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·Original Article·

Increased function of the TRPV1 channel in small sensory neurons after local inflammation or *in vitro* exposure to the pro-inflammatory cytokine GRO/KC

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Abstract: Objective Inflammation at the level of the sensory dorsal root ganglia (DRGs) leads to robust mechanical pain behavior and the local inflammation has direct excitatory effects on sensory neurons including small, primarily nociceptive, neurons. These neurons express the transient receptor potential vanilloid-1 (TRPV1) channel, which integrates multiple signals of pain and inflammation. The aim of this study was to characterize the regulation of the TRPV1 channel by local DRG inflammation and by growth-related oncogene (GRO/KC, systemic name: CXCL1), a cytokine known to be upregulated in inflamed DRGs. **Methods** Activation of the TRPV1 receptor with capsaicin was studied with patch clamp methods in acutely isolated small-diameter rat sensory neurons in primary culture. *In vivo*, behavioral effects of TRPV1 and GRO/KC were examined by paw injections. **Results** Neurons isolated from lumbar DRGs 3 days after local inflammation showed enhanced TRPV1 function: tachyphylaxis (the decline in response to repeated applications of capsaicin) was significantly reduced. A similar effect on tachyphylaxis was observed in neurons pre-treated for 4 h *in vitro* with GRO/KC. This effect was blocked by H-89, a protein kinase A inhibitor. Consistent with the *in vitro* results, *in vivo* behavioral responses to paw injection of capsaicin were enhanced and prolonged by pre-injecting the paw with GRO/KC 4 h before the capsaicin injection. GRO/KC paw injections alone did not elicit pain behaviors. **Conclusion** Function of the TRPV1 channel is enhanced by DRG inflammation and these effects are preserved *in vitro* during short-term culture. The effects (decreased tachyphylaxis) are mimicked by incubation with GRO/KC, which has previously been found to be strongly upregulated in this and other pain models.

Keywords: GRO/KC; CXCL1; TRPV1; inflammation

1 Introduction

Molecules produced during inflammation, including prostaglandins, acids, bradykinin, neurotrophic factors, and cytokines, may contribute importantly to pain states via their direct actions on sensory neurons. Although pain conditions and preclinical pain models are often categorized as either inflammatory or neuropathic (i.e., due to nerve injury), it has become clear that inflammatory processes may also contribute importantly to neuropathic pain conditions. In order to study the direct effects of inflammation on sen-

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sory neurons, in the absence of overt axon injury, we recently developed a pain model in which the L5 dorsal root ganglion (DRG) of the rat is inflamed by depositing over it a small amount of the immune activator zymosan in incomplete Freund's adjuvant (IFA). This local inflammation of the DRG (LID) causes a rapid (within 1 day) increase in mechanical pain behavior that lasts for over 5 weeks; increased excitability and spontaneous activity of sensory neurons; and large increases in several pro-inflammatory cytokines within the DRG[1,2]. We have been particularly interested in the cytokine growth-related oncogene (GRO/KC; systemic name: CXCL1; also called CINC-1) which is thought to play a role in the rat similar to that played by interleukin (IL) 8 in humans. This cytokine was noteworthy because of its very early and large upregulation in the LID[1] as well as in several other pain models, including the spinal nerve ligation model[3] which does involve axonal damage. Excitatory effects of LID on small-diameter, presumptive nociceptors could be preserved in short-term culture. Patch clamp experiments showed that both LID[4] and incubation with GRO/KC[5,6] caused large increases in voltage-gated Na^+ and K^+ currents, with the overall effect being an increase in excitability. These *in vitro* effects of GRO/KC were not acute, but required an incubation period and were blocked by protein synthesis inhibitors.

Another important channel that regulates nociceptor function is the transient receptor potential vanilloid-1 (TRPV1) channel. This channel, found in most nociceptive neurons, was originally cloned on the basis of its ability to be activated by capsaicin, the active ingredient in hot peppers. Because it can also be activated by temperatures near the threshold for noxious heat, TRPV1 was initially thought to be simply the receptor for detecting noxious heat. However, subsequent research has revealed a much broader role for this channel, particularly in the context of inflammation. Myriad endogenous inflammatory mediators can activate or sensitize this channel, either directly or indirectly, including acids, prostaglandins, bradykinin, substance P, lipoxygenase/cyclooxygenase products, nerve growth factor and other neurotrophins[7-9]. TRPV1 plays an important role in inflammation-induced thermal hyperalgesia. Of importance to the present study, TRPV1 may also play a role in mechanical allodynia and hyperalgesia, as indicated by the reduction of these behaviors by TRPV1 blockers. TRPV1 is found in both the peripheral and central terminals of sensory neurons and in some cases TRPV1 blockers work at the level of the spinal cord rather than at the peripheral terminals to relieve mechanical pain (see Discussion for references).

