



Influence of endometriosis on visceromotor and cardiovascular responses induced by vaginal distention in the rat

Hiroshi Nagabukuro, Karen J. Berkley *

Program in Neuroscience, Florida State University, Tallahassee, FL 32306-1270, USA

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Abstract

This study examined pseudoaffective responses elicited by vaginal distention in urethane-anesthetized rats, and tested hypotheses that responses would be increased by endometriosis (ENDO) and vary with the estrous cycle. Three groups were studied: ENDO, shamENDO, and Naïve. ENDO was induced by autotransplanting small pieces of uterine horn (or, for shamENDO, fat) on mesenteric arteries. Ten weeks later, rats in proestrus or metestrus were anesthetized with urethane. Distendable latex balloons were inserted into the vaginal canal. While an increasing series of vaginal distentions was delivered, changes in electromyographic activity of the external oblique musculature (visceromotor response, VMR) and mean arterial pressure (pressor) responses were simultaneously measured. Vaginal distention produced VMR and pressor responses in all groups. These responses were significantly greater in ENDO than in the other groups, and greater in proestrus than metestrus. Although the overall amount of cystic tissue was greater in proestrous than metestrous rats, there was no correlation between these amounts and VMR or pressor responses. Acute spinalization (T8–T9) and bilateral pelvic, but not hypogastric, neurectomy attenuated both VMR and pressor responses, supporting the hypothesis that vaginal nociception involves suprathoracic spinal processing of information conveyed by the pelvic nerve. These effects on VMR and pressor responses to vaginal distention parallel behavioral escape responses to the same stimuli reported previously. The findings encourage continued use of VMR and pressor responses for further investigation of mechanisms underlying pain associated with ENDO and its potential treatment.

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1. Introduction

Endometriosis is a poorly-understood condition defined by endometrial growths outside the uterus. Its symptoms include subfertility, severe dysmenorrhea, dyspareunia, dyschezia, and chronic pelvic pain (Giudice and Kao, 2004). In addition, endometriosis frequently co-occurs with other disorders such as interstitial cystitis, irritable bowel syndrome, vulvo-

dynia, repetitive kidney stones, and others (Berkley et al., 2005).

A rat model of endometriosis (ENDO) involves autotransplantation of pieces of uterus to ectopic sites (Jones, 1984; Vernon and Wilson, 1985). The transplants develop into cysts that share many features of the ectopic growths in women (Sharpe-Timms, 2002), including development of sensory and sympathetic innervation (Berkley et al., 2004, 2005; Tokushige et al., 2006). Like humans with endometriosis, ENDO rats are subfertile (Sharpe-Timms, 2002) and exhibit nociceptive symptoms. They develop vaginal hyperalgesia, whose severity correlates with estradiol levels during the rat's ovarian cycle (Cason et al., 2003), bladder overactivity (Morrison et al., 2006), and

* Corresponding author. Tel.: +1 850 644 5741; fax: +1 850 644 9874.

E-mail address: kberkley@psy.fsu.edu (K.J. Berkley).

exaggerated pain behaviors and muscle hyperalgesia in response to a kidney stone (Giamberardino et al., 2002). Thus, this rat model appears valid for studying mechanisms of endometriosis and its associated pains (Berkley et al., 2005).

So far, the effect of ENDO on nociception has been studied using behavioral psychophysical and videomonitoring techniques (Giamberardino et al., 2002; Cason et al., 2003). Other methods are needed. One possible method, used by others to study visceral nociception, involves measures of pseudoaffective responses such as referred muscle contractions (the “visceromotor response”; VMR) and blood pressure increases (“pressor” response) (Ness and Gebhart, 1988).

Although VMR and pressor responses elicited by colorectal (Ness and Gebhart, 1988), gastric (Ozaki et al., 2002), bladder (Ness et al., 2001; Cruz and Downie, 2006), and cervix (Sandner-Kiesling et al., 2002) distention have been well documented, studies of such responses to vaginal distention have not been done. Pseudoaffective responses likely would be elicited by these stimuli because they evoke behavioral escape responses (Berkley et al., 1995; Bradshaw et al., 1999). Furthermore, if VMR and pressor responses to visceral stimulation reflect behavioral visceral nociception, then ENDO – which produces behavioral vaginal hyperalgesia that is greatest in proestrus (Cason et al., 2003) – should increase VMR and pressor responses to vaginal distention, and the increases should be greatest in proestrus. Accordingly, here we examined whether vaginal distention would induce VMR and pressor responses in Naïve urethane-anesthetized rats, and assessed how ENDO, a sham surgery (shamENDO) and estrous stage affected them.

