Opposing viscerovisceral effects of surgically induced endometriosis and a control abdominal surgery on the rat bladder

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Objective: To determine, in rats, how surgically induced endometriosis and a control surgery (partial hysterectomy; sutures in abdomen) affects micturition thresholds and bladder vascular permeability.

Design: Two animal studies, each performed in three groups of urethane-anesthetized rats in proestrus.

Setting: Academic facility.

Animal(s): Seventy-three female, regularly cycling Sprague-Dawley rats studied in proestrus.

Intervention(s): Surgical induction of endometriosis (ENDO), surgical control (shamENDO), intact control (NoSURG), and bladder inflammation via intravesicular turpentine in all three groups.

Main Outcome Measure(s): [1] Micturition thresholds (MTs; volume voiding thresholds), as measured by repetitive transurethral cystometry before and after bladder inflammation and [2] bladder inflammation, as assessed by extravasation of Evans Blue dye.

Result(s): In the uninflamed bladder, MTs were significantly lower and dye extravasation significantly higher in ENDO rats than in shamENDO and NoSURG rats. Bladder inflammation increased dye extravasation in all groups and reduced MTs in the NoSURG and ENDO rats, but not in the shamENDO rats.

Conclusion(s): Endometriosis reduces MTs and produces signs of inflammation in the healthy bladder. Surprisingly, the control surgical procedure (partial hysterectomy; sutures on mesenteric blood vessels) protects bladder reflexes from the influence of bladder inflammation, a condition that is named silent bladder inflammation. Such cross-system inducing and masking effects have important clinical relevance. (Fertil Steril 2006;86(Suppl 3):1067–73. ©2006 by American Society for Reproductive Medicine.)

Key Words: Reproductive tract, lower urinary tract, interstitial cystitis, pain, cross-system, viscerovisceral interaction

Endometriosis is characterized by the presence of endometrial tissue in abnormal locations and is associated with two main symptoms, subfertility and severe pains, such as dysmenorrhea (menstrual pain), dyspareunia (coital pain, vaginal hyperalgesia), and chronic pelvic pain (1). In some women, the condition co-occurs with other painful disorders such as interstitial cystitis, irritable bowel syndrome, vulvodynia, repetitive kidney stones, migraine, fibromyalgia, temporomandibular disorder, and others (2–6). Mechanisms that underlie the pains of endometriosis, as well as their co-occurrence with painful symptoms from disorders of other visceral systems, are poorly understood (7).

Research that seeks to further our understanding of the processes underlying these cross-system effects has been recently reported. For example, regarding the bladder and uterus, it has been shown in rats that acute bladder inflammation increases the motility of the healthy uterus (8) and reduces the efficacy of drugs on the uterus (9). The reverse influence also has been observed; that is, that acute inflammation of the uterus in rats increases vascular permeability of the otherwise healthy bladder (10). Other studies have shown that acute irritation or inflammation of the colon or bladder produces signs of irritation or inflammation in the other organ (10–12).

Although three of the studies cited above showed that the hypogastric nerve contributes to the observed cross-system effects (8–10), an additional contribution likely comes from convergent responses of neurons in the central nervous system. For example, studies of neurons in the spinal cord and brain of female rats have shown that single neurons respond convergently to stimulation of several internal organs from different systems, such as uterus and vagina, colon and esophagus, bladder, and heart (7, 12–16). Some investigators reasoned that this convergence could provide a central nervous system substrate for cross-system effects (2, 12, 13).

One of the interesting features of the convergent responses in those studies is that neurons that were excited by stimulation of one visceral organ also were shown to be either...
excited or inhibited by stimulation of other organs. For example, in the spinal cord, gracile nucleus, and thalamus, single neurons that were excited by uterine distention were either excited or inhibited by colon distention (14–16). Similarly, in the spinal cord and thalamus, neurons responsive to stimulation of the bladder were either excited or inhibited by stimulation of the esophagus or colon (17, 18).

Such observations give rise to the possibility that events occurring in one organ have the potential not only to exacerbate but also to hide the effect of events occurring in another organ. Indeed, in addition to the co-occurrence of painful conditions associated with different organ systems that can exacerbate each other (6), another poorly understood situation is when visceral pathophysiology fails to give rise to symptoms. This dangerous clinical problem is well recognized for “silent myocardial ischemia” (19) but also can occur with other conditions, such as silent kidney stones and silent bladder infections (20–22).

