Vaginal hyperalgesia in a rat model of endometriosis

Karen J. Berkley*, Angie Cason, Heather Jacobs, Heather Bradshaw, Elizabeth Wood

Program in Neuroscience, Copeland Street, Department of Psychology, Florida State University, Tallahassee, FL 32306-1270, USA

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Abstract

This study examined whether a rat model of surgically-induced endometriosis that reduces fertility also evokes vaginal hyperalgesia along with changes in vaginal compliance. In nine rats trained to escape vaginal distention, percent escape responses to different volumes of vaginal distention were measured for 2.5 months before and after endometriosis or sham surgery. Vaginal pressures were also measured simultaneously to provide an estimate of vaginal compliance. Endometriosis (or sham) was induced by autotransplantation of small pieces of uterus (or fat) on mesenteric cascade arteries, abdomen, and ovary. Escape responses were significantly increased only in rats whose autotransplants had formed cysts. Vaginal pressures, however, remained unchanged. This vaginal hyperalgesia may involve a process of viscero-visceral referred hyperalgesia.

Keywords: Uterus; Referred pain; Fertility; Pelvic organ; Visceral pain

Endometriosis is a common clinical disorder in which abnormal growths of viable endometrial tissue are found outside the uterus, usually in the abdominal/pelvic cavity. Its main symptoms include reduced fertility, severe dysmenorrhea, dyspareunia (vaginal hyperalgesia [11]), and dyschezia [14,19]. Animal models involving surgical induction of endometriosis were developed in rodents nearly 20 years ago, and have been used successfully since then to study mechanisms of reduced fertility and fecundity [1,6,13,17]. No studies to date have assessed whether the models produce any increased pelvic nociception.

We have developed a procedure that enables behavioral measures of vaginal nociceptive sensitivity of awake rats [4,5]. The present study made use of this procedure to determine how surgical induction of endometriosis affects vaginal nociceptive sensitivity. Because one way in which vaginal nociceptive sensitivity could be influenced is by changes in compliance of the vagina, vaginal compliance was also measured at the same time [4,5].

Subjects were nine Sprague–Dawley virgin female rats, 200–225 g at the beginning of the study (325–350 g at end), housed individually in hanging cages, and maintained on a 12:12 h light:dark cycle. Estrous cycles were assessed by vaginal smears obtained daily ~2 h after light onset throughout the study [10]. Only data from rats with regular 4-day cycles were used. To control for possible effects of time of day, rats were always trained and tested 3–6 h after lights on. The study was approved by Florida State University’s Animal Care and Use Committee (protocol #9028) and carried out in compliance with the NIH Guide for Care and Use of Laboratory Animals and guidelines published by the International Association for the Study of Pain [20].

Using procedures to assess vaginal sensitivity identical to those previously described in detail [4,5], rats were trained to perform an operant response to terminate the distention of an inflatable latex balloon that had been temporarily placed in the vaginal canal (escape response). The uninflated balloon was ~10 mm long × 1.5 mm wide. During each testing session, three iterations of eight different volumes of distention were delivered in random order at 1–2 min intervals, and the percent escape response to each volume calculated. A 100% response to a particular distention volume meant that the rat responded every time that volume had been delivered; a 50% response meant that the rat responded on half of the delivery occasions [4,5]. Most aspects of the testing were computer-controlled, such as delivery of each distention volume, randomization, and response measures [4,5]. Sessions were run three times per week on non-consecutive days until three sessions in each of the rats estrous...
stages had been completed (~2.5 months). During all sessions, pressures produced by each of these volumes, corrected for compliance characteristics of the balloon, were measured through a small-volume pressure transducer [4,5].

Following this baseline assessment, rats were anesthetized and subjected to an endometriosis (ENDO, $n = 5$) or sham endometriosis (SHAM, $n = 4$) autotransplant surgical procedure using an endometriosis model previously established by researchers who study fertility [13,17]. These previous studies had shown that the endometrial autotransplants develop active cysts beginning ~2 weeks after the surgical induction that remain until the rat’s estrous cyclicity ceases [17]. These results were confirmed in our own laboratory with Dr Mark Vernon; unpublished data. Therefore, beginning 2 weeks post-surgery, escape responses and vaginal pressures were reassessed for ~2.5 months, again in thrice weekly sessions in different estrous stages. At the end of the study, an autopsy was performed to assess the autotransplants.

Surgical procedures were carried out as follows. Rats were anesthetized i.p. with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/kg). The uterus was exposed through a midline abdominal incision, and a 1-cm segment of the left uterine horn and associated fat tissue was removed. Five pieces of endometrium (~2.5 mm × 2.5 mm) or, for the sham procedure, five pieces of fat, were cut from this segment and sewn around blood vessels in various structures using nylon suture, as follows: one on left ovary, three on alternate cascade mesenteric arteries that supply the caudal small intestine starting from the caecum, and one on the internal lower abdominal wall-right side at approximately the level of the L2 dermatome. In 2 of the 5 ENDO rats, endometrial tissue was not sewn on the ovary. Instead, 6 pieces were sewn on alternate cascade mesenteric arteries starting from the caecum. Rats were closely observed at least twice daily during the entire post-surgical period. Regardless of which surgery had been performed, postoperative recovery was uneventful with no spontaneous pain behaviors, and regular estrous cyclicity resumed within ~1 week.

