Effects of agonists for estrogen receptor α and β on ovariectomy-induced lower urinary tract dysfunction in the rat

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1Division of Urology, Department of Surgery, Taichung Veterans General Hospital, Taichung, Taiwan, Republic of China; and 2Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

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Cheng CL, de Groat WC. Effects of agonists for estrogen receptor α and β on ovariectomy-induced lower urinary tract dysfunction in the rat. Am J Physiol Renal Physiol 306: F181–F187, 2014. First published November 20, 2013; doi:10.1152/ajprenal.00298.2013.—The postmenopausal hypoestrogen status induces various lower urinary tract dysfunctions. Ovariectomized (OVX) rats exhibit voiding abnormalities, including increased postvoiding residual urine (PVR), decreased voiding efficiency (VE), and altered coordination between the detrusor and external urethral sphincter (EUS). Estradiol replacement partially normalizes voiding function in OVX rats. We determined if selective agonists for estrogen receptor (ER)α and/or ERβ can reverse lower urinary tract dysfunction in OVX rats. Cystometry and EUS electromyograms (EMGs) were recorded 6 wk after bilateral OVX in urethane-anesthetized female Sprague-Dawley rats. Animals received daily subcutaneous injections of selective ERα [propylpyrazole triol (PPT)] or ERβ [diarylpropionitrile (DPN)] agonists or vehicle for 1 wk starting on the fifth week after OVX. PPT (1 mg·kg−1·day−1) decreased PVR, improved VE, and shortened the EUS EMG active period (AP) during voiding. DPN (2 or 5 mg·kg−1·day−1) did not alter cystometric parameters or EUS EMG activity. Combined PPT + DPN treatment elicited changes in PVR, VE, and AP, similar to those induced by PPT alone, but also increased the EUS EMG silent period and volume threshold for triggering micturition. PPT increased uterine weight fourfold and decreased body weight by 11%. DPN increased uterine weight 30–45% but decreased body weight by 3–5%. Reduced voiding efficiency in OVX rats can be reversed by 1-wk drug treatment that selectively targets ERα and reduces AP during EUS bursting. Combined pharmacological activation of ERα and ERβ further enhanced EUS bursting by increasing the EUS EMG silent period and also facilitated bladder storage mechanisms by increasing the volume threshold.

estrogen receptors; ovariectomy; lower urinary tract; bladder; external urethral sphincter

THE LOWER URINARY TRACT (LUT) and genital organs, which share a common embryological origin, express estrogen and progesterone receptors and show physiological responses to these hormones (25, 39, 43). The postmenopausal hypoestrogen status elicits anatomic and physiological changes in the LUT (15) and can be associated with various LUT dysfunctions, including frequency, urgency, urge incontinence, stress urinary incontinence, detrusor overactivity, and recurrent urinary tract infections (24). The influence of gonadal hormones on LUT function is likely mediated via multiple effects on neural control, vascular supply, detrusor muscle cell size and number, and connective tissue density and distribution (5, 15, 24, 46).

Our recent study (10) in adult female rats revealed that surgical ovariectomy (OVX) profoundly affects LUT function by increasing postvoiding residual urine volume (PVR), decreasing voiding efficiency (VE), shortening the silent period (SP), and increasing the active period (AP) of external urethral sphincter (EUS) electromyographic (EMG) activity during micturition. Estradiol replacement partially reversed these changes.

The goal of the present study was to determine the type of estrogen receptor (ER) involved in OVX-induced LUT dysfunctions. mRNA for both ER subtypes (ERα and ERβ) have been identified in bladder tissue, and ERβ is expressed at higher levels than ERα in the urothelium and smooth muscle of the rat and human bladder (1, 30, 35, 37). However, in the human bladder, only ERβ is translated into protein (39). Stimulation of ERβ with diarylpropionitrile (DPN), a selective agonist, increases the proliferation of urothelial cells in part by a mechanism involving nerve growth factor (36).

