was imposed to avoid tissue unnecessary damage. HPL was tested three times with a 15-min interval. The three measurements were averaged. The room temperature was maintained at 20 ± 1°C during the test.

CPZ Application in Behavioral Study

CPZ was purchased from Sigma–Aldrich Co. Ltd., USA and the stock solutions (0.1 mol/l) were prepared in methanol and diluted to appropriate concentration in saline on the day of experiment. One, 14 and 28 days after CFA injection, CPZ was intrathecally administered in methanol vehicle (methanol:saline = 1:25) in a volume of 10 μl followed by 15 μl of saline flush. CPZ was administrated subcutaneously in DMSO vehicle (DMSO:saline = 1:1) in a volume of 100 μl. The HPL and the thickness of the hind paws were measured 1, 2, 4, 24 and 48 h after CPZ administration.

Reversal of thermal hyperalgesia was calculated according to the following formula as described in our previous report [18]:

$$\%\ \text{Reversal of hyperalgesia} = \frac{\text{PeakHPLofpost} - \text{CPZ} - \text{HPLofpre} - \text{CPZ}}{\text{Cut off (60s)} - \text{HPL of pre-CPZ}} \times 100$$

Electrophysiological Recordings of the WDR Neuron Responses to Electrical Stimulation on the Sciatic Nerve

Electrophysiological recordings were carried out on normal rats or CFA-induced inflammatory pain rats 1, 14, or 28 days after CFA injection. Rats were anaesthetized with 20% urethane (ethyl carbamate C.P., 1.1 g/kg; *i.p.*) and a tracheal cannula was inserted for ventilation. The rats were secured in a stereotaxic frame by metal clamps attached to their vertebral columns. A laminectomy was performed to expose lumbar segments 3–6 (L3–L6). The dura was opened and the cord was bathed in warm paraffin liquid. The rats were paralyzed with 0.1% D-tubocurarine chloride (2.0 mg/kg; *i.p.*). During the experiment, the rectal temperature was monitored and maintained at 36°C with a feedback-controlled heater blanket.

Extracellular recordings were made with epoxylite-coated tungsten microelectrodes [19]. The action potentials were amplified, filtered and displayed on an oscilloscope and analyzed on-line by a CED 1401 computer interface using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). The sciatic nerve was dissected at the popliteal fossa and suspended on a pair of silver hook electrodes. Electrical stimulation (5 mA, 1 ms, 0.2 Hz) applied to the sciatic nerve was used as search stimuli and once a single unit was isolated and its response to electrical stimulation recorded, the neuron was then characterized with mechanical stimuli (light brushing, light pressure and pinch) and its receptive field was mapped. Only WDR neurons which had both Aδ- and C-fiber inputs and showed graded response to mechanical stimulation were included. The test stimulus was a train of 16 electrical stimuli (2–3 times the threshold of C-fiber response, 1 ms, 0.5 Hz) [20] and the interval of the test stimulus was 10 min.

Analysis of the data was based on the response of WDR neurons to different types of afferent inputs. From post-stimulus histogram, the Aβ-fiber, Aδ-fiber and C-fiber evoked responses were taken as the number of action potentials recorded 0–20 ms, 20–90 ms and 90–300 ms after stimulus, respectively. C-fiber mediated after-discharge were taken as the number of action potentials recorded 300–800 ms after stimulus [20].

A stable baseline (defined as less than 20% variation) was established for at least 20–30 min before CPZ administration. The effect of direct spinal application of CPZ (0.6 μg or 2.0 μg in 50 μl volume) and its vehicle on test stimulus evoked responses from dorsal horn WDR neurons were recorded at 10 min interval up to 1 h.

Electrophysiological recording of the WDR neuron responses to radiant heat stimulation on the receptive field skin

Single-unit extracellular recordings were carried out as described above. Radiant heat was given to the receptive field on the plantar skin. A train of three electrical stimuli (2.1 mA, 0.5 ms, 0.167 Hz) was applied to the sciatic nerve to demonstrate the amplitude, the waveform and the discharge pattern of the WDR neuron. Five minutes later, the number of the spontaneous spikes within 20 s was calculated every 30 s, which served as the background activity. After 10 recordings of background activity, the response of a WDR neuron to the noxious thermal stimulation was measured. Focused radiation heat from a projector bulb (12 V, 100 W) was applied to the receptive field. Data sampling was triggered by the lighting of the bulb and lasted for 20 s. After 10 painful thermal stimulation, 10 recordings were carried out at an interval of 30 s without any more stimulation to observe the recovery of the excited the WDR neuron.

