Brain Research 1698 (2018) 99–105

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Neonatal bladder inflammation alters the role of the central amygdala in hypersensitivity produced by Acute Footshock stress in adult female rats

Timothy J. Ness *, Cary DeWitte, Jennifer J. DeBerry, Alan Randich

Department of Anesthesiology and Perioperative Medicine, University of Alabama at Birmingham, Birmingham, Alabama, United States of America

ARTICLE INFO

Article history:
Received 24 November 2017
Received in revised form 26 May 2018
Accepted 28 June 2018
Available online 30 June 2018

Keywords:
Amygdala
Visceral
Hypersensitivity
Acute stress
Urinary bladder
Neonatal inflammation

ABSTRACT

There is increasing evidence that chronic pain may be associated with events that occur during critical periods of development. Recent studies have identified behavioral, spinal neurophysiological and spinal/peripheral neurochemical differences in rats that have experienced neonatal bladder inflammation (NBI): a putative model of the chronically painful bladder disorder, interstitial cystitis. Stress has been shown to exacerbate symptoms of interstitial cystitis and produces bladder hypersensitivity in animal models. We recently reported that Acute Footshock-induced bladder hypersensitivity was eliminated in otherwise normal rats by prior bilateral lesions of the central nucleus of the amygdala. Since the spinal and peripheral nervous systems of NBI-treated rats are known to differ from normal rats, the present experiments sought to determine whether a supraspinal nervous system structure, the central amygdala, is still necessary for the induction of Acute Footshock-induced hypersensitivity. The effect of bilateral amygdala electrolytic lesions on Acute Footshock-induced bladder hypersensitivity in adult female rats was tested in Control rats which underwent a control protocol as neonates and in experimental rats which experienced NBI. Consistent with our previous report, in Control rats, Acute Footshock-induced bladder hypersensitivity was eliminated by bilateral Amygdala Lesions. In contrast, Acute Footshock-induced bladder hypersensitivity in NBI-treated rats was unaffected by bilateral Amygdala Lesions. These findings provide evidence that NBI results in the recruitment of substrates of bladder hypersensitivity that may differ from those of normal rats. This, in turn, suggests that unique therapeutics may be needed for painful bladder disorders like interstitial cystitis.

1. Introduction

Acute and chronic pains originating from the urinary bladder are common clinical entities. Some conditions are easy to treat, but others, such as interstitial cystitis (IC), are conditions of bladder hypersensitivity that have proven resistant to diagnosis and treatment. IC is characterized by urinary frequency, urgency and pelvic pain (Bogart et al., 2007). Animal models of IC have generally lacked the main clinical features of IC. An exception to this generality is an animal model developed by our research group which involves a neonatal bladder inflammation (NBI) treatment. Animals treated in this manner have increased nociceptive responses to urinary bladder distension following an adult treatment, increased micturition frequency, decreased pressure/volume thresholds for activating micturition responses, nociceptive responses to intravesical ice water solutions, increased pelvic floor muscular tone and increased submucosal hemorrhages following sustained hydrodistension (Randich et al., 2006, 2009; DeBerry et al., 2007, 2010; Ness et al., 2014a). NBI is produced by infusing a yeast-derived inflammogen, zymosan, intravesically, during a critical period of development, the end of the neonatal period (P14-16). NBI experimentally mimicks the equivalent of childhood bladder infections in humans which is relevant to the pathophysiology of IC given epidemiological data (Peters et al., 2009) that suggests there were an increased number of childhood bladder infections during childhood in individuals who develop IC. Using standard histological stains, as adults, the bladders of rats given NBI look structurally normal, but neurochemical analyses of their bladders and spinal cords indicate increased nervous system neuropeptide content (DeBerry et al., 2010; Shaffer et al., 2011), opioid peptides (Shaffer et al., 2013a,b), altered GABA-A receptor expression/function (Sengupta et al., 2013; Kannampalli et al., 2017; Zhang et al., 2017) and altered spinal neuronal responses to bladder distension (Ness and Randich, 2010; Kannampalli et al., 2017). It is clear that both in IC and in our animal model system of IC we are working with an *altered* nervous system. To date, these

* Corresponding author at: BMR2-208, 901 19th Street South, Birmingham, Alabama 35294, United States of America.
E-mail address: tness@uabmc.edu (T.J. Ness).

https://doi.org/10.1016/j.brainres.2018.06.030
0006-8993/

alterations have been limited in scope to the spinal cord and periphery.

The neuroanatomical substrates controlling normal bladder function are well-researched, but those underlying bladder pain are poorly understood (Birder et al., 2010; Lovick, 2016). Bladder pain is not only evoked by physical perturbations to the bladder, such as bacterial infections, but also can be both induced by and exacerbated by stress. In humans, flares in bladder-related symptoms correlate with exposure to a laboratory stressor (Lutgendorf et al., 2000, 2004) and increased daily life stress (Rothrock et al., 2001). Stress is the most common exacerbating factor identified by IC subjects themselves with over 60% identifying it as a contributing factor to their flares of pain (Bogart et al., 2007). It is also a pain modifying factor that clearly involves supraspinal neurological structures.

