A neuroanatomical correlate of sensorimotor recovery in response to repeated vaginocervical stimulation in rats

Dina Conde1 and Barry R. Komisaruk2*

1 Department of Biology, Rutgers, The State University of New Jersey, Newark, NJ, USA
2 Department of Psychology, Rutgers, The State University of New Jersey, Newark, NJ, USA

INTRODUCTION

Vaginocervical stimulation (VCS) in rats acts at the spinal cord to block withdrawal reflexes to noxious stimulation. Hind leg and tail withdrawal reflexes, which persisted after surgical removal of a segment of the spinal cord at the mid-thoracic level, were still completely blocked by VCS (Komisaruk and Larsson, 1971). A possible mechanism for this effect is that foot-shock-induced release of substance P into spinal cord superfusate is inhibited by concurrent VCS (Steinman et al., 1994). The pelvic nerves, which provide sensory innervation of the vagina and cervix (Komisaruk et al., 1972; Peters et al., 1987; Berkley et al., 1990,1993) are the main mediator of this VCS-induced reflex inhibition, for transection of the pelvic nerves almost completely abolishes the ability of VCS to block withdrawal reflexes (Cunningham et al., 1991). Vasoactive intestinal peptide (VIP) is a possible mediator of this effect of VCS, for pelvic nerve contains VIP (Basbaum and Glazer, 1983), VCS releases VIP into spinal cord superfusates (Komisaruk et al., 1989), and VIP and certain of its fragments administered directly to the spinal cord, intrathecally, mimic the analgesia-producing effects of VCS (Komisaruk and Jordan, 1995). And in a different context, VIP exerts neurotrophic effects (Brenneman and Eiden, 1986; Gasser et al., 1993; Gressens et al., 1993; Brenneman et al., 1999).

These findings combined led us to hypothesize that VCS could promote recovery of spinal cord-mediated behavioral function. To test this hypothesis, we first reduced the inhibitory effect of VCS on the tail flick latency (TFL) to radiant heat test by transecting the pelvic nerve unilaterally. In order to maximize the unilateral genital neuroectomy effect, we also ipsilaterally transected the other genital sensory nerves, i.e., the pudendal and hypogastric (Komisaruk et al., 1972; Peters et al., 1987; Berkley et al., 1990,1993). Then we applied repeated VCS three times daily for 1 week. We reasoned that the daily repeated VCS would stimulate sprouting of the remaining intact pelvic nerve terminals, and thereby increase the effectiveness of the VCS. Thus, in the present study, we ascertained whether: (a) unilateral genital neuroectomy would reduce the magnitude of VCS-induced inhibition of the TFL, (b) daily repeated VCS would overcome this attenuation, and (c) the daily repeated VCS would induce sprouting of the remaining intact pelvic nerve terminals.

MATERIALS AND METHODS

EXPERIMENT 1. DOES DAILY REPEATED VCS IN GENITAL UNILATERAL-NEURECTOMIZED RATS INCREASE ITS INHIBITORY EFFECT ON TFL?

Subjects

Sprague Dawley female rats at least 90 days of age and weighing 300-400 g, were purchased from Charles River Breeding Laboratories. All animals were maintained on a reversed-light cycle...
All experimental procedures used in the present study received approval from the Rutgers University Institutional Animal Care and Use Committee. Nerve transections: rats were anesthetized with a solution of 0.5 ml Rompun/10 ml ketamine; dose: 0.07 ml/100 g body weight. A ventral abdominal incision, approximately 6 cm length, exposed the abdominal–pelvic region and facilitated the identification of the genitospinal nerves.

**Unilateral hypogastric neurectomy.** We identified the left ureter by its vigorous peristaltic activity, then visualized the left hypogastric nerve between the colon and the ureter as it courses parallel to the ureter, and removed a 4 mm segment of the nerve.

**Unilateral pelvic neurectomy.** The bifurcation of the vena cava into the common iliac veins was identified, the left common iliac vein was located unilaterally, and then each nerve was gently lifted with petroleum jelly (a 1 cc plastic syringe plunger). To provide cushioning during VCS, we placed the rubber tip of a 1 ml plastic syringe onto this modified tip.