Responses of WDR neurons to radiant heat stimulation were extracted in terms of: (1) *Background discharges:* the total numbers of spontaneous spikes before administration of noxious stimulus, which was obtained by calculating those in the 10 recordings of 20-s duration every half minute. (2) *Thermal responses:* the total numbers of spikes to a noxious radiant heat. The ten 20-s samplings were triggered by an initiation of the noxious irradiation of 8-s duration at an interval of 30 s. (3) *Recovery:* the total spike numbers of post-discharges when noxious heat was removed, which was obtained by calculating those in the 10 recordings of 20-s duration every 30 s. (4) *Cumulative latency:* the cumulative latency before a neuron was driven by the testing nociceptive stimulus.

Statistical Analysis

The behavioral results were expressed as mean ± SEM data were analyzed by one-way repeated ANOVA followed by Dunnett's Multiple comparison test, or unpaired *t* test or Mann–Whitney *U* test or Kruska–Wallis test. $p < 0.05$ was taken as statistically significant.

Results

CFA-induced Thermal Hyperalgesia

We observed thermal hyperalgesia development in CFA rats first. We found that decreased HPL occurred from day 1 to day 28 after CFA injection. In normal animals, the

average HPL was 21.2 ± 4.5 s, whereas on day 1, 3, 7, 14, 21 and 28 after CFA injection, the average HPL was shortened to 13.3 ± 2.2, 16.8 ± 5.0, 10.4 ± 4.7, 6.7 ± 1.6 and 8.2 ± 2.5 s, respectively. All HPLs in the CFA-injected were significantly decreased compared to those in normal animals ($p < 0.05$). These results suggest that thermal hyperalgesia occurred in CFA-injected rats.

Effect of Subcutaneous (*s.c.*) Administration of CPZ on Thermal Hyperalgesia

S.c. administration of CPZ was used to evaluate the function of peripheral TRPV1 in thermal hyperalgesia. In normal rats, *s.c.* administration of CPZ at the dose up to 1.0 mg did not change the basal HPL (data not shown). On day 1 and 14 after CFA injection, CPZ at 1.0 μg significantly inhibited the thermal hyperalgesia with the maximal effect at 4 h after administration ($p < 0.05$) (Fig. 1a and b), but not on day 28 (Fig. 1c). CPZ at 0.1 and 0.3 mg had no inhibitory effect on thermal hyperalgesia at any time-points ($p > 0.05$) (Fig. 1a–c). CPZ at all doses did not induce any significant change in inflammation represented by the difference of thickness between the two hind paws (Fig. 1d). In addition, CPZ did not change body weight neither (Fig. 1e).

Effect of *i.t.* administration of CPZ on thermal hyperalgesia

To observe the function of the spinal TRPV1, CPZ was applied intrathecally. In normal rats, *i.t.* administration of CPZ at dose up to 16.0 μg did not affect the basal HPL (data not shown). On day 14 after CFA injection, CPZ at 4.0 and 16.0 μg produced a reversal of thermal hyperalgesia with a maximal effect at 1 h ($p < 0.05$) (Fig. 2b). On day 1 (Fig. 2a) and 28 after CFA injection (Fig. 2c), CPZ had no inhibitory effect on thermal hyperalgesia. After CFA injection, CPZ at 1.0 μg had no effect on thermal hyperalgesia (Fig. 2a–c). In addition, CPZ had no effect on both inflammation itself (Fig. 2d) and the body weight of the animals (Fig. 2e). These results suggest that spinal TRPV1 may be involved in CFA-induced inflammatory pain rats 14 days after CFA injection.

Effect of CPZ Directly Applied to the Spinal Cord on the Responses of WDR Neurons to Noxious Electrical Stimulation and to Noxious Heat Stimulation

To prove whether TRPV1 in the spinal cord has function in CFA-induced inflammatory pain, we tested the effect of CPA on WDR neuron responses. The WDR neurons had similar characteristics in normal rats and in the CFA-injected rats at all time-points, including the depth of neurons, threshold and latency of C-fiber activation as well as A- and C-fiber evoked responses. In normal rats, CPZ at 0.6 and 2.0 μg did not influence the Aβ-fiber evoked responses (Fig. 3a), the C-fiber evoked responses and the after-discharges (Fig. 3c and d) of WDR neurons. In contrast, CPZ at 0.6 μg, but not 2.0 μg, significantly inhibited the Aδ-fiber evoked responses ($p < 0.05$) (Fig. 3b).

