Estrogen replacement reverses ovariectomy-induced vaginal hyperalgesia in the rat

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Abstract

Objectives: The loss of ovarian function in women through aging or oophorectomy is often associated with the development of vaginal hyperalgesia that can be alleviated with estrogen replacement. This study examined if ovariectomy in rats would similarly give rise to vaginal hyperalgesia, and, if so, whether estrogen replacement would alleviate it. Methods: Female rats were trained to perform an operant response to escape vaginal distention delivered by inflating a balloon located in mid-vaginal canal. Percent escape responses to eight different volumes of distention measured in normally cycling rats were compared with measures made in the same rats following ovariectomy (OVX) or sham ovariectomy (shamOVX), and then, in the OVX group, estrogen replacement (OVX + E2). Pressures exerted by the eight volumes on the vaginal wall were also measured, thereby permitting assessment of vaginal tone. Results: Whereas overall escape response percentages after OVX, but not shamOVX, were significantly higher to the largest six distention volumes compared with responses during cycling, there were individual differences in the amount of hyperalgesia. Following OVX + E2, escape response percentages decreased in all but one rat. Vaginal tone after OVX, shamOVX or OVX + E2 did not differ from overall vaginal tone in cycling rats. Conclusions: Ovariectomy in rats evokes a variable amount of vaginal hyperalgesia that can be alleviated by estrogen replacement in most cases. Thus, the ovariectomized rat appears to provide a useful model for the study of mechanisms underlying the dyspareunia that is associated with loss of ovarian function in women. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

The loss of ovarian function through aging or oophorectomy is often associated with an increase in vaginal sensitivity. For many women, dyspareunia (painful intercourse) accompanies this increase [1]. Of interest, however, is that not all women who experience a loss in ovarian function experience dyspareunia; likewise, not all women with dyspareunia that is associated estrogen deficiency improve with hormone replacement.

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Dyspareunia has long been considered a sexual dysfunction. Recently, however, more emphasis has been placed on reframing diagnosis and treatment of this condition as being in most cases a chronic pain syndrome, that is, vaginal hyperalgesia [2]. The mechanisms that underlie the vaginal hyperalgesia associated with loss of ovarian function are not completely understood. Strong evidence suggests that part of the mechanism is vaginal atrophy associated with the loss of ovarian hormones, particularly estrogen, because both vaginal atrophy and dyspareunia can be reversed by hormone or estrogen replacement therapy [3]. This mechanism may not be the entire explanation, however, because of the individual differences discussed above and because vaginal atrophy and dyspareunia do not always vary together [3].

Although there are studies in women using vaginal psychophysics to provide valuable information on sexual responsivity in the postmenopausal female [4], experimentation in women to improve understanding of the mechanisms underlying postmenopausal vaginal hyperalgesia has many limitations. An animal model would therefore seem of value. Berkley et al. [5] have developed a psychophysical testing strategy to assess vaginal sensitivity in rats. They demonstrated that rats detected low levels of distension of their vaginal canal, made escape responses when distention was above tolerable levels, and made increasingly more escape responses as the distention volumes increased. This protocol also included measurements of vaginal wall compliance that gave an estimate of vaginal tone.

The present study used this testing strategy to determine if it could provide an animal model for further study of the vaginal hyperalgesia that develops in women with loss of ovarian function. Vaginal sensitivity was measured in rats before and after ovariectomy (OVX), sham ovariectomy (shamOVX) and ovariectomy with long-term 17β-estradiol replacement (OVX + E2). Because changes in escape responding could be due to changes in vaginal tone, vaginal pressures were also measured in all groups.

2. Methods

2.1. Subjects

Subjects were eight adult virgin female Sprague–Dawley rats. They were housed individually in hanging cages, maintained on a 12-h light-dark cycle, and given food and water ad libitum. At the beginning of the study, they weighed 225–250 g (~3 months old). General hormonal status was assessed throughout the study by daily vaginal smears obtained ~2 h after light onset [6]. Only rats whose vaginal smears indicated that the rat had exhibited regular 4-day cycles (before and after shamOVX), that the rat had exhibited constant leukocytes (after OVX), and that the rat exhibited constant cornified cells (after OVX + E2) were used. This study was approved by Florida State University’s Animal Care and Use Committee (protocol #9028) and was carried out in compliance with the NIH Guide for Care and Use of Laboratory Animals as well as with guidelines published by the International Association for the Study of Pain [7,8].