Previous studies showed that the vagina is innervated by the pelvic, but not the hypogastric, nerve (Berkley et al., 1993; Berkley and Hubscher, 1995), suggesting that VMR and pressor responses to vaginal distention would require pelvic nerve input. Previous studies also showed that VMR and pressor responses to pelvic organ stimulation require supraspinal processing (Ness and Gebhart, 1988). Accordingly, here we tested these two hypotheses by examining the effect of pelvic and hypogastric neurectomy and spinalization on VMR and pressor responses produced by vaginal stimulation.

2. Methods

Female Sprague–Dawley rats weighing 200–250 g at the start of the study were housed individually and maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) They were assessed for their estrous stage daily by vaginal lavage, following traditional nomenclature (see Fig. 3 in Becker et al., 2005). Only rats that exhibited at least two complete regular four-day cycles before ENDO or shamENDO surgery and whose estrous cycling continued regularly until the day of the experiment were used. Three groups of rats were studied: (1) ENDO

surgery; (2) shamENDO surgery; (3) Naïve. All procedures were approved by Florida State University’s Animal Care and Use Committee.

2.1. ENDO and shamENDO surgery

Rats in diestrus were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/kg), and the surgery carried out using aseptic precautions. A midline abdominal incision was made to expose the uterus, and a ~1-cm segment of the left uterine horn and associated fat tissue was removed and placed in warm saline. Four pieces of uterine horn (~2 mm × 2 mm) or, for the sham procedure, four similarly-sized pieces of fat were cut from this segment. These pieces were sewn around alternate cascade mesenteric arteries that supply the caudal small intestine starting from the caecum using 4.0 nylon sutures. Rats were closely observed during the postsurgical period for potential complications. Postoperative recovery was uneventful, and regular estrous cyclicity resumed within one 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 autotransplants, followed by examining and measuring the largest and smallest diameter of any cysts formed at the sites (most cysts have an ovoid shape; Vernon and Wilson, 1985). Because the cysts produced by the ENDO surgery enlarge gradually over an 8-week period and then plateau (Vernon and Wilson, 1985), all rats were studied ~10 wks or more postsurgery.

2.2. VMR and pressor responses

2.2.1. Anesthesia

Rats in either proestrus or metestrus were anesthetized with an initial dose of urethane (0.8 g/kg, i.p.; Ness and Gebhart, 1988). Supplemental urethane was given until only a slight corneal reflex remained; the flexion reflex to strong but not gentle pinching of the foot also remained. Close attention was paid to maintain this level of anesthesia throughout the experiment.

2.2.2. Surgical preparation

The right carotid artery was catheterized with PE-50 tubing and connected to a pressure transducer (COBE Cardiovascular, Arvada, CO) for arterial pressure measurement. Teflon-coated stainless steel wires (AS633, Cooner Wire, Chatsworth, CA) were threaded into the left external oblique musculature and connected to recording equipment for measurement of its electromyographic (EMG) activity. A distendable latex balloon (2 mm long) tied to a catheter (1.0 mm OD, 0.6 mm ID) was inserted into the right uterine horn through a small incision to locate the balloon midway between the ovary and uterine cervix. Another distendable latex balloon (8 mm long) tied to a catheter (2.2 mm OD, 1.1 mm ID) lubricated with K–Y jelly was inserted into the middle of the vaginal canal. These two balloons were identical to those that had been used for behavioral studies in previous experiments (Bradshaw et al., 1999). Each balloon was connected via a catheter to a water-filled syringe and pressure transducer (COBE Cardiovascular). The transducer was connected to recording equipment for measurement of vaginal and uterine pressures during different

volumes of their manual distention using the syringe. Of importance to note is that before inserting these balloons in the rat, the pressures produced within the balloons (when they were located in approximately the same height relative to where they would be after placement inside the rat) by different volumes of distention were measured. As was done in previously published studies (e.g., Bradshaw et al., 1999; Cason et al., 2003), these “outside-the-rat” pressure values were subtracted from the pressures measured when the balloon was inside the rat to calculate actual pressures produced by each distention volume on the vaginal and uterine walls; i.e., vaginal tone (Figs. 1c, 3c and f).