Daily repeated VCS began 18 h after unilateral neurectomies and continued for 1 week. VCS (100 g force, 10 s on, 10 s off < 5 min) was applied three times daily, 2 h apart. The effect of acute VCS on TFL (100 and 300 g force) was ascertained 1 week after the daily VCS treatment ended, using a blind procedure.

**Behavioral testing**

Baseline TFL responses to VCS were obtained 1 week prior to neurectomy or control procedures. Each rat was placed in a hemi-cylindrical Plexiglas restrainer for approximately 5 min prior to testing. TFL responses before and during VCS (100 and 300 g force) were established prior to neurectomy or sham surgery.

**TFL test.** This measures the latency (in s) to flick the tail away from a focused regulated radiant heat source. The radiant heat source (ITT Inc., Model 33) was located 8 cm above and 4 cm proximal to the tip of the tail. The temperature of the heat lamp of the tail flick apparatus was adjusted to elicit a group mean approximate TFL of 3 s. The TFL reading displayed automatically when the rat withdrew the tip of its tail away from the radiant heat source, breaking a photocell circuit. To prevent tissue damage, the heat source was stopped at 9 s if the rat did not withdraw its tail. Each test consisted of three trials 15 s apart. The score for each test was calculated as the mean latency of three successive trials.

**Statistical analysis**

The data are expressed as mean ± SEM. Comparison among groups was made using repeated measures, two-way analysis of variance (ANOVA), and subsequent Fisher’s protected t-tests.

**CONCLUSIONS**

Daily repeated VCS induced sprouting in genital primary afferent nerve terminals? Sprouting of primary afferent nerve fibers was observed in the genitospinal nerves, pelvic nerve, and pudendal nerves after unilateral neurectomy. When the pelvic nerve was transected, the proximal end was dipped into horseradish peroxidase (HRP) (see details of method below). Two to three days after the nerve dip, the animals were sacrificed and the density of HRP particles in the terminal field of the nerve in the spinal cord was determined.

Pelvic nerve transaction and pelvic nerve dip

Fourteen days post lesion, the rats were anesthetized as above and the left pelvic nerve was exposed at the pelvic ganglion level and transected with microsissors. Approximately 5 μl HRP dissolved in saline (50 μM) was used to fill a small cup made by cutting PE-10 tubing to fit the nerve and closing one end with petroleum
As summarized in Figure 1, all groups showed equivalent baseline (pre-VCS; “0” g force) TFLs. As anticipated, the TFL in response to acute VCS (100 or 300 g force) was significantly lower in the unilateral genital neurectomy (GenX) group than in the intact group that did not receive daily repeated VCS (C). Thus, the unilateral neurectomy significantly reduced the ability of VCS to inhibit the tail flick response. The TFL elevation to 300 g (although not 100 g) VCS was significantly higher in the unilateral neurectomy + daily VCS group (GenX + VCS) than in the unilateral neurectomy group that did not receive daily VCS (GenX). However, in the intact group, daily repeated VCS (C + VCS) did not increase the ability of acute VCS to increase TFL any more than in the intact group that did not receive daily repeated VCS (C). Thus, the higher VCS force revealed the effect of daily repeated VCS to increase the ability of VCS to inhibit the tail flick response in the genital-neurectomized rats.

**EXPERIMENT 2. EFFECT OF UNILATERAL NERVE TRANSECTION AND DAILY REPEATED VCS ON HRP LABELING OF PELVIC NERVE TERMINALS**

HRP reaction product was located in the L6–S1 segments of the spinal cord. The primary afferent fibers enter this region through Lissauer’s tract, diverging into a medial and a lateral band surrounding the superficial border of the dorsal horn. The collaterals of some primary afferents that course along the lateral band approach, and form connections with, SPNs and dendrites. Other collaterals course among SPNs and dendrites to form a terminal field in the upper dorsal grey commissure with fewer fibers crossing to the contralateral side of the spinal cord. The primary afferent collaterals emerging from the medial band form...
Conde and Komisaruk

