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CB1 Cannabinoid Receptor Agonist Prevents NGF-Induced Sensitization of TRPV1 in Sensory Neurons

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Abstract

The transient receptor potential vanilloid type 1 channel (TRPV1) and nerve growth factor (NGF) are important mediators of inflammatory pain. NGF released during inflammation sensitizes TRPV1 in afferent nerve endings of peripheral nociceptors, increasing pain sensation. Cannabinoids, by activating CB1 G protein-coupled receptors, produce analgesia in a variety of pain models, though the exact mechanisms are not known. We tested the hypothesis that activation of the CB1 receptor by cannabinoids attenuates NGF-induced TRPV1 sensitization. TRPV1-mediated currents were measured in acutely isolated primary sensory neurons with the whole-cell patch clamp technique using capsaicin (100 nM) as the agonist. After the first capsaicin application, during which the baseline current was measured, cells were exposed to NGF (100 ng/mL), and the capsaicin application was repeated after 5 minutes. NGF sensitized TRPV1 in 31.0 % of cells (13 of 42), with a mean ($\pm$ SE) increase in the capsaicin-induced current of 262 ± 47 % over the baseline current. When the cannabinoid agonist ACEA (arachidonoyl-2'-chloroethylamide; 10 nM) was given before NGF, only 10.8 % of cells (4 of 37) were sensitized ($p < 0.05$). Neither this rate, nor the magnitude of the sensitization (198 ± 63 % of baseline) were different from that seen in cells not treated with NGF (3 of 25 cells sensitized (12.0 %), 253 ± 70 % of baseline). Pretreatment with the CB1 antagonist AM-251 (100 nM) prevented the effect of ACEA on NGF-induced sensitization. These results support the hypothesis that cannabinoids, acting through CB1 receptors, may produce analgesia in part by preventing NGF-induced sensitization of TRPV1 in afferent nociceptor nerve endings.

Keywords

Cannabinoids; analgesia; nociceptor; NGF; TRPV1; patch clamp

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Introduction

The transient receptor potential vanilloid type 1 channel (TRPV1) and nerve growth factor (NGF) are important mediators of somatic and visceral nociception and hyperalgesia [11, 19]. NGF is released from inflamed and damaged tissue and contributes to development of inflammatory pain, in large part by increasing activity of excitatory capsaicin- and heat-activated ion channel TRPV1 in afferent nerve endings of peripheral nociceptors. NGF and TRPV1 play particularly important roles in experimental cystitis models of visceral pain. We have previously shown that NGF released during experimental bladder inflammation contributes to mechanical hypersensitivity in this model, and that this hypersensitivity is reversed by administration of either anti-NGF antiserum or by K-252a, an inhibitor of the NGF receptor TrkA [13]. Furthermore, in TRPV1-KO mice, bladder inflammation fails to produce bladder mechanical hyperactivity and somatic mechanical hypersensitivity observed in wild-type animals [28].

Cannabinoids are lipophilic molecules with antinociceptive and antihyperalgesic properties. They activate specific G protein-coupled cannabinoid receptors, CB1 and CB2. CB1 cannabinoid receptors are present in both central and peripheral neurons and are thought to mediate most of the analgesic effects of cannabinoids [16, 24]. In experimental cystitis models, in which pain is largely NGF- and TRPV1-dependent [11, 13, 28], cannabinoids prevent inflammatory hyperalgesia via activation of CB1 receptors [9, 10]. Furthermore, local instillation of cannabinoids directly into the bladder attenuated hyperactivity of bladder afferent nerves seen after production of experimental cystitis, an effect prevented by a CB1 antagonist [27]. This suggests that cannabinoids may have effects directly on nociceptor nerve endings where NGF and TRPV1 are thought to produce their nociceptive and hyperalgesic effects, and is consistent with studies that show that the CB1 receptor is co-expressed with TRPV1 [1, 4, 6, 7, 17], and Substance P and CGRP, two neuropeptides that are highly correlated with NGF responsiveness [1, 2]. Moreover, expression of CB1 receptors is increased in sensory neurons after inflammation, particularly in those neurons that also express TRPV1 [5]. Levels of endogenous cannabinoids such as anandamide are also increased in the bladder after inflammation [13].