2.2.3. VMR and pressor response protocol

At least 30 min after surgical preparation, the vaginal balloon was inflated in a staircase manner every 30 s (0.2–1.3 ml), while simultaneously measuring the EMG and arterial pressure. This inflation was carried out manually at a slow steady rate (~0.05 ml/s) using the water-filled syringe attached to the balloon. Three or two sets of distentions were carried out, respectively, for the vaginal canal or uterine horn. The interval between each set was at least 10 min. EMG signals were amplified, filtered (high frequency, 3 kHz; low frequency, 10 Hz), converted to digital data using a Micro 1401 processor (Cambridge Electronics Design, Cambridge, UK) and sampled at 1 kHz using Spike2 software (Cambridge Electronics Design). The threshold for discriminating activity from the baseline EMG activity was pre-set at 200% of maximum amplitude of the baseline activity. Arterial pressure signals were also amplified and sampled at 100 Hz using Spike2.

Following vaginal distention, uterine distention was performed in the same staircase manner (0.02–0.20 ml every 30 s at ~5 μ l/s). Because a VMR was not detected in any of the groups, additional studies are underway to assess other muscle groups and stimulus parameters, and therefore these data will be presented later elsewhere.

2.3. Spinalization and pelvic or hypogastric neurectomy

2.3.1. Spinalization

A baseline assessment of VMR and pressor responses induced by vaginal distention was carried out as described in Section 2.2. Next, either the spinal cord was transected using a surgical knife at the level of T8–9 in 2 ENDO rats (one in P, one in M) and 4 shamENDO rats (3 in P and 1 in M) rats. At least 30 min after the transection, VMR and pressor responses to vaginal distention were remeasured. At the end of the experiment after the rat was sacrificed, the transection site was examined to confirm that the transection had been complete.

2.3.2. Neurectomy

For pelvic or hypogastric neurectomy, a 6–0 silk suture was tied around the left and right nerves so each suture could be used as a snare to sever each nerve during the experiment (Hubscher and Berkley, 1995). Next, baseline VMR and pressor responses were obtained as described in Section 2.2. The left and right pelvic nerves or hypogastric nerves were then transected by pulling the snare quickly through each nerve. VMR and pressor responses to vaginal distention were then remeasured a minimum of 15 min later.

2.4. Data analysis

For the VMR, the total number of spikes was measured and normalized to the number of spikes evoked by the 1.3-ml distention. For the pressor response, two-way ANOVAs showed that baseline values for the mean arterial pressure (MAP) did not differ across groups. Accordingly, the pressor response was calculated by subtracting the MAP during each distention step (30 s) from the baseline MAP measured during the 30 s period prior to the initiation of any distentions. Intra-balloon pressures were measured every 15 s post-distention. Average values of the two or three sets of measurements taken for each distention level were calculated.

In order to determine how the cysts might influence the effects observed, a calculation of a total “cyst burden” was performed for each rat, as follows: First, a value for the size of each cyst was calculated by multiplying its largest diameter times its smallest diameter. Thus, a cyst that measured 4 mm \times 5 mm would have a value of 20. Next, these values were totaled for all of the cysts in each rat to determine a final cyst burden for that rat. For example, if in a given rat, three of her four transplants had formed cysts, and these cysts measured, say, 20, 9, and 8, then the total cyst burden for that rat would be 37.

Area-under-the-curve (AUC) calculations were carried out using standard, trapezoid rule methods. Differences in the AUC between the Naïve group and the shamENDO or ENDO groups were calculated. Correlation analyses between the AUC values and cyst burdens for ENDO rats were performed. As appropriate, Student *t*-tests, One-way ANOVAs, repeated measures ANOVAs, and Pearson correlation coefficient statistics were carried out using SPSS v. 13 software. If significant, the ANOVAs were followed by post hoc Tukey tests. Significance was set at $P \leq 0.05$.

3. Results

3.1. VMR and pressor responses to vaginal distention in healthy rat: effect of estrous stage

As shown by the example in Fig. 1a, distention of the vaginal canal with volumes identical to those that evoked escape responses in awake rats (Bradshaw et al., 1999) also evoked both VMR and pressor responses in the urethane-anesthetized rats studied here. There were no significant differences either in the VMR [$F(1, 7) = 4.713$, $P = 0.067$] or in the pressor responses [$F(1, 7) = 0.167$, $P = 0.695$] between proestrus and metestrus (Fig. 1b and c), which paralleled a similar lack of effect of estrous stage on vaginal tone (Fig. 1d).