Stimulation-induced recovery of function

FIGURE 2 | In rats that received daily repeated VCS, there was increased HRP labeling in the terminal fields of the pelvic nerve at L6–S1 compared to rats that did not receive such VCS, suggesting that the daily repeated VCS induced sprouting (details in the text). The y-axes depict the number of HRP particles/1×10^4 μm². XR, HRP counts on the right side of the spinal cord; XRVCS, HRP counts on the right side of the spinal cord after 1 week of daily repeated VCS; L, HRP counts on the left side of the spinal cord; LVCS, HRP counts on the left side of the spinal cord after 1 week of daily repeated VCS. In general, daily repeated VCS increased the number of HRP granules compared to rats that did not receive such VCS. **p<0.02 (Mann–Whitney U-tests), *p<0.05 (Mann–Whitney U-tests). Notes: In all four groups, the right pelvic nerve was first transected 2 weeks earlier. Scales for HRP granule counts are different in the different groups.

| Lissauer’s tract | Medial collateral band | Dorsal gray commissure | Dorsal horn (central) |
|------------------|------------------------|------------------------|----------------------|
| XR, XRVCS, L, LVCS |
| XR, XRVCS, L, LVCS |
| XR, XRVCS, L, LVCS |
| XR, XRVCS, L, LVCS |

| Lateral collateral band | Laminae I and II | Lamina X | Dorsal horn (total) |
|------------------------|-----------------|----------|---------------------|
| XR, XRVCS, L, LVCS |
| XR, XRVCS, L, LVCS |
| XR, XRVCS, L, LVCS |
| XR, XRVCS, L, LVCS |

EVIDENCE THAT DAILY REPEATED VCS INCREASES HRP LABELING

The purpose of transecting the genitospinal nerves unilaterally (on the right side) was to clear a field for the ipsilateral and contralateral terminals of the remaining contralateral pelvic nerve to potentially proliferate. As can be seen in Figure 2, in the non-VCS group (XR and L), there was minimal HRP labeling in either the ipsilateral or the contralateral side of the spinal cord in the regions specified. By contrast, in the VCS group (XRVCS and LVCS), there was significantly more labeling than in the non-VCS group in six of the eight ipsilateral (i.e., left side) intact regions. And in six of the eight neurectomized (i.e., right side) regions, there was significantly more labeling in the XRVCS group than in the non-VCS (XR) group. Thus, whereas relatively minimal HRP labeling occurred in the terminals in the absence of VCS, there was a marked increase in HRP labeling on both the ipsilateral and the contralateral sides in the group that received daily repeated VCS. Table 1 summarizes the median number of HRP particles observed in each region of the spinal cord.

DISCUSSION

In the present study, unilateral denervation of the reproductive tract significantly reduced the magnitude of VCS-induced inhibition of the tail flick response to radiant heat. Daily repeated VCS for 1 week then significantly increased (restored) its analgesic effectiveness in these unilateral genital-denervated rats, whereas in the intact control rats, the same type of VCS did not increase further the analgesia magnitude. We tested a possible morphological basis for this effect by first transecting the pelvic nerve on the right side in order to vacate its terminal projection field. Subsequently, we applied HRP to the acutely cut proximal end of the remaining intact pelvic nerve on the left side, and then counted HRP reaction product in the pelvic nerve terminal projection zone at spinal cord level L6–S1 (Nadelhaft and Booth, 1984). The present findings provide support for the interpretation that daily repeated VCS increased the terminal proliferation, on the basis that the daily repeated VCS significantly and markedly increased the counts of a terminal field in the upper dorsal gray commissure. Primary afferent collaterals/terminals are also located in Laminae I, II, and X (Figure 3).
FIGURE 3 | Darkfield illumination photomicrograph (Neurolucida) of transverse 30–μm sections of the sacral 1 (S1) segment of the spinal cord. The right side of the figures is the left side of the spinal cord. (a) HRP labeling in a rat that received daily repeated VCS. Brightened image to emphasize appearance and location of HRP particles (white dots). (b) Same section as above showing how HRP counting was made. The white marker dots indicate the locations of HRP particles. Different types of marker dots were used to differentiate the selected regions of the spinal cord. (c) HRP labeling in a control rat that did not receive daily repeated VCS. Calibration bar = 200 μm.

HRP reaction product in the pelvic nerve terminal field in six of the eight ipsilateral terminal fields measured, as well as in six of the eight contralateral terminal fields measured (The pelvic nerve distributes its terminals ipsi- and contra-laterally; Babbaum and Glazer, 1983; Chung et al., 1993).

