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Influence of estradiol on micturition thresholds in the rat: involvement of the hypogastric nerve

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Abstract

Studies have shown that the severity of bladder hyperreflexia induced by acute bladder inflammation varies with the ovarian cycle. These results suggest that the hyperreflexia is modulated by ovarian hormones. Other studies have suggested that such modulation involves the bladder's sympathetic innervation. These hypotheses were tested by assessing the development of bladder hyperreflexia in urethane-anesthetized rats subjected to different hormonal manipulations with or without bilateral hypogastric neurectomy (HYPX). The groups included sham ovariectomy (sham OVX), ovariectomy (OVX), OVX with estradiol replacement (OVX+E), OVX+HYPX, and OVX+HYPX+E. Assessments were performed using repeated cystometrograms (CMGs) to measure micturition thresholds (MT) before and hourly for 3 h after intravesicular treatment with 50% turpentine oil (or olive oil in an OVX+E control group). In the uninflamed bladder, treatment with estradiol increased MTs in the OVX+E group compared with the OVX group. As expected, bladder inflammation induced bladder hyperreflexia in sham OVX rats (studied in estrus). This hyperreflexia was eliminated by OVX and restored by either estradiol replacement or HYPX. Combining estradiol replacement and HYPX (i.e., OVX+E+HYPX) did not increase the severity of bladder hyperreflexia compared with either manipulation alone. These results indicate that the bladder hyperreflexia that is induced by bladder inflammation requires the presence of estradiol and suggest that this hormonal modulation is exerted via the sympathetic control of the bladder, possibly via an increase of β -adrenergic inhibitory actions on the detrusor muscle. Similar mechanisms may contribute to bladder disorders in postmenopausal women.

Keywords

Inflammation; lower urinary tract; ovariectomy

ALTHOUGH BLADDER REFLEXES in healthy women remain stable across the menstrual cycle (37), symptoms and signs of bladder dysfunction are exacerbated perimenstrually (13, 33). Thus it appears that the rapid changes in ovarian hormones that occur perimenstrually can increase the severity of symptoms and signs of bladder dysfunction. This conclusion is consistent with findings that suppression of the menstrual cycle can alleviate symptoms (17).

The situation is somewhat different for postmenopausal women, whose bladders and urethras exhibit a loss of estrogen but not progesterone receptors (26). About one-third of postmenopausal women develop overactive bladder (25). Although it remains uncertain whether the loss of bladder and urethral estrogen receptors and reduction in plasma estrogens in postmenopausal women underlies their bladder overactivity, the conclusion is partially

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supported by the fact that estrogen treatment, particularly if delivered intravaginally rather than systemically, can alleviate symptoms (12,23,25,27,34).

These clinical findings indicate that the hormonal milieu influences bladder function. Evidence from animal research provides further support. In rodents as in women, bladder reflexes of the healthy bladder do not vary with the estrous cycle (8,15). On the other hand, the severity of bladder hyperreflexia induced by bladder inflammation does vary, being more severe in association with the rapid hormonal changes during the periovulatory period (proestrus/estrus) than in other stages of the cycle (metestrus/diestrus; Ref. 15).

Loss of ovarian hormones produced by ovariectomy (OVX) and estrogen replacement (OVX +E) in the rat produce distinct effects on bladder and urethral reflex activity (31). Depending on age, these manipulations also affect responses of the detrusor muscle to cholinergic stimulation (4). Furthermore, OVX+E increases water consumption and micturition volumes but not micturition frequency (19). In mice lacking either one or both estrogen receptor subtypes compared with wild types, there are no strain differences in urodynamic parameters in healthy bladders; but after treatment of the bladder with capsaicin, signs of bladder hyperreflexia fail to appear in mice lacking estrogen receptor- α (29).

Together, these results indicate that reproductive status and hormonal milieu exert potent influences on lower urinary tract function and on responses of the bladder to pathophysiology in both women and experimental animals. In particular, they suggest that loss (or suppression) of estrogens masks and the presence of estrogens increases the bladder's vulnerability to inflammation-induced bladder hyperreflexia.

