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Nitro-oleic acid desensitizes TRPA1 and TRPV1 agonist responses in adult rat DRG neurons ☆

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ABSTRACT

Nitro-oleic acid (OA-NO_2), an electrophilic fatty acid nitroalkene byproduct of redox reactions, activates transient receptor potential ion channels (TRPA1 and TRPV1) in primary sensory neurons. To test the possibility that signaling actions of OA-NO_2 might modulate TRP channels, we examined: (1) interactions between OA-NO_2 and other agonists for TRPA1 (allyl-isothiocyanate, AITC) and TRPV1 (capsaicin) in rat dissociated dorsal root ganglion cells using Ca^{2+} imaging and patch clamp techniques and (2) interactions between these agents on sensory nerves in the rat hindpaw. Ca^{2+} imaging revealed that brief application (15–30 s) of each of the three agonists induced homologous desensitization. Heterologous desensitization also occurred when one agonist was applied prior to another agonist. OA-NO_2 was more effective in desensitizing the response to AITC than the response to capsaicin. Prolonged exposure to OA-NO_2 (20 min) had a similar desensitizing effect on AITC or capsaicin. Homologous and heterologous desensitizations were also demonstrated with patch clamp recording. Deltamethrin, a phosphatase inhibitor, reduced the capsaicin or AITC induced desensitization of OA-NO_2 but did not suppress the OA-NO_2 induced desensitization of AITC or capsaicin, indicating that heterologous desensitization induced by either capsaicin or AITC occurs by a different mechanism than the desensitization produced by OA-NO_2. Subcutaneous injection of OA-NO_2 (2.5 mM, 35 μl) into a rat hindpaw induced delayed and prolonged nociceptive behavior. Homologous desensitization occurred with AITC and capsaicin when applied at 15 minute intervals, but did not occur with OA-NO_2 when applied at a 30 min interval. Pretreatment with OA-NO_2 reduced AITC-evoked nociceptive behaviors but did not alter capsaicin responses. These results raise the possibility that OA-NO_2 might be useful clinically to reduce neurogenic inflammation and certain types of painful sensations by desensitizing TRPA1 expressing nociceptive afferents.

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Introduction

Nitro-oleic acid (OA-NO_2) and related nitroalkenes are electrophilic fatty acid derivatives formed by nitric oxide- or nitrite-mediated redox reactions. These species are present in normal tissues at nM concentrations and can increase during inflammation to almost μM concentrations (Baker et al., 2005; Batthyany et al., 2006; Bonacci et al., 2012). Fatty acid nitroalkenes induce a variety of pharmacological effects including: (1) activation of peroxisome proliferator-activated receptor γ (PPARγ) (Baker et al., 2005), (2) activation of the Keap1–Nrf2 pathway (Villacorta et al., 2007), (3) upregulation of heme oxygenase 1 (HO-1) expression (Wright et al., 2006), (4) inhibition of NF-κB-dependent gene expression (Cui et al., 2006; Villacorta et al., 2013), (5) inhibition of platelet or neutrophil function and (6) inhibition of proinflammatory cytokine secretion by macrophages (Coles et al., 2002a,b). These actions can all be ascribed to the post-translational modification of functionally significant proteins by the reversible Michael addition reactions that nitroalkenes can undergo. OA-NO_2 may thus function as an endogenous anti-inflammatory mediator and contribute to resolution of inflammation (Schopfer et al., 2011).

OA-NO_2 also activates TRPA1 and TRPV1, which are nonselective cation channels expressed in nociceptive primary sensory neurons (Sculptoreanu et al., 2010; Taylor-Clark et al., 2009). Sensitization of these channels is involved in the development of hyperalgesia (hypersensitivity to noxious stimuli) in inflammatory pain models (da Costa et al., 2010; Davis et al., 2000); while desensitization is an important mechanism for down-regulation of channel activity and reducing nociceptor function. Capsaicin, a specific TRPV1 agonist, activates and subsequently desensitizes TRPV1 channels (homologous desensitization) and also reduces the effect of allyl isothiocyanate (AITC) on TRPA1 channels (heterologous desensitization) (Ruparel et al., 2008; Salas et al., 2009). AITC elicits similar homologous and heterologous (TRPV1) desensitizations (Ruparel et al., 2008; Salas et al., 2009).