3.2. Effects of spinalization and neurectomies on VMR and pressor responses to vaginal stimulation

In all rats examined, regardless of estrous stage or surgical condition, spinalization eliminated both the VMR and pressor responses to vaginal distention without changing either MAP or vaginal tone (Fig. 2a). Pelvic neurectomy also eliminated both the VMR and

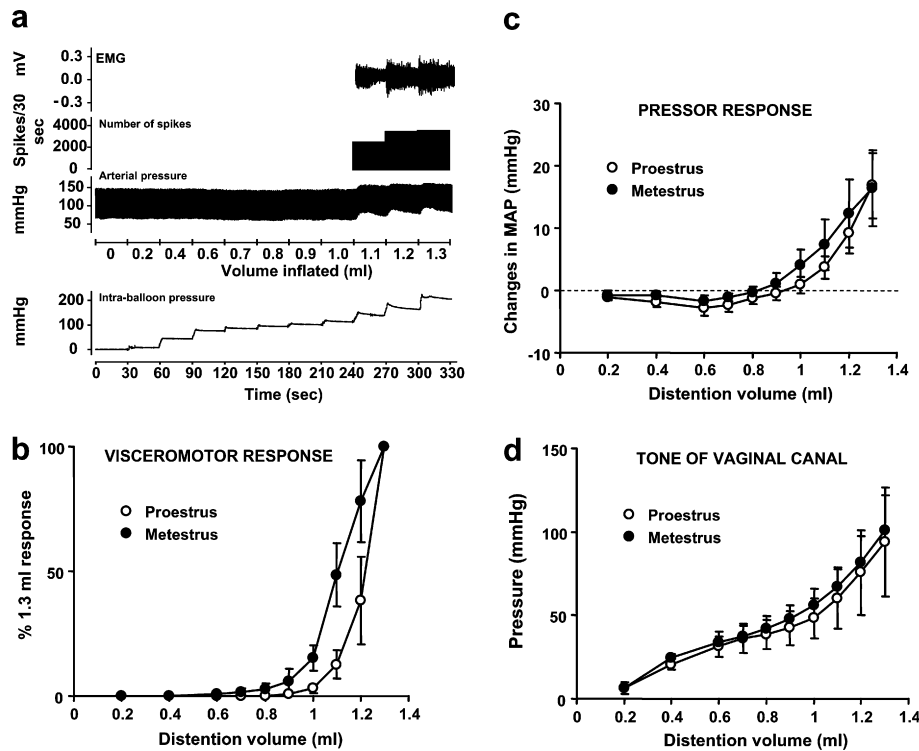


Fig. 1. Influence of estrous stage on VMR and pressor responses to vaginal distention in Naïve rats. (a) A representative example of results from a single Naïve rat in metestrus during increasing volumes of distention of the vaginal canal. This example shows traces of the abdominal EMG, the number of spikes of the EMG, the mean arterial pressure (MAP) and the pressure of the vaginal balloon (uncorrected, see Section 2). (b) Compares changes in the MAP from the baseline MAP in proestrus and metestrus during different volumes of vaginal distention. (c) Compares changes in the VMR in proestrus and metestrus during different volumes of vaginal distention. These changes were calculated after setting the response to a 1.3 ml vaginal distention volume as 100%. (d) Compares the vaginal pressure ("tone", corrected for pressure within the balloon, see Section 2) in proestrus and metestrus produced by different volumes of vaginal distention. Each plot displays mean \pm SEM. There were no significant estrous differences in any measure.

pressor responses to vaginal stimulation in all rats tested without changing either MAP or vaginal tone (Fig. 2b). Hypogastric neurectomy, in contrast, had no effect on the VMR, pressor responses, vaginal tone, or MAP in all rats tested (Fig. 2c).

3.3. Influence of ENDO on VMR or pressor responses to vaginal stimulation in different estrous stages

The influence of ENDO on the VMR and pressor responses to vaginal distention was greater in proestrus than it was in metestrus.

