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Prostaglandin levels, vaginal innervation, and cyst innervation as peripheral contributors to endometriosis-associated vaginal hyperalgesia in rodents

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ABSTRACT

Endometriosis is a painful condition characterized by growth of endometrial cysts outside the uterus. Here, we tested the hypothesis that peripheral innervation and prostaglandin levels contribute to endometriosis-associated pain. Female Sprague-Dawley rats (n = 16) were surgically instrumented by transplanting uterine tissue onto mesenteric arteries within the peritoneal cavity to create a model of endometriosis which forms extra-uterine endometrial cysts and vaginal hyperalgesia. Our results describe a significant positive correlation between endometriosis-induced vaginal hyperalgesia and cyst innervation density (sensory, r = 0.70, p = 0.003; sympathetic, r = 0.55, p = 0.03), vaginal canal sympathetic innervation density (r = 0.80, p = 0.003), and peritoneal fluid levels of the prostaglandins PGE2 (r = 0.65, p = 0.01) and PGF2α (r = 0.63, p = 0.02). These results support the involvement of cyst innervation and prostaglandins in endometriosis-associated pain. We also describe how sympathetic innervation density of the vaginal canal is an important predictor of vaginal hyperalgesia.

1. Introduction

Endometriosis is an estrogen-dependent condition characterized by extrauteral endometrial growths known as cysts. This condition affects approximately 10% of premenopausal women. Of those affected, 30–50% have severe pelvic pain (Giudice and Kao, 2004; Rogers et al., 2009). Painful symptoms include dyspareunia (vaginal hyperalgesia), dysmenorrhea (menstrual pain), dyschezia (pain on defecation), and chronic pelvic visceral and muscle pain; all which greatly reduce quality of life. Within the pelvic cavity, where endometriosis primarily occurs (Giudice and Kao, 2004; Stegmann et al., 2009), endometriosis can be subdivided into three categories: superficial peritoneal endometriosis, deeply infiltrating endometriosis, and ovarian (cystic) endometriosis (Ferrero et al., 2015; Raffi and Amer, 2011). In carefully documented studies, the location and amount of ectopic growth does not correlate with the presence or severity of pain symptoms except for that of deep infiltrating endometriosis (Fauconnier and Chapron, 2005; Stratton and Berkley, 2011; Vercellini et al., 2007). Hence, endometriosis is an “enigma” since how painful symptoms become associated with the condition is unclear. Due to this lack of knowledge, available pain treatments often only provide temporary relief, produce unwanted side effects, or are ineffective.

Further, similar to women, the amount of ectopic growth in endometriosis rats fails to correlate with symptom presence and pain severity; however, ectopic growth excision can provide long-term relief of painful symptoms (Alvarez et al., 2014; McAllister et al., 2009) suggesting some aspect of the growth contributes to the pain. One possibility is that the growth's sprouted sensory and sympathetic nerve supply opens a two-way line of communication between the growths and CNS that is capable of generating pain (Stratton and Berkley, 2011). Studies in the rodents (Alvarez et al., 2012; McAllister et al., 2009, 2012) and a few clinical studies support this hypothesis (Di Spiezio Sardo et al., 2015; Mechsner et al.,

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2009; Wang et al., 2010).

In women, the most commonly used drugs for the treatment of endometriosis-associated pain are nonsteroidal anti-inflammatory drugs (NSAIDs) hypothesized to work by suppressing prostaglandin levels via inhibition of cyclooxygenases (COX-1 and COX-2) that oxidize arachidonic acid (AA) (Streuli et al., 2013; Wu et al., 2010). However, NSAIDs must be used with caution due to their ability to produce significant adverse side effects including gastrointestinal bleeding, impaired renal function and interactions with other medications, making this a sub-optimal treatment systemically (Dhasmana et al., 2014). In women with endometriosis, peritoneal fluid levels of prostaglandin E2 (PGE2) and prostaglandin F2 alpha (PGF2α) are significantly greater than in women without the condition (Dawood et al., 1984; Karck et al., 1996; Wu et al., 2010). These prostaglandins are potent nociceptive activators, (Ray et al., 2015; Smith, 2006) key mediators in pain and nociception (Lousse et al., 2012), and likely influence cyst innervation directly or indirectly to contribute to endometriosis-associated pain.

In this study, we used a rat model of endometriosis where a piece of uterine horn (~1 cm) is removed and cut into four equal pieces (~2 mm × 2 mm) followed by transplantation onto cascading mesenteric arteries within the rat peritoneal cavity (Vernon and Wilson, 1985). These uterine transplants over time produce innervated and vascularized cysts (Berkley et al., 2004, 2005). The model produces symptoms similar to women including vaginal hyperalgesia (Berkley et al., 2007; McAllister et al., 2009, 2012) referred abdominal muscle hyperalgesia (Giamberardino et al., 2002; Nagabukuro and Berkley, 2007), and bladder hyperactivity (Morrison et al., 2006). Therefore, we examined the relationships between vaginal hyperalgesia, cyst sensory and sympathetic innervation density, and peritoneal fluid levels of PGE2 and PGF2α 4–6 wks after surgery when individual differences in hyperalgesic severity are greatest (McAllister et al., 2012). Further, we also analyzed the innervation density of the eutopic uterus as well as the vaginal canal. Since endometriosis-induced vaginal hyperalgesia was previously shown to be estrous-stage dependent we also examined how our results are influenced by estrous stage during development of endometriosis (Cason et al., 2003).