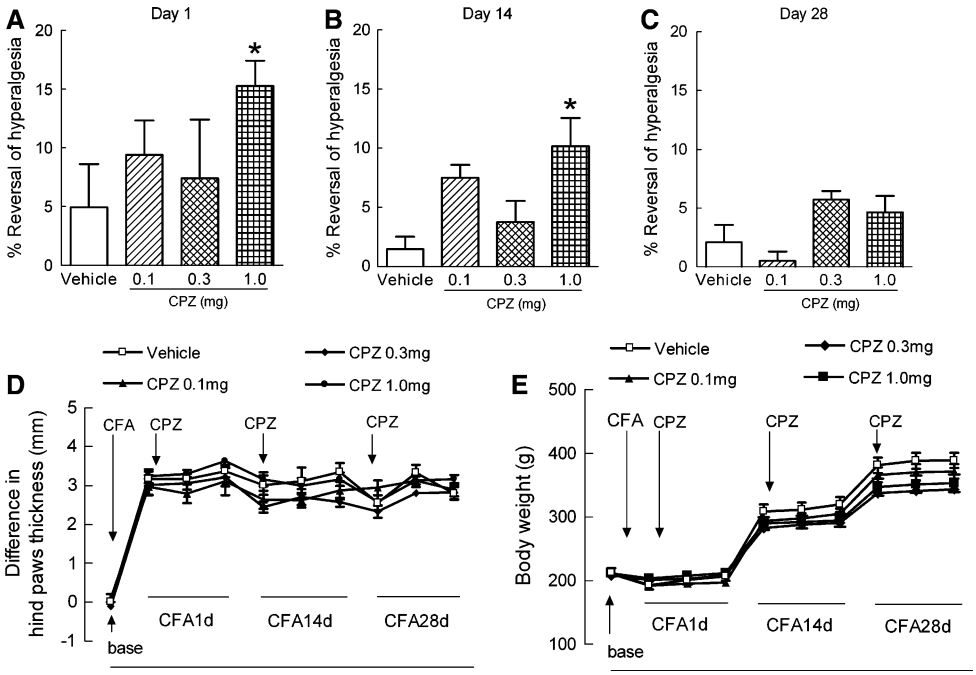

Fig. 1 The effect of subcutaneous application of CPZ on CFA-induced thermal hyperalgesia, inflammation and body weight. (**a** and **b**) On day 1 and on day 14 after CFA injection, CPZ at 1.0 mg produced a marked reversal of thermal hyperalgesia in rats. *$p < 0.05$ compared with the vehicle group. (**c**) On day 28, CPZ had no effect on thermal hyperalgesia. (**d**) CPZ at the three doses did not change the difference in thickness between the two hind paws. (**e**) CPZ at the three doses did not change body weight. $n = 6$–8