Many *in vitro* studies, including those in heterologous expression systems, have clarified the molecular aspects of TRPV1 function. When activated by capsaicin, TRPV1-mediated currents show both desensitization (defined here as a decline in the current during maintained application of capsaicin), and tachyphylaxis (a declining response to repeated brief applications of capsaicin). The two processes, though mechanistically distinct, both depend on calcium entry and are regulated by protein kinases and phosphatases[7,10,11].

In this study, we investigated the effects of DRG inflammation on the TRPV1 channel in rat sensory neurons. In view of the previous findings that GRO/KC is strongly upregulated in this and other pain models, here we also investigated the effects of GRO/KC on TRPV1 *in vitro* and *in vivo*.

2 Materials and methods

2.1 Reagents Capsaicin (Sigma, St. Louis, MO, USA) was prepared in ethanol at 10 mmol/L as a stock solution and diluted to the final concentration before use. GRO/KC (CXCL1), rat recombinant, from Prepro Tech (Rocky Hill, NJ, USA), was made from a 4.5 μmol/L stock solution in water and stored at -20°C. Other chemicals were from Sigma unless indicated.

2.2 Animals Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) were used for all experiments. Adult male rats (150–200 g) were used for behavioral experiments. Young adult females (60–80 g) were used for electrophysiological experiments. All the surgical procedures and the experimental protocol were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

2.3 Surgical procedure for LID LID was produced based on a previously published description[1] with modifications[2]. Briefly, rats were anesthetized with isoflurane, and an incision was made along the spine from vertebral levels S1 to L3. The L4/L5 intervertebral foramina were visualized after separating the paraspinal muscles. For behavioral experiments, only the L5 DRG was inflamed. To generate inflamed DRGs for acute culture, both L4 and L5 DRGs were inflamed. The immune activator zymosan (2 mg/mL, 10 μL) in IFA, was slowly injected on top of the L4/L5 DRGs, via a needle (30 gauge), which had been bent into a 90° angle 2–4 mm from the tip. During injection, the bent part of the needle was inserted into the intervertebral foramen beneath the transverse process covering the DRG and left for 1–2 min after injection to avoid leakage. This was a simplification of the previously published model which also involved drilling a hole through the transverse process. Both procedures induce marked mechanical allodynia as measured by von Frey filament testing in the hindpaw, beginning as early as postoperative day 1 and continuing for up to 5 weeks.

2.4 Injection of capsaicin and/or GRO/KC into the paw Animals were briefly anesthetized with isoflurane. The ventral surface of the paw was cleaned with alcohol. Capsaicin (30 μL, 0.05% in standard external bath solution) was subcutaneously injected into the heel region of one hindpaw (30-gauge needle). GRO/KC was injected into the same site (30 μL, 50 nmol/L in normal bath solution) 4 h prior to capsaicin injection. Control animals received injection of bath solution 4 h prior to capsaicin injection.

2.5 Behavioral testing To measure the response to capsaicin, paw flinches were counted for a 2-h period after capsaicin or vehicle injection, beginning 5 min after the injection when the brief isoflurane effect diminished. In some experiments, mechanical sensitivity was tested by applying a series of von Frey filaments to the heel region, using the up-and-down method[12]. A cutoff of 15 g was assigned to animals that did not respond to the highest filament strength used. Thermal sensitivity was measured using the method of Hargreaves *et al.*[13].

2.6 Primary culture of DRG neurons Animals were anesthetized with pentobarbital sodium. L4–L5 DRGs were dissected out and the nerves were trimmed at 4°C in oxygenated DMEM (high glucose - HEPES modification). The ganglia were then incubated at 37°C for 30–40 min in DMEM with 2 mg/mL collagenase (type IA) (Sigma). After three washes in 4°C standard external solution containing (in mmol/L): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES and 25 glucose (pH 7.4 adjusted with NaOH), cells were dissociated by trituration with a fire-polished Pasteur pipette and plated on glass coverslips coated with poly-*D*-lysine. Cells were incubated in the standard external solution at room temperature for 30 min, and then maintained at 37°C under 5% CO_2 in Neurobasal-A medium minus phenol red with B-27 serum-free supplement (Life Technologies Corp., Grand Island, NY, USA).

2.7 Patch clamp recording Recordings were made after 4–7 h in culture. Whole-cell voltage-clamp recordings of small DRG neurons (diameter 15–25 μm) were conducted at room temperature (20–22°C) with an AxoPatch-200B amplifier (Molecular Devices Corp, Union City, CA, USA). Patch pipettes (2.5–4.0 MΩ) were fabricated from borosilicate glass. The recording chamber was continuously perfused at room temperature with oxygenated bath solution at a flow rate of 1–2 mL/min. Capsaicin and other drugs as indicated were applied topically from a pressure controlled pipette positioned above the cell using the Valve Bank II (AutoMate Scientific, Inc., Berkeley, CA, USA), which can quickly change the local bath solution around individual cells.