On the other hand, both ER subtypes are functional in the spinal cord and brain, where they modulate dopaminergic, enkephalinergic, serotoninergic, and glutamatergic neurotransmission (2, 4, 20, 26, 38, 44). ERα immunoreactivity has also been identified in parasympathetic preganglionic neurons in the sacral spinal cord of the cat (40) and in ganglion cells in the pelvic autonomic ganglia in the rat (23). In addition, ERα and ERβ are synthesized in more than half of bladder afferent neurons in lumbosacral dorsal root ganglia of the rat (23), and both receptors are expressed in one-third of these neurons in combination with transient receptor potential vanilloid receptor (TRPV)1, the capsaicin-sensitive, nociceptive ion channel (6). Overnight exposure of cultured dorsal root ganglion cells to estradiol or DPN reduces the excitatory effects of capsaicin, suggesting that steroids might modulate pain (45). Thus, estrogen may act at multiple sites to influence voiding function. In this study, we examined the effects of selective agonists for ERα [propylpyrazole triol (PPT)] and ERβ (DPN) to determine the relative importance of ER subtypes in reversing OVX changes in LUT function.

MATERIALS AND METHODS

Experimental protocols. Bilateral OVX was performed via a midline abdominal approach using aseptic techniques under isoflurane anesthesia in female Sprague-Dawley rats (body weight: 250–320 g, n = 69), as previously described (10), and physiological experiments were performed 6 wk later. Animals were treated with antibiotic (150 mg/kg im ampicillin daily) for 2–3 days postsurgery. Body weights

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bladder after voiding; and 5) VE. VE was expressed as a percentage using the following formula: \( VE = \frac{\text{voided volume}}{\text{VT - PVR}} \times 100 \).

EUS EMG activity analysis was blinded to the status of the rat. As described in a previous paper (11), various EUS EMG parameters were measured, including average bursting duration (BD), SP, AP (Fig. 1), total SP (TSP) during each voiding, and the ratio of BD to CD (expressed as a percentage). As shown in Fig. 1, the bursting period was analyzed during the time when the EMG was completely quiescent during the interval between bursts. This occurred at the time when intravesical pressure began to decline during voiding. Before this time, some phasic EMG activity could be detected, but the SP was not obvious. At least three transvesical cystometrograms (CMGs) were obtained in each animal. All parameters were calculated with the aid of Acknowledge software (BIOPAC Systems). Computed data were compiled in spreadsheets and averaged using Excel (Microsoft).

**Drugs.** Animals were divided into six groups: one untreated group (group 1) and five groups treated by daily subcutaneous injection for 1 wk with either vehicle [10% DMSO in olive oil (group 2)], PPT [an ERα-specific agonist, 1 mg·kg⁻¹·day⁻¹ (group 3)], DPN [an ERβ-specific agonist, 2 mg·kg⁻¹·day⁻¹ (group 4)] or 5 mg·kg⁻¹·day⁻¹ (group 5), Tocris, Ellisville, MO, or combined PPT and DPN [1 mg·kg⁻¹·day⁻¹ PPT and 2 mg·kg⁻¹·day⁻¹ DPN (group 6)]. The receptor specificity of these agents has been shown in previous studies by other investigators (3, 7, 19, 31, 33, 34). The doses of PPT and DPN were in the range of doses (0.5–5 mg·kg⁻¹·day⁻¹) that are commonly used in the rat to activate ERα and ERβ, respectively (7, 17, 28). The Institutional Animal Care and Use Committee of Taichung Veterans General Hospital approved the experimental protocol.

**Statistical analysis.** All data are presented as means ± SE. Statistical significance was assessed by a Mann-Whitney U-test. P values of <0.05 were considered as significant.

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**Fig. 1.** Bladder (top traces) and external urethral sphincter (EUS) electromyogram (EMG) activity (bottom traces) recorded during a continuous transvesical infusion cystometrogram in a normal anesthetized rat. A: reflex bladder contraction, indicated by an abrupt, large increase in bladder pressure, was accompanied by large-amplitude EUS EMG activity. B: the same recording indicated by the asterisk in A shown at a faster timescale. C: the period of the recording indicated by the bracket above the record in B shown at a faster timescale. Tonic EUS EMG activity preceded the large rise in intravesical pressure and shifted to a bursting pattern at the peak of bladder contraction before the onset of voiding. D: the period of the recording indicated by the bracket above the record in C shown at a faster timescale. Recordings show individual EUS EMG bursts composed of active period (APs) and silent periods (SPs) and the small fluctuations in intravesical pressure accompanying each burst. Vertical calibration: intravesical pressure (in cmH₂O); horizontal calibration: time (in min or s). The arrow in A indicates the start of saline infusion. BD, bursting duration.
RESULTS