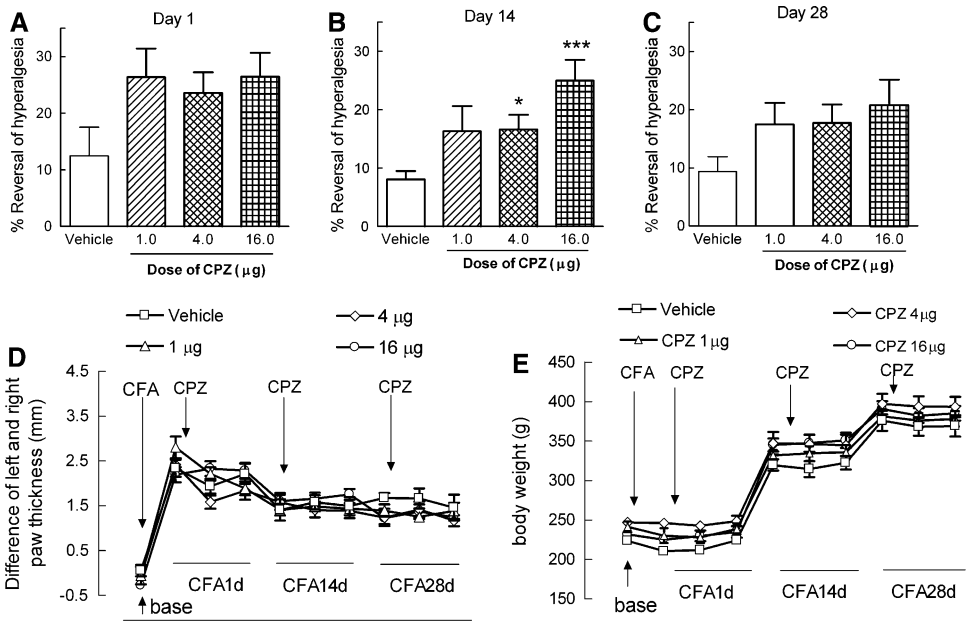

Fig. 2 The effect of intrathecal application of CPZ on the CFA-induced thermal hyperalgesia, inflammation and body weight. (**a** and **c**) On day 1 and day 28 after CFA injection, CPZ did not affect thermal hyperalgesia. (**b**) At day 14, CPZ at doses of 4.0 and 16.0 μg significantly reversed thermal hyperalgesia. $*p < 0.05$, $**p < 0.01$ compared with the vehicle group. (**d**) CPZ at the three doses did not change the difference of thickness between the two hind paws. (**e**) CPZ at the three doses did not change body weight. $n = 9$–11

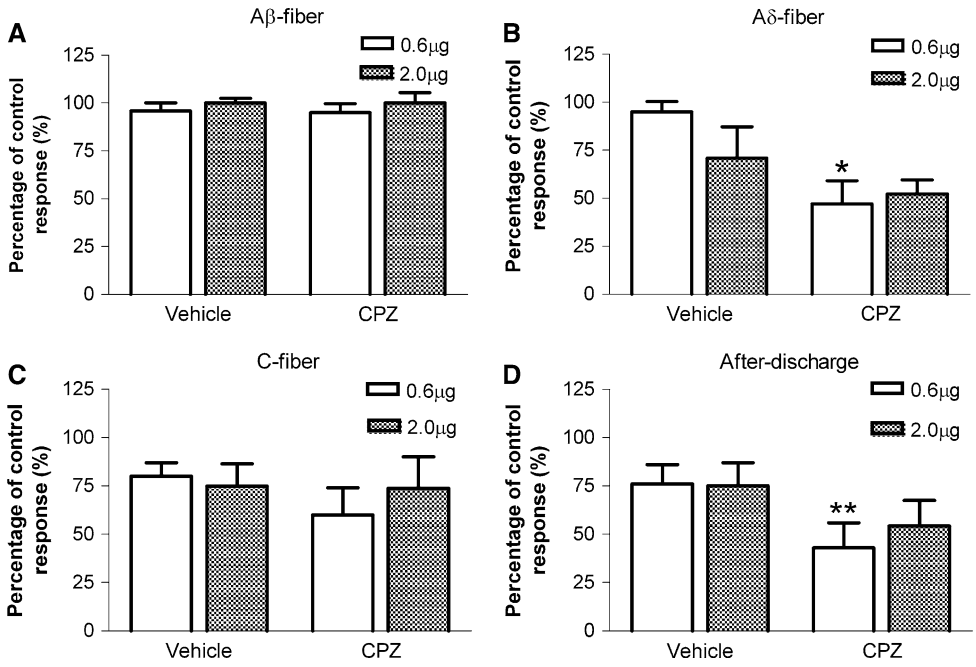

Fig. 3 The effect of CPZ directly applied to the spinal cord on the responses of WDR neurons to noxious electrical stimulation to sciatic nerve. (**a**) Aβ-fiber mediated discharge. (**b**) Aδ-fiber mediated discharge. (**c**) C-fiber mediated discharge. (**d**) C-fiber mediated after-discharge. The doses of CPZ were 0.6 μg (30.0 μmol/l) and 2.0 μg (100.0 μmol/l), and the stimulus was a train of 16 electrical stimuli (three times C-fiber threshold; 1 ms; 0.5 Hz). The data are expressed as percentage (%) of control (defined as 100%). CPZ at 0.6 μg significantly inhibited the Aδ-fiber mediated discharge than vehicle ($*p < 0.01$). $n = 7$ and 5 for 0.6 and 2.0 μg groups, respectively

One day after CFA injection, CPZ at 0.6 μg, but not 2.0 μg, significantly inhibited the Aδ- and the C-fiber evoked responses and the after-discharges without affecting the Aβ-fiber response (Fig. 4a–d). On day 14, CPZ at 2.0 μg decreased the responses of WDR neurons to Aδ- (Fig. 4b) and C-fiber inputs (Fig. 4c) ($p < 0.05$), while on day 28, CPZ produced no effect at any doses (Fig. 4a–d). Vehicle had no effect throughout the experiment (Fig. 4a–d).