After GΩ-seal formation, the whole-cell configuration was obtained at room temperature under voltage-clamp at a holding potential of -60 mV. The capacitance transient was cancelled and series resistance was compensated (>80%). The calculated liquid junction potential between the pipette solution (containing in mmol/L 108 KCl, 1.5 $MgCl_2$, 7.7 HEPES, 8.5 EGTA, 1.5 Mg-ATP, 0.8 Li-GTP, 7.7 NaOH, pH 7.4 adjusted with KOH) and the bath solution was -6.5 mV; the reported holding voltage has not been corrected. Data were low-pass filtered at 10 kHz and obtained only from small neurons (15–25 μm in diameter) that exhibited

resting membrane potentials more negative than -40 mV and an action potential overshoot above 0 mV.

Although larger, male animals were used for behavioral experiments for ease of surgical manipulations and the lack of confounding hormonal cycles, the younger, female animals were used for electrophysiological recording because they were found to have less connective tissue and provided a higher yield of cultured neurons. However, we have previously found that the behavioral response to DRG inflammation is similar in young females, as used for the electrophysiological recordings, and in older males as used for most behavioral experiments[2].

2.8 Quantitative PCR of neuron samples Isolated whole DRGs were observed with an upright BX70-WI (Olympus) microscope. Soma samples from individual neurons excluding surrounding satellite glial cells were collected with suction into a small glass pipette and expelled into lysis buffer. The SuperScript III CellsDirect cDNA synthesis system (Invitrogen, Grand Island, NY, USA) was used to process the cDNA from the samples. Each sample consisted of 5–10 cells identified as either small/medium or large diameter. Expression of TRPV1 and CXCR2 was examined with quantitative PCR using the MPx3005 instrument (Stratagene, Agilent Technologies, Inc., Santa Clara, CA, USA) and FastStart Universal SYBR Green Master mix (Roche Applied Science, Indianapolis, IN, USA). Primers crossing exon boundaries were designed using Primer-BLAST software to confirm specificity *versus* the *Rattus norvegicus* Refseq RNA database[14]: CXCR2 forward AGCCACTCCACTCCCAGCATCG, reverse CACCAG GGAGTTCCCCACGA; TRPV1 forward AGTAACTGC CAGGAGCTGGA, reverse GTGTCATTCTGCCCAT TGTG. The thermocycle protocol was as follows: activation of Taq polymerase at 95°C for 10 min, followed by 50 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 1 min, extension at 72°C for 1 min followed by 1 min at 76°C with fluorescence measurement at 516 and 610 nm (SYBER Green and ROX respectively). After completing 50 amplification cycles, a melting curve analysis was done. Samples were analyzed independently using LinRegPCR analysis software[15] which determines baseline, Cq values, and amplification efficiencies directly from the amplification curve.

2.9 Data analysis Data are presented as mean ± SEM. Comparison between different experimental groups was done using nonparametric methods for data that did not show a normal distribution based on the D'Agostino and Pearson omnibus normality test. The statistical test used in each case is indicated in the text or figure legend. P <0.05 indicated statistical significance. Levels of significance are indicated by the number of symbols, e.g., $^{*}P$ <0.05; $^{**}P$ < 0.01; $^{***}P$ <0.001.

3 Results

3.1 LID reduced tachyphylaxis of capsaicin responses Small-diameter sensory neurons were acutely cultured from either lumbar DRGs from normal animals, or from ganglia that had been locally inflamed with zymosan/IFA 3 days prior. Currents were evoked by short (10 s) pressure ejection applications of 0.5 μmol/L capsaicin. In control cells such applications induced a large inward current at -60 mV (Fig. 1). The average current density of the response was not significantly different between normal and LID cells. Responses declined when subsequent pulses were applied at 3-min intervals. This tachyphylaxis was quantified by normalizing the second and third responses to the first response of the same cell; on average, the second response was 38% of the first, and the third was only 28%. However, in cells isolated from inflamed DRGs, there was a striking reduction in tachyphylaxis, such that the second response was 74% of the first (Fig. 2).