Of relevance to this situation are recent studies using a rat model of surgically induced endometriosis in which a small segment of one uterine horn is removed, and small pieces of uterine tissue (ENDO), or of fat in controls (shamENDO), are autotransplanted onto abdominal mesenteric arteries (23). Over a period of 1–2 months, the uterine, but not the fat autotransplants, form cysts. This model exhibits many of the features of endometriosis in women and has been used successfully for >20 years to study potential mechanisms of endometriosis-associated subfertility (24). In addition, rats with ENDO, but not rats with shamENDO, develop vaginal hyperalgesia (25), which is one of the pain symptoms common in women with endometriosis (e.g., dyspareunia). Furthermore, the ENDO condition exacerbates pain behaviors associated with a ureteral stone (26), again similar to one of the painful conditions that co-occurs with endometriosis in women, interstitial cystitis (4).

Interestingly, however, the shamENDO surgical procedure in rats (i.e., partial hysterectomy plus four sutures in the abdomen) was found to eliminate nearly all of the pain behaviors induced by the ureteral stone, a process that the investigators called “silent stones” (26). This finding, although surprising at the time it was observed, likely reflects an example of the situation discussed above in which events occurring in one organ hide signs and symptoms of pathophysiology in other organs.

Given the above findings, here we tested the hypothesis that these two types of chronic pathophysiology associated with the reproductive tract—ENDO and shamENDO— influence bladder motility of and signs of bladder inflammation in the healthy and inflamed bladder in opposite directions. The study was performed by assessing the influence of ENDO and shamENDO on micturition thresholds and vascular permeability (an indicator of inflammation; Saria and Lundberg [27]) in healthy and inflamed bladders of urethane-anesthetized rats.

MATERIALS AND METHODS
Subjects
Subjects were 73 female Sprague-Dawley rats (Charles River, Kingston, NC), 200–300 g, housed individually in wood chip–lined cages, with free access to water and chow, and maintained on a 12:12-hour light–dark cycle, with lights on at 7 AM. Estrous stage was assessed daily by vaginal lavage and was defined using traditional nomenclature (28). Only rats that exhibited two complete regular 4-day cycles before surgery and whose estrous cycling continued regularly until the experimental day were used.

General Procedures
Three groups of rats were studied, all in the proestrous stage of their estrous cycle: no surgery (NoSURG), shamENDO surgery, or ENDO surgery. Because the cysts produced by the ENDO surgery enlarge gradually over an 8-week period and then plateau (23), all rats were studied approximately 10 weeks after surgery.

Two studies were performed in separate groups of rats, with both types of assessments made 3–6 hours after lights on. Study 1 (n = 39) assessed the influence of surgery on micturition (volume-voiding) thresholds (MTs) before and 3 hours after mild bladder inflammation with turpentine. Study 2 (n = 34) assessed the influence of surgery on Evans Blue dye extravasation in two sets of rats, one set with an uninflamed bladder and the other set 2–3 hours after inflaming the bladder. For both studies, experimenters were blinded to the rat’s surgical condition until after data had been collected.

Both studies followed US federal guidelines for the ethical treatment of experimental animals and were approved by Florida State University’s Animal Care and Use Committee.

Surgery for ENDO and ShamENDO Groups
Rats in diestrus were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/kg), and were placed on a heating pad to maintain body temperature at approximately 37°C. Surgery was performed with aseptic precautions. After a midline incision to expose the abdominal and pelvic cavities, a 1-cm segment midway between the ovary and uterus was excised and placed in 37°C sterile saline. For the ENDO group, the uterine segment was bisected along its longitudinal axis and cut into four approximately 2-mm × 2-mm pieces. With 4-0 nylon suture, the pieces were then sewn around alternate cascade mesenteric arteries that supply the caudal small intestine, starting from the cecum. For the shamENDO group, 2-mm pieces of fat trimmed from the excised piece of uterus were sewn around arteries in the same way. Rats were closely observed during the postsurgical period for potential complications. Postoperative recovery was uneventful, and regular estrous cyclicity resumed within 1 week.

At the end of the experiment, the state of the autotransplants was assessed at autopsy by locating each of the sutures...
that had been used to tie the uterine or fat transplants and then examining and measuring any cysts formed at the sites. For all of the rats whose data are presented here, none of the rats in the shamENDO (or NoSURG) group exhibited cysts, whereas all rats in the ENDO group exhibited at least three cysts larger than $2 \times 2$ mm in diameter.