In proestrus, repeated measures ANOVA showed that there were significant differences between the three experimental groups in both the VMR and pressor responses, but not for vaginal tone. (For the VMR: $[F(2, 15) = 7.835, P = 0.005]$. For the pressor response: $[F(2, 14) = 8.519, P = 0.004]$.) Post hoc tests showed that both the VMR (Fig. 3a) and the pressor responses (Fig. 3b) were significantly greater in the ENDO group compared with either the shamENDO or Naïve groups ($P < 0.05$), which did not differ significantly from each other. One-way ANOVAs showed that the distention volume at which the change in the EMG or MAP

became greater than that during the smallest distention volume (i.e., the "threshold") was lower in the ENDO group than in the shamENDO or Naïve groups (asterisks on the line graphs in Fig. 3a and b).

In metestrus, repeated measures ANOVA showed that there were significant differences between the three experimental groups only for the pressor response (Fig. 1e). Post hoc tests showed that the pressor response in the ENDO group was significantly greater than the pressor response in the shamENDO group ($P < 0.05$). One-way ANOVAs showed that the distention volume at which the change in EMG or MAP became greater than that during the smallest distention volume (i.e., the "threshold") was lower in the ENDO group than in the shamENDO group (asterisks on the line graphs in Fig. 3d and e).

Comparisons of the effects of ENDO on VMR and pressor responses in proestrus and metestrus were carried out by analyzing AUC measures (see Section 2). These analyses showed that, when compared with the shamENDO group, the VMR was significantly increased in the ENDO group only in proestrus (Fig. 3a and d, insets). Pressor responses were increased in the ENDO group in both proestrus and metestrus (Fig. 3b and e, insets).

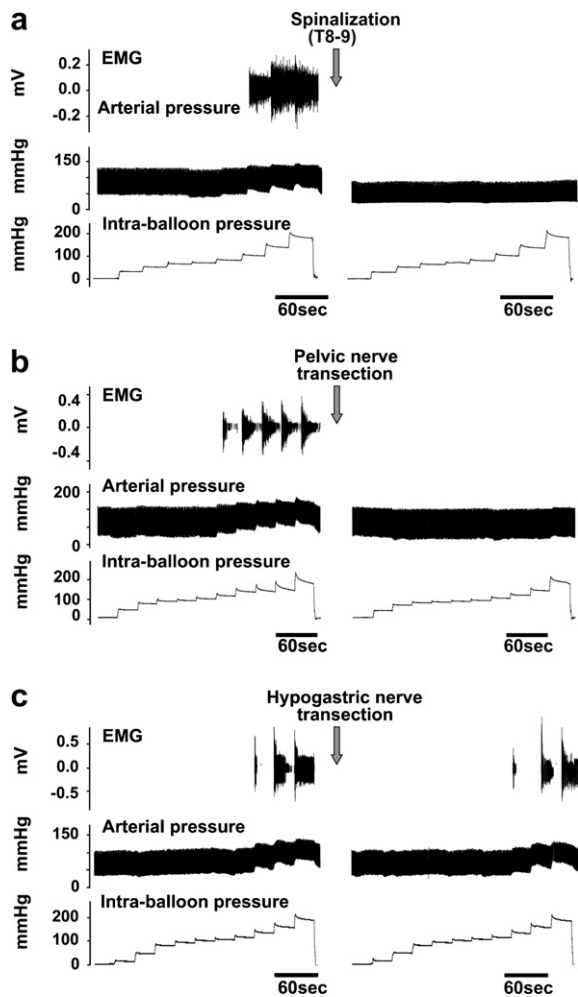


Fig. 2. Representative examples similar to the one shown in Fig. 1a, here showing the effects on VMR and pressor responses to distention of the vaginal canal of acute spinalization (a), bilateral pelvic neurectomy (b) or bilateral hypogastric neurectomy (c). The interval between spinalization (arrow in a) and remeasurement of the VMR and pressor responses was >30 min in all cases. The interval between neurectomy (arrows in b and c) and remeasurement of the VMR and pressor responses was >15 min in all cases.

3.4. Relationship between cyst burden and VMR or pressor responses to vaginal distention

Between two and four of the four transplants in each rat developed into measurable cysts. This number tended to be greater in rats tested and sacrificed in proestrus than in rats tested and sacrificed in metestrus (mean: 3.6 in proestrus, 2.9 in metestrus; $P = 0.07$). Similarly, the average size/cyst tended to be larger in proestrus than metestrus (mean: 19.2 in proestrus, 11.9 in metestrus; $P = 0.08$). Overall, however, the cyst burden was significantly greater in proestrus than in metestrus (mean: 65.9 in proestrus; 30.6 in metestrus; $P = 0.01$).