3.2 *In vitro* GRO/KC incubation reduced tachyphylaxis The effects of the pro-inflammatory cytokine GRO/KC (CXCL1) were assessed in sensory neurons isolated from normal DRGs and maintained in acute primary culture. Cells were pre-incubated with 1.5 nmol/L GRO/KC for at least 4 h before recording. GRO/KC was not present during the recording. This concentration and pre-treatment protocol were chosen based on our previous studies[5,6], which showed that GRO/KC at this concentration increases excitability in current clamp recordings and modulates Na^+ and K^+ currents if pretreatment is used, but that these

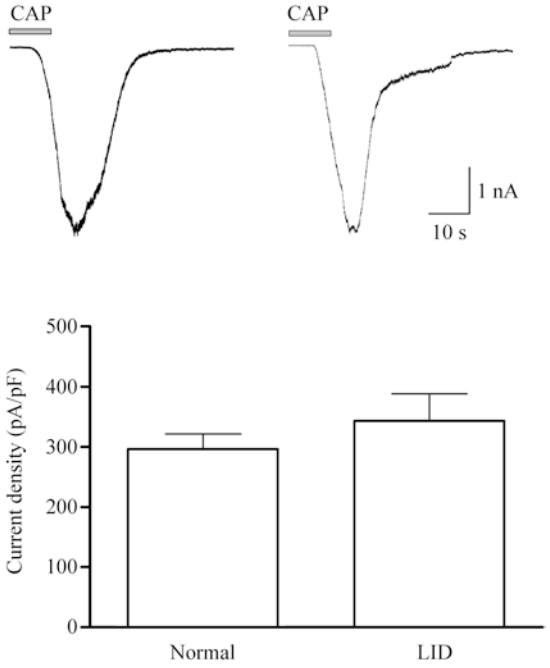

Fig. 1. Capsaicin (CAP)-induced current amplitude was not affected by local inflammation of the DRG (LID). Upper panel: Typical current tracings of capsaicin-induced currents at -60 mV in acutely cultured small DRG neurons isolated from normal (left) and 3 days after LID (right) DRGs. Capsaicin (0.5 μmol/L) was applied for 10 s as indicated by the bars. Lower panel: Average current density of capsaicin responses from normal (n = 28) and LID (n = 30) cells. The difference was not statistically significant (P = 0.36, Student's t-test). All data are from first exposures of the cell to capsaicin.

effects do not occur during acute application. GRO/KC pretreatment did not significantly affect the amplitude of the response to the first injection of capsaicin (Fig. 3), but reduced the tachyphylaxis observed during a subsequent application (Fig. 4).

The acute primary cultures mainly contained neurons with some smaller cells such as glial cells at low density, hence it was likely that the effects of GRO/KC were mediated by direct effects on neurons. For further confirmation of the previous reports that sensory neurons express the GRO/KC receptor (CXCR2), quantitative PCR was performed on soma samples extracted from small numbers (5–10) of small-diameter neurons. The cytoplasm was extracted from neurons in isolated whole DRGs by applying suction to a small glass pipette; microscopic observation confirmed that the satellite glial cells did not enter the