Effect of Subcutaneous Application of CPZ on the Responses of WDR Neurons to Noxious Heat Stimulation

In normal rats, CPZ at 0.3 mg had no effect compared with the vehicle group or the pre-drug control group (Fig. 5). On day 14 after CFA injection, CPZ significantly decreased the background activity of the WDR neuron responses to the noxious radiant heat compared with the vehicle group

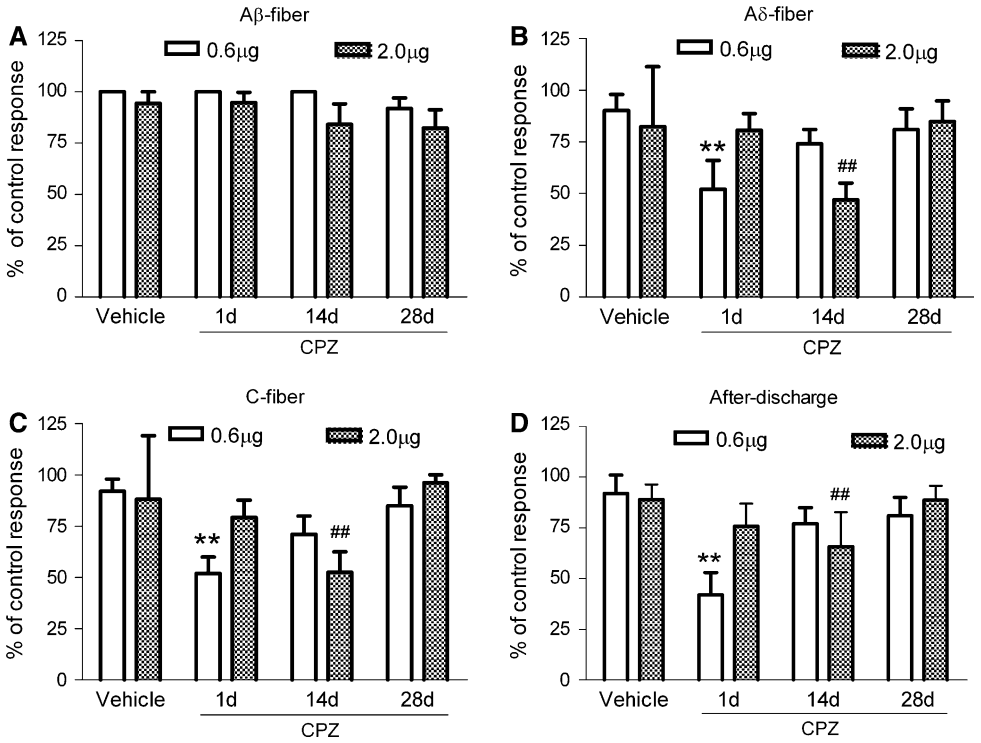

Fig. 4 The effect of CPZ directly applied to the spinal cord on the responses of WDR neurons to noxious electrical stimulation to sciatic nerve. (**a**) Aβ-fiber mediated discharge. No significant difference was found at both 0.6 μg (30.0 μmol/l) and 2.0 μg (100.0 μmol/l). (**b**) Aδ-fiber mediated discharge. The inhibitory effect of CPZ at 0.6 μg on day 1 after CFA injection was significantly stronger than that of vehicle ($^{*}p < 0.05$). The inhibitory effect of 2.0 μg CPZ on day 14 after CFA injection was significantly stronger than that of vehicle ($^{\#\#}p < 0.05$). (**c**) C-fiber mediated discharge. The inhibitory effect of 0.6 μg CPZ on C-fiber mediated discharge on day 1 after inflammation was significantly stronger than that of vehicle ($^{*}p < 0.05$). The inhibitory effect of CPZ at 2.0 μg on day 14 after CFA injection was also observed compared with vehicle group ($^{\#\#}p < 0.05$). (**d**) C-fiber mediated after-discharge. The inhibitory effect of CPZ at 0.6 μg on day 1 after CFA injection is significant compared with that of vehicle ($^{*}p < 0.05$). $n = 8, 6, 8, 9$ for groups of 0.6 μg, $n = 8, 5, 5, 6$ for groups of 2.0 μg