As can be seen in scatterplots showing the correlation between cyst burden and AUC values for VMR (Fig. 4a) and pressor responses (Fig. 4b), there was no significant

correlation between cyst burden and either outcome measure regardless of estrous stage. For the relationship between cyst burden and the VMR, for all rats, $r = 0.11$ ($P = 0.70$). For proestrous rats, $r = -0.43$ ($P = 0.33$), and for metestrous rats, $r = 0.46$ ($P = 0.22$). For the relationship between cyst burden and pressor responses, for all rats, $r = -0.08$ ($P = 0.78$). For proestrous rats, $r = -0.07$ ($P = 0.89$), and for metestrous rats, $r = -0.17$ ($P = 0.71$).

4. Discussion

These results showed that vaginal distention in healthy rats or in rats with shamENDO surgery evoked visceromotor responses in abdominal muscles and increased MAP. Surgically-induced ENDO increased the VMR and pressor responses in proestrus, whereas in metestrus only pressor responses were increased. Although the cyst burden was greater in rats studied in proestrus than in metestrus, there was no correlation between cyst burden and VMR or pressor responses to vaginal stimulation in either stage. Both the VMR and pressor responses were eliminated by T8–T9 spinalization and pelvic neurectomy, but not by hypogastric neurectomy.

4.1. Effects of spinalization and pelvic or hypogastric neurectomy

The fact that pelvic, but not hypogastric, neurectomy eliminated VMR and pressor responses reflects previous electrophysiological and dye-tracing studies showing that sensory innervation of the vaginal canal is via the pelvic and not the hypogastric nerve (Berkley et al., 1993; Berkley and Hubscher, 1995). The fact that T8–T9 spinalization eliminated VMR and pressor responses is consistent with previous studies showing that these responses to colorectal distention also do not occur in T6 spinalized rats (Ness and Gebhart, 1988), suggesting that these reflexes require supraspinal processing.

4.2. Effect of ENDO

Both VMR and pressor responses increased as vaginal distention volume increased in a manner similar to increases in VMR and pressor responses elicited by colorectal (Ness and Gebhart, 1988), gastric (Ozaki et al., 2002) or bladder (Ness et al., 2001) stimulation. VMR and pressor responses to vaginal distention also paralleled behavioral escape responses in awake rats (Bradshaw et al., 1999; Cason et al., 2003) in two ways. First, VMR and pressor responses in Naïve rats were similar in proestrus and metestrus (Bradshaw et al., 1999). Second, VMR and pressor responses were greater in ENDO than in shamENDO or Naïve groups, and the severity of ENDO's effect was greater in proestrus than