Given the close correlation between CB1 receptor and TRPV1 expression and the important roles of TRPV1 and NGF in nociception and inflammatory hyperalgesia, we wondered whether cannabinoids might produce analgesia in part by interfering with NGF-induced sensitization of TRPV1. In this study, we quantified the effect of NGF on TRPV1 activation in sensory neurons in vitro and tested whether a selective CB1 receptor agonist could attenuate the sensitizing effect of NGF.

Methods

Sensory neurons were isolated from rat dorsal root ganglia using standard procedures in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of the University of Wisconsin. Briefly, weanling (3-4 week old) rats were euthanized with sodium pentobarbital (0.15-0.2 mg/g i.p.). Ganglia were collected in DMEM, treated with collagenase (2 mg/ml) and trypsin (2.5 mg/ml) for 30-45 min at 35-37°C, and mechanically dissociated by trituration with fire-polished Pasteur pipettes. Cells were resuspended in Neurobasal medium containing B27 supplement, 50 U/mL penicillin and 50 ug/mL streptomycin, plated on poly-D-lysine-coated coverslips, and maintained in an incubator with 5 % CO₂ atmosphere at 37°C until the cells adhered to the coverslip, about 1-2 hours. All experiments were performed within 1-36 hours after isolation.

Capsaicin-induced currents were measured at room temperature from sensory neurons using the patch clamp technique with an Axopatch 200B patch clamp amplifier, a Digidata 1322 A/D converter and pClamp 9 software (Axon Instruments, Foster City, CA). Currents were filtered at 1 kHz and acquired at 2 kHz. Cell capacitance and series resistance were read from the dials of the patch clamp amplifier after cancellation of the capacitive current transient obtained during a small depolarizing test pulse. Patch pipettes were made from borosilicate and filled with a solution containing (in mM): KCl, 130; CaCl₂, 0.5; EGTA, 5; HEPES, 10; MgATP, 2; LiGTP, 0.3; and KOH, 20 (pH 7.2 with KOH; Osmolality 290 mOsm with sucrose). The external solution contained (in mM): NaCl, 130; KCl, 5; MgCl₂, 1; CaCl₂, 2; HEPES, 10; NaOH, 4; and glucose, 10 (pH 7.4 with NaOH; Osmolality 310 mOsm with sucrose). The membrane potential was clamped at -60 mV.

Stock solutions of capsaicin, the selective CB1 agonist arachidonoyl-2'-chloroethylamide (ACEA), and the CB1 antagonist N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM-251) were made in ethanol, kept at -20°C, and diluted with external solution on the day of the experiment. Vehicle treatment consisted of ethanol (1:10⁶ v:v) in external solution as this was the concentration of ethanol present in the highest concentration of ACEA tested (10 nM). Stock solutions of NGF were kept in aliquots at -80°C and diluted with external solution on the day of the experiment to a final concentration of 100 ng/mL. Cells were gravity perfused with solutions flowing through one of three large capillary tubes glued together in a row. For rapid and precise capsaicin application, the tubes were moved with a motor (Fast Stepper, Warner, Hamden CT, USA) controlled by a computer.

The first capsaicin application started 2 minutes after achieving whole cell configuration. Subsequent capsaicin applications were separated by 5 minutes. NGF application began after the first capsaicin application. ACEA or vehicle was applied continuously for 2 minutes before the first capsaicin application. AM-251 was applied continuously beginning 2 minutes before application of ACEA.

Data are represented as mean ± SE, or as numbers and percentages. Capsaicin current amplitude was defined as the difference between the current measured over the last 10 seconds of the 30 second application and the baseline current. A cell was considered to be sensitized by NGF if the magnitude of the current measured during the second capsaicin application (after 5 minutes of NGF exposure) was greater than the magnitude of the current measured during the first capsaicin application (baseline, before NGF exposure). Three data points were excluded from analysis after being shown to be statistical outliers using Grubb's test. Differences in mean values between various treatments were analyzed by ANOVA with Tukey's post-hoc test or by t-test, while differences in response rates were analyzed using Fisher's exact test. Changes in the magnitude of sensitization and desensitization among groups over time were analyzed using twoway ANOVA followed by Dunnett's test. The significance level was 0.05.