2. Materials and methods

2.1. General description of rodents

Sixteen adult virgin female Sprague Dawley rats (175–200 g at arrival; Charles River, Raleigh, NC) were used in this experiment. Animals were housed individually in plastic cages lined with chip bedding and *ad libitum* access to rat chow and water. Housing was in environmentally controlled conditions (room temp temperature ~22 °C; 12-h light/dark cycle, with lights on at 07:00). This study conformed to the NIH Guidelines for the Care and Use of Laboratory Animals. The Florida State University Institutional Animal Care and Use Committee approved the experimental protocols of this study as #9028, #1212, and #0913.

In all rats, reproductive status was assessed daily by vaginal lavage ~2 h after lights on. Traditional nomenclature was used for the four rat estrous stages of proestrus, estrus, metestrus, and diestrus (McLean et al., 2012). All rats maintained normal four-day estrous cycles throughout the study. All behavioral assessments (training and testing) were done ~3–8 h after lights on.

2.2. Surgical induction of endometriosis (endometriosis)

Endometriosis was induced following the protocol develop by Vernon and Wilson (1985). Briefly, using aseptic techniques, rats in diestrus were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/kg) and placed on a heating pad to maintain body temperature ~37 °C. A midline abdominal incision was made to expose the uterus and a ~1-cm segment of the left uterine horn and associated fat tissue were removed and placed in warm sterile saline. The uterine horn, including the endometrium and myometrium, was then cut into 4 equal pieces (~2 mm × 2 mm) and sewn (4.0 nylon suture) onto cascading mesenteric arteries within the pelvic cavity that supply the caudal small intestine beginning at the ceacum. The incision was closed in layers. Rats were monitored closely after surgery for potential complications. The postoperative recovery period was uneventful, regular estrous cyclicity resumed within a few days, and assessment of vaginal nociception resumed ~1 wk after surgery.

2.3. Behavioral assessment of vaginal nociception

The behavioral training, testing, and assessment procedures were identical to those described in detail previously (McAllister et al., 2009, 2012). Rats were trained to perform an escape response to terminate vaginal distention produced by an inflatable latex balloon. During each testing session, eight different distention volumes were delivered three times each at random ~60 s apart and the percent escape response to each volume given was assessed.

2.3.1. Behavioral apparatus and stimulator

The training and testing apparatus was a Plexiglas® chamber allowing movement but preventing the rat from turning around. In the front of the chamber, a hollow tube is extended containing a light-emitting diode and photo sensor. When a rat extended her nose into this tube, a light beam is broken and the stimulus is terminated. In other words, the rat breaking the light beam constituted an escape response. An opening in the rear of the chamber allowed the catheter (attached to the vaginal stimulator) to be connected to the computer-controlled stimulus-delivery device.

The vaginal stimulator consisted of a small latex balloon (~10 mm long × 1.5 mm wide when uninflated) tied to a thin catheter with silk suture. Immediately prior to the training or testing session, the uninflated balloon was lubricated with K-Y® jelly, inserted into the mid-vaginal canal, and located to ensure the cervix was not touched (even when inflated). The vaginal canal was then distended by the delivery (computer-controlled) of different volumes of water to the balloon. A small-volume Cobe pressure transducer measured the pressures produced by the volumes of distention (corrected for compliance characteristics of the balloon).

2.3.2. Behavioral training

Rats were first adapted to the testing chamber by being placed in the box for 10 min daily for 3–4 days. Then, rats were trained to perform an “escape response” which involves the rat extending her head into the hollow tube to interrupt the light beam. To do this, the trainer pinches the rat's tail with padded forceps and used a forcep release to shape the required “escape response.” These training sessions, done 3/week on non-consecutive days, consisted of 10 tail “pinches” delivered at ~1 min-intervals. This “tail pinch” training was completed (>80% escape behavior) in 3–5 sessions.

Rats were then trained to make an identical escape response to terminate vaginal distention stimuli (the balloon). In these sessions, ten large distention volumes (0.80 ml–1.0 ml, inflation rate 1 ml/s) were delivered for a maximum of 15 s at ~1-min intervals. All rats showed some behavioral response to these stimuli, which allowed the experimenter to use balloon deflation to shape the rat's escape response. This “balloon training” was done 3/wk on non-consecutive days and completed in 3–5 sessions. Once trained,