Estrous influences on micturition thresholds of the female rat before and after bladder inflammation

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Johnson, O. L., and K. J. Berkley. Estrous influences on micturition thresholds of the female rat before and after bladder inflammation. Am J Physiol Regulatory Integrative Comp Physiol 282: R289–R294, 2002.—Recent evidence suggests that lower urinary tract functions may be influenced by reproductive status, particularly under pathophysiological conditions. This study used repeated cystometrograms via a transurethral catheter to investigate the influence of estrous stage on micturition thresholds before and after turpentine-induced bladder inflammation in urethane-anesthetized female rats. Whereas there were no estrous influences on micturition threshold in the uninflamed bladder, micturition thresholds after bladder inflammation were significantly lower in rats in proestrus or estrus than in rats in metestrus or diestrus. Furthermore, the risk that the initial urethral catheterization and preinflammation cystometrogram would produce hematuria was significantly lower in estrus than in the other stages. These estrous influences are not readily explicable by levels of ovarian hormones at the time of testing and may relate instead to dynamic interactions between these hormones and other neuroactive molecules. In addition, the results here have relevance to interpretations of cystometrographic findings in the clinic and basic research.

NATURAL VARIATIONS in the reproductive status of females, such as those associated with the ovarian cycle, can influence functions of the lower urinary tract (25). For example, ~40% of premenopausal women with regular menstrual cycles report cyclical changes in their urinary symptoms (12). In addition, detrusor instability measured by cystometry increases with time after menstruation in a manner that reflects increasing progesterone levels (12). Furthermore, under inflammatory conditions of the bladder, such as interstitial cystitis, urinary urge and frequency symptoms are exacerbated perimenstrually (36).

Some studies related to this issue have been done using rodents. Spontaneous and nerve stimulation-induced muscular activity of the urinary bladder vary during the estrous cycle of the guinea pig (18), and the susceptibility of the bladder wall to cyclophosphamide-induced inflammation is greatest for rats in the estrous period after ovulation (3).

Cystometric studies in rats have shown that when a mild irritant such as turpentine is applied intravesicularly, the result is long-lasting bladder hyperreflexia (22). As the experimental bladder inflammation progresses over the course of several hours, so does the associated hyperreflexia, which is recognized by a decrease in micturition threshold (MT) and an increase in the number of contractions during a fixed amount of fluid infusion (7). None of the studies using cystometry, however, has examined the influence of reproductive status.

The female rat typically has a 4-day estrous cycle, divided into metestrus, diestrus, proestrus, and estrus. Estrogen and then progesterone levels surge during proestrus (in preparation for ovulation) and by midestrus (after ovulation) return to basal levels (31). The purpose of the present study was to assess the influence of estrous stage on the capacity of the uninflamed bladder as well as on the development of hyperreflexia after turpentine-induced inflammation.

METHODS

Animals. Female Sprague-Dawley rats (200–250 g body wt) were individually housed in hanging cages and maintained on a 12:12-h light-dark schedule with free access to chow and water. Estrous stage was assessed by daily vaginal smears 2 h after lights on. Only rats that had at least two consecutive regular 4-day cycles before the day of the experiment were used, and assessments of bladder capacity were made 3–7 h after lights on.

Experimental and control groups. There were four experimental groups and two control groups: the bladders of experimental animals were infused with 50% turpentine in olive oil after the baseline cystometrograms (CMGs) had been run, and the bladders of the control animals were infused only with oil. Experimental groups consisted of rats in metestrus, diestrus, proestrus, or estrus. The two oil control groups, tested to assess the effects of repeated CMGs as opposed to chemically induced inflammation, consisted of rats in metestrus and estrus. Subsequently, an additional baseline study was added (see Additional study).

Experimental procedures. The general experimental protocol was similar to that of Dmitrieva and colleagues (7). Repeated CMGs were done in each rat to compare MTs before and during a 3-h period after bladder inflammation had been induced by intravesicular turpentine. Representative CMGs...
from rats in each of the estrous stages are shown in Fig. 1. Experimenters were blind to the rat's estrous stage until after the data had been analyzed.

Surgery and CMGs. For each experiment, the rat was anesthetized with urethane (1.2 g/kg ip). Body temperature was maintained at ~37°C by a heating pad and warming lamp. The bladder was catheterized transurethrally with 1.1-mm-diameter polyethylene tubing, with the tip of the catheter placed ~2 mm within the bladder lumen so that it did not contact the bladder walls. A suture was tied around the skin where the catheter enters the urethra to secure the position of the catheter and prevent fluid leakage from around the catheter. This single catheter was used to fill the bladder and to measure bladder pressure. A 2- to 3-cm incision was made on the ventral midline to expose the bladder, so that it could be emptied completely before each CMG. The bladder was kept moist throughout the study with saline-dampened pads covering the incision. The bladder was catheterized transurethrally with saline-moistened cotton-tipped applicators. The bladder was kept moist throughout the study with saline-dampened pads covering the incision. The signal from the catheter was amplified and relayed to a videotape recorder and, after analog-to-digital conversion, to a computer that had been programmed locally for data collection. The urethral catheter was then disconnected from the transducer so the bladder could be emptied by natural drainage, gentle suction with absorbent cotton pledgets, and gentle pressure on the bladder with a moist cotton-tipped applicator.