Protocol for MTs
Micturition thresholds were measured by repetitive cystometrograms (CMG) performed before and after the bladder was inflamed with turpentine, as described elsewhere in detail (29).

Rats were anesthetized with urethane (1.25 g/kg, IP). The bladder was catheterized transurethrally with 1.1-mm-diameter polyethylene tubing, with the tip of the catheter placed within the bladder lumen so it did not contact the bladder wall. A small (approximately 1 cm) ventral midline incision was made to expose the bladder so that it could be emptied completely before each CMG with gentle pressure. The bladder was kept moist with warm saline-dampened pads, and the rat’s body temperature was maintained at approximately 37°C with a heating pad and warming lamp (as determined by thermometer in previous studies; Berkley and Hubscher [30]).

Each CMG provided a measure of the pressure within the bladder while the bladder was slowly infused with saline via the transurethral catheter. Before beginning each CMG, the bladder was emptied, and saline was infused at a steady rate (0.05 mL/min) for 20 minutes (1.0 mL). Pressure was measured via a small-volume pressure transducer (Cobe), which was placed in-line with the catheter. The MT was defined as the volume at which the first bladder contraction began.

Three baseline CMGs were performed at approximately 10-minute intervals. The last baseline CMG was used as the uninflamed-baseline measure for data analysis. The bladder was then filled with 0.5 mL of 50% turpentine in olive oil. One hour later, the bladder was emptied, and CMGs were repeated hourly until 3 hours after bladder inflammation. The CMG that was performed 3 hours after inflammation was used as the postinflammation measure for data analysis.

Protocol for Evans Blue Dye Extravasation
The rats were anesthetized and the bladder catheterized as for CMGs. The jugular vein was also catheterized. The bladder was filled with 0.5 mL of either saline or 50% turpentine in olive oil. One hour later, the bladder was emptied. One to two hours after that, a dose of Evans Blue dye was delivered through the jugular vein. Thirty minutes after dye delivery, the rat was perfused transcardially with saline. The bladder was harvested, rinsed briefly in saline, patted dry, weighed, and placed in 2 mL of formamide for incubation at 55°C for 24 hours. The optical density of the formamide solution was measured spectrophotometrically ($\lambda = 620$ nm; Shimadzu, Columbia, MD), and a calibration curve was used to assess the amount of dye per gram of tissue. This procedure is similar to that developed by Saria and Lundberg (27).

Data Analysis
Statistical analyses were performed using Statistical Package for the Social Sciences software, version 12 (SPSS, Chicago, IL). For study 1, repeated measures analyses of variance were performed, followed as appropriate by one-way analyses of variance and, if significant, by post hoc Tukey’s honestly significant difference tests. For study 2, a two-way ANOVA was performed, followed as appropriate by one-way analyses of variance and post hoc Tukey’s honestly significant difference tests. Significance was set at $P \leq .05$. Data are presented as means and SEs.

RESULTS
Micturition Thresholds
Micturition thresholds varied significantly as a function of both the surgical condition [$F(2,36) = 8.194, P < .01$], and bladder inflammation [$F(1,36) = 48.222, P < .001$], with a significant interaction effect [$F(2,36) = 3.414, P < .05$]. Thus, as shown in Figure 1A, the effect of bladder inflammation on MTs was influenced significantly by the surgical treatment.

For the uninflamed bladder, MTs were significantly reduced in the ENDO condition, compared with either the shamENDO ($P = .03$) or the NoSURG conditions ($P = .04$). Micturition thresholds of the shamENDO and NoSURG groups did not differ significantly ($P = .89$).

However, whereas bladder inflammation significantly reduced MTs in both the ENDO and NoSURG groups ($P = .001$ for both comparisons), it did not do so in the shamENDO group ($P = .153$). The net result of bladder inflammation was that MTs of the inflamed bladder in the shamENDO group were significantly greater than those of both the NoSURG ($P = .01$) and the ENDO ($P = .001$) groups, which did not differ significantly from each other ($P = .42$).

