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TRPV1 in Experimental Autoimmune Prostatitis

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Abstract

Background: Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) is a disorder that is characterized by persistent pelvic pain in men of any age. Although several studies suggest that the transient receptor potential vanilloid 1 (TRPV1) channel is involved in various pathways of chronic pain, the TRPV1 channel has not been implicated in chronic pelvic pain associated with CP/CPPS.

Methods: Male C57BL/6J (B6) and TRPV1 knockout (TRPV1 KO) mice (5–7 weeks old) were used to study the development of pelvic allodynia in a murine model of CP/CPPS called experimental autoimmune prostatitis (EAP). The prostate lobes, dorsal root ganglia (DRG), and spinal cord were excised at day 20. The prostate lobes were assessed for inflammation, TRPV1 expression, and mast cell activity. DRG and spinal cord, between the L6–S4 regions, were analyzed to determine levels of phosphorylated ERK1/2 (p-ERK 1/2). To examine the therapeutic potential of TRPV1, B6 mice with EAP received intraurethral infusion of a TRPV1 antagonist at day 20 (repeated every two days) and pelvic pain was evaluated at days 20, 25, 30, and 35.

Results: Our data showed that B6 mice with EAP developed pelvic tactile allodynia at days 7, 14, and 20. In contrast, TRPV1 KO mice with EAP do not develop pelvic tactile allodynia at any time point. Although we observed no change in levels of TRPV1 protein expression in the prostate from B6 mice with EAP, there was evidence of significant inflammation and elevated mast cell activation. Interestingly, the prostate from TRPV1 KO mice with EAP showed a lack of mast cell activation despite evidence of prostate inflammation. Next, we observed a significant increase of p-ERK1/2 in the DRG and spinal cord from B6 mice with EAP; however, p-ERK1/2 expression was unaltered in TRPV1 KO mice with EAP. Finally, we confirmed that intraurethral administration of a TRPV1 antagonist peptide reduced pelvic tactile allodynia in B6 mice with EAP after day 20.

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Conflict of interest statement

The authors do not have any conflict of interest to report with regard to the study reported in this manuscript.

Conclusions: We demonstrated that in a murine model of CP/CPPS, the TRPV1 channel is key to persistent pelvic tactile allodynia and blocking TRPV1 in the prostate may be a promising strategy to quell chronic pelvic pain.

Keywords

Vanilloid 1; Pelvic pain; CPPS; Mast cells; Inflammation

1. INTRODUCTION

Men diagnosed with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) experience chronic pain in the prostate, urethra, and genitals with urinary symptoms.^{1,2} The lack of effective therapies in CP/CPPS patients severely impacts the patient's quality-of-life.³ We have previously used multiple mouse models of CP/CPPS to study the initiation and maintenance of CP/CPPS symptoms.^{4–6} Experimental autoimmune prostatitis (EAP) is the most common murine model that recapitulates several pathological features reported in CP/CPPS patients^{7–10} and has been used to discover novel targets and improved treatment strategies.⁹

Since chronic pain is predominantly treated with opioids that generate undesired side effects (e.g., addiction), the research community is desperately seeking non-opioid drugs to treat chronic pain.^{11,12} There is a large body of literature indicating that the non-selective cation channel known as transient receptor potential vanilloid 1 (TRPV1) mediates the effects of some stimuli that elicit pain, hyperalgesia, and inflammation.^{13–15} The TRPV1 channel is found in neurons, epithelial cells, mast cells, and several other immune cells.^{16,17} Since prostate biopsies are not required to diagnose CP/CPPS patients, the expression and role of TRPV1 in the prostate is understudied. Nevertheless, human prostate biopsies obtained from prostate cancer patients showed that the TRPV1 protein is located predominantly in epithelial cells.¹⁸ Despite evidence that TRPV1 is involved in multiple chronic pain conditions, CP/CPPS patients have not been evaluated as potential candidates to benefit from targeted TRPV1 treatments.¹⁹

