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

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Cheng CL, de Groat WC. Effects of agonists for estrogen receptor α and/or 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 ureter weight fourfold and decreased body weight by 11%. DPN increased ureter 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.

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were measured 5 and 6 wk post-OVX. Uterine weights were also measured at the end of the study. 

Physiological investigation. Experiments were performed under urethane anesthesia (1.1 g/kg sc). A femoral vein was catheterized for fluid administration. Body temperature was maintained between 36 and 38°C with a heating lamp. The urinary bladder was exposed via a midline abdominal incision, and the rostral half of the pubic symphysis was removed to expose the midurethra and EUS. Fine insulated silver wire electrodes (0.05-mm diameter) with exposed tips were inserted into the EUS on the lateral sides of the midurethra. EUS EMG activity was displayed on an oscilloscope and a paper recorder along with bladder pressure and also recorded on a computer. The EUS EMG was attributed to striated muscle because activity was eliminated after neuromuscular blockade with pancuronium bromide (9). A polyethylene tube 60 (1.0-mm inner diameter and 1.5-mm outer diameter) was inserted into the bladder lumen and tied in place, and the abdominal wall was closed. The polyethylene tube was, in turn, connected via a three-way stopcock to an infusion pump and a three-way stopcock to a transvesical catheter, and the system was filled with physiological saline solution. Urodynamic examination usually began 3–4 h after the induction of anesthesia. After the bladder was emptied, transvesical cystometrogram was performed at an infusion rate of 0.123 ml/min with saline at room temperature. Fluid release from the urethral orifice was recorded with a video camera to determine the beginning and end of voiding. The infusion pump was turned off after the induction of a voiding contraction, and residual volume was measured by empyting the bladder by pressure on the abdominal wall. The following parameters were measured: 1) volume threshold (VT), the volume of saline sufficient to induce bladder contractions exceeding a pressure of 15 cmH2O; 2) contraction amplitude (CA), the maximal intravesical pressure during voiding; 3) contraction duration (CD), duration of a voiding contraction; 4) PVR, the volume of saline withdrawn from the bladder after voiding; and 5) VE, VE was expressed as a percentage using the following formula: VE = voided volume × (VT – PVR)/VT × 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 EUS 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 computer 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.
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-
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 ERβ, also reversed the changes in PVR, VE, and AP, whereas a 1-wk treatment with DPN, an agonist for ERα, was ineffective. Combined therapy with PPT and DPN elicited similar changes in PVR, VE, and AP, whereas 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

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

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).

Fig. 2. Effects of different treatments on intravesical pressure (top traces) and EUS EMG activity (bottom traces) during constant infusion cystometrograms in 5 rats (A–E) 6 wk after ovariectomy (OVX). On the left (A1–E1), the records are at a slow timescale, and the middle tracings show baseline intravesical pressure. Part of the same recordings illustrating the period during voiding are shown at two faster timescales in the records on the right (A2–E2). Recordings were obtained in an OVX untreated rat (A), a vehicle-treated rat (B), after 1-wk treatment with propylipyrazole triol (PPT; C), after 1-wk treatment with diarylpropionitrile (DPN; D), and after 1-wk treatment with combined PPT and DPN (E). Vertical calibration: intravesical pressure (in cmH₂O); horizontal calibration: time (in min or s). Note treatment with PPT decreased the duration of the AP of the EUS EMG bursting (C3), whereas combined treatment with PPT and DPN increased the volume threshold (E1), increased the duration of the SP (E3), and decreased the duration of the AP of EUS EMG bursting activity (E3).
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. 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 activation 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. conception and design of research; C.-L.C. performed experiments; C.-L.C. analyzed data; C.-L.C. and W.C.d.G. interpreted results of experiments; C.-L.C. prepared figures; C.-L.C. and W.C.d.G. drafted manuscript; C.-L.C. and W.C.d.G. revised manuscript; C.-L.C. approved final version of manuscript.

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