Results

A total of 272 neurons were studied. In most cells capsaicin (100 nM) produced an inward current that developed slowly with peak magnitude occurring towards the end of the 30 s application, and then diminished quickly (see Fig 1). Overall 187 of 272 cells (68.8 %) responded to capsaicin application. Capsaicin-responsive cells were small, with membrane capacitance of 22 ± 1 pF (n=186) and resting membrane potential of -51 ± 1 mV (n=186). Capsaicin response rates and baseline current magnitudes for each group are shown in Table 1.

With no intervening treatment, a second capsaicin application 5 minutes after the first typically produced a current of smaller magnitude (desensitization or tachyphylaxis). However, in 3 of 25 untreated cells (12.0 %), the capsaicin current magnitude was greater on the second application than the first (sensitization). In a separate group of cells that were exposed to NGF between the first and second capsaicin applications, the percentage of cells demonstrating sensitization increased to 31.0 % (13 of 42 cells, $p < 0.05$; Fig 1). NGF-induced sensitization lasted up to 20 minutes during continuous perfusion of NGF in 6 cells (data not shown). Cells that were not sensitized by NGF exhibited tachyphylaxis with repeated exposure to capsaicin which was not different from that seen in cells that were not exposed to NGF (Fig 2).

Exposure to the CB1 receptor agonist ACEA (10 nM) prevented NGF-induced sensitization of the capsaicin current, with only 4 of 37 cells (10.8 %) showing sensitization (Fig 3A; $p < 0.05$). This was similar to the 12 % response rate of cells not exposed to NGF. ACEA (1-10 nM) induced no currents on its own, and had no effect on the magnitude of the capsaicin current (Table 1). Treatment with either vehicle (6 of 20 cells sensitized, 30.0 %) or a lower concentration of ACEA (1 nM; 6 of 17 cells sensitized, 35.3 %) had no effect on NGF-induced sensitization. Pretreatment with the CB1 receptor antagonist AM-251 (100 nM) partially prevented the effect of ACEA on NGF-induced sensitization (5 of 25 cells sensitized, 20.0 %).

The magnitudes of NGF-induced sensitization of the capsaicin current for each treatment are shown in Fig 3B. There were no differences among the groups in the magnitude of sensitization among cells that were sensitized. Within each treatment group, cells with smaller capsaicin currents were more likely to be sensitized by NGF. Averaging values across all six groups, sensitized cells had a smaller baseline capsaicin current magnitude (-142 ± 40 pA, $n=37$) than those that were not sensitized (-318 ± 35 pA, $n=128$; $p=0.01$). There were no differences in resting membrane potential or membrane capacitance between cells that were sensitized and those that were not.

Discussion

Cannabinoids are effective analgesics, though their exact mechanism of action is not known. The peripheral nociceptor seems to be the most likely cellular target for these agents, but the important molecular targets remain elusive. In vitro, cannabinoid agonists have been shown to have direct, receptor-independent effects on a variety of voltage- and ligand-gated ion channels important for nociceptor activation, including TRPV1 channels [16, 18]. However, these direct cannabinoid effects are generally observed only at relatively high agonist concentrations, while activation of CB1 receptors occurs at agonist concentrations several orders of magnitude lower [3, 22]. We did not see any currents during application of ACEA, nor did ACEA have any effect on capsaicin current magnitudes at the concentrations we tested (1-10 nM). The importance of nociceptor-expressed CB1 receptors in the analgesic action of cannabinoids was clearly shown in a recent study by Agarwal et al. using mice with a conditional deletion of the CB1 receptor from peripheral nociceptive neurons [1]. They found that nociceptor-specific loss of CB1 caused baseline hyperalgesia and allodynia in standard behavioral testing, suggesting loss of endogenous cannabinoid signaling, and eliminated the analgesic effects of systemically and peripherally administered cannabinoids in both inflammatory and neuropathic models of pain.

In the present study we have shown that the selective CB1 receptor agonist ACEA prevents NGF-induced sensitization of TRPV1 in isolated peripheral sensory neurons in vitro, and that this effect of ACEA was mediated by activation of the CB1 receptor. We hypothesize that this interaction between CB1, NGF, and TRPV1 occurs at nociceptor afferent terminals

in vivo, where NGF released during inflammation sensitizes the noxious heat transducer TRPV1. The idea that CB1 receptors reside on afferent nerve endings and that their activation there has analgesic actions is supported by several studies of both somatic and visceral pain. Subcutaneous intraplantar injection of cannabinoid agonists at the site of experimental inflammation suppressed pain responses [1, 14, 15, 20] and decreased the activation of cutaneous nerve fibers innervating inflamed skin [20]. In a model of interstitial cystitis pain, intravesical administration of a cannabinoid agonist also reduced the sensitization of bladder afferent nerves in a CB1-dependent manner [27].