The means by which these reproductive and hormonal influences occur are poorly understood. One possibility is that regulation of detrusor activity by the hypogastric nerve is involved. Studies have shown that activation of the hypogastric nerve inhibits the detrusor muscle (3) and that acute hypogastric neurectomy (HYPX) increases motility of the healthy bladder (6). Furthermore, relaxation of the detrusor muscle by β_3 -adrenergic agents, whose major source is the hypogastric nerve (3,24), is increased by hormonal manipulations such as OVX and decreased by OVX+E (21,32,40). Estrogen treatment influences hypogastric nerve actions on the bladder (28).

It is therefore possible that when the bladder is inflamed, the presence of sufficient estradiol levels reduces the hypogastric nerve's inhibition of detrusor reflexes and that depletion of estradiol augments the inhibition. The present study tested this hypothesis in urethane-anesthetized rats by assessing the influence of OVX and OVX+E with or without HYPX on volume voiding thresholds (micturition thresholds, MTs) measured using cystometry before and after bladder inflammation with intravesicular turpentine (7,22). If the hypothesis is correct, OVX should reduce the severity of inflammation-induced bladder hyperreflexia only when the hypogastric nerve is intact, and estradiol replacement should prevent this effect.

METHODS

Animals

Adult female Sprague-Dawley rats weighing 250–300 g were used. They were individually housed in chip-bedded plastic cages and maintained on a 12:12-h light-dark cycle. Each rat's reproductive status was assessed by daily vaginal smears for at least 3 wk before the experiment. Estrous stage was defined using the traditional stage assessment protocol (see Fig. 3 in Ref. 2). The study was approved by Florida State University's Institutional Animal Care and Use Committee.

Experimental groups

Seven to 10 days before the day of the experiment, rats that had exhibited at least two regular 4-day estrous cycles were subjected to OVX, sham OVX, or OVX+E. Some rats in the OVX and OVX+E groups also underwent HYPX performed as described previously by Temple et al. (36). During the experiment, the bladder was treated with either turpentine (inflammation) or olive oil. These preparations resulted in six experimental groups of 8–13 rats/group: 1) sham OVX, bladder inflammation; 2) OVX only, bladder inflammation; 3) OVX + HYPX, bladder inflammation; 4) OVX+E, bladder inflammation; 5) OVX+E+HYPX, bladder inflammation; and 6) OVX+E, olive oil treatment.

Surgical procedures for OVX, sham OVX, OVX+E, and HYPX rats

Rats were anesthetized intraperitoneally with ketamine and xylazine anesthesia (73 and 9 mg/kg, respectively). A ventral midline incision was made to expose the ovaries, which were then removed (OVX) or identified and left in place (sham OVX). In some OVX rats, a silastic capsule (Dow Corning, Midland, MI) filled with 17β -estradiol was inserted subcutaneously into the nape of the neck. The capsule was 5 mm in length with a 1.57-mm internal diameter. Capsules of this size have been shown to maintain a steady ~50–60 pg/ml 17β -estradiol plasma level (18). In some OVX and OVX+E rats, the left and right hypogastric nerves were identified as they exited the inferior mesenteric ganglion, and a 5-mm section of each nerve was cut out. The abdominal incision was then sutured in layers. Hormonal status was verified by daily vaginal smears after the surgery. OVX rats produced a continued daily presence of leukocytes (diestrus-like smear pattern); OVX+E rats produced a continuous presence of cornified cells (estrus-like smear pattern); in sham OVX rats, the 4-day cyclical pattern did not change.

Cystometrograms

MTs were measured by obtaining repetitive cystometrograms (CMGs) before and after treatment of the bladder with turpentine or olive oil. The methods have been described in detail elsewhere (5). Briefly, on the day of the experiment, the rats were anesthetized with urethane (1.2 g/kg intraperitoneal injection). Rats in the group that cycled (sham OVX) were studied on the day of estrus. Body temperature was maintained at $\sim 37^{\circ}\text{C}$ throughout the experiment using a heating pad and warming lamp. The bladder was catheterized transurethraly with polyethylene tubing (outer diameter, 0.97 mm) with the tip placed within the lumen so that it did not touch the bladder wall. A suture was tied around the surrounding skin to prevent leakage. An abdominal incision was made in the lower abdomen to expose the bladder so that it could be emptied by gentle pressure and suction (using a cotton wick placed in the open end of the catheter) before each CMG was obtained. The incision was covered by a gauze pad soaked in warm saline. The catheter was connected to a small-volume pressure transducer whose signals were amplified and relayed to strip chart and videotape recorders for offline analyses.

