Neuroscience 288 (2015) 37–50

NOVEL SPINAL PATHWAYS IDENTIFIED BY NEURONAL C-FOS EXPRESSION AFTER URETHROGENITAL REFLEX ACTIVATION IN FEMALE GUINEA PIGS

S. Y. YUAN,[a]* P. I. VILIMAS,[a] V. P. ZAGORODNYUK[b] AND I. L. GIBBINS[a]

[a] *Anatomy and Histology and Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia*

[b] *Human Physiology and Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia*

Abstract—Pudendal nerve-spinal pathways are involved in urethrogenital sensation, pain and sexual activity. However, details of these pathways and their modulation are unclear. We examined spinal pathways activated by the urethrogenital reflex (UGR) and visualized by c-Fos immunoreactivity in reflexly activated neurons within spinal cord. In anesthetized female guinea pigs, a balloon was inserted into the urethra and inflated with short-repeat or long-continuous distension to activate the UGR. A second balloon recorded reflex contractions of the vagina and uterus. Two control groups had either no balloon or a vaginal balloon (VB) only. Ninety minutes after UGR activation, c-Fos immunoreactivity in L3 and S2 spinal segments was examined. Reflex activated c-Fos immunoreactivity also was investigated in some animals with acute spinal transections at either L4 or T12 levels. There was no significant difference in spinal c-Fos expression between the control groups. Short-repeat distension reliably induced a UGR and a two- to threefold increase in c-Fos-expressing neurons throughout dorsal, intermediate and lateral spinal gray matter at S2 and about twofold increase in superficial dorsal horn at L3. T12 transection had little effect on c-Fos expression at either spinal level. However, after L4 transection, UGR generation was associated with a four- to sixfold increase in c-Fos-expressing neurons in lateral horn (LH) and central canal areas at S2, and but only 20–30% increase at L3. Thus, UGR activates preganglionic neurons projecting to pelvic viscera in both sacral and lumbar spinal cord. The reflex also must activate ascending and descending spinal inhibitory circuits that suppress c-Fos-expression in neurons at both sacral and lumbar spinal levels.

Key words: pudendal nerve, spinal reflex, pre-ganglionic nerves, female sexual function, c-Fos.

INTRODUCTION

Female sexual dysfunction (FSD) affects a high proportion of women at some stage in their lives (Verit et al., 2006; Jha and Thakar, 2010; Bergeron et al., 2011). Some elements of FSD involve peripheral neural pathways. For example, vulvar vestibulitis syndrome (VVS) or dyspareunia can involve hypersensitivity of genital sensory nerves, while reduced vaginal lubrication ultimately is a failure of autonomic secretomotor and vasodilator pathways (Pukall et al., 2005). The spinal cord represents the first stage of processing genital sensory input and the final stage in generating autonomic motor output to the genital tract (Giraldi et al., 2004). Furthermore, the lumbo-sacral spinal cord has a well known ability to support sexual responsiveness at some level of the reflex in the absence of descending central control, as seen after spinal lesions (Chapelle et al., 1980; Sipski et al., 2004). Nevertheless, the spinal pathways activated during stimulation of the genital tract, especially in females, are poorly known.

In general, it is not feasible to identify spinal circuits in humans. However, spinal circuitry is well conserved across mammalian species, so that pathways identified in laboratory mammals are highly likely to have close homologs in humans. A spinal reflex response to mechanical stimulation of the urethrogenital region, known as urethrogenital reflex (UGR), has been reported in both male and female rats (McKenna et al., 1991; Vathy and Marson, 1998; Marson et al., 2003; Marson and Gravitt, 2004). The responses to the UGR activation in female animals resemble those seen during sexual activity, including significant increases in pelvic blood flow and rhythmic contractions of the vagina and uterus (Bohlen et al., 1982a,b; Sipski et al., 2001). Afferent input arises from the pudendal nerves and the visceral responses themselves are produced by activation of autonomic pathways comprising spinal preganglionic neurons, the hypogastric and pelvic nerves, with final motor neurons in the paracervical (anterior pelvic) ganglia (Morris and Gibbins, 1987; Keast, 1999; Jobling et al., 2003; Morris et al., 2005a; Wiedey et al., 2008). In guinea pigs, pudendal nerves project from sacral spinal cord mainly at S2 segment, hypogastric from rostral lumbar (eg. L3), pelvic nerves from caudal lumbar and sacral segments (Yuan et al., 2011). Most of them contain both afferents and efferents in the nerve bundles. In rats, hypogastric nerves mainly come from T13-L2 segments, pelvic nerves

*Corresponding author. Tel: +61-882046637; fax: +61-882770085.
E-mail address: shiyong.yuan@flinders.edu.au (S. Y. Yuan).
Abbreviations: CC, central autonomic nuclei; CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; FSD, female sexual dysfunction; GLM, general linear model; IR, immunoreactive; IML, intermediolateral; LH, lateral horn; UB, urethral balloon; UGR, urethrogenital reflex; VB, vaginal balloon; VH, ventral horn.

http://dx.doi.org/10.1016/j.neuroscience.2014.12.031
0306-4522/

from L6 and S1, pudendal nerves from sacral segments (de Groat and Booth, 1993a). In humans, it may be possible that there are some variations comparing with animals, but humans most likely conserve a similar anatomical arrangement for these pathways (Wesselmann et al., 1997). Although the UGR has been regarded as a surrogate for sexual stimulation, it seems more likely that reflexes activated by mechanical stimulation of the urethra, especially its distension, are components of pain detection and response pathways.