2.2. General

The training and testing procedures were identical to those described in detail in previous reports [5,9]. The following text abbreviates that description. The rats were first trained to perform an operant escape response to terminate a noxious stimulus, tail pinch. Distendable latex balloons were then placed in the rat’s vaginal canal and a series of assessments were made in the four different stages of estrous, after shamOVX, OVX, and OVX + E2. The percentage of the escape responses performed to a series of vaginal distention volumes was measured in each rat. Pressures produced by the different volumes of distension were also measured to obtain pressure–volume functions that provided estimates of vaginal tone. All training and testing was carried out 2–6 h after light onset.

2.3. Apparatus

The training and testing apparatus was a transparent rectangular plexiglass chamber sized to fit...
the rat gently, but snugly enough to prevent her from turning around (Fig. 1A). A hollow tube (nose cone) equipped with light-emitting diodes and a photosensor extended from the front end of the chamber (Fig. 1B).

2.4. Stimulation of vaginal canal

The stimulator was a small latex balloon tied to a thin catheter with silk suture (uninflated: ~10 mm long × 1.5 mm; Fig. 1C). The stimulus was a distension of the vaginal canal using a computer-controlled pump to inflate the balloon with different volumes of water. The pressure produced by each volume of distension (corrected for compliance characteristics of the balloon) was measured through a small-volume Cobe pressure transducer. The lubricated stimulator was temporarily inserted into the middle of the rat’s vaginal canal for the duration of each testing session, positioned so that, even when inflated, contact to the cervix was minimized.

2.5. Training

Following 3–4 days of 10-min sessions in which the rat was acclimated to the chamber, training sessions began in which the trainer pinched the rat’s tail with a padded forceps, using release of the pinch to ‘shape’ the required escape response, which consisted of the rat’s extending her head briefly into the nosecone, thereby interrupting the light beams (Fig. 1B). Training sessions of 15 pinches delivered at ~1-min intervals were performed three per week. Training was usually completed (>80% correct escape behavior) in 4–8 sessions.

Fig. 1. Psychophysical testing chamber and vaginal stimulator: (A) female rat resting during inter-trial interval in 7 × 7 × 20 cm Plexiglas testing chamber (see Section 2); (B) rat performing an escape response by entering the nosecone containing a light-emitting diode thereby disrupting the light-beam; (C) latex balloon attached to water-filled catheter (shown at control distention volume; 0.01 ml).
The rat was then trained to make identical escape responses to terminate (escape from) the vaginal distension stimuli. These sessions were run on non-consecutive days three per week for 3–5 sessions. To shape the behavior to vaginal distension, 10 large stimuli (0.8–1.0 ml, inflation rate 1 ml/s) were delivered during each session at intervals of ~1 min, each for a maximum of 15 s, making careful observations of the rat’s behavior during each stimulus. All rats showed some behavioral reaction to stimuli between 0.8 and 1.0 ml, which allowed the experimenter to use deflation of the balloon to shape the rat’s responses. All rats generalized from the tail pinch and responded appropriately to the vaginal distension (i.e. extended their heads into the tube) within 2–4 sessions.

2.6. Testing

A series of computer-controlled escape trials were run at ~1-min intervals (range 50–70 s). Each trial consisted of rapid inflation of the balloon (1 ml/s) to a fixed volume, where it remained until the rat performed an escape response or until a maximum of 15 s had elapsed, at which time it was rapidly deflated. Eight different volumes, including a control (0.01 ml) were used. These volumes were delivered three times each in random order. The computer recorded the response latency, stimulus volume and stimulus pressure for each trial. The experimenter was blind to the volumes being delivered. After the escape trials had been run, the rat was removed from the chamber, disconnected from the stimulation apparatus, and sometimes given a small treat (a dab of peanut butter on a small stick) before being returned to her home cage. Rats were tested for 4–6 weeks during normal cycling prior to any surgery (n = 6). Testing began 7–10 days after the shamOVX (n = 4) and OVX (n = 8) surgeries and continued for 4–6 weeks. Estrogen replacement capsules were implanted subcutaneously between the shoulder blades. These capsules were provided by Gorospe and Freeman [10], and deliver a dose of 17β-estradiol that maintains a plasma level of 70–100 pg/ml, which mimics periovulatory levels [10]. Blood levels of hormones were not measured directly because the stress of the sampling procedures could have influenced behavioral responses.