A large proportion (65-70 %) of capsaicin-responsive neurons in our study were not sensitized by NGF. This is similar to the proportion of nociceptors that do not express the TrkA receptor [21], and is consistent with the study by Galoyan et al. that showed that only those sensory neurons that expressed TrkA demonstrated NGF-induced sensitization of TRPV1 [12]. Cells in our study that were not sensitized exhibited tachyphylaxis with repeated capsaicin applications, a phenomenon that has been well described. Calcium that enters the cell through the opened TRPV1 channel is thought to activate the protein phosphatase calcineurin, which rapidly dephosphorylates the TRPV1 channel and decreases its sensitivity to capsaicin and heat [25]. The degree of tachyphylaxis we observed in cells that were treated with, but not sensitized by, NGF was thus not different from that observed in cells that were not treated with NGF at all.

CB1 agonists may interfere with several signaling pathways thought to be responsible for NGF-mediated TRPV1 sensitization. The NGF receptor TrkA is coupled to the PLC, PI3K, and MAPK pathways that have all been shown to participate in TRPV1 sensitization [19, 26]. Activation of PLC leads to hydrolysis of PIP₂, a possible inhibitor of TRPV1, and to activation of PKC, which phosphorylates and sensitizes TRPV1 [8, 12]. The PI3K pathway also increases TRPV1-dependent currents [30, 31], at least partly by increasing TRPV1 channel density through translocation of TRPV1 channels to the cell surface [23, 29]. The MAPK pathway can lead to both short term sensitization and longer term increases in TRPV1 channel expression [30, 31]. CB1 activation may alter multiple sites in these pathways, including interfering with TrkA activation, inhibiting intermediate kinases, or through direct effects on TRPV1 such as dephosphorylation of the channel. Our recent experiments suggest PI3K signaling may be disrupted by CB1 receptor activation (Wang et al., submitted).

Acknowledgments

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References

1. Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ, Rubino T, Michalski CW, Marsicano G, Monory K, Mackie K, Marian C, Batkai S, Parolaro D, Fischer MJ, Reeh P, Kunos G, Kress M, Lutz B, Woolf CJ, Kuner R. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci.* 2007; 10:870–879. [PubMed: 17558404]
2. Ahluwalia J, Urban L, Bevan S, Capogna M, Nagy I. Cannabinoid 1 receptors are expressed by nerve growth factor- and glial cell-derived neurotrophic factor-responsive primary sensory neurones. *Neuroscience.* 2002; 110:747–753. [PubMed: 11934481]
3. Ahluwalia J, Urban L, Bevan S, Nagy I. Anandamide regulates neuropeptide release from capsaicin-sensitive primary sensory neurons by activating both the cannabinoid 1 receptor and the vanilloid receptor 1 in vitro. *Eur J Neurosci.* 2003; 17:2611–2618. [PubMed: 12823468]
4. Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. *Neuroscience.* 2000; 100:685–688. [PubMed: 11036202]