The administration of TRPV1 agonists or antagonists in animal pain models and in clinical trials have generated mixed results based on the delivery site of the compound.^{20–23} For example, intra-articular injection of the TRPV1 agonist, resiniferatoxin (RTX), in canines with osteoarthritis provided long-term pain relief and intrathecal injections in mice blunts mechanical allodynia.^{21,24} However, clinical trials conducted in patients with interstitial cystitis (IC)/painful bladder syndrome (PBS) that received intravesicular administration of RTX showed no benefit.²² In a mouse model of neuropathic pain, a TRPV1 antagonist administered intrathecally reduced mechanical allodynia.²⁵ Moreover, clinical trials performed in healthy volunteers showed that a TRPV1 antagonist applied intradermally can mitigate heat evoked pain.^{23,26} The viability of TRPV1 targeting for chronic pelvic pain and the optimal route of administration therefore require further exploration.

Despite the mixed research results on TRPV1 compounds, studies have demonstrated that TRPV1 deficient mice show decreased pain in models of arthritis, bone cancer, and inflammatory bowel disease.^{27–29} However, it is unclear whether TRPV1 plays a role in the

mouse model of CP/CPPS. Therefore, this study will (i) explore the role of TRPV1 in the maintenance of pelvic tactile allodynia, (ii) establish the relevance of TRPV1 to prostate inflammation and mast cell activation, and (iii) determine whether blocking the TRPV1 channel reduces pelvic tactile allodynia in mice with EAP.

2. MATERIALS AND METHODS

2.1 Animals and Treatments

All animal studies were approved by the Northwestern University Animal Care and Use Committee. Animal experiments were conducted with following strains of mice: C57BL/6J (B6; 000664) and TRPV1 deficient mice (TRPV KO; B6.129X1-Trpv1^{tm1Jul/J}). Mice were obtained from Jackson Laboratory (Bar Harbor, ME) at 5-7 weeks old. All experiments were performed on adult male mice that were age matched littermates. Rat prostate antigen was diluted (10mg/ml) with adjuvant and subcutaneously injected into the shoulder of mice to induce EAP as previously described by our laboratory.⁵ In therapeutic experiments, a lubricated (sterile) polyethylene (PE-10) (BD Medical; cat.63019-004) catheter tube was inserted intraurethrally and the end of the tube was placed in an area adjacent to the prostate. The length (~1.5cm) and placement of the catheter was determined with Evan's blue dye applied intraurethrally to littermate mice not used for data analysis. The catheter was attached to a 30-gauge needle mounted on a gastight micro syringe (Hamilton; No.80301) and 10µl of either 0.9% saline (sterile) or TRPV1 antagonist peptide (L-R₄W₂) (1mM; Tocris; No.1577) was infused into mice with EAP and control cohorts at day 20. The delivery of the peptide or control was repeated every two days for 15 days.

2.2 Behavioral assay

We examined the development of pelvic tactile allodynia in mice with EAP using methodology described in detail with video recordings at: (<https://www.jove.com/video/50158/measurement-tactile-allodynia-murine-model-bacterial>).^{4,30} Briefly, mice were habituated for one hour by placing them in individual plexiglass chambers (6×10×12cm) on top of a wire mesh floor and elevated 2 feet via a metal platform rig. Then, von Frey filaments with calibrated forces of 0.04, 0.16, 0.4, 1, and 4g were used to determine the emergence and maintenance of pelvic tactile allodynia. The filaments were applied in increasing force order to the pelvic area considered in close proximity to the prostate with a brief resting period between filaments (approximately 5 seconds). Each filament was applied 10 times by an observer blinded to all groups. The behavioral response(s) to the filament were established as follows: 1) licking or scratching of the pelvic area, 2) retraction of the abdomen, and 3) avoidance (jumping). Behavioral assays were conducted at day 0 (baseline), 7, 14, and 20 after inducing EAP. However, mice treated with either saline or the TRPV1 antagonistic peptide intraurethrally at day 20 were examined an additional 15 days. Data were analyzed as the mean percentage of response frequency ± SEM.

2.3 Inflammation scoring and mast cell counts

Mice used to assess prostatic inflammation and mast cell counts were euthanized on day 20 via cervical dislocation and the following prostate lobes were excised and placed in 10% formalin: ventral prostate (VP), anterior prostate (AP), dorsal and lateral prostate (DLP).