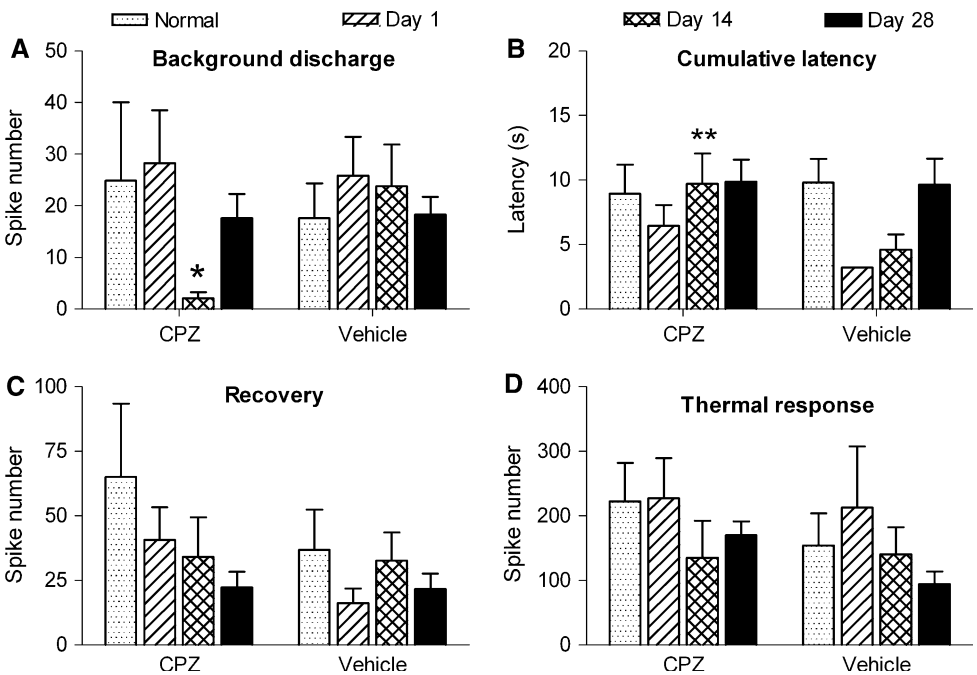

Fig. 5 The effect of CPZ directly applied to the spinal cord on the responses of WDR neurons to noxious heat stimulation to the receptive field skin. (**a**) Background discharges, (**b**) Thermal responses, (**c**) Recovery, (**d**) Cumulative latencies. CPZ significantly inhibited the background activities (**a**) and cumulative latencies (**b**) on day 14, but not day 1 or day 28. CPZ did not change recovery spikes (**c**) and thermal responses (**d**) at all time points. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the vehicle group of the corresponding day. $n = 4$–10

($p < 0.05$) (Fig. 5a). CPZ also significantly increased the cumulative latencies ($p < 0.05$) (Fig. 5d). No significant change was observed on day 1 and day 28.

Discussion

Functional Roles of TRPV1 in CFA-induced Inflammatory Pain: Peripheral Versus Central, Early Stage Versus Late Stage

In our previous report, we systematically assessed the expression of TRPV1 protein in the DRG and the spinal dorsal horn of CFA-induced inflammatory pain rats within a long period, from day 1 to day 28 after CFA injection. We found that TRPV1 protein was significantly increased in ipsilateral DRG and dorsal horn on day 1 after CFA injection, peaked on day 7–14, and gradually returned to the normal level on day 28 [13].

