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TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS IN CAPSAICIN SENSITIVE AFFERENT NERVES OF RAT URINARY BLADDER ARE TARGETS FOR NITRO-OLEIC ACID.

Hypothesis / aims of study

Nitro-oleic acid (OA-NO_2), an endogenous nitrated fatty acid generated by oxidative and nitrative stress, has been shown to inhibit inflammatory signaling [1]. OA-NO_2 has electrophilic properties and reacts with cysteine residues on a variety of target proteins including transient receptor potential (TRP) channels. Previous studies have demonstrated that OA-NO_2 activates TRP channels in sensory neurons of the dorsal root and nodose ganglia [2,3]. TRP channels are expressed in the bladder and have a role in afferent mechanisms. The aim of the present study was to test the hypothesis that OA-NO_2 activates TRP channels on afferent nerve terminals, thereby increasing bladder activity.

Study design, materials and methods

Bladders from adult female (200 – 250g) Sprague Dawley rats were removed under isoflurane anesthesia, cut into four longitudinal strips (~1.5 mm x 8-10 mm) and mounted in a vertical double jacketed organ bath in oxygenated Krebs' solution (15 ml volume). The muscle strips were kept at 37º C via a circulating warm water bath for tension recording. Tissue was allowed to equilibrate for 1-2 h prior to drug testing.

Capsaicin pretreatment: For some experiments, one group of animals was pretreated with capsaicin (125 mg/kg subcutaneous injection; dissolved in 10% ethanol, 10% Tween 80 and 80% physiological saline) to desensitize C-fiber afferent neurons and another group was treated with vehicle control. Capsaicin was administered under isoflurane anesthesia in 3 injections, divided in 25, 50 and 50 mg/kg doses over a two day period (at ~12 h intervals), and the experiments were performed 4 days after the last injection.

Results

The TRPV1 agonist capsaicin (CAPS; 1µM) and the TRPA1 agonist allyl isothiocyanate (AITC; 30µM) elicited excitatory effects on bladder strip activity, increasing basal tone and the amplitude of spontaneous contractions. OA-NO_2 mimicked these effects in a concentration-dependent manner (1µM – 33µM). None of these agents significantly changed the frequency of spontaneous contractions. The effect of OA-NO_2 (15µM) was reversible and repeatable. The TRPA1 antagonist HC3-030031 (HC3; 30µM) did not reduce the effect of OA-NO_2, but suppressed the excitatory effect of AITC. However, pretreatment of bladder strips with the TRPV1 antagonist diaryl piperazine (DP, 1µM), which blocked the excitatory effect of CAPS, significantly reduced the effect of OA-NO_2 on basal tone by 50%, but did not prevent the increase in contraction amplitude. The combination of HC3 and DP was not more effective than DP alone. The universal TRP channel blocker, ruthenium red (30µM) was significantly more effective at preventing OA-NO_2 effects than DP. Pretreatment of bladder strips with ruthenium red reduced the effects of OA-NO_2 on basal tone by 75% and the effects on amplitude of spontaneous contractions by 85%. In bladder strips from CAPS treated rats, the effect of CAPS on contraction amplitude was eliminated and the effect of OA-NO_2 was reduced by 65%, while the effects of CAPS and OA-NO_2 on basal tone were reduced by 80% and 60%, respectively. Additionally, pretreatment of bladder strips with a combination of neurokinin receptor antagonists (NK1 selective antagonist CP 96345; NK2 selective antagonist MEN 10,376; NK3 selective antagonist SB 234,375; 1µM each) reduced the effect of OA-NO_2 on basal tone, but not contraction amplitude.