Two baseline CMGs were obtained before inflammation, with a 5-min interval between them. The second measure was used as the preinflammation baseline. If the fluid removed from the bladder before or after either of the baseline measures was bloody (i.e., obvious hematuria), the data were not used in the final analysis. Of a total of 106 rats, this elimination process created a final group of 72 (experimental groups: 14 in metestrus, 13 in diestrus, 12 in proestrus, and 12 in estrus; oil control group: 9 in metestrus, 16 in diestrus, 8 in proestrus, and 11 in estrus). In these rats there was no limit on saline infusion; instead, saline was infused until two micturition contractions occurred. Although, as expected, the overall means in the extra baseline group were slightly higher than those in the experimental baseline group (the MT of the 4 estrous stages of the additional baseline group averaged 0.74 ml while the MT of the 4 estrous stages of the original group averaged 0.62 ml), the MTs in the two baseline groups did not differ significantly from each other when the estrous stages were grouped together or analyzed separately (P = 0.951, grouped together; P = 0.917 during metestrus; P = 0.220 during diestrus; P = 0.336 during proestrus; P = 0.849 during estrus).

Baseline CMGs. There were no significant differences in MTs across the estrous cycle in either of the two baseline groups (Fig. 2; P = 0.43 for the group in which maximum volume was limited to 0.75 ml; P = 0.336 during proestrus; P = 0.220 during diestrus; P = 0.917 during metestrus; P = 0.951, grouped together).

Fig. 1. Representative cystometrograms (CMGs). Each column shows results of 4 CMGs from 1 rat. Top to bottom: baseline, 1 h after inflammation, 2 h after inflammation, and 3 h after inflammation.
Fig. 2. Average micturition threshold before and after intravesicular infusion of turpentine (metestrus, diestrus, proestrus, and estrus groups) or the oil solvent [oil control metestrus (M) and oil control proestrus (P) groups]. *Significantly lower than that stage's baseline MT. †Significantly lower than that stage at 1 h postinflammation. ‡Significantly lower than that stage at 1 h postinflammation. ‡‡Significantly lower than that stage at 1 h postinflammation.

Hematuria. Whereas before inflammation 27.5–37.5% of rats in metestrus, diestrus, and proestrus developed severe hematuria, only 11.1% of rats in estrus developed severe hematuria, demonstrating a significantly greater risk for hematuria in metestrus, diestrus, and proestrus than in estrus ($\chi^2$, $P = 0.025$).

**DISCUSSION**

Whereas MTs in the uninflamed bladder were not influenced by estrous stage, there were significant estrous differences in the vulnerability of the bladder to become hyperreflexic after turpentine-induced inflammation. Specifically, rats in proestrus and estrus developed more severe hyperreflexia than did rats in metestrus and diestrus. In addition, the risk that the preinflammation CMG would produce hematuria was lower in estrus than in the other estrous stages.

**Estrous influences on inflammation-induced hyperreflexia.** The estrous variations in hyperreflexia induced by intravesicular turpentine observed here are not easily predicted by estrous variations in ovarian hormone levels. The amount of hyperreflexia was similar in proestrus and estrus, yet estrogen levels are high in proestrus but very low in regularly cycling rats in estrus (31). Similarly, the amount of hyperreflexia in diestrus and metestrus was low, yet there are moderately low estrogen levels in diestrus but moderately low levels of estrogen and progesterone levels in metestrus (31). This situation indicates that the mechanisms underlying estrous differences in hyperreflexia vulnerability are not a direct result of current ovarian hormone levels. Instead, the mechanisms may relate to differences in how ovarian hormones and neuroactive molecules interact over time throughout the ovarian cycle.

**Nerve growth factor.** One possible mechanism that could underlie inflammation-induced hyperreflexia during proestrus is hormonal modulation of the actions of nerve growth factor (NGF) on afferent fibers supplying the bladder or on the bladder smooth muscle itself. The NGF receptor trkA is expressed by 75–90% of sensory afferents innervating the urinary bladder (2), as well as by bladder mast cells; bladder mast cells invade the inflamed bladder, and their degranulation by NGF causes further inflammation (13). Furthermore, instillation of NGF into the bladder results in bladder hyperreflexia that is similar to the hyperreflexia produced by turpentine inflammation (7), although this effect may not occur in all rodent species (4). Furthermore, turpentine-induced hyperreflexia, as well as the referred hyperalgesia it produces, is reversed by systemic pretreatment with trkA-IgG fusion proteins that interfere with NGF’s action (16, 23).

Recent evidence suggests that NGF, trkA, and p75 levels are hormonally regulated. Studies on dorsal root ganglion cells show that estrogen receptors (ERs) colocalize with trkA and p75 receptors and that trkA and p75 mRNA is increased during proestrus relative to other estrous stages (32), decreased after ovariectomy, and increased after estrogen replacement (24). Finally,
increased numbers of ER-positive mast cells, which have the capability of releasing NGF in multiple systems (6, 9, 38), as well as elevated NGF levels are two characteristics of the disease interstitial cystitis, an inflammatory disease of the bladder that affects mainly women and has symptoms that fluctuate with the menstrual cycle (36).