In males and females, sacral parasympathetic pathways play a dominant role in producing vasodilation to increase blood flow to the reproductive organs (Dail et al., 1985; de Groat and Booth, 1993b; Papka and Traurig, 1993; Traurig and Papka, 1993; Sato et al., 1996; Cai et al., 2008). However, in several species, including humans, lumbar sympathetic pathways also can contribute to the change of pelvic blood flow and sexual arousal (Chapelle et al., 1980; Fahrenkrug and Ottesen, 1982; de Groat and Booth, 1993b; Sato et al., 1996; Sipski et al., 2004; Cai et al., 2008). Indeed, many vasodilator neurons in guinea-pig paracervical ganglia receive their dominant preganglionic input from mid-lumbar (L3) spinal cord, often with convergent sacral spinal inputs (Jobling et al., 2003; Morris et al., 2005a).

Expression levels of an immediate-early proto-oncogene protein, c-Fos, in spinal cord neurons are increased by activation of nociceptive sensory neurons, providing a valuable tool to identify neurons within spinal nociceptive pathways (Morgan et al., 1987; Marson et al., 2003; Marson and Gravitt, 2004; Coggeshall, 2005; Wiedey et al., 2008; Gao and Ji, 2009; Wang et al., 2010). Indeed, immunohistochemical mapping of c-Fos expression has allowed the identification of several populations of spinal neurons activated by stimulation of pelvic and pudendal nerves as well as the UGR (Marson et al., 2003). This work showed that the UGR activates neurons across most of the lumbar and sacral spinal cord, including autonomic preganglionic neurons and a wide range of neurons that must process different levels of afferent input. Such activation of c-Fos expression by the UGR further supports the interpretation that this reflex involves stimulation of nociceptive sensory pathways from the urinogenital tract.

Our recent electrophysiological studies in female guinea pigs demonstrated that activation of pudendal sensory nerves stimulates pelvic autonomic neurons controlling blood flow to the female genital tract via ascending spinal circuits projecting to both sacral and lumbar levels (Yuan et al., 2011). Many of these pelvic neurons in guinea pigs also receive descending central inputs from lumbar levels (L3) in addition to inputs from the sacral spinal cord (Jobling et al., 2003; Morris et al., 2005a; Yuan et al., 2011). However, outputs of the lumbar pathways to pelvic vasodilator and uterine motor neurons appear to be inhibited by spinal neurons acting via $GABA_A$ receptors (Yuan et al., 2011). To date, the contributions of these neurons to pathways activated by the UGR reflex are not known. Building on our previous studies in guinea pigs (which confirm and extend observations made in other species, such as rats) (Jobling et al., 2003; Morris et al., 2005a; Yuan et al., 2011; Vilimas et al., 2011), we have adapted the UGR model for female guinea pigs. We used c-Fos immunohistochemistry to identify neurons that are activated by UGR in animals with intact spinal cord, and in animals following lesions of the spinal cord at different levels.

EXPERIMENTAL PROCEDURES

Young adult female guinea pigs (pre-estrous 6–8 weeks old; 250–280 g body weight; Hartley-IMVS, Adelaide, Australia) were anesthetized with 50% urethane (up to 1.8 g/kg i.p.) and placed in a prone position on a heating pad to maintain body temperature at 37 °C. Oxygen was supplied continuously with a facemask during the experiment. Urethane provided stable anesthesia to allow for repeated applications of stimuli to an animal *in vivo* and subsequent perfusion of the spinal cord for c-Fos immunohistochemistry. Animals were handled gently to avoid any overt activation of nociceptors that could increase background c-Fos expression in the spinal cord. Control animals were anesthetized but otherwise had no other procedures in order to obtain baseline c-Fos expression with these anesthesia and handling conditions. All experimental procedures employed in this study were approved by the Flinders University Animal Welfare Committee in accordance with national guidelines.

In vivo UGR activation to induce spinal c-Fos expression

Two experimental groups were setup with urethral balloon (UB) distension to examine reflex-induced c-Fos expression in the spinal cord of anesthetized animals. A small rubber balloon (UB: size 3F Fogarty arterial embolectomy catheter, Edwards Lifesciences, USA) was inserted into the urethra 1–2 mm away from the external orifice and without stretching the wall of urethra. The balloon was inflated with 120-μl distilled water for 30 s every 10 min over 90 min (short-repeat distension) to activate the UGR. The degree of balloon distension was controlled to produce only limited increase in the diameter of the urethra. Such relatively mild distension was sufficient to induce UGR, which was used as threshold volume for urethral distension. In one group of animals with thoracic cord transection (T12, see below), the UB was inflated continuously for 30 min (continuous distension) to test for maintained activation of the UGR. A second balloon (vaginal balloon (VB): size 4F Fogarty arterial embolectomy catheter, Edwards Lifesciences, USA) was inserted into the area of vagina/lower uterus, but did not across the cervix, and then inflated with 200-μl distilled water to detect reflex contractions in response to urethral distension. Different sizes of the balloons used in urethra (3F) and vagina (4F) were determined by the sizes of luminal diameters of the urethra and vagina. Unlike the balloon in the urethra, VB was distended to the level just filling up the lumen without significant stretching the wall of the organ, while sufficient to detect any vaginal spontaneous or