Evans Blue Dye Extravasation
Dye extravasation in the bladder varied significantly as a function of both surgical condition [$F(2,28) = 7.68, P < .01$] and bladder inflammation [$F(1,28) = 19.56, P < .001$], with an insignificant interaction effect [$F(2,28) = 1.01, P = .38$]. Thus, as shown in Figure 1B, for the saline-treated bladder, post hoc tests showed that dye extravasation was greater in the ENDO condition than in the NoSURG condition. Although the amount of dye extravasation in the shamENDO condition was between that of the ENDO and NoSURG conditions, it did not differ significantly from either group. For the inflamed bladder, there were no significant differences between groups [$F(2,15) = 2.8, P = .10$].
DISCUSSION

These results showed that MTs of the uninflamed bladder were reduced in rats in the ENDO condition compared with either the NoSURG or shamENDO conditions. In concordance, protein extravasation in the uninflamed bladder was greater in the ENDO condition than in the NoSURG group. Thus, chronic pathology of the reproductive system and abdomen (partial hysterectomy plus abdominal cysts and sutures) produced signs of pathophysiology in the otherwise healthy bladder.

On the other hand, even though bladder inflammation increased protein extravasation in the bladder in all three conditions, it failed to reduce MT for rats in the shamENDO condition as it did in both the ENDO and NoSURG conditions. Thus, a milder pathology of the reproductive system (partial hysterectomy plus abdominal sutures) prevented bladder inflammation from influencing bladder physiology; that is, it induced a "silent" bladder inflammation.

Before discussing these two effects, it is important to point out that recent studies have shown that the effects of inflammation on the bladder are influenced by the rat's reproductive status (31, 32). Thus, even though estrous stage was controlled in the present study (i.e., all rats were studied in proestrus when E2 levels are high; Freeman [33]), it is possible that the effects observed here differ in other reproductive conditions.

The Excitatory Influences of the ENDO Procedure on the Healthy Bladder

The effect of ENDO on both MTs and protein extravasation in the bladder is consistent with a growing body of evidence showing cross-system effects in which pathophysiology in one organ produces signs of pathophysiology in another otherwise healthy organ. Thus, ENDO produces vaginal hyperalgesia in an otherwise healthy vaginal canal (25), uterine inflammation produces signs of inflammation in an otherwise healthy bladder (10), and bladder or colon irritation or inflammation produce signs of irritation or inflammation in the otherwise healthy other organ (10 – 12).

Mechanisms underlying these relatively newly discovered cross-system excitatory effects are not yet understood. With respect to the present results, we recently have shown that the cysts in rats with ENDO develop their own sensory and sympathetic nerve supply (7, 34) whose fibers travel in the splanchnic and vagus nerves (35). Regarding the splanchnic nerve, its sensory fibers travel mainly to the T8 – T12 spinal segments (36). Input from the healthy bladder is conveyed mainly to the L6 – S1 segments and, to a lesser extent, to the T13 – L2 segments (37). One possibility, therefore, is that the excitatory effects of ENDO on the bladder are mediated via intersegmental spinal interconnections in which input from the cysts via the splanchnic nerve to T8 – T12, together with input from neurons supplying the damaged area of the uterus to T13 – L1 (38), exert an excitatory influence via interspinal connections to the L6 – S1 segments (as well as within the T13 – L2 segments) on how neurons respond to input from the bladder. In support of such a possibility are results of a study by Wall and colleagues (39) that show that intersegmental connections descending from the T13 – L2 to the L6 – S1 segments are part of the mechanism by which input

FIGURE 1

(A) Micturition thresholds before and 3 hours after bladder inflammation in rats in the NoSURG (n = 16), shamENDO (n = 9), or ENDO condition (n = 16). Within the same group, *significantly different from the uninflamed bladder. Across groups, #significantly different from the uninflamed bladder in the NoSURG group. (B) Evans Blue dye extravasation in the uninflamed or inflamed bladder from rats in the NoSURG (uninflamed, n = 6; inflamed, n = 6), shamENDO (uninflamed, n = 6; inflamed, n = 5), or ENDO condition (uninflamed, n = 6; inflamed, n = 5). Within the same group, *significantly different from the uninflamed bladder. Across groups, #significantly different from the uninflamed bladder in the NoSURG group.
from the uterus to the T13–L2 segments influences the responses of neurons in the more caudal L6–S1 segments to stimulation of different pelvic organs.