Prostate lobes were sectioned and processed at the Northwestern pathology. Prostates were embedded in paraffin, sectioned (5µm thickness), and stained with hematoxylin & eosin (H&E). Prostate sections were scored for inflammation using criteria established by Nickel and colleagues.³¹ Briefly, the tissue-section score can range from 0 to 3, a score of 0 indicated no inflammation and a score of 3 signaled severe inflammation with a high number of infiltrating cells (e.g., leukocytes) and visible destruction of either the stroma or duct/gland epithelium or both. In a separate set of prostate sections, we stained with toluidine blue (0.02%) and counted the number of mast cells under a 20X objective. The selected prostate area for mast cell counts was random. The dispersal of the toluidine blue stained granules in and around the cell membrane was used to determine whether the mast cells were resting or activated, as previously described.³⁰ Slides were covered with mounting medium (VectaMount; cat.H-5000) and sealed with a coverslip. Images were viewed and collected with a Leica DMI 6000B inverted microscope. Experiments were conducted on consecutive sections (4/animal; n=6-7) and prostate areas were imaged randomly by observers blinded to experimental groups. Percentage differences were determined relative to respective controls.

2.4 Tissue procedures and Western blots

After mice were euthanized as described above, the lumbosacral region (L6-S4) of the spinal cord and corresponding dorsal root ganglia (DRG) were excised, lysed with RIPA buffer (Sigma; cat.R0278), and homogenized. A Bradford protein kit assay (Thermo Scientific; cat.23225) was used to determine total protein and 20µg of protein were loaded onto a 10% SDS-PAGE gel (Bio-Rad; cat.3450010). Proteins were then transferred to PVDF membranes (Bio-Rad; cat.1620177). Membranes were blocked with 1% milk diluent (SeraCare; cat.50-82-01) for 1 hour and incubated with primary rabbit polyclonal anti-phosphorylated-ERK1/2 (p-ERK1/2) (1:1,000; Cell Signaling; cat.9101) antibody overnight at 4°C. In a separate experiment, prostate proteins (20µg) were transferred to PVDF membranes, blocked, and incubated with rabbit polyclonal anti-TRPV1 (1:500; Novus Biologicals; cat.NBP1-97417) antibody overnight at 4°C. The next day the membranes were washed with 1X Tris-Buffered Saline mixed with 0.1% Tween[®] 20 (TBST) and placed in goat anti-rabbit IgG attached to horseradish peroxidase (HRP) (1:3,000; Bio-Rad; cat.170-6515) secondary antibody and proteins were detected using chemiluminescent HRP substrate (ThermoFisher; cat.34580). Then membranes were rinsed with stripping buffer for 5 minutes, washed, and placed in blocking buffer at room temperature for 1 hour. The membranes were relabeled with either rabbit polyclonal anti-total-ERK1/2 (T-ERK1/2) (1:1,000; Cell Signaling; cat.9102) or rabbit monoclonal anti-β-actin (1:1,000; Cell Signaling; cat.8457) antibody and incubated with goat anti-rabbit IgG bound to HRP. Again, proteins were detected with ECL substrate. Image J software was used to determine optical density of protein bands in the immunoblot.

2.5 Statistical analysis

GraphPad Prism software (La Jolla, CA, version 7) was used to apply two tailed (Welch's) t-tests to experiments comparing two groups. Behavior assays and mast cell studies were analyzed using a two-way ANOVA test followed by either Bonferroni's or LSD Fisher's

multiple comparison post-hoc test, respectively. Quantitative data were expressed as mean $\pm$ SEM and considered statistically significant different if $P < 0.05$.

3. RESULTS

3.1 TRPV1 deficient mice with EAP do not develop pelvic tactile allodynia

Several studies conducted on TRPV1 KO mice demonstrated changes to synaptic plasticity and a higher tolerance for pain.^{32–34} Hence, we explored whether TRPV1 KO mice with EAP develop pelvic tactile allodynia at days 0, 7, 14, and 20. Our study revealed that TRPV1 KO mice with EAP (TRPV1 KO-EAP) do not show evidence of sensitization or changes in response frequency at any time point compared to naive TRPV1 KO (TRPV1 KO-Naive) mice (Figure 1A). In contrast, B6 mice with EAP have an increased response frequency compared to naive cohorts at days 7, 14, and 20 (Figure 1B) with this response known to be chronic beyond day 20 (an additional 17 days).³⁵ The present data suggests that TRPV1 channels play an essential role in the development of pelvic tactile allodynia in our mouse model of CP/CPPS.