Choline acetyltransferase. One possible mechanism that could underlie the inflammation-induced hyperreflexia of estrus is the influence of estrogen on choline acetyltransferase (ChAT) in central neural pathways that control micturition. The ChAT gene contains an estrogen response element that binds ER-α and ER-β (14). In some areas of the basal forebrain, ChAT mRNA levels fluctuate across the estrous cycle, are downregulated by ovariectomy, and are upregulated by exogenous estrogen treatment (10, 11, 24), suggesting that estrogen and acetylcholine increase and decrease together. A recent study showed that acetic acid-induced bladder inflammation results in increased bladder reflexes, which are inhibited by the muscarinic agonist oxotremorine, and that this inhibition is reduced by the central muscarinic antagonist atropine but not by the peripheral muscarinic antagonist scopolamine methylbromide (34). These authors further showed that the central cholinesterase inhibitor physostigmine inhibited bladder reflex activity, but the peripheral cholinesterase inhibitor neostigmine did not. Together, these data suggest that inflammation-induced bladder reflexes could be inhibited centrally via the muscarinic acetylcholine system. Because estrogen and, therefore, acetylcholine are at low circulating levels during estrus, the ability of this system to counter the hyperreflexic effects of bladder irritation is limited, and the result would be hyperreflexia in estrus.

Proestrus and diestrus. The lack of hyperreflexia that was seen during metestrus and proestrus suggests that a mechanism exists to protect the bladder during these stages. Unlike the periovulatory time of proestrus and estrus, when estrogen, progesterone, luteinizing hormone, and follicle-stimulating hormone first surge to stimulate ovulation and then rapidly drop to basal levels, there are no large and rapid hormonal fluctuations during metestrus and diestrus. Instead, hormone levels of estrogen and progesterone (metestrus) or estrogen (diestrus) are slowly rising during these periods. It is therefore possible that gradual increases in estrogen or estrogen and progesterone protect the bladder during metestrus and diestrus while rapid hormonal fluctuations associated with proestrus and estrus contribute to the hyperreflexia. Consistent with this possibility is that long-term, but not short-term, estrogen replacement after ovariectomy decreases trkA mRNA levels in dorsal root ganglion neurons (19, 20). Similarly, in women with urinary symptoms of frequency or urgency, abnormal CMG findings are least likely to be observed during the luteal phase of their cycle (30), i.e., when estrogen and progesterone levels are slowly rising.

Lack of estrous influence on MTs in the uninflamed bladder. The finding here that bladder capacity of the uninflamed bladder does not vary with estrous stage indicates that, in the absence of an inflammatory challenge, the rapid changes in hormone levels associated with the ovarian cycle are not sufficient to influence the central or peripheral mechanisms that control bladder reflexes.

Risk of hematuria during CMGs. The risk of hematuria in the uninflamed bladder sometime during the initial catheterization and baseline CMG procedure was lower in estrus than in metestrus, diestrus, or proestrus. The latter three stages are characterized by higher circulating estrogen levels than in estrus (31). One possible explanation for the lower risk of hematuria in estrus could be estrogen’s ability to promote vascularization (17). ERs have been found in the lower urinary tract of female rats, rabbits, and humans, specifically in the areas of the urethra and trigone, the entry point of the catheter (15, 28, 29). Furthermore, it has been shown in rats that estrogen administration after ovariectomy provokes metaplasia and hyperplasia as well as increased vascularization throughout most of the urinary tract, including the proximal urethra (8, 33). It is therefore possible that the urethra and trigone are less vascularized during estrus than during the other stages and that this lowered vascularization made the tissue less vulnerable to damage by the catheter.

Influence of anesthesia. The relevance of the estrous effects observed here to the conscious animal is uncertain. A wide range of drugs have been reported to affect micturition reflexes in rats (39, 26), whereas others do not (26). With respect to urethane, some studies have shown that it reduces bladder hyperreflexia induced by occlusion of the middle cerebral artery (40, 41). However, other studies using chronically implanted bladder catheters [rats (27)] or transurethral catheters [guinea pigs (21)] have shown that urethane has little effect on CMG parameters. In the present study, all groups were anesthetized similarly. Therefore, any influence of urethane would apply equally to all of them, thus eliminating urethane as a confounding variable, unless of course urethane’s pharmacokinetics or pharmacodynamics are influenced by reproductive status, a question that remains to be addressed for many drugs (37).

Perspectives

Cystometry is one of the main clinical assessments carried out during urodynamic studies to aid diagnosis and treatment of symptoms of bladder dysfunction, such as urinary urgency and frequency. However, CMG findings can be inconsistent and do not always correlate with symptoms, as when MT or bladder capacity is found to be normal, despite the woman’s report of severe urgency and frequency (1, 5). The findings here that the urethane-anesthetized female rat’s vulnerability to inflammation-induced bladder hyperreflexia varies with estrous stage encourage fur-
Estrous influences on bladder capacity in rats