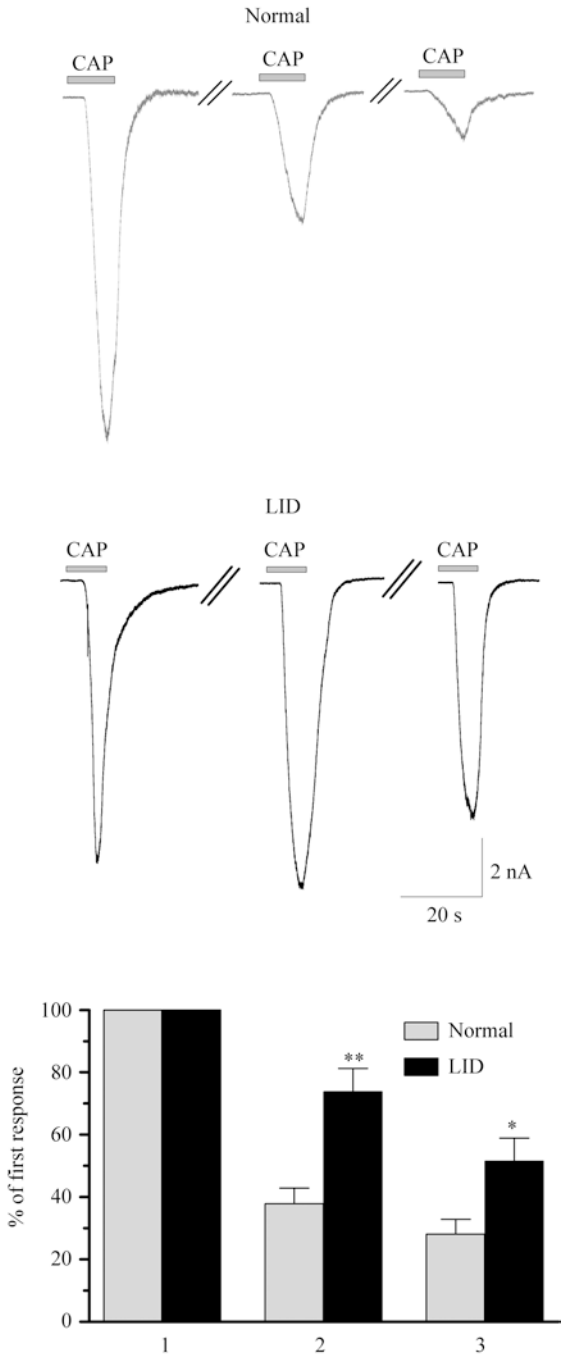

Fig. 2. Tachyphylaxis of capsaicin (CAP)-induced currents was reduced in cells from locally inflamed DRGs. Examples of 3 sequential 10-s applications of 0.5 μmol/L capsaicin given 3 min apart, in one cell from a normal DRG (upper panel) or a DRG 3 days after local inflammation (middle panel). Lower panel: Summary of tachyphylaxis data from normal cells and those from inflamed DRGs. $^{*}P$ < 0.05, $^{**}P$ <0.01 *vs* normal group (Kruskal-Wallis test with Dunn's posttest). Data are from the same cells as those of Fig. 1. LID, local inflammation of the DRG.

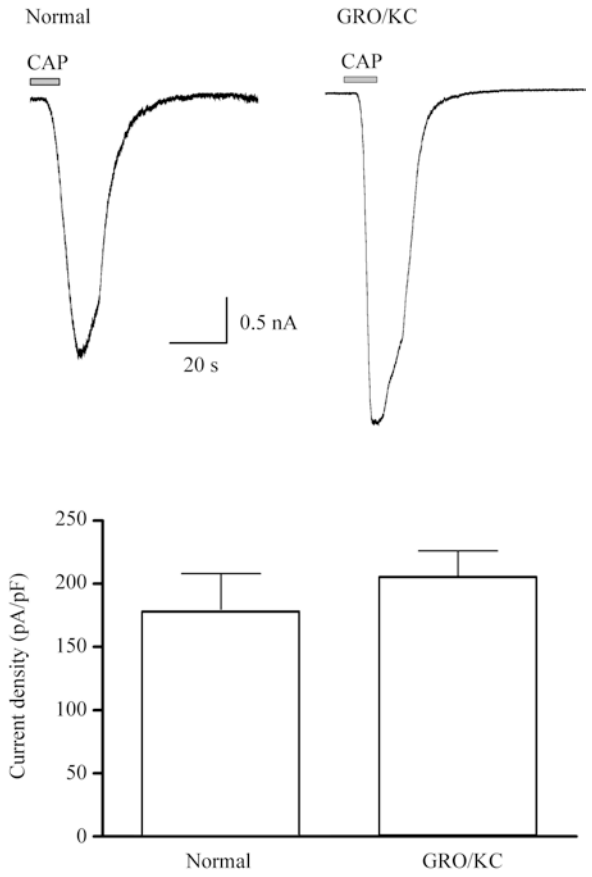

Fig. 3. The amplitude of capsaicin (CAP)-evoked inward currents was not affected by GRO/KC incubation. Upper panel: Examples of capsaicin-induced currents from cells pre-incubated for at least 4 h with or without 1.5 nmol/L GRO/KC before recording. Lower panel: There was no significant difference in the average current density of the response to a first application of capsaicin (P = 0.43, Student's t-test). n = 42 GRO/KC-treated cells and 22 normal cells. Cells were isolated from normal DRGs.