Effect of PPT and DPN treatment on uterine and body weight in OVX animals. OVX animals treated with vehicle did not exhibit a significant change in body weight during the course of the 1-wk treatment and had uterine weights that were not significantly different from untreated control OVX animals. However, in both groups of OVX animals, the uterus and fallopian tubes were atrophied, and uterine weights (107–120 mg) were 20–25% of those (500–550 mg) of control animals of similar age and body weight (10). Treatment with PPT (1 mg·kg⁻¹·day⁻¹) for 1 wk produced a significant fourfold increase in uterine weight but also produced a significant 11% reduction in body weight (Table 1). Treatment with DPN (2 or 5 mg·kg⁻¹·day⁻¹) for 1 wk produced a smaller (30–45%) but still significant increase in uterine weight and a smaller (3–5%) decrease in body weight. Combined PPT and DPN treatment for 1 wk elicited changes that were comparable with the changes produced by PPT alone (Table 1).

Effect of PPT and DPN treatment on bladder activity. Transvesical CMGs performed 1 wk after the various treatments did not reveal significant differences in VT, micturition CA, or CD between vehicle and treatment groups with individual drugs, although VT was slightly larger (21% and 26%, respectively) in PPT (1 mg·kg⁻¹·day⁻¹)-or DPN (5 mg·kg⁻¹·day⁻¹)-treated animals. However, combined treatment with PPT and DPN significantly increased VT by 58%. PPT and combined PPT and DPN treatments significantly reduced PVR by ~50% and significantly improved VE by 35–40% (Table 2). DPN (2 or 5 mg·kg⁻¹·day⁻¹) alone did not alter RV or VE.

Effect of PPT and DPN treatment on EUS EMG activity. During continuous infusion CMGs, the EUS EMG usually consisted of low-amplitude, tonic activity during the filling phase (Fig. 1), although, in some animals, this tonic activity increased gradually as infusion volume approached the micturition VT. During a bladder contraction, EUS EMG activity markedly increased, consisting of an initial period of tonic activity interspersed with phasic activity followed by a bursting pattern of activity characterized by clusters of high-frequency spikes (AP) separated by SPs. Durations of AP and SP, BD, and TSP as well as the ratio of BD to CD were measured. Neither vehicle nor DPN (2 or 5 mg·kg⁻¹·day⁻¹) changed any of these parameters. However, after PPT or combined PPT and DPN treatment, the duration of the AP decreased (12.8–15.7%) significantly (P < 0.05). In addition, the SP, which was not altered by either drug alone, was significantly increased 17.5% by the combined PPT and DPN treatment (Table 3 and Fig. 2).

DISCUSSION

Our previous experiments revealed that OVX of 6-wk duration induced several voiding abnormalities in urethane-

Table 1. Body and uterine weight in rats treated with vehicle, PPT, or DPN

<table>
<thead>
<tr>
<th></th>
<th>Number of Rats</th>
<th>Before treatment (with vehicle, PPT, or DPN)</th>
<th>Body Weight After Treatment – Before Treatment, g</th>
<th>Uterus Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6-wk OVX)</td>
<td>15</td>
<td>383.7 ± 27.6</td>
<td>6.83 ± 3.64</td>
<td>120.6 ± 24.5</td>
</tr>
<tr>
<td>Vehicle (10% DMSO in olive oil, 7 days)</td>
<td>14</td>
<td>375.8 ± 41.6</td>
<td>11.57 ± 7.33</td>
<td>107.6 ± 11.4</td>
</tr>
<tr>
<td>PPT (1 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>11</td>
<td>382.7 ± 32.7</td>
<td>-44.73 ± 7.66*</td>
<td>431.4 ± 61.0*</td>
</tr>
<tr>
<td>DPN (2 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>12</td>
<td>370.8 ± 22.6</td>
<td>12.67 ± 5.93</td>
<td>155.4 ± 19.7*</td>
</tr>
<tr>
<td>PPT value vs. vehicle treatment</td>
<td>0.647</td>
<td>0.166</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>DPN value vs. vehicle treatment</td>
<td>0.899</td>
<td>0.781</td>
<td>0.462</td>
<td>0.000</td>
</tr>
<tr>
<td>P value vs. vehicle treatment</td>
<td>0.082</td>
<td>0.238</td>
<td>0.006</td>
<td>0.003</td>
</tr>
<tr>
<td>Combined PPT (1 mg·kg⁻¹·day⁻¹) and DPN (2 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>9</td>
<td>385.1 ± 21.9</td>
<td>43.22 ± 9.40*</td>
<td>358.8 ± 35.34*</td>
</tr>
<tr>
<td>P value vs. vehicle treatment</td>
<td>0.439</td>
<td>0.159</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are means ± SE. VT, volume threshold; CA, contraction amplitude; CD, contraction duration; PVR, postvoiding residual urine; VE, voiding efficiency [VE = (VT − PVR)/VT]. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (by Mann-Whitney U-test).