Interpretation of results

These results indicate that OA-NO_2 increases spontaneous activity and increases basal tone in the rat urinary bladder. The universal TRP channel blocker was more effective at preventing the effects of OA-NO_2 than a TRPV1 channel antagonist, suggesting that TRPV1 and additional TRP channels mediate the effects. However, the TRPA1 antagonist was not effective in preventing OA-NO_2 effects, indicating that TRPA1 channels are not a target of OA-NO_2 in the afferent terminals. Activation of afferents by OA-NO_2 triggers the release of neurokinins which, in turn act on the detrusor muscle to stimulate bladder activity. The primary site of action of OA-NO_2 is the CAPS-sensitive afferent nerve terminals, as pretreatment with CAPS to deplete neurokinin stores &/or desensitize TRPV1-containing nerves significantly reduced the effects of OA-NO_2.

Concluding message

Nitrated fatty acids can activate TRP channels on CAPS-sensitive afferent nerve terminals, leading to increased bladder contractile activity. Nitrated fatty acids produced endogenously by the combination of fatty acids and nitric oxide released from the urothelium &/or afferent nerves may play a physiological role in modulating bladder activity.

Figure 1 Figure 2

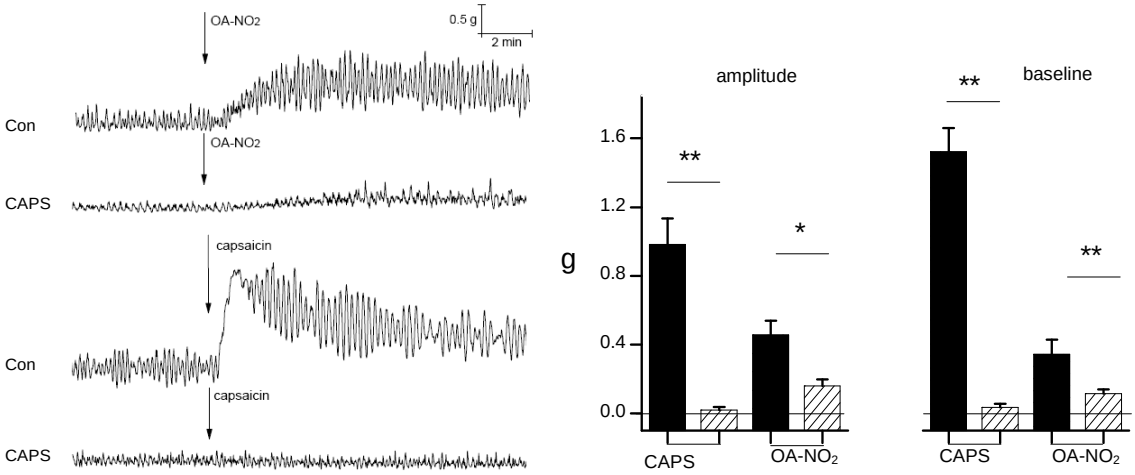

Figure 1. Examples of CAPS and OA-NO_2-evoked responses in bladder strips from control (Con) and CAPS-pretreated (CAPS) rats. Arrows show time of drug application.

Figure 2. Summary data of CAPS and OA-NO_2-evoked responses on the amplitude of spontaneous contractions and basal tone in bladder strips from control (black bars; n = 14) and CAPS-pretreated (hatched bars; n = 12) rats. * $p < 0.05$; ** $p < 0.05$ by 2-tailed t-test.

References

1. Baker, PR, Schopfer, FJ, O'Donnell, VB, Freeman, BA. Convergence of nitric oxide and lipid signaling: Anti-inflammatory nitro-fatty acids. Free Radic Biol Med. (2009): 989-1003
2. Sculptoreanu A, Kullmann FA, Artim DE, Bazley FA, Schopfer F, Woodcock S, Freeman BA, de Groat W. Nitro-oleic acid inhibits firing and activates TRPV1- and TRPA1-mediated inward currents in DRG neurons from adult male rats. J Pharmacol Exp Ther. 2010 Mar 22
3. Taylor-Clark TE, Ghatta S, Bettner W, Undem BJ. Nitrooleic acid, an endogenous product of nitrative stress, activates nociceptive sensory nerves via the direct activation of TRPA1.Mol Pharmacol. 2009 Apr;75(4):820-9.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee**