Effects of hypogastric neurectomy on escape responses to uterine distention in the rat

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Abstract

Anatomical data indicate that the rat uterine horn is innervated primarily by afferent fibers in the hypogastric nerves, suggesting that hypogastric neurectomy, but not pelvic or pudendal neurectomy, should eliminate behavioral responses to uterine horn stimulation. To test this hypothesis, detection and escape responses of rats to different volumes of uterine horn distention (via an indwelling intrauterine balloon) were compared before and after bilateral hypogastric (n = 9), sham-hypogastric (n = 3), pelvic (n = 3), or pudendal (n = 2) neurectomies. As predicted, sham-hypogastric, pelvic, and pudendal neurectomies had no effect on the rats' responses. However, although hypogastric neurectomy completely eliminated responses in five rats whose postmortem evaluation revealed no signs that the uterine balloons had evoked any pelvic pathophysiology, the neurectomy had no effect on the responses of an additional four rats. Postmortem evaluation of these rats revealed gross signs of severe pathology in the vicinity of the balloon in two rats, and evidence that the balloon had shifted caudally so that it was stimulating the cervix rather than the uterine horn in a third. In the fourth rat, pathophysiology had been deliberately induced by the prior implantation of a small pellet that released ~1 μg/day of prostaglandin PF2α over the uterine horn. Similar findings have been reported in clinical studies on the efficacy of hypogastric ('presacral') neurectomy for dysmenorrhea. Together, the findings support the hypothesis that the major source of afferent innervation of the uterine horn in healthy rats and women is the hypogastric nerve but that the situation changes under conditions of pelvic pathology. Such changes could include additional activation of afferent fibers in nerves that supply other pelvic organs, activation by the uterine pathophysiology of latent uterine innervation from afferent fibers in the pelvic, vagus or ovarian plexus nerves, or some form of central sensitization. © 1999 International Association for the Study of Pain. Published by Elsevier Science B.V.

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1. Introduction

Dysmenorrhea (menstrual pain) is a recurrent pain condition estimated to occur in ~50% of all menstruating women (Ylikorkala and Dawood, 1978). The most common hypothesis is that dysmenorrhea results from abnormally high intrauterine pressures during the perimenstrual period that are produced by abnormally strong uterine contractions induced by a hyperproduction of eicosanoids, particularly the prostaglandin PGFα (Smith, 1990; Rapkin et al., 1997). Information arising from such hypercontractility of the uterus is presumed to be conveyed to the CNS via uterine afferent fibers located in the hypogastric nerves (Rogers, 1998).

Consistent with this hypothesis, the main medical treatment for dysmenorrhea includes non-steroidal anti-inflammatory medication or supplemental hormones that reduce prostaglandin synthesis (Rapkin et al., 1997). When these treatments fail, and the dysmenorrhea remains severe and intractible, surgical treatments are considered, usually either hysterectomy or, for women who wish to remain fertile, presacral neurectomy (i.e. bilateral hypogastric neurectomy; Hammond and Riddick, 1999).

Regardless of whether the dysmenorrhea is classified as primary (without evident pelvic pathology) or secondary (accompanied by pelvic pathology such as adenomyosis, endometriosis, polyps, myomea, infection, etc.), presacral neurectomy has consistently proven quite beneficial (75–94%), with a substantial proportion of patients (>75%) continuing to experience relief for many years postoperatively (Metzger, 1998; Nezhat et al., 1998). However, presacral neurectomies are less effective (but not totally ineffective) in patients with laterally-located, rather than (or in addition to) midline pain, in patients with secondary dysmenorrhea if resections of the pelvic pathology (such as...
endometriotic implants) are not or cannot be also done, and in patients with additional chronic pelvic pains not associated with menstruation (Chen and Soong, 1997; Metzger, 1998). One explanation for the lesser effectiveness of presacral neurectomy in these situations has been that the nociceptive information is being conveyed to the CNS via afferent fibers in nerves other than the hypogastrics that supply the pelvic region (Nezhat and Nezhat, 1992).

Studies in animals would be of value for improving our understanding of how information conveyed to the CNS via afferent fibers in the several nerves supplying pelvic organs might give rise to dysmenorrheic or other pains. One such animal is the rat. Previous studies in our laboratory have shown that the unanesthetized rat can detect distention of her uterine horn and that the probability of her performing escape responses to such stimulation increases as the distention volumes and pressures increase (Berkley et al., 1995; Bradshaw et al., 1999). Furthermore, the primary afferent innervation of the rat reproductive tract is now well understood and appears analogous to that in women (Bonica, 1990; Rogers, 1998). As illustrated in Fig. 1, the rat’s vaginal canal is supplied by afferent fibers in the pelvic nerve, the cervix by fibers in both the pelvic and hypogastric nerves, the uterine horns by fibers in the hypogastric nerve, and the oviduct and ovary by fibers in the superior olivary, ovarian plexus and vagus nerves (Peters et al., 1987; Berkley et al., 1993; Papka and Traurig, 1993; Traurig and Papka, 1993; Berkley and Hubscher, 1995). Of potential importance is recent evidence for additional afferent innervation of the entire reproductive tract by the vagus nerve in both female rats and women (Komisaruk et al., 1996; Komisaruk et al., 1997).

Here we tested the hypothesis that, in the healthy rat, the afferent innervation of the uterine horn is derived primarily from the hypogastric nerve. If so, it would be expected that escape responses evoked by uterine distention would be eliminated by hypogastric neurectomy, but not by sham hypogastric neurectomy, nor by pudendal neurectomy (all bilateral).

2. Materials and methods

Twelve virgin female rats were used. They weighed 200–250 g at the start of the study and were housed in individual hanging cages and maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). All had regular 4-day estrous cycles (assessed daily by vaginal smears). Because the training and testing procedures used were identical to those previously described (Berkley et al., 1995; Bradshaw et al., 1999), these procedures are described here briefly. All studies were approved by Florida State University’s Animal Care and Use Committee (protocol #9028).

2.1. Apparatus

The training and testing apparatus was a transparent rectangular plexiglass chamber, sized to fit the rat snugly but not with excessive restraint. A hollow tube fitted with light emitting diodes on one side and a photosensor on the other was located at the front end of the chamber. The distention stimulus was delivered via a computer-controlled stepper motor-driven pump that inflated the balloon with various pre-selected volumes of water and deflated it either when the rat made an escape response (see below) or after 15 s had elapsed. A small-volume pressure transducer attached to the stimulus delivery apparatus measured the pressure of the uterine horn with each distention.

2.2. Initial training

Each rat was acclimated to the testing chamber by being placed in it for 10–15 min each day for 3–4 days. The rat was then trained to terminate a noxious tail pinch stimulus (maximum duration 15 s), by extending her head into the tube located at the front end of the chamber, thereby interrupting light-emitting diodes and activating the photosensor. This action constituted an escape response. After this initial training, a distensible balloon was permanently implanted into the uterus.

2.3. Uterine balloon implant

The balloon was a piece of latex condom attached to the open end of thin catheter tubing. When deflated, the balloon was ~5 mm long and ~1.5 mm wide. Each tail-pinched-trained rat was anesthetized IP with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/kg) while in either proestrus or estrus. A longitudinal midline abdominal incision was made to expose the uterine horns. The left uterine

![Diagram of reproductive tract](image-url)
horm remained quietly in the chamber. The rat was then given a
experiencer noted whether an escape response was made
experiments (mmHg) and escape responses to uterine distention
series of three tail pinch stimuli at 30-s intervals and the
pressure of uterine disten-
reworked 100% escape responding during training. The computer recorded the time
elapsed and the volume at which the balloon was actually
deflated (real trial) or would have deflated (sham trial). This
strategy produced two measures: (1) the likelihood of a rat
making a detection behavior during experimental and sham
trials, and (2) the range of actual or potential volumes at
which detections were made. The chance of a rat making a
detection behavior during sham trials provided an estimate
of the likelihood of false-positive detection. False positive
probability was always less than that during real trials,
except after hypogastric neurectomies that reduced escape
responses (see Section 3).

2.5.2. Detection of uterine distention

The rat was next given five ‘detection’ trials at ~60-s
intervals. These trials, maximum duration 15 s, involved
either distending the uterus at a slow, pre-programmed
rate of 0.025 ml/s (‘real trial’) or no stimulus (‘sham
trial’), ~50% each (randomly programmed), with the experi-
menter unaware of which type of trial was occurring. The
experiencer observed the rat’s behavior and signalled the
computer to deflate the balloon whenever the rat’s behavior
changed (e.g. repositioning, stretching, or grinding teeth,
etc.). These changes were considered as an indication the
rat ‘detected’ the stimulus. The computer recorded the time
elapsed and the volume at which the balloon was actually
deflated (real trial) or would have deflated (sham trial). This
strategy produced two measures: (1) the likelihood of a rat
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except after hypogastric neurectomies that reduced escape
responses (see Section 3).