The validity of the quantitative HRP method used in the present study is supported by our finding of a distribution of primary afferent fiber terminals consistent with the distribution described by others (Nadelhaft and Booth, 1984). According to those authors, pelvic nerve primary afferent fibers enter Lissauer’s Tract in the spinal cord in the same segments (L6–S2) as those in which the preganglionic neurons are located that enter the pelvic nerve efferents. This supports our criterion for selecting, for quantitative HRP analysis, sections with SPN neurons showing maximal HRP label, since these sections also contain primary afferent fibers and terminals of the pelvic nerve.

We speculate that the marked quantitative increase in HRP reaction product in the pelvic nerve terminal fields in the daily repeated VCS group represents VCS-induced proliferation, i.e., sprouting, of pelvic nerve primary afferent terminals in the spinal cord, thereby mediating the ability of repeated daily VCS to increase its tail flick response-inhibiting potency.

A mechanism for this effect may be the release of neurotrophic substances into the dorsal horn of the spinal cord, inducing sprouting of intact pelvic nerve fibers into the denervated region. Sprouting occurs spontaneously within 2 weeks of trauma (Woollf et al., 1995); the present findings suggest that daily repeated VCS can markedly intensify this process. Several other mechanisms could play a role in functional recovery of the CNS in response to injury, e.g., a rearrangement of spared neuronal circuits, an upregulation of neuropeptides with neurotrophic abilities (Muir and Steeves, 1997), and/or an acceleration of transport of neurotrophic substances to the terminal fields, perhaps represented by HRP accelerated transport of HRP particles. VCS, which activates the pelvic nerve, releases VIP into the spinal cord (Komisaruk et al., 1989). Evidence that VIP is a neurotrophic factor is that it induces neurite growth in vitro and protects neurons from cell death (Brekenrem and Eidem, 1986; Pincus et al., 1990; White and Mansfield, 1996). VIP increases neuronal survival indirectly, via the release of several neurotrophic substances from glial cells.

| Spinal cord region | XR | XRVCS | L | LVCS |
|--------------------|----|-------|---|------|
| Lissauer’s tract    | 0  | 1.88  | 0 | 61.33|
| Medical collateral band | 0  | 4.47  | 0 | 37.76|
| Dorsal gray commissure | 1.15 | 21.38 | 0.90 | 30.88|
| Dorsal horn (centr) | 0.70 | 5.19  | 3.39 | 7.73 |
| Lateral collateral band | 0  | 0     | 0 | 138.09|
| Laminae I and II   | 1.20 | 4.33  | 0.90 | 8.55 |
| Lamina X           | 0.86 | 6.64  | 1.04 | 7.43 |
| Dorsal horn (total)| 4.04 | 44.01 | 5.12 | 273.47|

This is a numerical summary of the data shown in Figure 2. In the control group (n = 5), the right genitospinal (hypogastric, pelvic, pudendal) nerves were transected (XR) 2 weeks before HRP administration to the intact contralateral pelvic nerve (left side of the spinal cord L). In the experimental group (n = 3) rats were treated with a daily regimen of VCS following unilateral right side genitospinal nerve transaction (XRVCS). Following daily VCS treatment HRP was administered to the intact contralateral pelvic nerve (LVCS). HRP counts were done in both sides of the spinal cord (see text for details).
VIP antisera or with VIP receptor antagonists resulted in neuronal blockade spinal neurons (Brenneman and Eiden, 1986; Brenneman et al., 1999). Also, blocking the action of VIP with neutralizing VIP antisera or with VIP receptor antagonists resulted in neuronal blockade spinal neurons.

REFERENCES

Brenneman, D. E., Hauser, J., Phillips, T. M., Doraisom, A., Basun, M., and Green, I. (1989). Vasoactive intestinal peptide: a neurotrophic releasing agent and an astroglial mitogen. J. Neurosci. Res. 25, 386–394.

Brenneman, D. E., Nicol, T. D., Warren, D., and Brown, L. M. (1988). Vasoactive intestinal peptide: a neurotrophic releasing agent and an astroglial mitogen. J. Neurosci. Res. 25, 386–394.

Gozes, I., McCann, S. K., Jacobson, L., Warren, D., Meeks, T. W., Fradin, M., and Brenneman, D. E. (1991). An Antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. J. Pharmacol. Exp. Ther. 257, 386–390.