5. Amaya F, Shimosato G, Kawasaki Y, Hashimoto S, Tanaka Y, Ji RR, Tanaka M. Induction of CB1 cannabinoid receptor by inflammation in primary afferent neurons facilitates antihyperalgesic effect of peripheral CB1 agonist. *Pain*. 2006; 124:175–183. [PubMed: 16709443]
6. Anand U, Otto WR, Sanchez-Herrera D, Facer P, Yiangou Y, Korchev Y, Birch R, Benham C, Bountra C, Chessell IP, Anand P. Cannabinoid receptor CB2 localisation and agonist-mediated inhibition of capsaicin responses in human sensory neurons. *Pain*. 2008; 138:667–680. [PubMed: 18692962]
7. Binzen U, Greffrath W, Hennessy S, Bausen M, Saaler-Reinhardt S, Treede RD. Co-expression of the voltage-gated potassium channel Kv1.4 with transient receptor potential channels (TRPV1 and TRPV2) and the cannabinoid receptor CB1 in rat dorsal root ganglion neurons. *Neuroscience*. 2006; 142:527–539. [PubMed: 16889902]
8. Bonnington JK, McNaughton PA. Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. *J Physiol*. 2003; 551:433–446. [PubMed: 12815188]
9. Farquhar-Smith WP, Jaggar SI, Rice AS. Attenuation of nerve growth factor-induced visceral hyperalgesia via cannabinoid CB(1) and CB(2)-like receptors. *Pain*. 2002; 97:11–21. [PubMed: 12031775]
10. Farquhar-Smith WP, Rice AS. Administration of endocannabinoids prevents a referred hyperalgesia associated with inflammation of the urinary bladder. *Anesthesiology*. 2001; 94:507–513. discussion 506A. [PubMed: 11374613]
11. Frias B, Lopes T, Pinto R, Cruz F, Cruz CD. Neurotrophins in the lower urinary tract: becoming of age. *Curr Neuroparmacol*. 2011; 9:553–558. [PubMed: 22654715]
12. Galoyan SM, Petruska JC, Mendell LM. Mechanisms of sensitization of the response of single dorsal root ganglion cells from adult rat to noxious heat. *Eur J Neurosci*. 2003; 18:535–541. [PubMed: 12911749]
13. Guerios SD, Wang ZY, Bjorling DE. Nerve growth factor mediates peripheral mechanical hypersensitivity that accompanies experimental cystitis in mice. *Neurosci Lett*. 2006; 392:193–197. [PubMed: 16203088]
14. Gutierrez T, Farthing JN, Zvonok AM, Makriyannis A, Hohmann AG. Activation of peripheral cannabinoid CB1 and CB2 receptors suppresses the maintenance of inflammatory nociception: a comparative analysis. *Br J Pharmacol*. 2007; 150:153–163. [PubMed: 17160008]
15. Johanek LM, Simone DA. Activation of peripheral cannabinoid receptors attenuates cutaneous hyperalgesia produced by a heat injury. *Pain*. 2004; 109:432–442. [PubMed: 15157704]
16. Kress M, Kuner R. Mode of action of cannabinoids on nociceptive nerve endings. *Exp Brain Res*. 2009; 196:79–88. [PubMed: 19306092]
17. Mittrirattanakul S, Ramakul N, Guerrero AV, Matsuka Y, Ono T, Iwase H, Mackie K, Faull KF, Spigelman I. Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain. *Pain*. 2006; 126:102–114. [PubMed: 16844297]
18. Patwardhan AM, Jeske NA, Price TJ, Gamper N, Akopian AN, Hargreaves KM. The cannabinoid WIN 55,212-2 inhibits transient receptor potential vanilloid 1 (TRPV1) and evokes peripheral antihyperalgesia via calcineurin. *Proc Natl Acad Sci U S A*. 2006; 103:11393–11398. [PubMed: 16849427]
19. Pezet S, McMahon SB. Neurotrophins: mediators and modulators of pain. *Annu Rev Neurosci*. 2006; 29:507–538. [PubMed: 16776595]
20. Potenziari C, Brink TS, Pacharinsak C, Simone DA. Cannabinoid modulation of cutaneous Delta nociceptors during inflammation. *J Neurophysiol*. 2008; 100:2794–2806. [PubMed: 18784270]
21. Priestley JV, Michael GJ, Averill S, Liu M, Willmott N. Regulation of nociceptive neurons by nerve growth factor and glial cell line derived neurotrophic factor. *Can J Physiol Pharmacol*. 2002; 80:495–505. [PubMed: 12056559]
22. Roberts LA, Christie MJ, Connor M. Anandamide is a partial agonist at native vanilloid receptors in acutely isolated mouse trigeminal sensory neurons. *Br J Pharmacol*. 2002; 137:421–428. [PubMed: 12359623]
23. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE. Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. *J Gen Physiol*. 2006; 128:509–522. [PubMed: 17074976]

