

INVOLVEMENT OF TRPA1 CHANNELS IN EXCITATORY EFFECTS OF NITRO-OLEIC ACID ON RAT BLADDER FUNCTION

Hypothesis / aims of study

Nitro-oleic acid (9- and 10-nitro-octadecenoic acid, OA-NO₂), an endogenous nitrated fatty acid generated by oxidative and nitrative stress, has been shown to modulate inflammatory signaling and activate transient receptor potential (TRP) channels in sensory neurons. Although oxidative stress has been considered as a pathophysiological basis of bladder dysfunction in various disease conditions such as diabetes or bladder outlet obstruction (1, 2), it is not known whether OA-NO₂ can modulate bladder function in vivo. We therefore examined the effect of OA-NO₂ administered intrathecally or intravesically on bladder activity in normal rats and rats with capsaicin pretreatment, which induces desensitization of capsaicin-sensitive C-fiber afferent pathways. We also tested the antagonists of TRPV1 and TRPA1 channels to elucidate the receptor type involved in the mechanism of OA-NO₂.

Study design, materials and methods

Urodynamic studies were conducted under urethane anesthesia (1.2g/kg, s.c.) in female SD rats.

(1) Expt #1 (intrathecal injection): Rats were implanted with a polyethylene (PE-10) catheter at the level of the L6-S1 spinal cord 2 days before the experiment. A PE-50 catheter was inserted into the bladder through the dome. After evaluating 3 pre-injection cystometrograms (CMGs), either 1μl of vehicle (N=6) or 100μM of OA-NO₂ dissolved in artificial cerebrospinal fluid was injected through the intrathecal catheter (N=6). Post-injection data were then obtained immediately and 30min after the injection to evaluate changes in CMG parameters (intercontraction interval: ICI, maximum voiding pressure: MVP, basal pressure: BP, and threshold pressure: TP).

(2) Expt #2 (intravesical injection): A PE-50 catheter was inserted through the urethra and tied in place by a ligature around the urethral orifice, and single CMGs were recorded. First, OA-NO₂ was injected intravesically (50μM in 200μl saline, retained in the bladder for 30 min) in rats with (OA-NO₂ w Cap group, N=4) or without C-fiber desensitization induced by capsaicin pretreatment (OA-NO₂ w/o Cap group, N=9). Secondly, the effects of TRPA1 antagonist (HC-030031) (OA-NO₂ w anti-TRPA1 group, N=4) or TRPV1 antagonist (BCTC) (OA-NO₂ w anti-TRPV1 group, N=3) on OA-NO₂-induced bladder overactivity were examined in comparison to vehicle (polyethylene glycol: PEG)-treated groups (OA-NO₂ w/o anti-TRPA1 group, N=5 or OA-NO₂ w/o anti-TRPV1 group, N=3, respectively). Changes in CMG parameters (time to first contraction, BP and PT) after the intravesical OA-NO₂ injection were compared with those in vehicle (PAG)-treated control rats (N=5).

Results

(1) Expt #1: There was a tendency to increase in ICI 30 min after intrathecal OA-NO₂ compared to vehicle controls. There were no significant differences in any other parameters.

(2) Expt #2: There was a significant decrease in the time to first contraction in the OA-NO₂ w/o Cap group compared to vehicle (saline) controls (post/pre ratio: 87.2±8.8 vs. 107.7±7.3%, p= 0.014). However, there was no significant decrease in the time to first contraction in the OA-NO₂ w Cap group compare to the control (saline) group (p=0.261). In the experiments with TRPA1 or TRPV1 antagonists, the reduction in the time to first contraction after OA-NO₂ application was significantly suppressed in the OA-NO₂ w anti-TRPA1 group compared to the vehicle (PEG) control group (post/pre ratio: 96.03±4.70 vs. 79.76±7.93%, p=0.01, Fig. 1). In contrast the time to first contraction after OA-NO₂ was similarly reduced in the OA-NO₂ w anti-TRPV1 group when compared to the PEG control group (p=0.128).

Interpretation of results

Intrathecal injection of OA-NO₂ (100μM, 1μL) tended to increase ICI but the difference was not significant when compared with the control value. Intravesical injection of OA-NO₂ (50μM, 200μL) for 30 min caused a significant decrease in the time to first contraction, indicative of bladder overactivity. Pretreatment with capsaicin reduced the excitatory effects of OA-NO₂ on bladder activity, indicating that capsaicin-sensitive C-fiber afferent pathways are involved in OA-NO₂-induced bladder overactivity. In addition, TRPA1 channels, but not TRPV1, contribute to excitation of C-fiber afferent pathways to induce bladder overactivity following OA-NO₂ application.