Each CMG was obtained by slowly filling the bladder (0.055 ml/min). The MT was defined as the intraluminal volume that produced the first micturition contraction.

Two or three CMGs obtained at 10-min intervals were run to establish a baseline MT, and then the bladder was filled with 0.7 ml of 50% turpentine oil (in olive oil) or olive oil. The bladder was emptied 1 h later, and the first posttreatment CMG was obtained. CMGs were then obtained twice more at hourly intervals.

Data analyses

Comparisons of the effects of the various manipulations on baseline MTs were assessed using one-way ANOVA. When significant, ANOVA were followed by Bonferroni post hoc tests. Differences in MTs before and after turpentine or olive oil treatment were analyzed using

repeated-measures ANOVA followed, as appropriate, by Bonferroni post hoc tests. Significance was set at $P < 0.05$.

RESULTS

Uninflamed bladder (baseline)

MTs in the sham OVX group (run in estrus) were similar to those reported previously in intact, normally cycling rats in estrus (15). As shown in the comparison of baseline MTs among four groups in Figs. 1A and 2A, OVX had no influence on MTs of the uninflamed bladder compared with the sham OVX condition. In contrast, OVX+E rats demonstrated significantly increased MTs compared with the OVX and OVX+HYPX groups ($P < 0.05$).

Influence of OVX and OVX+E on bladder inflammation

As shown in Fig. 1, treatment of the bladder with turpentine induced a significant, progressive decrease in MTs in sham OVX rats in estrus that was similar in time course and magnitude to the decrease previously reported from regularly cycling rats in estrus (15). MTs in OVX rats, however, did not decrease significantly after turpentine treatment (Fig. 1A). As a result, the reduction in MTs in OVX rats was significantly less than that in sham OVX rats ($P < 0.005$) (Fig. 1B).

Estrogen replacement restored the inflammation-induced reduction in MTs to a level that did not differ significantly from that in the sham OVX group (Fig. 1, A and B).

Influence of HYPX on bladder inflammation

As shown in Fig. 2, HYPX, like estradiol replacement, restored bladder hyperreflexia in OVX rats, so that the reduction in MTs in OVX+HYPX rats did not differ significantly from that observed in sham OVX rats (Fig. 1). Combining HYPX and estradiol replacement with ovariectomy did not affect the inflammation-induced reduction in MTs produced by estradiol replacement. However, differences in MTs in OVX+E and OVX+E+HYPX rats were significantly different from those in OVX rats ($P < 0.005$) (Fig. 2B).

Control treatment of bladder

As expected, MTs after treatment of the bladder with olive oil did not change significantly over time (Fig. 1).

DISCUSSION

The present results show that treatment of the ovariectomized rat with 17β -estradiol for 7–10 days increased MTs of the uninflamed bladder. The results also show that the bladder hyperreflexia that is produced by treating the bladder with turpentine (i.e., reduction in micturition thresholds) in cycling rats in estrus is eliminated after ovariectomy and restored by estrogen replacement. The results suggest that the hypogastric nerve contributes to these effects.

Uninflamed bladder

OVX has been found to decrease the responsiveness of isolated bladder muscle strips to cholinergic stimulation, to decrease maximum urine flow rate and voiding frequency, and to increase urethral opening pressure (4). These results led the authors to conclude that OVX compromises either the urethral wall musculature or urethral innervation. In the present study, we found that OVX had no effect on baseline MTs compared with regularly cycling rats. Because the intraluminal bladder pressure in the present study was measured via a transurethral

catheter, the MT depended mostly on the motility of the bladder itself. Thus the influence of OVX may be less on the detrusor muscle than on urethral activity. In contrast to the lack of effects of OVX, however, estradiol replacement significantly increased baseline MTs, which is consistent with the report that estradiol increases micturition volumes compared with OVX and sham OVX rats (19). Furthermore, long-term estrogen replacement has been shown to increase bladder contractile responsiveness to carbachol compared with sham OVX rats (19). These findings are consistent with urodynamic assessments in postmenopausal women with and without estrogen replacement that indicate that estrogen replacement increases bladder sensory threshold (9).