3.2 TRPV1 KO mice with EAP experience prostate inflammation but mast cell activity is suppressed during inflammation

Although TRPV1 KO mice have been studied in various models of inflammation and mast cell activation, the results are not clear due to conflicting data from different tissues.^{36–40} Thus, we examined whether TRPV1 KO mice or the B6 background strain develop inflammation in the prostate after EAP induction. Prostate sections stained with H&E and scored for inflammation showed that B6 mice with EAP developed significantly higher levels of inflammation (72%) in the DLP lobes compared to control cohorts at day 20 (Figures 2Aa,b–2B). Moreover, we observed abundant leukocyte infiltration in the stroma and near epithelial cells (red arrows) in the DLP lobes from B6 mice with EAP (Figure 2Ab). Interestingly, our TRPV1 KO mouse data was consistent with studies that suggest that the absence of the TRPV1 channel does not diminish tissue inflammation,^{41–43} since we observed that the DLP lobes from TRPV1 KO mice with EAP showed a significantly higher level of inflammation (69%) and leukocyte infiltration (red arrow) compared to respective controls (Figures 2Ca,b–2D). In addition, we confirmed that the ventral (Figures 2Ac,d; 2Cc,d) and anterior (Figures 2Ae,f; 2Ce,f) prostates lobes obtained from B6 and TRPV1 KO mice with EAP do not show significant inflammation at day 20 (Figures 2B,2D; respectively). Taken together, our data shows that leukocyte infiltration of the prostate is not dependent on TRPV1 expression in the EAP model of chronic prostatitis.

Since inflammation was predominantly observed in the DLP lobes at day 20, we asked whether TRPV1 expression was altered in the DLP lobes from B6 mice with EAP. Our results revealed that the level of TRPV1 expression in the DLP lobes was unchanged between B6 mice with EAP and respective controls (Figures 3A–B). Next, we investigated changes in mast cells located in the prostate via toluidine blue staining and we observed no difference in total number of mast cells (8% difference) between B6 mice with EAP and control mice (Figures 4Aa,b–4B). However, B6 mice with EAP showed a significant increase (103%) in the number of activated mast cell (red arrows) and a significant decrease

(62%) in the number of resting mast cells compared to control cohorts at day 20 (Figures 4Aa,b–4B). We then assessed the status of mast cells in TRPV1 KO mice with and without EAP. At day 20, the total number of mast cells in the prostates obtained from TRPV1 KO mice with EAP was not significantly different from naive TRPV1 KO mice. In contrast to B6 mice with EAP, TRPV1 KO mice with EAP showed no significant difference in resting or activated mast cell numbers at day 20 compared to naive TRPV1 KO mice (Figures 4Ca,b–4D). These results suggest that the lack of TRPV1 diminishes the capability of mast cells in the prostate to undergo activation in response to EAP.

3.3 TRPV1 mediates ERK1/2 phosphorylation in DRG and spinal cord

A previous study conducted by our laboratory showed that the DRG from B6 mice with EAP had increased p-ERK1/2 compared to control mice.⁸ Therefore, we sought to determine whether the absence of the TRPV1 channel changed the expression of p-ERK1/2 in the DRG and spinal cord (L6-S4 regions) excised from mice with EAP. The data showed that B6 mice with EAP had a significantly (97%) higher level of p-ERK1/2 expression in the lumbosacral region of the DRG (Figures 5A–5B) and spinal cord (Figures 5D–5E) compared to control cohorts at day 20. However, TRPV1 KO mice with EAP showed a similar level (<8% difference) of p-ERK1/2 expression in the lumbosacral region of the DRG (Figures 5A,5C) and spinal cord (Figures 5D,5F), compared to control TRPV1 KO mice. Collectively, the data indicates that TRPV1 expression is important for initiating the MAP kinase signaling pathways via the DRG in mice with EAP.