Concluding message

Intravesical injection of OA-NO₂ enhances the micturition reflex by decreasing bladder capacity via activation of TRPA1 receptors and capsaicin-sensitive C-fiber afferent pathways. Therefore, nitrated fatty acids produced endogenously by the combination of fatty acids and nitric oxide may play a physiological role in modulating C-fiber-dependent bladder activity, and this process might contribute to bladder dysfunction induced by oxidative stress. Moreover, as TRPA1 channels seem to be involved in C-fiber activation to induce bladder overactivity, TRPA1 antagonists could be useful for the treatment of overactive bladder (OAB) conditions.

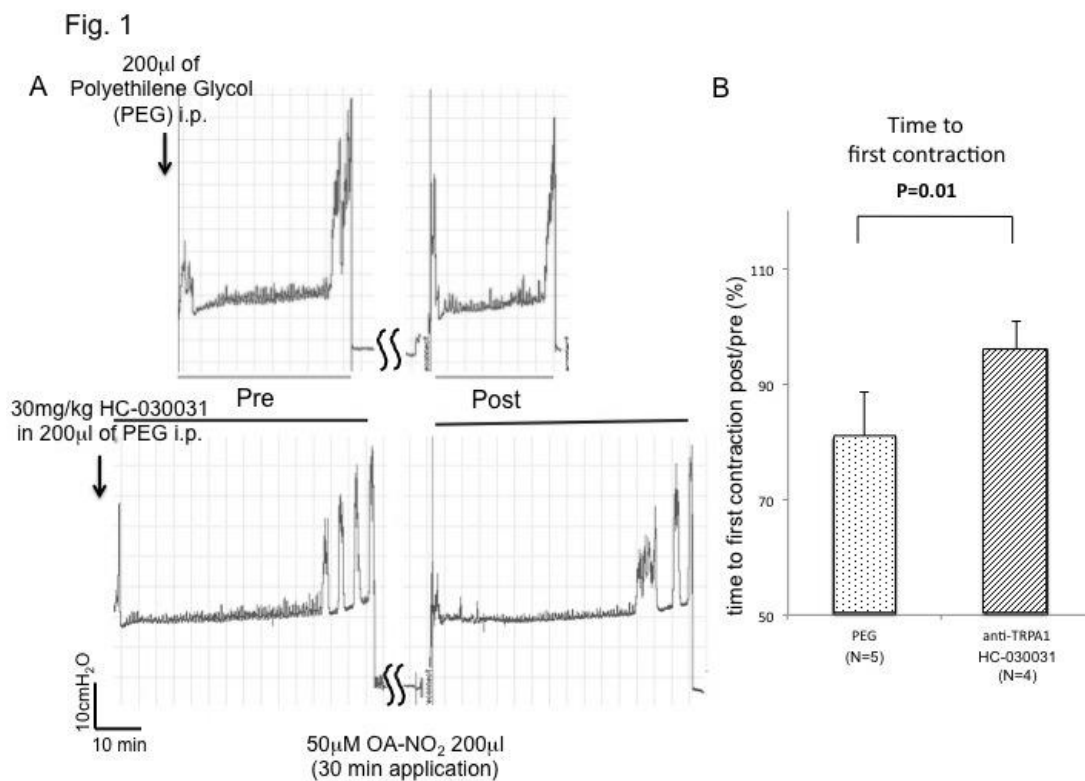


Fig. 1 TRPA1 antagonist (HC-030031) prevented OA-NO₂-induced bladder overactivity. (A) Representative figures of CMG before and after the instillation of 50µM OA-NO₂ for 30 minutes with PEG i.p. pretreatment (upper) or with 30mg/kg HC-030031 i.p. pretreatment (lower) (B) The reduction in the time to first contraction after OA-NO₂ application was significantly decreased in the OA-NO₂ w anti-TRPA1 group compared to the vehicle (PEG) control group

References

1. Oxidative stress plays a role in diabetes-induced bladder dysfunction in a rat model. Beshay E, Carrier S. Urology. 2004 Nov;64(5):1062-7.
2. The role of free radicals and nitric oxide in the ischemia-reperfusion injury mediated by acute bladder outlet obstruction. Yildirim A, Onol FF, Haklar G, Tarcan T. Int Urol Nephrol. 2008;40(1):71-7.

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Is this a clinical trial?	No
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