2.7. Surgical procedures

For OVX and shamOVX procedures, rats were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/kg). For OVX, a 2-cm, midline incision was made to expose the abdominal cavity. The uterine horns were visualized and followed rostrally to the fallopian tubes where a hemostat was clamped at the caudal end of the fallopian tubes. The ovaries were removed via a cut on the rostral side of the hemostat. The abdominal muscle and fascia were closed with 3.0 chromic gut and the skin closed with 3.0 silk. The procedure was identical during shamOVX excluding clamping the fallopian tubes and removal of the ovaries. For 17β-estradiol replacement, rats were anesthetized with a gaseous mixture of 2/3 nitrous oxide–1/3 oxygen and 3% halothane. 17β-estradiol-filled Silastic capsules were implanted subcutaneously between the shoulder blades. These capsules were provided by Gorospe and Freeman [10], and deliver a dose of 17β-estradiol that maintains a plasma level of 70–100 pg/ml, which mimics periovulatory levels [10]. Blood levels of hormones were not measured directly because the stress of the sampling procedures could have influenced behavioral responses.

2.8. Data analysis

The following measures were available from each session: Escape response percentages, which measured the probability and latency of escape response to different volumes of distension of the vaginal canal. Pressure-volume functions, which measured the pressure exerted by the vaginal wall on the balloon by each distention volume. Previous data had shown no differences in percent escape response across the estrous cycle to vaginal distention [9]. Those results were replicated here (data not shown). Therefore, all escape response data collected during normal cycling were combined. Pressure data were also presented combined during normal cycling as well as in different stages of the estrous cycle. Differences in escape responses and pressures produced by different distention volumes during normal cycling, after
Fig. 2. Escape responses to vaginal distension: (A) percent escape response as a function of vaginal distention volume during cycling and OVX. * = $P \leq 0.05$; (B) percent escape response as a function of vaginal distention volume during cycling and shamOVX; (C) percent escape response as a function of vaginal distention volume during OVX and OVX + E2. # = $P \leq 0.05$; (D) percent escape response as a function of vaginal distention volume during cycling and OVX + E2. Arrows indicate distention volume at which escape response percentages were significantly greater than responses to the control volume (0.01 ml).

shamOVX, OVX and OVX + E2 were analyzed statistically using ANOVAs for repeated measures, followed by post hoc Fisher's least significant difference comparisons and $t$-tests, with alpha levels set at 0.05.

3. Results

3.1. Escape response percentages

Escape response percentages after OVX were significantly higher to distention volumes 3–8 (0.25–0.85 ml) compared to responses during cycling (Fig. 2A) and during OVX + E2 (Fig. 2C). No differences were observed between escape response percentages during cycling with those after shamOVX or OVX + E2 (Fig. 2B, D).

3.2. Individual differences

While Fig. 2A shows that the overall change in escape response percentages to the top six volumes increased with OVX, there was a substantial range in the increases across rats. To illustrate this variability, Fig. 3A–C shows examples of three of the rats during normal cycling, after OVX and OVX + E2. Of the six rats tested here, one showed an exaggerated increase in responses (Fig. 3A), and four showed significant but moderate increases in responses after OVX like the example in Fig. 3B. After OVX + E2, all five showed decreases like that in Fig. 3B. One rat, # 1258 (Fig. 3C) showed only marginal increases in response to OVX and no change with E2 replacement.

3.3. Pressure–volume functions

Vaginal tone after either OVX, shamOVX or OVX + E2 did not differ from overall vaginal tone in cycling rats (Fig. 4A, B, D). Notably, however, despite the fact that OVX produced vaginal hyperalgesia and OVX + E2 reversed this hyperalgesia, vaginal tone in OVX rats was sig-
significantly lower than that in OVX + E2 rats (Fig. 4C).

As in previous reports [9] vaginal tone in the present study varied with the estrous cycle. Specifically, vaginal tone was lowest during diestrus and highest during estrus (Fig. 5). Vaginal tone in OVX rats was equivalent to that measured during diestrus, while vaginal tone in OVX + E2 was equivalent to that measured during estrus (Fig. 5).

4. Discussion

The present study demonstrated that ovariectomy induces vaginal hyperalgesia in most but not all rats, and that it is reversible with estrogen replacement. The hyperalgesia was evoked by loss of the ovaries, but not by abdominal surgery alone. Although ovariectomy and estrogen replacement affected vaginal tone, the tone changes did not predict the changes in vaginal sensitivity.