The present experiments used Ca^{2+} imaging and patch clamp techniques to examine: (1) desensitizing interactions between OA-NO_2,

Abbreviations: AITC, allyl-isothiocyanate; DRG, dorsal root ganglion; OA-NO_2, nitro-oleic acid.

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capsaicin and AITC in dissociated dorsal root ganglion (DRG) neurons of adult rats and (2) the interactions between these agents on sensory nerves in the rat hindpaw. Our results revealed that pretreatment with OA-NO_2 desensitized TRPA1 and TRPV1 responses in vitro as well as the TRPA1 response in vivo. These findings raise the possibility that anti-inflammatory signaling actions of OA-NO_2 can also be related in part to modulation of TRP channels in sensory neurons. This suggests that electrophilic fatty acids such as OA-NO_2 might be clinically useful in reducing neurogenic inflammation and certain types of painful sensations by desensitizing nociceptive afferents.

Materials and methods

Experiments were performed on adult Sprague–Dawley female rats (200–250 g). The stage of the estrous cycle at the time of the experiments was not determined. All experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee (Protocol approval # IACUC 1201539) and were consistent with the guidelines of the National Institutes of Health and the International Association for the Study of Pain.

DRG neuron culture

After a laminectomy under isoflurane anesthesia L4–L6 DRGs were removed bilaterally, enzymatically treated (collagenase type 4, 2 mg/ml and trypsin, 1 mg/ml; Worthington Biochemical, Lakewood, NJ) and mechanically dissociated as described elsewhere (Zhang et al., 2011). The cells were plated on poly-L-lysine-coated glass coverslips (Sigma, St. Louis, MO) and incubated at 37 °C in 5% CO_2 and 90% humidity for at least 3–4 h before Ca^{2+} imaging or patch clamp recording. Because trypsin treatment has been reported to reduce TRPA1 responses, cells were studied 4–12 h after dissociation to allow for recovery from the effects of dissociation. The percentage of AITC responsive neurons obtained in our Ca^{2+} imaging (33%) and patch clamp recording (43%) were comparable with the percentages reported in other studies (Dai et al., 2007; Kobayashi et al., 2005) suggesting that this time period is sufficient for return of TRPA1 responses.

Ca^{2+} imaging

DRG cells were loaded with Fura 2-AM (2 μM; Molecular Probes, Eugene, OR) for 30 min at 37 °C in an atmosphere of 5% CO_2. Fura 2-AM was dissolved in Hank's balanced salt solution (HBSS) containing (in mM): 138 NaCl, 5 KCl, 0.3 KH_2PO_4, 4 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 5.6 glucose, pH 7.4. Ca^{2+} imaging was performed as previously (Zhang et al., 2011). Briefly, coverslips were placed on an epifluorescence microscope (Olympus IX70) and continuously perfused (2–3 ml/min) with HBSS. Fura 2 was excited alternately with ultraviolet light at 340 and 380 nm; and the fluorescence emission was detected at 510 nm using a computer-controlled monochromator. Image pairs were acquired every 1–30 s using illumination periods between 20 and 50 ms. Wavelength selection, timing of excitation, and the acquisition of images were controlled using the program C-Imaging (Compix, Cranberry Township, PA) running on a personal computer. Digital images were stored on hard disk for off-line analysis. One coverslip usually contained 20–40 DRG neurons/microscopic field at 40× magnification. OA-NO_2 was synthesized as described previously (Baker et al., 2004). Capsaicin and allyl-isothiocyanate (AITC) were obtained from Sigma Aldrich (St. Louis, MO). On the day of the experiment, stock solutions of OA-NO_2 (70 mM in 100% ethanol), capsaicin (10 mM in 100% ethanol) and AITC (100 mM in DMSO) were diluted in HBSS and delivered via bath application using a gravity-driven system (infusion rate was 2–3 ml/min). Vehicles applied via the same method were inactive.