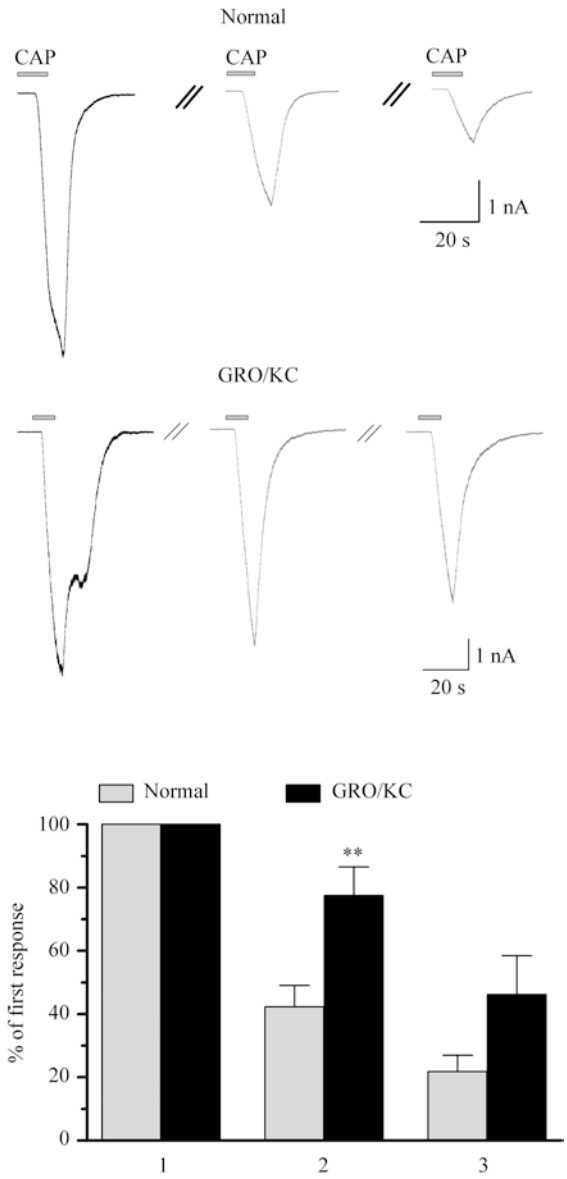

Fig. 4. GRO/KC incubation reduced tachyphylaxis. Examples of capsaicin (CAP)-induced currents during repeated applications in cells from normal DRGs without (upper panel) or with (middle panel) 4-h pre-incubation with 1.5 nmol/L GRO/KC. Lower panel: The amplitude of the second response normalized to the first response was significantly higher in GRO/KC-treated cells (Kruskal-Wallis test with Dunn's posttest). n = 42 GRO/KC-treated cells and 22 normal cells. Cells were the same as those of Fig. 3.

pipette. Co-expression of CXCR2 with TRPV1 was confirmed in small cells from normal, GRO/KC-incubated (4 h) and inflamed (postoperative day 1) DRGs; the geometric average ratio of CXCR2 to TRPV1 was 0.09. CXCR2 was also amplified from large cells though these had lower TRPV1 expression. In separate experiments using microarray analysis of samples from whole DRGs isolated 3 days after inflammation *vs* sham operation (n = 6 animals per group, examined with Affymetrix GeneChip Rat Gene 1.0 Exon Array), no significant regulation of TRPV1 or CXCR2 by DRG inflammation was found. TRPV1 upregulation of 1.1 fold (P = 0.125) and CXCR2 upregulation of 1.3 fold (P = 0.055) were not statistically significant.

3.3 Protein kinase A (PKA) inhibitor blocked the GRO/KC effect on tachyphylaxis As an initial examination of

the possible mechanisms underlying the *in vitro* effect of GRO/KC on tachyphylaxis of the capsaicin response, we determined the effects of the PKA inhibitor H-89. Experiments were conducted as described in section 3.2, except that H-89 (1 μmol/L) was added to the incubation medium at the last 30 min of the 4-h GRO/KC pre-incubation. Neither GRO/KC nor H-89 was present during the recording period. The results showed that H-89 restored the tachyphylaxis during the second capsaicin application to a value not significantly different from normal (Fig. 5). Previous studies have demonstrated that H-89 has no effect on the initial capsaicin response or degree of tachyphylaxis when applied alone, in the absence of PKA activators[16,17].

3.4 Pre-injection of GRO/KC into the paw enhanced and prolonged the behavioral response to injected capsaicin The above results suggested that GRO/KC treatment might increase the behavioral response to capsaicin by priming the TRPV1 channels. This was tested by measuring the number of paw flinches after subcutaneous injection of capsaicin into the hindpaw (30 μL 0.05% in normal bath solution). To match the *in vitro* experiments, GRO/KC (30 μL 50 nmol/L in normal bath solution) was pre-injected into the same site 4 h prior to capsaicin injection. Control animals received either no pre-injection (n = 5) or normal bath solution pre-injection (n = 5). The two control groups did not differ in the capsaicin response at any

