Increased Skin TRPM8 by Estrogen Deficiency may Induce Detrusor Overactivity Mediated Alpha1D-Adrenergic Receptors Triggered by Sudden Cold Stress

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Aims of study
Our previous studies showed that cold stress significantly decreased voiding interval, micturition volume, and bladder capacity in conscious rats. The transient receptor potential channel melastatin member 8 (TRPM8) expressing on the skin partially mediated the micturition responses. In addition, we indicated that alpha1D-adrenergic receptor antagonists prevented the decreases of voiding interval and bladder capacity. Clinically, LUTS of the postmenopausal women are often developed by the cold stress. However, the mechanisms are not well known.
In this study, we determined if alpha1D-adrenergic receptor blockers inhibited the cold stress-induced detrusor overactivity of ovariectomized rats, and expression levels of TRPM8 receptors on the skin were increased with the ovariectomy.

Materials and Methods
Twenty-four Sprague-Dawley rats at postnatal week 30 were randomly selected for ovariectomy (OVX, n=16) or sham ovariectomy (n=8). Five weeks later, cystometric measurements of the conscious, free-moving rats were performed at room temperature (RT, 28±2°C) for 20 min. Eight OVX rats were intravenously administered 1.0 mg/kg naftopidil, and the other OVX and sham-operated rats (n=8 each) were given naftopidil-free vehicle. Five min later, they were transferred to a low temperature (LT) environment (4±2°C) and micturition patterns were again recorded. TRPM8 channel expression in lumbar skin was estimated by real-time reverse-transcriptase polymerase chain reaction and immunohistochemistry.

Results
The serum estradiol level of the OVX rats was significantly lower than that of the sham rats (P <0.01). The TRPM8 mRNA expression level of the OVX rats (2.61±0.61) was significantly higher than that of the sham operated rats (0.83±0.12, P<0.01, Figure 1).

In immunohistochemistry, the proportion of TRPM8 channel-positive area in the OVX rats (Figure 2B) was significantly higher than that in the sham rats (Figure 2A, 2C).

Figure 2. Expression of TRPM8 on the skin. (A) Sham rats, (B) OVX rats. Red: TRPM8 receptors; Green: S100-positive nerve fibers; Blue: nuclei. (C) The proportion of area occupied by TRPM8 channels in the OVX rats was significantly higher than that in the sham-operated rats.

Micturition parameters were similar for sham-operated (Figure 3A) and OVX (Figure 3B) rats at RT (Table 1). At LT, both sham-operated and OVX rats exhibited cold stress-induced detrusor overactivity, but the increased micturition frequency and decreased bladder capacity were significantly greater for OVX rats. Treatment of OVX rats with naftopidil (Figure 3C) inhibited the cold stress-induced detrusor overactivity.

Table 1. Micturition Parameters under RT and LT

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<thead>
<tr>
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<th>Voided interval (min)</th>
<th>Bladder capacity (ml)</th>
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<tr>
<td></td>
<td>RT</td>
<td>LT</td>
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<tr>
<td>Sham</td>
<td>4.16±0.36</td>
<td>2.98±0.48*</td>
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<tr>
<td>Saline-OVX</td>
<td>4.30±0.54</td>
<td>2.02±0.26***</td>
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<tr>
<td>Naftopidil-OVX</td>
<td>4.18±0.59§</td>
<td>4.30±0.79††#</td>
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*P<0.05, **P<0.01; compared to RT in each temperature.
†P<0.05, ††P<0.01; compared to saline-administered sham rats transferred to LT.
#P<0.05, ##P<0.01; compared to saline-administered OVX rats transferred to LT.

Conclusions
1. The expression of TRPM8 channel protein and mRNA in the lumbar skin of OVX rats was significantly higher than that of sham-operated rats.
2. At LT, the OVX rats exhibited cold stress-induced detrusor overactivity that was significantly more pronounced than that found in the sham-operated rats.
3. Cold stress-induced detrusor overactivity may be mediated, at least in part, by nerve pathways that utilizes alpha1D-adrenergic receptors.