In the present study, the function of TRPV1 in periphery and the spinal dorsal horn was tested by pharmacological and behavioral approaches. The behavioral results show that TRPV1 has functional significance in the relatively early stage (up to 14 days after CFA injection) but not in late stage of CFA inflammatory pain in rats. On day 1 after CFA injection when the expression of TRPV1 was up-regulated in the ipsilateral DRG [13], *s.c.* injection of CPZ into the paw significantly reversed the thermal hyperalgesia whereas no effect was observed after *i.t.* administration of CPZ even at the highest dose of 16.0 μg (Figs. 1a and 2a). These results indicate that it is the peripheral TRPV1 synthesized and transported from DRG, but not central TRPV1 in the spinal dorsal horn that has more important functional significance on the thermal hyperalgesia at this time-point after CFA injection. On day 14 after CFA injection when TRPV1 protein in the DRG and the dorsal horn was at its highest level [13], subcutaneous and intrathecal application of CPZ could significantly reverse thermal hyperalgesia (Figs. 1b and 2b). These results indicate both peripheral and central TRPV1 participate in the development or maintenance of thermal hyperalgesia at this time. On day 28 after CFA injection, neither *s.c.* nor *i.t.* application of CPZ did not have any effect on thermal hyperalgesia (Figs. 1c and 2c). This correlated well with our previous morphological study in which TRPV1 was down-regulated to normal level at this time point [13]. It suggests that TRPV1 might loss its function at the late stage of CFA-induced inflammatory pain.

In addition, neither *s.c.* nor *i.t.* application of CPZ influenced the inflammation itself. No difference of thickness between two hind paws or body weight was observed after CPZ administration (Figs. 1d and 2d), indicating a specific anti-hyperalgesic effect of TRPV1 under inflammatory pain (Figs. 1e and 2e).

TRPV1 Involves in WDR Neuron Responses to Noxious Electrical and Heat Stimulation in CFA-induced Inflammatory Pain

It is well known that WDR neurons are very important in nociception transmission in the spinal cord. Our results clearly showed that TRPV1 blockade in periphery or in the spinal cord weaken the responses of WDR neurons to noxious electrical or heat stimulation of sciatic nerve. In normal rats, CPZ directly applied to the spinal cord significantly reduced Aδ-fiber evoked responses of WDR neurons (Fig. 3b). On day 1 after CFA injection, CPZ at 0.6 μg significantly inhibited Aδ- and C-fiber evoked responses as well as C-fiber mediated after-discharges (Fig. 4b–d). On day 14 after CFA injection, CPZ at 2.0 μg significantly reduced WDR neuron response to Aδ- and C-fiber inputs (Fig. 4b and c), whereas on day 28, CPZ produced no effect (Fig. 4a–d). These results support that activation of spinal TRPV1 contributes to spinal nociceptive transmission under normal conditions and in the early stage of the peripheral inflammatory pain. Combined with results from our previous morphological study [13] and the behavioral results of the present study, we speculate that activation of TRPV1 in the spinal dorsal horn contributes to thermal hyperalgesia in CFA-induced inflammatory pain rats.

Several early studies showed that noxious stimulation activates TRPV1 in the spinal cord. The extent of such activation appears, however, to be variable across these experiments. In normal condition, we observed an effect on Aδ-fiber-mediated response, but not on C-fiber response nor after-discharges whereas Kelly and Chapman [7] found an effect of CPZ on all three responses (Aδ-, C- and after-discharges). An earlier study by Dickenson and Dray [21] did not observe an effect of CPZ on electrical stimulation-evoked C-fiber response, although it did block the capsaicin effect on C-fiber.

A previous study showed that in carrageenan-induced short-term inflammatory pain TRPV1 mRNA increased in the central terminals of the DRG neurons [10]. Our recent morphological study showed an up-regulation of TRPV1 in nerve fibers in the superficial laminae of dorsal horn in CFA induced inflammatory pain rat [13]. Thus, a presynaptic mechanism for the anti-hyperalgesic effect of *i.t.* CPZ under inflammatory pain is most likely the case in the present situation even though the possibility of the postsynaptic mechanism may exist. Recently, it has been reported that TRPV1 is also expressed in rat spinal dorsal horn astrocytes [22]; thus the role of TRPV1 on spinal glial cells should not be overlooked.

Similarly, *s.c.* application of CPZ could significantly extend the latencies of WDR responses to noxious heat and lessened background activity (Fig. 5). In the peripheral thermal stimulation, changes of latency and background activity paralleled thermal hyperalgesia. In our behavioral study, thermal hyperalgesia was obvious on day 1 and day 14. These findings were consistent with our previous histochemical results that TRPV1 expression in dorsal root ganglion was up-regulated with a peak on day 14 after CFA injection.

Increased WDR spontaneous activity was involved in inflammatory and neuropathic pain. In the present study, CPZ alleviated thermal hyperalgesia and reversed the increase of spontaneous activity in the inflammatory pain rats. This suggests that TRPV1 could promote or maintain peripheral sensitization in inflammatory pain and might be essential for spontaneous discharges during inflammatory pain.