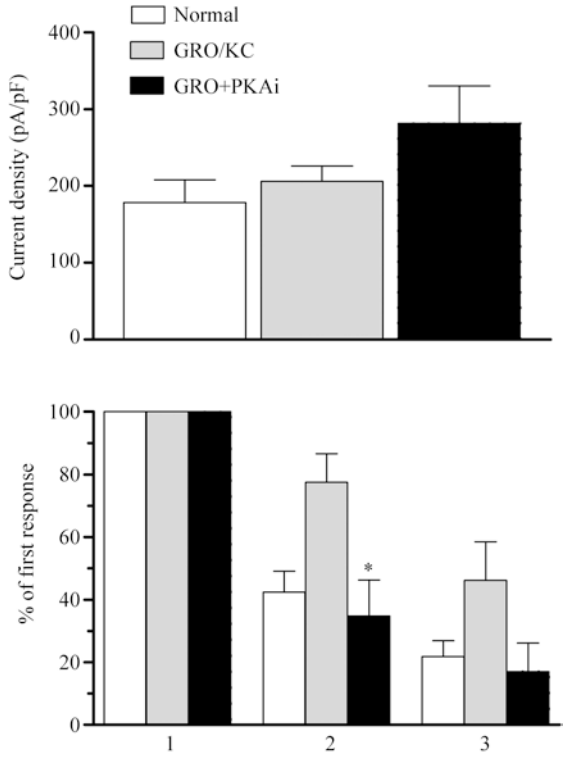

Fig. 5. The protein kinase A inhibitor (PKAi) H-89 blocked the effect of GRO/KC on tachyphylaxis *in vitro*. Upper panel: The response to a first application of capsaicin did not differ significantly between the three groups of cells (ANOVA with Tukey's posttest). Lower panel: H-89 restored the tachyphylaxis during the second capsaicin application. There was no significant difference between normal and PKAi-treated cells. $^{*}P$ <0.05 *vs* GRO/KC. n = 9 cells treated with GRO/KC + H-89; other data were re-plotted from Figs. 3 and 4.

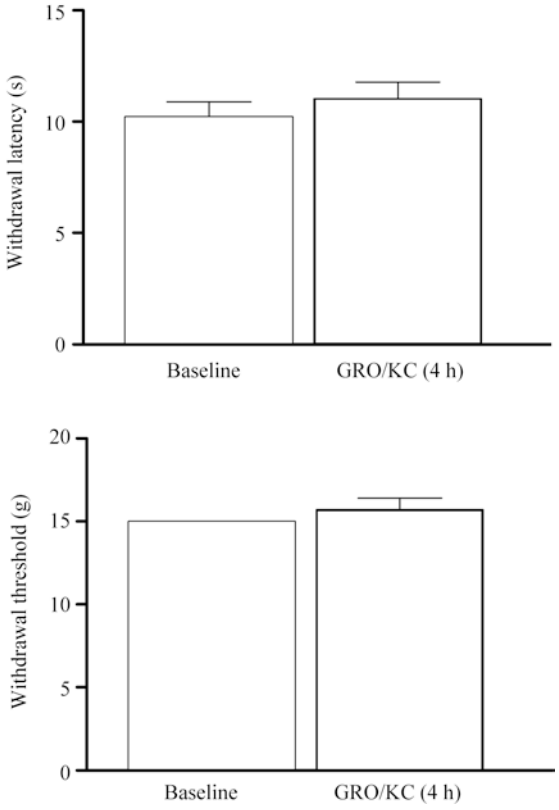

Fig. 6. GRO/KC injection into the paw did not affect thermal or mechanical sensitivity. Upper panel: The withdrawal latency to a thermal stimulus was not significantly changed 4 h after injection of GRO/KC into the paw (n = 6; Wilcoxon signed rank test). Lower panel: The withdrawal threshold to von Frey filaments applied to the paw was not significantly changed 4 h after injection of GRO/KC into the paw (n = 4). The SEM was zero for the baseline mechanical threshold.

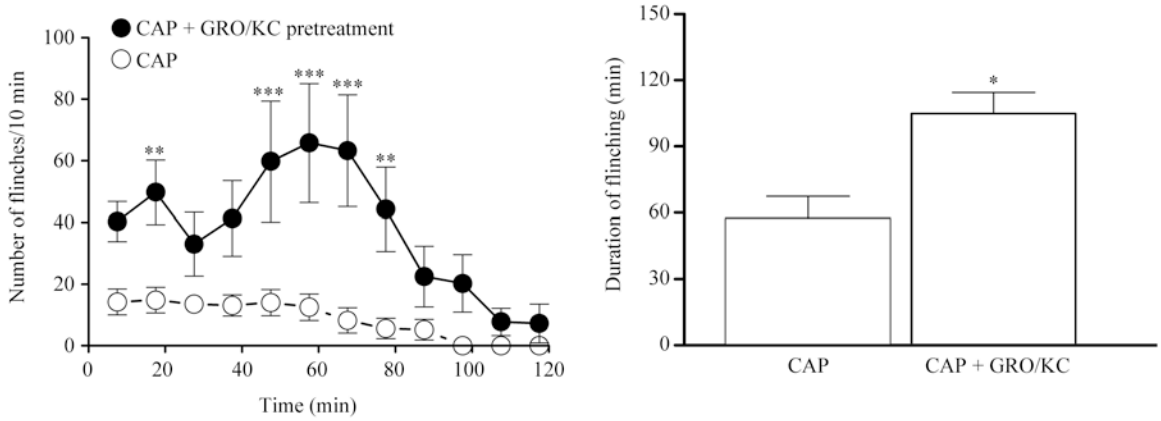

Fig. 7. Pre-injection of GRO/KC into the paw enhanced the behavioral response to subsequent capsaicin (CAP) injection. Left panel: Number of paw flinches per 10 min plotted *versus* time after capsaicin injection. Control group received either capsaicin injection only, or capsaicin injection 4 h after GRO/KC vehicle injection (data were combined; see text). $^{**}P$ <0.01, $^{***}P$ <0.001 *vs* control (CAP) (two-way ANOVA with Bonferoni posttest). *n* = 4 animals in CAP + GRO/KC pretreatment group; *n* = 10 animals in control group (CAP). Right panel: Average duration of paw flinching behavior from the same animals. $^{*}P$ <0.05 *vs* control group (CAP) (Mann-Whitney test).