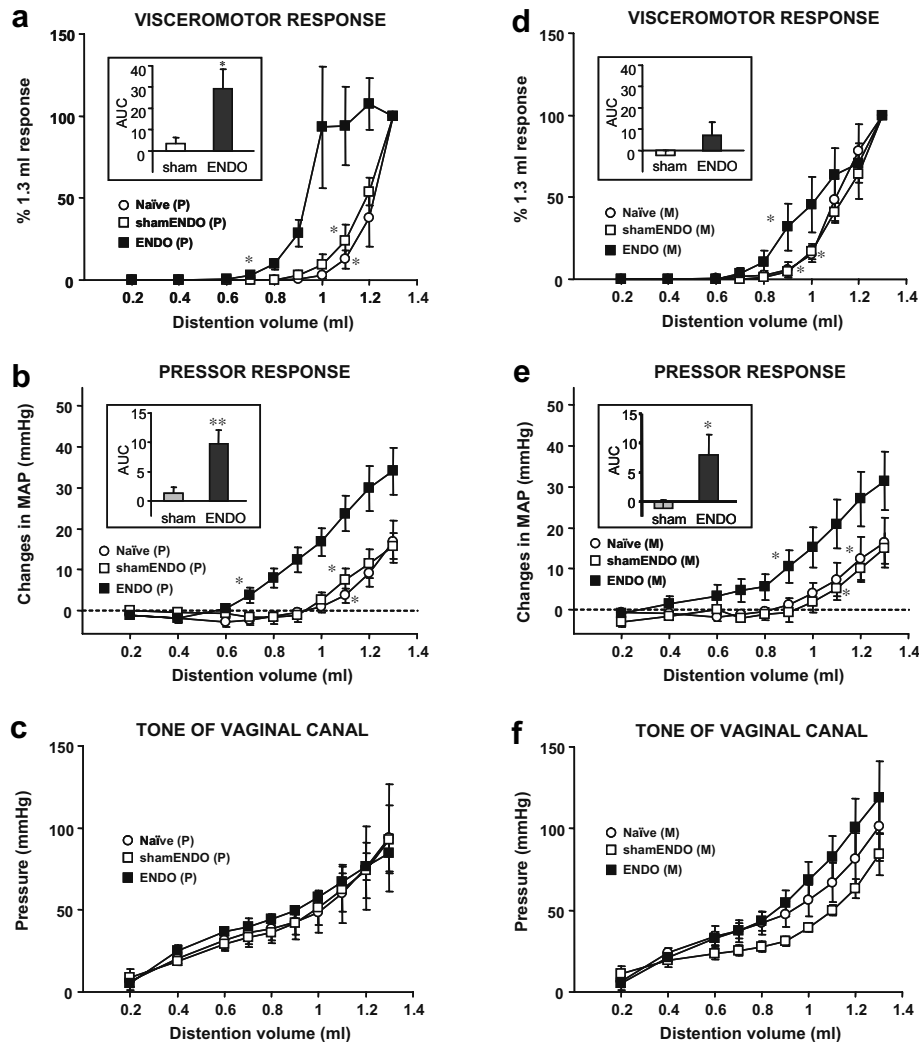


Fig. 3. Effects of ENDO on VMR (a and d), pressor (b and e) responses, and vaginal tone (c and f) in proestrus (a–c) and metestrus (d–f) to different volumes of vaginal distention. See legend in Fig. 1 for details. The asterisks on each graph show, for each group, the lowest volume at which the response was significantly greater than the response to the smallest distention volume (i.e., the response “threshold”). The bar graphs inserted in (a), (b), (d) and (e) show differences in the AUC between the Naïve group and the shamENDO or ENDO groups. Asterisks over the bars indicate that the AUC of that group differed significantly from AUC for the Naïve group (* $P > 0.05$; ** $P > 0.01$).

in metestrus (Cason et al., 2003). These similarities with the behavioral data suggest that the influence of the uterine balloon in the present study was likely negligible.

The main difference between the results from previous behavioral studies and those here was that VMR and pressor response thresholds were higher ($> \sim 0.7$ ml) than escape response thresholds ($> \sim 0.4$ ml; Bradshaw et al., 1999). This difference could be due to anesthesia. Of importance, however, is that, during proestrus, the threshold for both VMR and pressor responses was lowest in the ENDO group. Similarly, during metestrus, the threshold for the pressor response was lowest in the ENDO group.

This situation indicates that VMR and pressor responses indeed reflect vaginal hyperalgesia, even in the anesthetized rat. It should be noted, however,

that although the VMR was observed in anesthetized animals by others, not only in urethane-anesthetized rats to colorectal distention (Barkova et al., 2005), but also in pentobarbital- or α -chloralose-anesthetized rats to cervix distention (Sandner-Kiesling et al., 2002), urethane was reported to abolish VMR and pressor responses elicited by colorectal distention (Ness and Gebhart, 1988). Accordingly, here we were extremely attentive to maintaining a steady level of anesthesia throughout the assessment period in which flexor reflexes to pinching the foot could just be evoked. However, given that many aspects of anesthesia are likely to affect responses, we are currently developing methods to assess pseudoaffective responses to vaginal or uterine distention in awake rats.