The state of the autotransplants was assessed at autopsy before behavioral and pressure data were analyzed (i.e. experimenters were blind to changes in vaginal sensitivity and compliance). Rats were anesthetized with ketamine/xylazine as above. After a wide abdominal incision, the pelvic cavity was examined to assess the autotransplants and general health of pelvic and abdominal organs. Sutures that had tied the endometrial or fat autotransplants to abdominal tissue were located, and cysts, if any, dissected free of connective tissue and fat and measured. The rat was killed with an overdose of intracardiac ketamine/xylazine.

Data were analyzed by repeated measures ANOVA using SPSS for Windows, 10.0 (SPSS Inc., Chicago, IL) to compare escape responses and pressures produced by the different distention volumes before and after the autotransplantation. If significant ($P < 0.05$), the ANOVA was followed by post-hoc Fisher’s least significant difference comparisons and paired t-tests, with alpha levels set at 0.05. Data were collapsed across the cycle because previous studies have shown that escape response percentages do not vary with estrous stage [5]. Future studies will assess how estrous condition and hormonal manipulations influence the effects of the autotransplantation and whether the hyperalgesia changes across time after surgery.

Autopsy findings from the ENDO rats revealed the presence of fluid-filled, oval-shaped cysts that surrounded the sutures and were often embedded in substantive quantities of fat and connective tissue that sometimes adhered to nearby structures. When dissected free, the cysts formed by the piece of endometrial tissue that had been sewn to the mesenteric vessels usually measured ~6 mm × 4 mm, whereas the cysts on the ovary were about twice that size. Endometrial autotransplants on the abdominal wall rarely produced cysts, and those that did form were small (1 mm × 2 mm). In SHAM rats, it was possible to locate all of the sutures, but, in 3 of the 4 rats, no cysts were identified, nor any significant fat or connective tissue deposition. In one SHAM rat, however, a large, fluid-filled cyst was found on the ovary surrounded by considerable fat and connective tissue; i.e. identical in appearance to the cyst formed by endometrial tissue.

Escape responses to vaginal distention before surgery did not differ significantly between groups. However, responses after surgery were significantly increased in the five ENDO rats (Fig. 1A; all five rats showed increases), but were unchanged in the three SHAM rats whose autopsy results had revealed no cysts (Fig. 1B; none showed increases). In the SHAM rat with the large ovarian cyst, post-surgical escape responses were significantly greater than those prior to surgery (repeated measures ANOVA, $P = 0.006$; data not shown). The amount of increase in escape responding was approximately the same for ENDO rats whose autotransplants had or had not been sewn on the ovary, but the number of subjects was too few for appropriate statistics. The increased escape responding was not associated with changes in compliance of the vaginal wall (Fig. 1C,D).

These results demonstrate that surgical induction of endometriosis in the rat in a manner that reduces fertility and fecundity [1,17] also gives rise to vaginal hyperalgesia. This hyperalgesia does not appear to be due to the surgical manipulation itself, unless the manipulation produces cystic formations, because identical surgical procedures in the non-cystic SHAM cases, which involved autotransplantation of fat instead of endometrium, did not evoke vaginal hyperalgesia.

Mechanisms by which cystic autotransplants could bring about vaginal hyperalgesia are undetermined. One possibility involves the fact that the cysts, but not the fat autotransplants, release endometrial and other substances, including algogenic prostaglandins, into the abdomen [15,17] and increase the concentration of interleukin-6 in peripheral
serum [9]. But how such secretions might influence sensory input from the vaginal canal is problematic. One of the several ways they could act is by somehow reducing vaginal compliance so that volumes of vaginal distention would produce greater pressures on the vaginal wall. However, vaginal compliance was unchanged in both the ENDO and SHAM cases. Another way they might act is on vaginal afferents, sensitizing them. But, again, it is difficult to conceptualize how such sensitization could occur because the active cysts in most cases were in the upper abdomen (and sometimes on one ovary), which are areas that are remote from the vagina.

It therefore seems possible that central neural mechanisms could be involved. One of several possibilities involves the concept of ‘viscero-visceral referred hyperalgesia’, which refers to a process of central sensitization in which increased input to the nervous system from one visceral domain can sensitize neurons that receive convergent input from another visceral domain [7]. Such sensitization could give rise to pain behaviors that are associated with the other visceral organ, i.e. referred visceral hyperalgesia.

Regions where the grafts produced cysts (ovary and mesenteric arterial cascade vessels) are innervated by splanchnic nerve fibers traveling via the superior mesenteric, inferior mesenteric and coeliac ganglia to lower thoracic/upper lumbar segments [8,12,16]. The vaginal canal in the rat is innervated by fibers in the pelvic nerves that enter the spinal cord via the L6, S1 and S2 dorsal roots [3]. The vaginocervix is additionally innervated by the hypogastric nerve whose fibers enter spinal cord via the T12, T13, L1 and L2 dorsal roots [3]. Neurons within both sets of segments have been shown to respond convergently to stimulation of the uterus, colon and vagina [2], and considerable interactions exist between these two separated sets of caudal spinal segments [18]. It is therefore conceivable that sensitized afferents innervating regions surrounding the cysts created a central sensitization within the caudal spinal cord that was referred to the vaginal canal.

There are of course other possible explanations, involving changes induced by the cysts on the vagina or somatic structures surrounding it, as well as other central effects. Whatever the mechanism(s), however, the fact that surgical
induction of endometriosis in rats not only affects fertility but also produces a vaginal hyperalgesia that bears some semblance to clinical symptoms (dyspareunia) encourages the use of this model for further study to improve understanding of the etiology of painful symptoms associated with endometriosis in women.

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