In summary, we have previously reported the morphological plasticity of TRPV1 during CFA inflammation [13]. The present study demonstrated the functional significance of this change. Both behavioral and electrophysiological studies suggested that at the early stage, but not the late stage, of the CFA-induced inflammatory pain, the activation of TRPV1 could produce excitatory effect. Thus, specific antagonist of TRPV1 may be useful as novel analgesics in the treatment of chronic inflammatory pain.

Acknowledgements This work was supported by the grants from National Natural Science Foundation of China (30600173, 30570566 and 30470559), "111" Project of the Ministry of Education of China, "973" program of the Ministry of Science and Technology of China (2007CB512501).

References

1. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
2. Guo A, Vulchanova L, Wang J, Li X, Elde R (1999) Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. Eur J Neurosci 11:946–958
3. Valtschanoff JG, Rustioni A, Guo A, Hwang SJ (2001) Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. J Comp Neurol 436:225–235
4. Hayes P, Meadows HJ, Gunthorpe MJ, Harries MH, Duckworth DM, Cairns W, Harrison DC, Clarke CE, Ellington K, Prinjha RK, Barton AJ, Medhurst AD, Smith GD, Topp S, Murdock P, Sanger GJ, Terrett J, Jenkins O, Benham CD, Randall AD, Gloger IS, Davis JB (2000) Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. Pain 88:205–215
5. Farquhar-Smith WP, Egertova M, Bradbury EJ, McMahon SB, Rice AS, Elphick MR (2000) Cannabinoid CB(1) receptor expression in rat spinal cord. Mol Cell Neurosci 15:510–521
6. Hwang SJ, Valtschanoff JG (2003) Vanilloid receptor VR1-positive afferents are distributed differently at different levels of the rat lumbar spinal cord. Neurosci Lett 349:41–44
7. Kelly S, Chapman V (2002) Spinal administration of capsazepine inhibits noxious evoked responses of dorsal horn neurons in non-inflamed and carrageenan inflamed rats. Brain Res 935:103–108
8. Kelly S, Chapman V (2002) Effects of peripheral nerve injury on functional spinal VR1 receptors. NeuroReport 13:1147–1150
9. Ohkubo T, Shibata M, Takahashi H (1993) The analgesia induced by intrathecal injection of ruthenium red. Pain 54:219–221
10. Tohda C, Sasaki M, Konemura T, Sasamura T, Itoh M, Kuraishi Y (2001) Axonal transport of VR1 capsaicin receptor mRNA in primary afferents and its participation in inflammation-induced increase in capsaicin sensitivity. J Neurochem 76:1628–1635
11. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183–187
12. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313
13. Luo H, Cheng J, Han JS, Wan Y (2004) Change of vanilloid receptor 1 expression in dorsal root ganglion and spinal dorsal horn during inflammatory nociception induced by complete Freund's adjuvant in rats. NeuroReport 15:655–658
14. Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CS, Yeats JC (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br J Pharmacol 107:544–552
15. Jerman JC, Brough SJ, Prinjha R, Harries MH, Davis JB, Smart D (2000) Characterization using FLIPR of rat vanilloid receptor (rVR1) pharmacology. Br J Pharmacol 130:916–922
16. Maggi CA, Bevan S, Walpole CS, Rang HP, Giuliani S (1993) A comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens. Br J Pharmacol 108:801–805
17. Storkson RV, Kjorsvik A, Tjolsen A, Hole K (1996) Lumbar catheterization of the spinal subarachnoid space in the rat. J Neurosci Methods 65:167–172
18. Wan Y, Wilson SG, Han J, Mogil JS (2001) The effect of genotype on sensitivity to electroacupuncture analgesia. Pain 91:5–13
19. Merrill EG, Ainsworth A (1972) Glass-coated platinum-plated tungsten microelectrodes. Med Biol Eng 10:662–672
20. Dickenson AH, Sullivan AF (1990) Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurones in the rat. Brain Res 506:31–39
21. Dickenson AH, Dray A (1991) Selective antagonism of capsaicin by capsazepine: evidence for a spinal receptor site in capsaicin-induced antinociception. Br J Pharmacol 104:1045–1049
22. Doly S, Fischer J, Salio C, Conrath M (2004) The vanilloid receptor-1 is expressed in rat spinal dorsal horn astrocytes. Neurosci Lett 357:123–126