Bladder inflammation and estradiol

Consistent with our earlier findings in regularly cycling intact rats in estrus (15), bladder inflammation in the present study induced significant bladder hyperreflexia in sham OVX rats in estrus. In the same earlier study, we also found that the severity of hyperreflexia induced by bladder inflammation decreased during the 2 days following the acute fall in ovarian hormones, that is, during metestrus and diestrus (15). This reduction is consistent with our finding in the present study that 7–10 days after OVX, inflammation-induced bladder hyperreflexia was significantly reduced.

The β_3 -adrenoreceptor has recently been identified in the rat bladder (10). In the bladder of the rat and other species, activation of this receptor relaxes smooth muscles (10,20,30,38,39). Thus, in a rat model of bladder hyperactivity, the β_3 -adrenoreceptor agonist CL316,243 inhibits bladder hyperreflexia and detrusor instability (38), increases micturition interval, and decreases micturition volume in rats with prostaglandin E₂-induced bladder hyperreflexia (35).

Recent studies by others have shown that OVX increases the relaxant effect of a β_3 -adrenoreceptor agonist on detrusor muscles and that estradiol replacement abolishes this increase (21,32). Thus one possible means by which the low ovarian hormone levels that result from OVX could decrease inflammation-induced bladder hyperreflexia is via an increase in β_3 -adrenergic activity. Because the opposite condition created by estradiol replacement restored hyperreflexia, it is possible that estradiol tonically suppresses β_3 -adrenergic activity.

Bladder inflammation and HYPX

Similarly to OVX+E, OVX+HYPX also restored inflammation-induced bladder hyperreflexia compared with OVX alone. That HYPX added to OVX+E did not increase the hyperreflexia even further than either manipulation alone suggests that estradiol and HYPX could reinstate inflammation-induced bladder hyperreflexia in the OVX rats via the same mechanism, i.e., by decreasing β_3 -adrenergic activity. The converse might also apply, i.e., that inflammation-induced activity in hypogastric nerve fibers relaxes the estrogen-depleted bladder by increasing β_3 -adrenergic activity. Consistent with this possibility is that application of noradrenaline inhibits contractions of guinea pig bladder strips that are induced by stimulating the hypogastric nerve (11).

Acute activation of hypogastric nerve activity has been shown to inhibit micturition (3), while acute HYPX reduces micturition thresholds (6). It is therefore possible that the development of inflammation-induced bladder hyperreflexia involves activity in hypogastric nerve fibers, particularly sensory afferents. Thus OVX could reduce the bladder hyperreflexia induced by bladder inflammation by influencing hypogastric nerve activity. However, the fact that OVX did not reduce baseline MTs (i.e., MTs in the uninflamed bladder), an effect that one would expect if the sensitivity of the hypogastric nerve to sensory stimulation changed, is inconsistent with this possibility.

Clinical relevance

These results may be relevant to women's health. Postmenopausal women have a higher risk of urinary tract infections (14). Despite such a risk, there is evidence to suggest that the bladder in postmenopausal women may not respond to inflammation as it does in young females. Thus postmenopausal women have been found to void with weaker detrusor contractions compared with premenopausal women (16), which may at least be partly due to a decreased intramural bladder thickness in postmenopausal women, which positively correlates with the circulating estradiol level (1). As the present results suggest, hormonal depletion may also lead to a decreased response of the bladder to bladder inflammation, possibly because of an abnormal relaxant response to hypogastric nerve activation. If so, then postmenopausal women may be at risk of having bladder inflammation go unnoticed because bladder hyperreflexia is a major problem associated with this condition.

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Fig. 1.

Effects of ovariectomy (OVX) with or without estradiol replacement (OVX+E) on the development of inflammation-induced bladder hyperreflexia. *A*: micturition thresholds (MTs) measured before and 1, 2, and 3 h after treating the bladder with turpentine (inflammation) or olive oil. $*P < 0.05$ compared with baseline in the OVX+E group. *B*: decreases in micturition thresholds (MTs) before and after inflammation as specified in *A*. $*P < 0.05$; $**P < 0.01$, compared with oil control; $\#P < 0.05$, $\##P < 0.01$, and $\###P < 0.005$, compared with OVX rats.

Fig. 2.

Effects of hypogastric neurectomy (HYPX) on the development of inflammation-induced bladder hyperreflexia. *A*: MTs measured before and 1, 2, and 3 h after treating the bladder with turpentine. * $P < 0.05$, compared with baseline in OVX+E group. *B*: differences in the MTs before and after inflammation as specified in *A*. # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.005$, compared with OVX rats.