Table 2. Parameters of bladder activity in transvesical cystometrograms in rats treated with vehicle, PPT, or DPN

<table>
<thead>
<tr>
<th></th>
<th>Number of Rats</th>
<th>VT, ml</th>
<th>CA, cmH₂O</th>
<th>CD, min</th>
<th>PVR, ml</th>
<th>VE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6-wk OVX)</td>
<td>15</td>
<td>0.64 ± 0.25</td>
<td>35.29 ± 4.39</td>
<td>0.35 ± 0.07</td>
<td>0.26 ± 0.17</td>
<td>59.97 ± 17.64</td>
</tr>
<tr>
<td>Vehicle (10% DMSO in olive oil, 7 days)</td>
<td>14</td>
<td>0.61 ± 0.16</td>
<td>32.89 ± 4.54</td>
<td>0.33 ± 0.05</td>
<td>0.23 ± 0.09</td>
<td>63.22 ± 8.97</td>
</tr>
<tr>
<td>PPT (1 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>11</td>
<td>0.74 ± 0.30</td>
<td>31.53 ± 5.04</td>
<td>0.34 ± 0.05</td>
<td>0.11 ± 0.07*</td>
<td>85.17 ± 5.34*</td>
</tr>
<tr>
<td>P value vs. vehicle treatment</td>
<td>0.252</td>
<td>0.649</td>
<td>0.776</td>
<td>0.002</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>DPN (2 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>12</td>
<td>0.48 ± 0.16</td>
<td>34.38 ± 3.72</td>
<td>0.34 ± 0.06</td>
<td>0.18 ± 0.07</td>
<td>62.48 ± 9.64</td>
</tr>
<tr>
<td>P value vs. vehicle treatment</td>
<td>0.106</td>
<td>0.331</td>
<td>0.865</td>
<td>0.150</td>
<td>0.361</td>
<td></td>
</tr>
<tr>
<td>DPN (5 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>8</td>
<td>0.77 ± 0.29</td>
<td>30.44 ± 5.72</td>
<td>0.35 ± 0.08</td>
<td>0.33 ± 0.18</td>
<td>58.47 ± 15.02</td>
</tr>
<tr>
<td>P value vs. vehicle treatment</td>
<td>0.238</td>
<td>0.456</td>
<td>0.804</td>
<td>0.238</td>
<td>0.301</td>
<td></td>
</tr>
<tr>
<td>Combined PPT (1 mg·kg⁻¹·day⁻¹) and DPN (2 mg·kg⁻¹·day⁻¹ sc, 7 days)</td>
<td>9</td>
<td>1.01 ± 0.35*</td>
<td>29.20 ± 6.06</td>
<td>0.34 ± 0.07</td>
<td>0.12 ± 0.07*</td>
<td>88.38 ± 4.63*</td>
</tr>
<tr>
<td>P value vs. vehicle treatment</td>
<td>0.004</td>
<td>0.051</td>
<td>1.000</td>
<td>0.001</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. VT, volume threshold; CA, contraction amplitude; CD, contraction duration; PVR, postvoiding residual urine; VE, voiding efficiency [VE = (VT − PVR)/VT]. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (by Mann-Whitney U-test).
anesthetized rats, including 1) an increase in PVR, 2) a decrease in VE, and 3) an alteration of EUS EMG bursting activity characterized by a decrease in the duration of the SP and an increase in the duration of the AP during micturition. These changes were reversed by treatment with 17β-estradiol (E2) for 3 wk (10). The present experiments showed that 1-wk treatment of OVX rats with PPT, an agonist for ERα, also reversed the changes in PVR, VE, and AP, whereas a 1-wk treatment with DPN, an agonist for ERβ, was ineffective. Combined therapy with PPT and DPN elicited similar changes in PVR, VE, and AP, but also increased SP as well as VT for triggering micturition. These results indicate that the major deficits in LUT function after OVX are due to reduced activation of ERα leading to changes in the neural control of the EUS. However, an interaction between ERβ and ERα must be necessary for the control of SP because neither PPT nor DPN alone reversed the decrease in SP after OVX, whereas the combined treatment was effective.