In rats, the stressors foot shock and water avoidance evoke stress responses which have been demonstrated to augment bladder nociceptive reflexes and other measures of bladder hypersensitivity (Lee et al., 2015; Robbins and Ness, 2008; Robbins et al., 2007; DeBerry et al., 2015; Smith et al., 2011). Acute footshock alters spinal neuronal responses to noxious bladder stimulation in a fashion which requires supraspinal mechanisms since spinal transection abolishs augmentation of responses (Robbins et al., 2011). Footshock also activates the stress-responsive hypothalamic-pituitaryadrenal (HPA) axis (DeBerry et al., 2015: Robbins and Ness, 2008) in ways which parallel the effects of stress observed in humans. We have reported that the central amygdala is required for the expression of Acute Footshock-induced bladder hyperalgesia in healthy adult female rats (DeBerry et al., 2015; Randich et al., 2017). DeBerry has also shown that acute central amygdala activation by corticosterone microinjection was sufficient to drive bladder hyperalgesia in the absence of Acute Footshock (DeBerry et al., 2015) giving further support for a role for the amygdala in Acute Footshock-related effects. However, no published studies have examined whether stress alters bladder sensitivity in adult rats experiencing prior NBI, nor has the necessity of an intact amygdala been examined in this context. Neuroablative manipulations in which lesions can be confirmed in location and extent are standard manipulations for determining the role of central nervous system structures in behavioral phenomena.

Therefore, the current set of neuroanatomical lesion studies examined this issue by asking the question of whether the central amygdala is required for the exacerbation of bladder pain produced by Acute Footshock in adult female rats which have experienced NBI in order to determine whether neural structures altered by NBI include typical stress-related neural mechanisms. Female rats were exclusively studied since painful bladder disorders such as interstitial cystitis affect women more than men by an order of magnitude (Bogart et al 2007).

2. Results

2.1. Amygdala Lesions have no significant effects on visceromotor responses in rats receiving No Footshock treatments.

Visceromotor responses to graded urinary bladder distension were graded in a monotonic, accelerating fashion. Fig. 1A demonstrates group data for the stimulus-response functions (SRFs) obtained from the four groups of rats which received No Footshock treatments as their final experimental manipulation. Comparison of their data allows an assessment of the effect of Amygdala Lesions on baseline bladder sensory processing. A repeated-measures ANOVA analysis of these SRF data demonstrates that there were no significant differences between any of the No Footshock groups as a function of the Amygdala Lesion versus Sham Lesion pretreatment [overall ANOVA with $p = 0.2417$; there was a significant distension pressure effect with $p < 0.001$ but no pressure × group interaction]. This was true in focused statistical comparison of both the Control groups and in the NBI groups. These data suggest no tonic modulatory effects are apparent when both central amygdalae are intact. A typical example of graded visceromotor responses of an individual rat to urinary bladder distension is given in Fig. 1B.

2.2. Acute Footshock produces bladder hypersensitivity in Control rats that is mediated by the amygdalae

Fig. 2 shows the results obtained in rats receiving neonatal Control treatments. In the Area-Under-the-Curve (AUC) analyses shown in the inset of Fig. 2A, the mean AUC of the Control groups

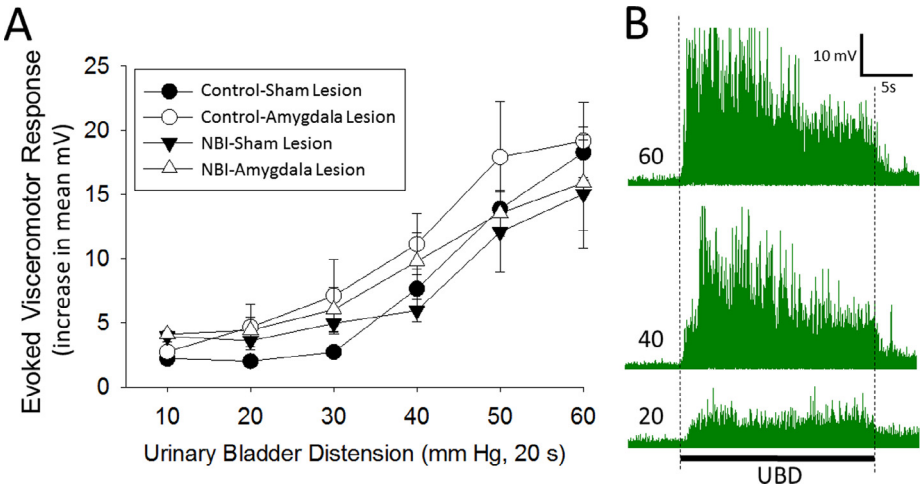

Fig. 1. Graded visceromotor responses to urinary bladder distension in No Footshock rats are not affected by Amygdala lesions. Abdominal electromyographic responses to graded urinary bladder distension (UBD; 10–60 mmHg) in No Footshock rats were quantified as Evoked Visceromotor Responses in rats which received either Control procedures or Neonatal Bladder Inflammation (NBI) and subsequently received Sham lesions or bilateral amygdalar lesions. In A, data are presented as group means ± SEM. The amygdalar lesions did not significantly alter the visceromotor responses to UBD in either Control or NBI groups. N = 6–8/group. Note: these data serve as control data for Figs. 2 and 3. In B, a typical example of graded visceromotor responses to UBD in a individual rat presented as peristimulus rectified electromyograms. Graded pressure in mm Hg is indicated at left. Evoked Visceromotor Response activity was calculated as the mean rectified myoelectric activity during the 20 s of UBD minus the mean ongoing myoelectric activity immediately prior to UBD.