Another factor that could contribute to the excitatory effects of ENDO on the bladder involves input from the vagal afferent fibers supplying the cysts. In support of this possibility is that vagal afferents activated by inflammation can contribute to symptoms of visceral pathophysiology (40). Thus, the effects observed here might have been produced by way of vagal inputs to the solitary nucleus, whose neurons then project to the caudal spinal cord (41), where they would exert excitatory influences on input from the bladder. In support of this mechanism, excitatory effects have been observed for other visceral organs (42). Vagal influences within the other areas of the brainstem also could be involved. For example, Hubscher and colleagues (43) have shown that neurons in the medullary reticular formation can respond by excitation to stimulation of both the vagus nerve and pelvic organs.

**The Inhibitory Influences of the ShamENDO Procedure on the Inflamed Bladder**

Mechanisms underlying cross-system inhibitory effects are even more poorly understood than the excitatory effects. The results here are consistent with those of earlier studies in which it was observed that the shamENDO condition nearly eliminates pain behaviors that are produced by a ureteral stone (26), a situation that was dubbed “silent stones.” It appears likely that both of these inhibitory effects, like the excitatory ones discussed above, are centrally mediated. Thus, the intraspinal effects observed by Wall and colleagues (39) were both excitatory and inhibitory. Therefore, input from sensory fibers in the splanchnic nerve supplying the abdominal areas that had been damaged by the sutures and sensory fibers in the hypogastric nerve that was supplying the area of the uterus that was damaged by hysterectomy could have reduced the influence of bladder inflammation on responses of neurons in the caudal spinal cord to distention of the inflamed bladder. A more likely possibility, however, may be inputs from the vagus nerve, both from the abdomen (44) and from the uterus (45), that have the potential for exerting anti-inflammatory effects (46).

**Induction Compared With Masking**

The main question here then concerns mechanisms that underlie the opposing effects. In other words, what determines whether the effects of pathophysiology in one organ system are excitatory or inhibitory on another organ system? Foreman (13) has suggested that viscerovisceral interactions are more likely to be inhibitory the further away that two organs are from each other. With regard to the present results, however, the pathophysiology in both the ENDO and shamENDO conditions were in the same regions (uterine horn and abdomen). Thus, the main difference between the ENDO and the shamENDO conditions is not distance but that the ENDO condition gives rise to an extra neural input to the spinal cord that likely does not exist in the shamENDO condition. As shown by Berkley and colleagues (34), this extra input is via sensory fibers that extend into the epithelial layer lining the lumen of the cysts and are immunoreactive for substance P and calcitonin gene–related peptide. Thus, these sensory fibers likely are continuously conveying nociceptive input to the spinal cord from the cysts (whose lumens are replete with inflammatory cells; Berkley et al. [34]). It may be that this extra nociceptive drive in the ENDO, compared with the shamENDO condition, is what shifts putative inhibitory effects to excitatory ones.

**Clinical Relevance**

Regardless of the mechanisms discussed above, the present observations likely have clinical relevance. The studies here were performed in female rats, and clearly female rats are not women. The rat ENDO model, however, appears reasonable for assessing processes involved in the signs and symptoms of endometriosis in women (7, 24). Thus, if the effects of ENDO that are observed here in rats also occur in women, they likely contribute to the co-occurrence and frequent confusion of endometriosis with troublesome bladder conditions such as interstitial cystitis (6, 47). In other words, it may be that interstitial cystitis in some patients actually is a manifestation of endometriosis, and therefore that treatment aimed at endometriosis may alleviate the bladder urgency and frequency problems associated with interstitial cystitis. Indeed, bladder symptoms in women with endometriosis can be treated successfully with hormonal manipulations that often are used for endometriosis (48).

However, the effects of the shamENDO condition suggest the existence of another process that is of equal, and perhaps more important, clinical relevance than the excitatory interactions that are associated with ENDO. This other process is one in which pathology in one part of the body can prevent, or mask, signs and symptoms of pathology in another part of the body. In the context of pain, the situation in which a noxious stimulus in one part of the body inhibits a noxious stimulus in another part of the body from producing pain has been referred to as *counterirritation* or diffuse noxious inhibitory controls (49). Diffuse noxious inhibitory controls has been well described in human beings and experimental animals for somatosomatic heterotopic interactions and for viscerosomatic inhibitory influences (50–52). In both situations, inhibition of spinal convergent neurons takes place. It is possible therefore that viscerovisceral interactions such as those observed here are based on a similar process. This process could underlie potentially lethal clinical problems such as, among others, silent myocardial ischemia (53), silent kidney stones (54), and silent bladder inflammation (55).

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