3.4 TRPV1 antagonist blunts persistent pelvic tactile allodynia

Although several TRPV1 antagonists have been developed and tested on animal models with chronic pain, the efficacy of TRPV1 antagonists targeted to a particular visceral organ (e.g., prostate) have not been studied.^{44–46} Thus, we considered whether an arginine-rich hexapeptide (RRRRWW-NH₂; L-R₄W₂), a non-competitive TRPV1 antagonist, administered via intraurethral instillation to the prostate inhibits the maintenance of pelvic tactile allodynia in mice with EAP. Our results showed that mice with EAP that received L-R₄W₂ (every two days) at days 20, 22, 24, 26, 28, 30, 32, and 34 (Figure 6A) developed a pain response similar to B6 mice without EAP that received either saline or L-R₄W₂ treatment (Figure 6B). In contrast, mice with EAP that received intraurethral infusion of saline developed tactile allodynia from day 7 to 35 (Figure 6B). In line with the role of TRPV1 as a pain receptor, the data highlights the importance of TRPV1 activation in sustained pelvic tactile allodynia and indicates that TRPV1 antagonists may have a role in the treatment of chronic pelvic pain.

4. DISCUSSION

Within the transient receptor potential vanilloid channel subfamily, TRPV1 is the most well-known receptor and is linked to thermosensation, vasodilation, inflammation, and nociception.^{47–50} However, studies that examined the functional relevance of TRPV1 during inflammation seem to yield conflicting results due to different outcomes observed in various models of inflammation targeting specific organs. Since the role of TRPV1 is not fully explored in a validated murine model of CP/CPPS (e.g., EAP) that elicits chronic pelvic pain

and inflammation of the prostate via autoimmune mechanisms, we investigated whether TRPV1 is required for the development of pain and inflammation and whether targeted inhibition of the TRPV1 channel might be a useful therapy to mitigate symptoms associated with CP/CPPS.

Previously, our laboratory published data indicating that B6 mice with EAP develop sustained pelvic tactile allodynia and prostate inflammation by day 30.⁸ Here, we scrutinized our CP/CPPS mouse model at day 20 and demonstrated that the absence of TRPV1 disrupts the development of pelvic tactile allodynia in mice with EAP (Figures 1A–1B). Hence, our results align with other pain studies that showed a blunted pain response in TRPV1 KO mice.^{27,51,52} Then, we assessed whether inflammation is diminished in TRPV1 deficient tissue; as reported by several studies.^{53,54} Our data revealed that global deletion of the TRPV1 channel did not quench prostate inflammation after the induction of EAP at day 20 (Figures 2C–2D). Thus, our results were consistent with studies that observed inflammation of visceral tissue in TRPV1 KO mice.^{41,55,56} Although the mechanisms that cause differences in inflammation between tissues lacking TRPV1 is not known, a study reported that TRPV1 KO mice may have a compensatory mechanism to elicit inflammation.⁵⁷

To add to the complexity, the level of TRPV1 expression in inflamed tissue remains a controversial issue; some studies suggest that TRPV1 expression in human visceral tissue with inflammation is upregulated, whereas others maintained that TRPV1 levels remain the same.⁵⁸ Biopsies from prostate cancer patients showed increased expression of TRPV1 in the epithelium; however, a direct link between CP/CPPS and subsequent development of prostate cancer is not currently supported by the literature.¹⁸ As a result, the level of TRPV1 expression in the prostate is not well defined in CP/CPPS patients or relevant murine models. In the present study, we confirmed that the level of TRPV1 expression is stable in the prostate of mice with EAP (Figures 3A–3B). Although we attempted to identify the cellular location of TRPV1 expression in the DLP lobes via immunohistochemistry, we were unsuccessful due to nonspecific labeling by the TRPV1 antibody (unpublished).

Prior work from our group demonstrated that mast cell activity was enhanced in the prostates from mice with EAP and we confirmed that intraurethral instillation of an ortholog of tryptase- β (predominantly released by mast cells) in mice induced pelvic tactile allodynia.^{8,30} In the same study, we reported high levels of tryptase- β in the expressed prostatic section (EPS) fluid collected from CP/CPPS patients.⁸ Therefore, in our present study, we explored whether deletion of the TRPV1 channel from prostate tissue altered mast cell activity in mice with EAP. The data revealed that the absence of the TRPV1 channel restricted degranulation of mast cells in the prostates collected from mice with EAP at day 20 (Figures 4Cb–4D). Differences between our data and other inflammation models can be explained by studies that suggest mast cells can express different cytokines, proteases, and receptors (subtypes) depending on the tissue that they reside.^{59–61} Here we determined that in a murine autoimmune model of CP/CPPS, the TRPV1 channel influences the activity of mast cells found in the prostate while not having any effect on overall prostate inflammation.