2.5.3. Escape response from uterine distention

A series of escape trials were run at ~90-s intervals
(range, 80–100 s). Each trial consisted of computer-
controlled rapid inflation of the balloon (0.2 ml/s) to a
fixed volume until the rat performed an escape response
or for a maximum of 15 s. The six volumes chosen during
the training period were delivered three times each in a
random order, with the experiencer blind to which volume
was being delivered. For each trial, the computer recorded
the stimulus volume and the pressure. Following this testing
period, uterine contractions were rerecorded for another 10
min. The rat was given a bit of peanut butter, disconnected
from the stimulus delivery apparatus, and returned to her
cage.

2.6. Neurectomies

The animals were anesthetized as above, and a slightly
off-midline abdominal incision was made to expose the
pelvic organs. For the hypogastric neurectomy (n = 9),
the left and right nerves were identified about 1 cm caudal
to the point where the nerves exit the inferior mesenteric
ganglion and begin to run parallel with the ureter. For the
pelvic neurectomy (n = 3), the internal branch of the left
and right pelvic nerves were isolated deep within the muscle
layer dorsal to the internal iliac artery on each side. For the
pubdernal neurectomy (n = 2), the sensory branches on each
side were identified as they branched from the L6-S1 trunk.
For each neurectomy, a 1-cm section of the nerve was care-
fully dissected free from surrounding tissues, cut out and
removed. Sham hypogastric neurectomy (n = 3) was identi-
tical to the real neurectomy in every respect except that the
nerves were not cut. Following recovery (~1 week), the rats were tested for 8–10 more sessions (again only in the metestrus or diestrus phases of their cycle) using the same testing protocol described above.

Some rats were subjected to two neurectomy procedures, with 8–10 testing sessions following recovery from each neurectomy. Thus, the 12 rats whose data are reported here were treated as follows: hypogastric neurectomy only (n = 4; includes rat with PGF2α pellet, see below); pudendal neurectomy only (n = 2); pelvic neurectomy only (n = 1); sham hypogastric neurectomy followed by real hypogastric neurectomy (n = 3); pelvic neurectomy followed by hypogastric neurectomy (n = 2).

2.7. Sacrifice and observation

Rats were killed with an overdose of pentobarbital (IP). Shortly after the pentobarbital was injected (i.e. during the brief period when the rat was anesthetized but not yet dead), the rat’s abdominal cavity was opened so that the uterine horns could be examined to determine the extent, if any, of ‘pathology’ in addition to the implanted balloon. Such examination was carried out by an individual blind to the effectiveness of the neurectomy that had been performed. Pathology consisted of any grossly visible evidence of inflammation (severely reddened uterine wall), or signs of damage to the uterus in the vicinity of the balloon (tears, abscess), significant scar tissue, adhesions to other abdominal organs and/or excessive fat growth. The placement of the uterine balloon was also checked to make sure it had remained where it had been implanted. In addition, the nerves were examined to insure that the two ends had remained separated (in the real neurectomies) or undamaged (if sham).

2.8. PGF2α implant

To examine the influence of excess pathology further, one rat was treated as follows: Following testing to obtain baseline response data to distention of the uterine balloon, the rat was anesthetized and a small abdominal incision made so that a small, continuous-release pellet containing PGF2α (~2 mm diameter; sufficient to deliver a dose of ~1 μg/day for 21 days; Innovative Research of America) could be tucked into an abdominal area on the surface of the uterine horn near the uterine balloon. Following recovery from surgery, the rat was then retested during the 21-day period of pellet activity. A hypogastric neurectomy was then carried out, and again following recovery, the rat retested once more.

2.9. Data analysis

Escape responses to tail pinches, detection responses, and escape responses to the six volumes of uterine distention during testing sessions in the experimental and control conditions were statistically compared using ANOVA for repeated measures, followed by post-hoc Fisher’s least significant difference comparisons, with alpha levels set at 0.05.

3. Results

3.1. Responses to tail pinch

Before surgery, all rats responded to all tail pinch stimuli (i.e. 100% response), with a mean latency of 1.5 ± 0.5 s, SE). There were no significant changes in escape response probability or latency after hypogastric neurectomy, sham hypogastric neurectomy, pelvic neurectomy, or pudendal neurectomy.

3.2. Detection of uterine distention

Before surgery, rats made detection responses during 89% of real trials and 35% of sham trials (P(0.001) at real volumes of 0.04 ± 0.007 ml SE. After ‘successful’ hypogastric neurectomy (see below), detection responses were reduced to 38% (real stimuli) and 20% (sham stimuli) with no significant difference between real and sham. Furthermore, uterine detection volumes during real trials were significantly increased to 0.11 ± 0.005 ml (P(0.001). There were no significant changes in detection responses after sham hypogastric neurectomy, ‘ineffective’ hypogastric neurectomy (see below), pelvic neurectomy or pudendal neurectomy.