4.1. Mechanisms

Loss of ovarian hormones in women is often associated with an increase in vaginal sensitivity [1,11,12]. The present study demonstrates that this increase also occurs in rats. In women, vaginal atrophy that is associated with declining estrogen levels (and vaginal hyperalgesia) is characterized by an overall thinning of the vaginal wall, an increase in the proportion of vaginal parabasal cells, a decrease in superficial cells and a significant increase in vaginal pH [3]. Animal studies have demonstrated that ovariectomy in rats and mice produce vaginal atrophy, with tissue changes similar to those in women described above [13–16]. Estrogen replacement has been shown to substantially reverse vaginal atrophy in both women and rodents [3,14,16]. The changes observed here in vaginal sensitivity in rats after OVX and OVX + E2 may result in part from the changes that take place in the vaginal epithelium.

The loss of ovarian hormones has also been implicated in the postmenopausal decline of vulvovaginal sensorimotor responses associated with a decreased intensity of vaginal contractility and an increase in incontinence [17]. Vulvovaginal sensorimotor reflexes, blood volume and vascular compliance appear to play a role in the maintenance of vaginal tone, which may be involved, in vaginal sensitivity. Studies on rats have demonstrated that although the compliance of the saphenous artery does not change after ovariectomy, it
is increased after hormone replacement [18]. The present study showed the same trend for overall vaginal tone. However, when the phase of the estrous cycle was considered, there were significant differences observed in vaginal tone between cycling and OVX and cycling and OVX + E2 (Fig. 5). Thus, vaginal tone is equally high after OVX + E2 or on the morning of estrus, which is a period less than 12 h after the hormonal surges that initiate ovulation. Likewise, vaginal tone is equally low after either OVX or on the morning of diestrus, which is ~60 h after ovulation.

What is surprising, however, is that the changes in vaginal tone were opposite to those that would be expected from the changes in vaginal sensitivity. One would expect that vaginal sensitivity would increase if vaginal tone increased because, for any given distention volume, the pressure produced by that volume would be greater. However, despite the fact that vaginal tone was greater after OVX + E2 than after OVX, escape response percentages were lower after OVX + E2 than after OVX.

On the other hand, a similar phenomenon was observed in the cycling animal. Whereas no differences in escape response percentages were observed with any particular volume as function of estrous stage, it was found that rats tolerated higher pressures before responding to vaginal distention during estrus when vaginal tone is normally higher than during diestrus when vaginal tone is normally lower [9]. This trend may become exaggerated with a chronic loss in vaginal tone after OVX in that the rat is able to tolerate even less pressure over time. That long-term estrogen replacement increases vaginal tone and increases
the amount of pressure on the vaginal wall that is tolerated before an escape response mimics what is seen in the normal cycling rat.

4.2. Individual differences

The fact that at least one rat (Fig. 3C), showed only slight increases in responses with OVX and little to no decreases with OVX + E2 demonstrates that individual variability in the psycho-physical response to the loss of ovarian hormones exists for rats as well as humans. One possible explanation may be individual differences in hormonal levels associated with OVX and OVX + E2, which were not measured here because the stress of taking blood samples could have affected responses. However, the majority of rats in the study showed a significant increase in sensitivity to vaginal distention after OVX with one rat demonstrating a substantially larger than average increase in responsiveness. In addition, all increases in response to vaginal distention seen in this study were reduced by estrogen replacement with the exception of the one rat mentioned above. Therefore, this model could be used for further study of the mechanisms underlying changes in vaginal sensitivity seen with modulations in ovarian hormones as well as individual differences in these changes.

4.3. Significance

The present study introduces an animal model that mimics the changes in vaginal sensitivity seen in some women with the loss of ovarian hormones. While there are obviously considerable differences between rats and humans, these findings provide a justification for further animal studies to address individual differences and the relationship between the changes in physiological parameters such as vaginal atrophy and vascular compliance with changes in perception. Because these studies were limited to adult virgin rats, there are no data to compare how these manipulations might differ in sexually active or parous rats. Of relevance is that postmenopausal changes in vaginal sensitivity have been shown to be influenced by the level of sexual activity of the individual [3]. Further studies using this testing strategy in rats could address the issues of the effects of mating, mating frequency and pregnancy on postovariectomy vaginal sensitivity. Taken together, these results indicate that the ovariectomized rat appears to be a useful model for the study of menopause-associated dyspareunia.

References