Studies on pain mechanism(s) show that TRPV1 activation in neuronal cells can influence the MAP/ERK1/2 signaling pathway.^{27,62} In previous studies, we established that mice with

EAP have increased ERK1/2 phosphorylation in the DRG.⁸ Thus, we investigated whether p-ERK1/2 expression changed in the lumbosacral region of the DRG and spinal cord excised from mice with EAP that lack TRPV1. Of note in our present research, we demonstrated that TRPV1 deficient mice with EAP do not have altered expression levels of p-ERK1/2 in either the DRG or spinal cord lumbosacral region (Figure 5A), which correlated with the absence of pelvic tactile allodynia (Figure 1B) and suppressed mast cell activity at day 20 (Figures 4C–4D). These results confirmed that TRPV1 is important for ERK1/2 signaling in regions of the DRG and spinal cord that receive sensory information from the prostate; hence, TRPV1 is required to induce chronic pelvic pain in mice with EAP.

Although there has been controversy regarding the role of TRPV1 agonists and antagonist to treat chronic pain due to conflicting results and undesired secondary effects (e.g., hyperthermia or hypothermia), the TRPV1 channel remains a promising target due to its crucial function in neuronal sensitization and pain.⁶³ Moreover, advances in drug development showed that a new generation of TRPV1 antagonists will be more selective with fewer side effects and may offer better outcomes if applied directly to the affected organ.¹⁹ Since the newer TRPV1 antagonist(s) were not commercially available during the execution of our experiments, we used an older (less specific) TRPV1 antagonist peptide called L-R₄W₂ to determine the therapeutic potential of TRPV1 antagonism in our model. To diminish any potential systemic side effects of L-R₄W₂, the TRPV1 antagonist was delivered intraurethrally into the prostate of mice with established pelvic tactile allodynia at days 20, 22, 24, 26, 28, 30, 32, and 34 (Figure 6A). Notably, our data confirmed that treatment with L-R₄W₂ reversed the level of pelvic tactile allodynia in mice with EAP after initial infusion at day 20 (Figure 6B). Our results therefore parallel data that supports TRPV1 antagonists as a viable analgesic to treat chronic pain.^{20,64} Future studies will address whether L-R₄W₂ or similar TRPV1 antagonists mitigate chronic visceral pain via other delivery methods (e.g., intrathecal, intra-articular, or intra-cisternal infusion).

5. CONCLUSIONS

In this study, our findings provide a framework for understanding the role of the TRPV1 channel during the development of chronic pelvic pain in an animal model of CP/CPPS called EAP. We demonstrated that 1) the absence of the TRPV1 channel disrupts the progression of pelvic tactile allodynia, 2) inflammation of the prostate in mice with EAP is not dependent on TRPV1 activation and TRPV1 expression levels do not change, 3) global TRPV1 deletion reduced mast cell activation in mice with EAP, 4) the lumbosacral region of the DRG and spinal cord tissue excised from TRPV1 deficient mice with EAP showed normal levels p-ERK1/2, and 5) pharmacological targeting of TRPV1 with an antagonist peptide alleviated pelvic tactile allodynia in mice with EAP. Altogether, our study suggests that blocking the activation of the TRPV1 channel in conjunction with anti-inflammatory compounds might be a suitable treatment option to lessen chronic pelvic pain associated with CP/CPPS.

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Synopsis

The TRPV1 channel is a key mediator in persistent pelvic pain in EAP and targeted therapy with a TRPV1 antagonist may alleviate CP/CPPS symptoms.

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FIGURE 1.