3.3. Escape responses and pressures produced by uterine distention

3.3.1. Before neurectomy

In all rats (n = 13), percent escape response increased as distention volume increased (Fig. 2). Uterine pressures also increased as the distention volume increased, but the data are not shown here because none of the neurectomies had significant effects on the pressure-volume function. (Such pressure-volume functions have been illustrated in two earlier publications; Berkey et al., 1995; Bradshaw et al., 1999).

3.3.2. After hypogastric neurectomy

Postmortem examination of nine rats after hypogastric neurectomy revealed that five of them had no gross abnormalities associated with the uterus or within the pelvic cavity (other than the intrauterine balloon). In two other rats, excessive fat growth around the uterine horn containing the balloon, gross evidence of inflammation, or extensive adhesions to the abdominal wall and intestines were found. In a third rat, the one with an implanted PGF2α pellet, some excess fat was observed around the balloons with some adhesions to the adjacent abdominal wall, but this pathology was not as severe (extensive) as that observed in the other two rats. In a fourth rat, the balloon had moved caudally within the uterine horn so that the stimulus to which the rat was responding was one that distended the uterocervix.
region rather than just the uterine horn (i.e. in a part of the uterus that receives both hypogastric and pelvic nerve innervation; Fig. 1).

Escape responses were eliminated following hypogastric neurectomy in the five rats without gross abnormalities (Fig. 2A, Fig. 3A). In other words, responses were significantly reduced to such low levels after hypogastric neurectomy that there were no significant differences between escape percentages to control and any of the other volumes. In contrast, hypogastric neurectomy had no significant effect on escape responses in four rats. These four included the two rats with evidence of pelvic pathology, the rat whose balloon was distending the uterocervix region, and the rat that had received the PGF2α pellet (Fig. 2B, Fig. 3B). (Note also that there was no significant difference in escape responding before and after implantation of the PGF2α pellet; data not shown).

3.3.3. After sham hypogastric neurectomy

Postmortem examination of the three rats that underwent sham hypogastric neurectomy revealed that two rats had no gross pelvic abnormalities and one rat had extensive adhesions to surrounding structures and excessive fat growth around the uterine horn containing the balloon. Escape responses in all three rats were unaffected by the sham neurectomy (Fig. 2C, Fig. 3C).

3.3.4. After pelvic or pudendal neurectomy

No gross pelvic abnormalities were noted in the two rats that underwent pudendal neurectomy as well as in the three rats that underwent pelvic neurectomy. Escape responses in all five rats were unaffected by the neurectomies (Fig. 2D, Fig. 3D).

4. Discussion

Hypogastric neurectomy eliminated escape and detection responses to uterine distention in rats that had not developed pelvic pathology, but had no affect on responses in two rats that had developed additional pelvic pathology, in one rat that had received an intraabdominal PGF2α-releasing
pellet, and in one rat whose balloon distended the cervix as well as the uterine horn. Responses were also unaffected by sham hypogastric, pelvic, or pudendal neurectomies.

These results are consistent with clinical observations summarized in the Section 1 indicating the remarkable and consistent effectiveness of presacral neurectomy to reduce severe dysmenorrhea in women who have no signs of pelvic pathology and its lesser effectiveness in women who do have such pathology. Together, the findings support two hypotheses; firstly, that the major source of afferent innervation for the uterine horn in healthy rats and women is the hypogastric nerve (Fig. 1), and, secondly, that this situation changes under conditions of additional pelvic pathology or when the potentially algogenic stimulation affects other parts of the reproductive tract (e.g. cervix) or pelvis.

Before discussing the nature of these changes, it is important to consider the possibility that the nerves were incompletely severed in those rats in whom a neurectomy failed to reduce escape responding. This possibility seems unlikely because (a) the nerves are readily identified in the rat, (b) a full 1 cm segment of each nerve was removed, and (c) completeness of the neurectomy was confirmed postmortem.

There are at least four potentially-complementary effects of pelvic pathology that could serve to reduce the effectiveness of hypogastric neurectomy on escape responding to uterine distention. One obvious effect of the additional pathology might be that it extends into regions that are innervated by nerves other than the hypogastric. Such a situation would apply for the rat whose balloon stimulated the cervix, as well as to the rat with adhesions/excess fat that had grown between the uterine horn, bladder, colon and abdominal wall. It also fits clinical findings showing that the effectiveness of presacral neurectomy is increased when sources of such other pathology are removed at the same time the neurectomy is performed (such as endometrial growths in the pelvic/abdominal cavity; Nezhat and Nezhat, 1992; Chen and Soong, 1997; Nezhat et al., 1998).