| Table 3. Parameters of external urethral sphincter electromyogram activity in rats treated with vehicle, PPT, or DPN |
|-----------------------------------------------|----------|----------|----------|----------|-----------------|
| Number of Rats | SP, s | AP, s | BD, s | TSP, s | BD/CD, % |
| Control (6-wk OVX) | 15 | 0.093 ± 0.020 | 0.069 ± 0.005 | 5.06 ± 1.74 | 2.88 ± 1.16 | 24.02 ± 7.35 |
| Vehicle (10% DMSO in olive oil, 7 days) | 14 | 0.103 ± 0.019 | 0.070 ± 0.007 | 5.05 ± 1.18 | 2.98 ± 0.87 | 25.64 ± 5.03 |
| PPT (1 mg·kg⁻¹·day⁻¹ sc, 7 days) | 11 | 0.106 ± 0.019 | 0.061 ± 0.003* | 4.72 ± 1.65 | 2.98 ± 1.07 | 23.26 ± 6.55 |
| P value vs. vehicle treatment | 0.776 | 0.001 | 0.331 | 0.910 | 0.252 |
| DPN (2 mg·kg⁻¹·day⁻¹ sc, 7 days) | 12 | 0.103 ± 0.025 | 0.071 ± 0.008 | 5.18 ± 1.80 | 3.08 ± 1.28 | 25.47 ± 5.27 |
| P value vs. vehicle treatment | 0.691 | 0.910 | 0.569 | 0.865 | 0.608 |
| DPN (5 mg·kg⁻¹·day⁻¹ sc, 7 days) | 8 | 0.098 ± 0.021 | 0.069 ± 0.007 | 5.13 ± 1.60 | 3.02 ± 1.10 | 25.07 ± 7.82 |
| P value vs. vehicle treatment | 0.547 | 0.860 | 0.972 | 0.916 | 0.916 |
| Combined PPT (1 mg·kg⁻¹·day⁻¹) and DPN (2 mg·kg⁻¹·day⁻¹ sc, 7 days) | 9 | 0.121 ± 0.020* | 0.059 ± 0.004* | 5.73 ± 1.60 | 3.84 ± 1.07 | 28.18 ± 6.78 |
| P value vs. vehicle treatment | 0.043 | 0.000 | 0.357 | 0.096 | 0.471 |

Values are means ± SE. Silent period (SP) and active period (AP) denote the average duration of quiescent and tonic external urethral sphincter electromyogram activity during the bursting period [bursting duration (BD)] shown in Fig. 1. TSP, total duration of SPs during each voiding; CD, contraction duration. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (by Mann-Whitney U-test).
The correlation between the effects of combined ER agonist treatment on voiding and their effects on EUS EMG bursting support the conclusions of our earlier experiments (9, 10) demonstrating that EUS activity is a major determinant of VE in female rats. EUS EMG bursting reflects the rhythmic opening and closing of the urethral outlet to produce a pulsatile flow of urine. The two components of bursting, SP and AP, represent the periods of maximal urethral relaxation and contraction, respectively. Thus, a decrease in SP or an increase in AP, both of which occur after OVX (10), could contribute to the OVX-induced decrease in VE. Similar changes in SP and AP occur in chronic spinal cord-injured rats, which exhibit detrusor-sphincter dyssynergia and reduced VE (11). Treatment of these rats with capsaicin, which improves VE, increased SP, reduced AP, and increased total BD.

The changes in LUT function in OVX rats after treatment with PPT alone or with the combination of PPT and DPN were equivalent to those induced by E2 in the previous study (10) even though the selective agonists were administered for a shorter period (1 wk) than the E2 treatment (3 wk). This indicates that the OVX deficits in LUT function are rapidly reversible. The reversal of OVX-induced uterine atrophy was also similar after 1-wk PPT treatment (4-fold increase in uterine weight; Table 1) or 3-wk E2 treatment (3.6-fold increase), whereas DPN treatment had a much smaller effect on uterine weight (33–50% increase). This increase in uterine weight was the only parameter significantly changed by DPN alone. A recent study by another laboratory (32) showed that ERα and ERβ are expressed in uterine tissue in OVX rats and that 3-day treatment with PPT (0.5 mg/kg ip) almost doubled uterine weight. On the other hand, 3-day DPN treatment (1 mg/kg ip) did not change uterine weight or alter the effect of PPT.