TRPV1 is required for pelvic pain. (A-B) Graphs show response frequencies measured with von Frey filaments applied to C57BL/6J (B6) mice and TRPV1 KO mice at days 0, 7, 14, and 20. (A) B6 mice with EAP (n=10) develop increased pelvic tactile allodynia compared to naive (n=10) mice at days 7, 14, and 20; (B) however, mice lacking the TRPV1 channel with EAP (TRPV1 KO-EAP; n=7) do not show a change in pelvic tactile allodynia at any time point, compared to respective control (TRPV1 KO-Naive; n=8). Data represents the mean $\pm$ SEM and was generated from three independent experiments. (*) denotes $P < 0.05$.

FIGURE 2.

Absence of TRPV1 does not change prostate inflammation. (A,C) Representative images and (B,D) relative quantification of prostate inflammation in the dorsal and lateral prostate (DLP), ventral prostate (VP), and anterior prostate (AP) lobes stained with H&E. (A-B) The DLP lobes excised from mice with (Ab) EAP showed a significant increase in inflammation compared to (Ac) control cohorts. (C-D) Likewise, (Cb) the DLP lobes excised from TRPV1 KO mice with EAP (TRPV1 KO-EAP) demonstrated robust inflammation and leukocyte infiltration (red arrows), compared to respective (Ca) controls (TRPV1 KO-Naive). (A-D) In

contrast, the (Ac,d; Cc,d) VP and (Ae,f; Ce,f) AP lobes lacked significant inflammation in all mice. Scale bars indicate 100 microns. Data represents the mean $\pm$ SEM. (*) denotes $P < 0.05$.

FIGURE 3.

TRPV1 expression is unaltered in the prostate of mice with EAP. (A-B) Protein analysis and immunohistochemistry of the DLP lobes showed no differences in expression of TRPV1 between B6 mice with EAP and respective controls. (A) Representative western blots and (B) densitometry data comparing TRPV1 expression in B6 mice with EAP (n=3) to control (n=3) cohorts. Data represents the mean $\pm$ SEM.

FIGURE 4.

Active mast cells increase after prostate inflammation and the absence of TRPV1 hinders activity. (A-D) Panels show representative images of the prostate stained with toluidine blue and excised from B6 and TRPV1 KO mice with or without EAP; graphs depict quantification of mast cell numbers in the prostate for each group. (A) Although B6 with EAP showed no significant changes in total number of mast cells (white arrows) compared to control at day 20, (B) B6 mice with EAP showed an increased number of active mast cells (red arrows) compared to controls. (C-D) Prostates from mice with EAP that lack TRPV1 channels (TRPV1 KO-EAP) showed no changes in mast cell numbers or activity, compared to respective controls (TRPV1 KO-Naive). Scale bars indicate 100 microns. Data represents the mean $\pm$ SEM. (*) denotes $P < 0.05$.

FIGURE 5.

ERK1/2 phosphorylation is linked to TRPV1 expression. (A) Western blot analysis and quantification of ERK1/2 phosphorylation in the lumbosacral region of the DRG (n=3) and (B) spinal cord (n=3) from mice with EAP revealed a significant increase in p-ERK1/2 expression compared to control littermates at day 20. However, (C) DRG and (D) spinal cords obtained from TRPV1 deficient mice with EAP showed no changed in ERK1/2 phosphorylation compared to control cohorts. Densitometry data represents the mean $\pm$ SEM. (*) denotes $P < 0.05$.

FIGURE 6.

Pelvic tactile allodynia is diminished by TRPV1 antagonist peptide. (A) Schematic shows mice treatment regime. Mice (B6) with or without EAP received intraurethral (IU) instillation (10 μ l) of either sterile 0.9% saline or a TRPV1 antagonist peptide (L-R₄W₂; 1mM) from day 20 (indicated by black arrows) to 35. (B) After initial treatment at day 20, mice with EAP that received L-R₄W₂ (EAP+Antagonist; n=3) treatment showed a similar response frequency to mice without EAP that received either saline (Naive+Saline; n=3) or L-R₄W₂ (Naive+Antagonist; n=3) at days 25, 30, and 35. However, mice with EAP that

received intraurethral saline (EAP+Saline; n=3) showed increased response frequency between days 7-35. Data represents the mean $\pm$ SEM from 3 independent experiments. (*) denotes $P < 0.05$.