24. Talwar R, Potluri VK. Cannabinoid 1 (CB1) receptor--pharmacology, role in pain and recent developments in emerging CB1 agonists. *CNS Neurol Disord Drug Targets*. 2011; 10:536–544. [PubMed: 21631407]
25. Touska F, Marsakova L, Teisinger J, Vlachova V. A “cute” desensitization of TRPV1. *Curr Pharm Biotechnol*. 2011; 12:122–129. [PubMed: 20932251]
26. Vay L, Gu C, McNaughton PA. The thermo-TRP ion channel family: properties and therapeutic implications. *Br J Pharmacol*. 2012; 165:787–801. [PubMed: 21797839]
27. Walczak JS, Cervero F. Local activation of cannabinoid CB(1) receptors in the urinary bladder reduces the inflammation-induced sensitization of bladder afferents. *Mol Pain*. 2011; 7:31. [PubMed: 21554718]
28. Wang ZY, Wang P, Merriam FV, Bjorling DE. Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. *Pain*. 2008; 139:158–167. [PubMed: 18445509]
29. Zhang X, Huang J, McNaughton PA. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *EMBO J*. 2005; 24:4211–4223. [PubMed: 16319926]
30. Zhu W, Oxford GS. Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1. *Mol Cell Neurosci*. 2007; 34:689–700. [PubMed: 17324588]
31. Zhuang ZY, Xu H, Clapham DE, Ji RR. Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. *J Neurosci*. 2004; 24:8300–8309. [PubMed: 15385613]

Highlights

We demonstrate how peripheral administration of cannabinoids can produce analgesia
Currents mediated by TRPV1 are increased by nerve growth factor in sensory neurons
A CB1 cannabinoid agonist attenuates sensitization of TRPV1 by nerve growth factor

Figure 1.

Representative traces of capsaicin-induced currents from two different cells exposed to NGF (100 ng/mL). In both A and B, the left panels show the baseline responses to capsaicin in the absence of NGF, the middle panels show the responses after 5 minutes of NGF exposure, and the right panels show the responses after 10 minutes of NGF exposure. The time of capsaicin (100 nM; 30 s) application is indicated by the bar above each trace. The capsaicin current did not increase after NGF treatment in the cell shown in A, while it did increase at both 5 and 10 minutes after NGF in the cell shown in B. Vertical scale bars denote 50 pA in both A and B.

Figure 2.

NGF increased capsaicin currents in some cells (Sensitized, NGF) but not in others (Desensitized, NGF). The rate and magnitude of desensitization were not different from those seen in cells that were not exposed to NGF (Desensitized, no Rx). Rx, treatment; CAP, capsaicin. Values are means $\pm$ SE. Within groups, changes in CAP current magnitudes during the second and third CAP applications were significantly different from baseline values (first CAP application) where indicated (*, $p < 0.01$). Relative CAP current magnitudes in the Sensitized, NGF group were significantly different from those in either of the other two groups at both time points (†, $p < 0.01$). For the Sensitized, NGF group, $n=13$; for the Desensitized, NGF group, $n=27-29$; for the Desensitized, no Rx group, $n=20-22$.

Figure 3.

ACEA (10 nM) reduced the % of cells sensitized by NGF but did not significantly change the magnitude of the sensitization of the capsaicin-induced current. The percentage of sensitized cells is shown in A for each group. NGF treatment (NGF +) increased the percentage of sensitized cells ($\dagger$, $p < 0.05$ vs. NGF -). ACEA at 10 nM, but not 1 nM, prevented NGF-induced sensitization ($*$ $p < 0.05$ vs. NGF +). This effect was partially prevented by pretreatment with the CB1 receptor antagonist AM-251 (100 nM). For those cells in which the second capsaicin application was greater than the first (sensitized cells only), the magnitude of the capsaicin current sensitization is shown in B (means $\pm$ SE).

There were no significant differences among the groups. The number of cells in each group is given in the text of the Results section.

Table 1

Baseline Values for Capsaicin Responsiveness

	No Treatment	NGF	NGF + ACEA (10 ⁻⁹ M)	NGF + ACEA (10 ⁻⁸ M)	NGF + vehicle	NGF + ACEA (10 ⁻⁸ M) + AM-251
Cells tested (n)	47	80	26	53	27	39
Cells with CAP response (n (%))	31 (66.0 %)	49 (61.3 %)	19 (73.1 %)	42 (79.2 %)	21 (77.8 %)	25 (64.1 %)
Baseline CAP current (-pA)	226.4 ± 48.4	385.1 ± 74.5	296.3 ± 91.6	183.7 ± 30.2	248.0 ± 87.4	205.2 ± 36.8

CAP, capsaicin; values are numbers, (percent), or means ± SE.