The present data indicate that the E2-sensitive LUT dysfunctions that were identified in our previous study (10) in OVX rats can be divided into two groups: 1) ERα dependent and 2) ERα/ERβ dependent. ERα-dependent mechanisms affect the neural control of the EUS and, in turn, voiding, whereas ERα/ERβ-dependent mechanisms affect the neural control of the bladder as well as the EUS and influence storage and voiding.

The ERα-dependent mechanisms elicited by PPT treatment decreased PVR by 52%, increased VE by 34%, and decreased AP by 12.8%. On the other hand, E2 treatment, which should activate ERα-dependent mechanisms, produced similar changes in VE and AP but did not significantly change PVR or SP. It is likely that the lack of effect of E2 on PVR was due to the almost doubling of bladder capacity, which led to a corresponding increase in PVR despite the increase in VE. Because PPT increased VE without changing VT, the reduction in PVR was detectable in the present experiments.

It seems reasonable to attribute the effect of PPT on VE and PVR to a change in the urethral outlet because PPT did not change the amplitude or duration of bladder contractions. This is consistent with the previous finding that ERα is not expressed in smooth muscle of the female rat urinary bladder (22). However, the effect of PPT on the outlet may be mediated by multiple mechanisms in addition to the small reduction in the duration of the AP. For example, effects on the neural control of urethral smooth muscle may also contribute to the improved voiding. A recent study by Kitta et al. (18) revealed that OVX of 6-wk duration induces stress urinary incontinence associated with a reduction in EUS activity. At an earlier time point (3 wk post-OVX), urethral smooth muscle activity was also reduced; this effect was reversed by E2 treatment. However, ERα is not expressed in urethral smooth muscle of the female rat (22). Thus, any additional effect of PPT on urethral smooth muscle activity that might contribute to its effects in OVX rats would have to be mediated by a change in neural control rather than a direct effect on the muscle.

On the other hand, longer survival times after OVX may influence bladder smooth muscle as well as the innervation of the bladder. Zhu et al. (46) reported that OVX of 4-mo duration in 13- to 14-mo-old female Fisher rats induced pathological changes in the detrusor muscle, axonal degeneration in the detrusor muscle, and a 40–50% reduction in carbachol-induced contractions of bladder smooth muscle. These changes would be expected to enhance the defect in VE observed in the present experiments.

The ERα/ERβ-dependent mechanisms activated by combined PPT-DPN treatment produced a significant 17.5% increase in the duration of the SP (Table 3), an effect that exceeded the 12.5% increase elicited by E2 (10). These data indicate that the enhancement of SP requires a synergistic interaction between the two types of ER because neither agent alone altered SP. However, combination treatment did not enhance the effect of PPT on VE or AP.

Combination therapy also elicited a marked increase (66%) in VT for triggering micturition. This effect, which mimicked the effect of E2, is unusual because OVX did not change VT. This suggests VT is not normally controlled by ovarian hormones but that supraphysiological doses of E2 acting simultaneously on ERα and ERβ either raise the set point of the central micturition gating circuit or suppress the bladder afferent activity that activates voiding. ERs are expressed in primary afferent neurons and at sites in the central nervous system involved in the control of the bladder (6, 12, 13, 23, 27, 40–42). Estrogen treatment is known to inhibit TRPV1 (45) and P2X3 (21) channel currents that have an important excitatory role in bladder sensory pathways (8, 14, 16, 29) and also modulate central monoaminergic neurotransmitter mechanisms that are known to play a role in voiding function (2, 4, 20, 26, 38, 44). Thus, the change in VT induced by activity of ERα/ERβ could be mediated by complex actions at multiple sites in the nervous system.

In summary, our results indicate that the reduced VE in OVX rats can be reversed by 1-wk hormone replacement therapy that selectively targets ERα and reduces the duration of the AP during EUS bursting. Combined pharmacological activation of ERα/ERβ further enhances EUS bursting by increasing the SP and also facilitates bladder storage mechanisms by increasing VT.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

Author contributions: C.-L.C. and W.C.d.G. contributed to the draft manuscript; C.-L.C. and W.C.d.G. contributed to the revised manuscript; C.-L.C. and W.C.d.G. contributed to the performed experiments; C.-L.C. and W.C.d.G. contributed to the data analysis; C.-L.C. and W.C.d.G. contributed to the intellectual content of the manuscript; C.-L.C. and W.C.d.G. contributed to the final version of the manuscript.

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