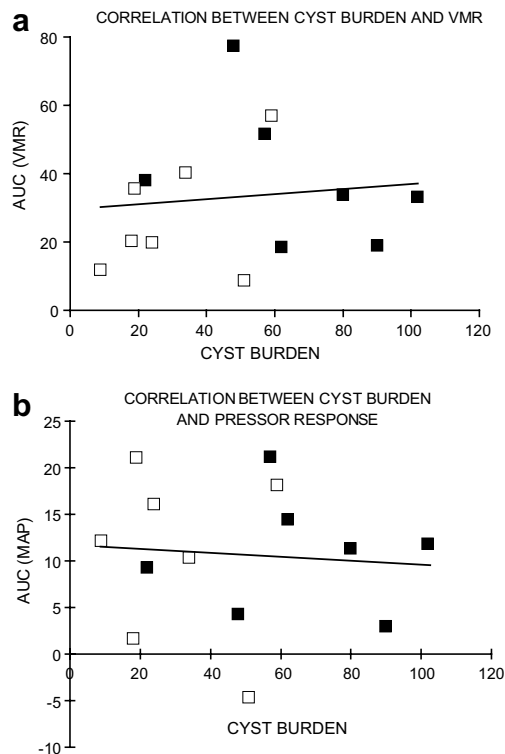


Fig. 4. Correlation between cyst burden and the VMR or pressor response in ENDO rats. Graphs are scatterplots containing trendlines comparing cyst burden and AUC values for VMR (a) or MAP (b). Values for rats in proestrus are shown as filled squares; values for rats in metestrus are shown as unfilled squares. Note that although the cyst burden was greater for proestrous than metestrous rats, there was no significant correlation between the values for rats in either stage (or all rats).

4.3. Influence of estrous stage

The fact that the vaginal hyperalgesia in the ENDO group was more severe in proestrus, when serum estradiol levels are at their peak (Freeman, 1994), supports the general conclusion that endometriosis is an “estrogen-dependent” condition (Giudice and Kao, 2004). Thus, in women, the ectopic growths express estrogen receptors (Matsuzaki et al., 2000). Furthermore, drugs that induce a hypoestrogenic state reduce the size or growth of the ectopic endometrial implants and alleviate pain (Olive, 2004; Garcia-Velasco and Quea, 2005). In the rat model used here, the ectopic endometrial growths disappear following ovariectomy and reappear with estradiol replacement (Vernon and Wilson, 1985; Rajkumar et al., 1990). In addition, locally-applied danazol (an antigonadotropin) decreases cyst size (Nomura et al., 2006). Furthermore, in the present study, the overall cyst burden was greater in rats studied and sacrificed in proestrus (when estradiol levels are at their peak; Freeman, 1994) than in rats studied and sacrificed in metestrus (when estradiol levels are low; Freeman, 1994). It is possible, therefore, that estradiol enhances

sensory information conveyed by the sensory afferents that have sprouted to supply the ectopic cysts in both women and rats (Berkley et al., 2005). This conclusion is consistent with a recent study demonstrating that estradiol enhances the VMR induced by colorectal distention in the colonic inflammation model (Ji et al., 2005). As reviewed in Berkley et al., 2005 (shown in Fig. 1 of that article), information from the cysts in the rats studied here would arrive in thoracic spinal segments which could then modify, via descending intraspinal and supraspinal interactions, nociceptive information that arrives in the L6/S1 spinal cord from the vaginal canal (Berkley et al., 1993).

4.4. Relationship between cyst burden and VMR or MAP responses to vaginal distention

The fact that both the cyst burden and ENDO’s effect on the VMR and MAP were greater in proestrus than metestrus suggests that the amount of cystic material contributes to the severity of ENDO’s influence. However, the fact that there was no correlation between cyst burden and the AUC values for either the VMR or MAP responses in either stage (or overall) suggests that it is not entirely the size or amount of abnormal tissue that determines ENDO’s effects, but rather some other factor(s) associated with the cysts. This situation parallels the clinical one, in which one major dilemma is the fact that there is so little correlation between anatomical aspects of the ectopic growths and clinical symptoms (Giudice and Kao, 2004; Berkley et al., 2005; Kennedy et al., 2005). This conclusion raises the issue of what factors could be involved. One possibility involves the fact that the ectopic growths in both rats and women develop their own sensory and sympathetic nerve supply (Berkley et al., 2005). Indeed, recent studies from our group in rats and women suggest that differences in this innervation and associated contents of growth factors in the cysts may contribute to differences in symptoms (Berkley et al., 2006; McGinty et al., 2006; Zhang et al., 2006).

4.5. Conclusions

Persistent visceral pains in women, such as dysmenorrhea, dyspareunia and dyschezia, are a major health problem, but their medical treatments are too often unsatisfactory. Hindering the development of new approaches is that only a few animal models are available. Furthermore, even in those models, visceral nociception has not yet been extensively investigated in part because assessing behavioral escape responses (e.g., Cason et al., 2003) and coding of videotaped responses (e.g., Wesselmann et al., 1998) are time- and labor-intensive processes. It would therefore be valuable to develop additional and more efficient assessment

strategies. The results of the present study support previous studies indicating that pseudoaffective responses represent one such strategy and encourage their continued use (eventually in awake rats) for further investigation of these pains, their association with endometriosis, and their treatment.

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