time point (two-way ANOVA), so their data were combined (Fig. 7). Neither GRO/KC nor normal saline injection alone caused any paw flinching. In addition, GRO/KC injection did not affect the thermal or mechanical sensitivity when tested 4 h after injection (Fig. 6). However, GRO/KC pre-injection caused a marked enhancement and prolongation of the response to subsequent capsaicin injection (Fig. 7).

4 Discussion

In this study, the effects of localized inflammation on DRG neurons were preserved *in vitro*, after isolation and culture: cells cultured from inflamed DRGs had reduced tachyphylaxis in response to repeated applications. Decreased tachyphylaxis was also reported in sensory neurons isolated from rats in another pain model, the diabetic neuropathy model[18]; this study also reported an increased amplitude of the first capsaicin response in contrast to our study. Increased current density was reported in sensory neurons isolated from a cat model of interstitial cystitis pain[19]. That study did not examine tachyphylaxis but found reduced desensitization as measured by prolonged capsaicin exposures. Taken together, these studies suggest that altered desensitization and tachyphylaxis occur *in vivo* in different pain models and that these alterations can be preserved in short-term culture. In most studies of tachyphylaxis and desensitization, the phenomena have been induced *in vitro*. A short-term (20 min) exposure to the chemokine CCL3 (macrophage inhibitory protein 1α) increases TRPV1 responses in cultured DRG neurons as well as when the two molecules are co-expressed in a heterologous expression system[20]. Acute application of nerve growth factor (NGF), an important pro-nociceptive factor that is upregulated in pain models, causes decreased tachyphylaxis in cultured DRG neurons to a degree quite similar to that reported here[21]. This effect is likely dependent on protein kinases though there is some controversy about which kinases are most important. NGF also regulates TRPV1 density on a more long-term basis, possibly by regulating trafficking[22]. Other nerve growth factors can also reduce the tachyphylaxis to capsaicin when applied acutely[23]. In the present study, we focused on the effects of longer-term incubation with GRO/KC, as this may more closely mimic the *in vivo* situation in which such cytokines can be elevated for at least several days[1]. We previously showed that longer (hours) incubations with GRO/KC cause changes in membrane excitability and voltage-gated Na^+ and K^+ channels which are not mimicked by acute (minutes) applications[5,6].

Activation of PKA has previously been shown to reduce the tachyphylaxis of capsaicin responses in both neurons and expression systems[7,24]. This prompted us to test the effect of the PKA inhibitor H-89, which we found blocked the GRO/KC-mediated reduction in tachyphylaxis. The primary receptor for GRO/KC is CXCR2[25]. This receptor is generally considered to act via phospholipase C (PLC)- or G_i-coupled pathways, not by direct stimulation of adenylate cyclase via $G_{\alpha s}$. However, there are several pathways by which CXCR2 might ultimately activate the PKA pathway. For example, some subtypes of adenylate cyclase can be activated through released $G_{\beta\gamma}$ subunits. In the chemotaxis pathway in neutrophils, CXCR2-activated phosphorylation of the receptor-interacting protein VASP is mediated through PKA[26]. Perhaps most relevant to this study, in mouse DRG neurons, reduction of TRPV1 tachyphylaxis by the metabotropic glutamate receptor 5 (another receptor generally considered to activate PLC rather than adenylate cyclase) has also been shown to be dependent on PKA; in this case the PKA activation is several steps downstream of PLC, going through the prostaglandin E2 pathway[27].

Enhanced activation of the TRPV1 receptor in the context of inflammation not only is important for thermal hyperalgesia, but may also play a role in mediating mechanical allodynia. This is relevant to the current study because the LID model, which enhances TRPV1 responses, shows marked mechanical hypersensitivity and allodynia but little thermal hypersensitivity[1]. One mechanism by which TRPV1 may regulate mechanical pain is through TRPV1 receptors in the central terminals of the sensory neurons. Hence, intrathecal application or release of endogenous TRPV1 activators in the spinal cord has been demonstrated to evoke mechanical allodynia[28] while intrathecal TRPV1 antagonists relieve mechanical allodynia[29-31]. More generally, pharmacological antagonism of TRPV1 attenuates mechanical pain in several models, albeit with some differences between species[32-35]. Hence it is possible that the decreased TRPV1 tachyphylaxis after inflammation of the DRG as demonstrated here contributes to the mechanical pain induced by this model.

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