Gozes, I., McCann, S. K., Jacobson, L., Warren, D., Meeks, T. W., Fradin, M., and Brenneman, D. E. (1991). An Antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. J. Pharmacol. Exp. Ther. 257, 386–390.

Gozes, I., McCann, S. K., Jacobson, L., Warren, D., Meeks, T. W., Fradin, M., and Brenneman, D. E. (1991). An Antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. J. Pharmacol. Exp. Ther. 257, 386–390.

Gozes, I., McCann, S. K., Jacobson, L., Warren, D., Meeks, T. W., Fradin, M., and Brenneman, D. E. (1991). An Antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. J. Pharmacol. Exp. Ther. 257, 386–390.

Gozes, I., McCann, S. K., Jacobson, L., Warren, D., Meeks, T. W., Fradin, M., and Brenneman, D. E. (1991). An Antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. J. Pharmacol. Exp. Ther. 257, 386–390.

Gozes, I., McCann, S. K., Jacobson, L., Warren, D., Meeks, T. W., Fradin, M., and Brenneman, D. E. (1991). An Antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. J. Pharmacol. Exp. Ther. 257, 386–390.
vaginal stimulation in rats: neuro-physiological and behavioral studies. Brain Res. 117, 85–107.

Komisaruk, B. R., and Whipple, B. (1984). Evidence that vaginal self-stimulation in women suppresses experimentally-induced finger pain. Soc. Neurosci. Abstr. 10, 475.

Komisaruk, B. R., Whipple, B., Crawford, A., Grimes, S., Liu, W.-C., Kahn, A., and Mosser, K. (2004). Brain activation during vaginocervical self-stimulation and orgasm in women with complete spinal cord injury: BOLD evidence of mediation by the vagus nerves. Brain Res. 1024, 27–36.

Mesulam, M. M., and Brushart, T. M. (1979). Transganglionic and anterograde transport of horseradish peroxidase across dorsal root ganglia: a tetramethylbenzidine method for tracing central sensory connections of muscles and peripheral nerves. Neuroscience 4, 1107–1117.

Muir, G. D., and Stoevers, J. D. (1997). Sensomotor stimulation to improve locomotor recovery after spinal cord injury. Trends Neurosci. 20, 72–77.

Nadkarni, J., and Broth, A. M. (1991). The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study. J. Comp. Neurol. 298, 238–248.

Peters, C. L., Kratal, M. B., and Komisaruk, B. R. (1987). Sensory innervation of the external and internal genitalia of the female rat. Brain Res. 408, 199–204.

Pincus, D. W., DiCicco-Bloom, E. M., and Black, I. B. (1990). Vasoactive intestinal peptide regulates mitosis, differentiation and survival of cultured sympathetic neuroblasts. Nature 343, 564–567.

Steinman, J. L., Hoffman, S. W., Baras, C., and Komisaruk, B. R. (1994). Vaginocervical stimulation attenuates hindpaw shock-induced substance P release into spinal cord superfusates in rats. Brain Res. 647, 204–208.

Whipple, B., and Komisaruk, B. R. (1985). Elevation of pain threshold by vaginal stimulation in women. Pain 21, 357–367.

White, D. M., and Mansfield, K. (1996). Vasoactive intestinal peptide and neuropeptide Y act indirectly to increase neurite outgrowth of dissociated dorsal root ganglion cells. Neuroscience 73, 881–887.

Woolf, C. J., Shortland, P., Renold, M., Ridings, J., and Dubell, T. (1995). Rearrangements of central terminals of medullated primary afferents in the rat dorsal horn following peripheral axotomy. J. Comp. Neurol. 360, 121–134.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 07 January 2012; paper pending published: 30 January; accepted: 30 March 2012; published online: 19 April 2012.

Citation: Conde D and Komisaruk BR (2012). A neuroanatomical correlate of sensorimotor recovery in response to repeated vaginocervical stimulation in rats. Front. Physio. 3:100. doi: 10.3389/fphys.2012.00100

This article was submitted to Frontiers in Integrative Physiology, a specialty of Frontiers in Physiology. Copyright © 2012 Conde and Komisaruk. This is an open-access article distributed under the terms of the Creative Commons Attribution Non Commercial License, which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.