Patch clamp recording

Voltage-clamp data were acquired with conventional whole cell patch clamp techniques with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale CA) controlled with a PC running pClamp Software (V 10.3, Molecular Devices). Current traces were sampled at 5–10 kHz and filtered at 1–2 kHz. Patch electrodes were pulled from borosilicate glass (WPI, Sarasota FL) on a horizontal puller (Sutter Inst. Novato CA), and had resistances of 2–5 MΩ when filled with an electrode solution containing (in mM): K-methanesulfonate 110, KCl 30, NaCl 5, $CaCl_2$ 1, $MgCl_2$ 2, HEPES 10, EGTA 11, Mg-ATP 2, Li-GTP 1, pH 7.2 (adjusted with Tris-base), and 310 mOsm (adjusted with sucrose). Currents were recorded in HBSS solution. The junction potential associated with all test solutions was measured and was less than 5 mV, and therefore, junction potential was not corrected. Series resistance compensation was >80%. Whole-cell capacitance and series resistance were compensated with amplifier circuitry. Neurons were held at −60 mV and OA-NO_2, capsaicin and AITC were applied with a piezo-driven perfusion system (Warner Instruments, Hamden CT). Current data were analyzed with pClamp software in combination with SigmaPlot (Systat, Chicago, IL). Current density was determined by dividing peak-evoked current by membrane capacitance (as determined with a 5 mV voltage step prior to compensation).

Behavioral tests

On the day of experiments, stock solutions of chemicals: capsaicin (10 mM in 100% ethanol), AITC (1 M in DMSO) and OA-NO_2 (70 mM in 100% ethanol) were diluted with normal saline. The concentrations of all agents used in vivo were 100 times higher than those tested in vitro on DRG neurons. Rats were put in a transparent box at least 30 min before behavioral testing. Then 35 μl of capsaicin (100 μM), AITC (10 mM) or OA-NO_2 (2.5 mM) or a vehicle control was injected subcutaneously into the plantar surface of the right hindpaw. The concentrations and the amounts of injected capsaicin and AITC were similar to those used in other behavioral studies (Ruparel et al., 2008; Schmidt et al., 2009). The nociceptive responses were measured for 20–30 min by an observer blinded to treatments by counting the time spent licking or withdrawing the hindpaw during 5 min periods post-injection. For homologous or heterologous desensitization experiments, 10–30 min after the first injection the rats received a second injection (35 μl) at the same site as the first injection and the behavioral test was repeated.

Data analysis

In Ca^{2+} imaging studies, data were analyzed using program C-Imaging (Compix). Background was subtracted to minimize camera dark noise and tissue autofluorescence. An area of interest was drawn around each cell, and the average value of all pixels included in this area was taken as one measurement. The ratio of fluorescence signal measured at 340 nm divided by the fluorescence signal measured at 380 nm was used to measure the increase of intracellular Ca^{2+}. All data are expressed as mean ± SEM. Paired or unpaired *t*-test or one way ANOVA followed by Dunnett's post hoc test was used to assess statistical significance. Chi square tests were used when % responsive neurons was compared, $p < 0.05$ was considered statistically significant.

Results

Comparison of OA-NO_2, AITC and capsaicin induced Ca^{2+} transients

Concentrations of the three agonists (15 or 30 μM for OA-NO_2, 100 μM for AITC and 500 nM for capsaicin) were selected based on our previous study and reports in literature to be near the concentrations for eliciting maximal TRPA1 or TRPV1 activation (Caterina et al., 1997; Macpherson et al., 2007; Sculptoreanu et al., 2010). In agreement