This explanation does not apply, however, for one of the rats with ineffective hypogastric neurectomies whose gross pathophysiology appeared to be restricted to the uterine horn in the vicinity of the balloon (growth of excess fat, inflammation, but no adhesions). Similarly, it does not apply to the lesser effectiveness of presacral neurectomy for women with secondary dysmenorrhea associated with adenomyosis (growth of endometrium into uterine musculature; Chen and Soong, 1997). A second effect that could have been happening in these cases is that the abnormal tissues are releasing excess algogenic substances such as

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Fig. 3. The effect of various bilateral neurectomies on escape responses: single case examples. Figure format is identical to that of Fig. 2.
PGF2α into the pelvic cavity. Such release could then activate fibers in other nerves supplying the pelvic/abdominal region. The ineffectiveness of hypogastric neurectomy to reduce escape responding in the rat with a PGF2α-releasing pellet supports this possibility, as well as clinical data showing increased production of prostaglandins and interleukin-6 by abnormal tissue (Buyalos et al., 1992; Koike et al., 1992b) and a correlation of this increased release with dysmenorrheic severity (Koike et al., 1992a).

A third possible effect of pelvic pathology involves the prospect that the uterine horn is supplied by afferent fibers traveling in nerves other than the hypogastric and that these fibers convey sensory information to the nervous system by way of action potentials only after pathophysiologic events have occurred; i.e. the fibers are ‘silent’ under healthy conditions. There are three potential sources of such sensitizable, latent innervation; they include fibers in the ovarian plexus, pelvic and vagus nerves. For the ovarian plexus nerve, Berkley and Sato showed that ovarian plexus nerve fibers that were previously responsive only to gentle mechanical stimulation of the oviduct became additionally responsive to stimulation of the rostral 2/3 of the adjacent uterine horn after several such stimuli were delivered (described in Berkley and Hubsher, 1995). In other words, the innervation territory of the ovarian plexus nerve extended from the oviduct into a large segment of the adjacent uterine horn after irritation of the uterine horn tissue. For the pelvic nerve, Berkley et al. (1993) showed that irritation of the body of the uterus or cervix can increase the sensitivity of the pelvic nerve afferents so that their apparent innervation territory expands rostrally from the cervix into the caudal portions of the uterus. For the vagus nerve, Komisaruk et al. (1997) have recently shown that vagal innervation of caudal reproductive tract becomes evident in rats and paraplegic women.

If it was indeed the case that activation of silent afferents in any of these three nerves had anything to do with the results here, then it is actually surprising that hypogastric neurectomy had any affect at all on escape responding, because it would seem that the invasive surgery of intrauterine balloon implantation would likely have activated some of these afferents. It would therefore be important to determine what it was about the additional pathology, but not implantation of the balloon itself, that could activate the fibers.

A fourth possible consequence of pelvic pathology that could reduce the effectiveness of a hypogastric neurectomy is that the pathology induced some form of central sensitization so that spinal or brainstem neurons receiving input from the uterus had become excitable to peripheral stimulation either of the uterus itself or of other regions that had previously elicited no response (McMahon et al., 1993; Wall et al., 1993; Berkley and Hubsher, 1995). This explanation seems at most minimally applicable here, however, because, similar to activation of silent afferent fibers, it would seem that implantation of the balloon would itself be enough to induce some form of central sensitization. In that case, it would be expected that hypogastric neurectomy would never eliminate escape responding to distention of an implanted intrauterine balloon, unless, as some have argued, central sensitization requires continued input from the affected organs (Devor, 1997).

In conclusion, whatever the explanation for the ineffectiveness of some hypogastric neurectomies, the fact that hypogastric neurectomy can eliminate escape responding to distention of the uterine horn in uncomplicated cases supports anatomical and physiological studies indicating that the uterine horn is supplied mainly by afferents in the hypogastric nerve. Such results are similar to clinical results indicating that presacral neurectomy can alleviate uncomplicated cases of otherwise intractable severe dysmenorrhea. Together, the findings support clinical assumptions that perimenstrual uterine hyperactivity is one of the main etiological factors for dysmenorrhea and indicate that the rat provides a useful model for its investigation.

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References

Berkley KJ, Hubsher CH. Visceral and somatic sensory tracts through the neuroaxis and their relation to pain: lessons from the rat female repro-


Devor M. Central versus peripheral substrates of persistent pain: which contributes more? Behav Brain Sci 1997;20:446.


