Brain, Behavior, and Immunity 25 (2011) 443–460

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Ascending caudal medullary catecholamine pathways drive sickness-induced deficits in exploratory behavior: Brain substrates for fatigue?

Ronald P.A. Gaykema, Lisa E. Goehler *

Center for the Study of Complementary and Alternative Therapies, University of Virginia School of Nursing, Charlottesville, VA 22908, USA

ARTICLE INFO

Article history:
Received 17 September 2010
Received in revised form 5 November 2010
Accepted 5 November 2010
Available online 12 November 2010

Keywords:
Dopamine beta-hydroxylase
Saporin toxin
Dorsal vagal complex
Ventrolateral medulla
Hypothalamus
Noradrenergic
Lipopolysaccharide
Behavioral arousal

ABSTRACT

Immune challenges can lead to marked behavioral changes, including fatigue, reduced social interest, anorexia, and somnolence, but the precise neuronal mechanisms that underlie sickness behavior remain elusive. Part of the neurocircuitry influencing behavior associated with illness likely includes viscerosensory nuclei located in the caudal brainstem, based on findings that inactivation of the dorsal vagal complex (DVC) can prevent social withdrawal. These brainstem nuclei contribute multiple neuronal projections that target different components of autonomic and stress-related neurocircuitry. In particular, catecholaminergic neurons in the ventrolateral medulla (VLM) and DVC target the hypothalamus and drive neuroendocrine responses to immune challenge, but their particular role in sickness behavior is not known. To test whether this catecholamine pathway also mediates sickness behavior, we compared effects of DVC inactivation with targeted lesion of the catecholamine pathway on exploratory behavior, which provides an index of motivation and fatigue, and associated patterns of brain activation assessed by immunohistochemical detection of c-Fos protein. LPS treatment dramatically reduced exploratory behavior, and produced a pattern of increased c-Fos expression in brain regions associated with stress and autonomic adjustments paraventricular hypothalamus (PVN), bed nucleus of the stria terminalis (BST), central amygdala (CEA), whereas activation was reduced in regions involved in exploratory behavior (hippocampus, dorsal striatum, ventral tuberomammillary nucleus, and ventral tegmental area). Both DVC inactivation and catecholamine lesion prevented reductions in exploratory behavior and completely blocked the inhibitory LPS effects on c-Fos expression in the behavior-associated regions. In contrast, LPS-induced activation in the CEA and BST was inhibited by DVC inactivation but not by catecholamine lesion. The findings support the idea that parallel pathways from immune-sensory caudal brainstem sources target distinct populations of forebrain neurons that likely mediate different aspects of sickness. The caudal medullary catecholaminergic projections to the hypothalamus may significantly contribute to brain mechanisms that induce behavioral "fatigue" in the context of physiological stressors.

1. Introduction

Physiological and psychological challenges, including inflammation and stress, can induce a constellation of symptoms referred to as "sickness behavior". This behavioral pattern involves reductions in motivated behavior including food and water intake, social and sexual behavior, exploratory behavior, and motor activity, and an increase in stress hormones. Sickness behavior supports host defense and recuperation by conserving energy, and is considered to be a motivated and adaptive response to physiological challenges (Dantzer and Kelley, 2007; Miller, 2009). However, in the context of chronic disease, sickness symptoms can lead to prolonged fatigue or symptoms of behavioral depression.

Although the precise neural substrates responsible for the behavioral manifestations of sickness are still emerging, studies using the activation marker c-Fos have indicated that activity in brain regions that support "positive motivation", including the nucleus accumbens, and several cortical areas including secondary motor, cingulate and piriform, is reduced in animals treated with the immune stimulant lipopolysaccharide (LPS; Stone et al., 2006). Conversely, this inhibition of neuronal activity marker expression occurs concomitant with activation in stress-sensitive, viscerosensory and autonomic control regions including the caudal brainstem, hypothalamus, and extended amygdala (Wan et al., 1994; Stone et al., 2006; Gaykema et al., 2007, 2008; Elmquist and Saper, 1996; Elmquist et al., 1996; Frenois et al., 2007). This pattern of inhibition of "positive motivation"-related brain regions

* Corresponding author. Address: Laboratory of Neuroimmunology and Behavior, Center for the Study of Complementary and Alternative Therapies, University of Virginia, School of Nursing, P.O. Box 800782, Charlottesville, VA 22932, USA. Fax: +1 434 243 9938.
E-mail address: goehler@virginia.edu (L.E. Goehler).

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doi:10.1016/j.bbi.2010.11.005

and activation of stress-related regions likely contributes to behavioral and mood-related sequelae of infection or inflammation.

The mechanisms by which peripherally generated immune signals are able to influence forebrain neurocircuitry involved in sickness behavior has not been firmly established. Because sickness behavior has been shown to be dependent upon the interaction of cytokines with the brain, constituents of the network of brain regions that subserve sickness behavior must interface with brain regions that can detect central or peripheral cytokines, such as circumventricular organs, vascular endothelium, or viscerosensory relay nuclei.

Of the brain regions that consistently show evidence of activation following immune challenge, the sensory dorsal vagal complex (DVC) located in the dorsomedial caudal medulla (consisting of the nucleus of the solitary tract (NTS) and area postrema), serves as one interface by which peripheral immune-related information influences the brain. The DVC receives immune-sensitive inputs from neural pathways (vagal and spinal) and circulating immune signals via the weak blood barrier in the area postrema (a circumventricular organ). Immune cells within the area postrema that produce interleukin-1 following immune challenge make direct contact with neurons, providing a potential pathway mediating cytokine-neural interface (Goehler et al., 2006). Additionally, the DVC is interconnected with the caudal ventrolateral medulla (VLM), which relays immune sensory information via activation of vascular endothelial cells within the nucleus predominantly by its catecholaminergic projections to the forebrain (Ericsson et al., 1997). Together with the VLM, the DVC projects to forebrain regions that mediate stress responses, autonomic control and arousal. Thus, the caudal brainstem is uniquely situated to transduce and propagate immune-related signals to brain regions likely involved in initiation of sickness behavior.

Functional evidence that the DVC mediates components of sickness behavior derives from findings that inactivation of the DVC with a local anesthetic prior to administration of LPS prevented both social withdrawal behavior and the characteristic brain activation pattern typically that occurs in association with immune activation in resting animals (Marvel et al., 2004). Further, DVC inactivation prevented the suppression of histamine neurons that are normally activated during an exploratory behavior task (Gaykema et al., 2008). These findings support the idea that activation of the DVC and VLM contribute to brain and behavioral responses to immune challenge. However, the specific links (neuronal pathways) between immune-related activation of brainstem viscerosensory regions and the forebrain nuclei that mediate sickness behavior have not been established.

Neuronal projections from the DVC and VLM to the forebrain follow multiple trajectories of catecholaminergic and non-catecholaminergic neurons (Gaykema et al., 2007). The catecholamine pathway arises in both the VLM and the DVC. It projects heavily to the hypothalamus and is well established to activate neuroendocrine responses in context of physiological challenges (e.g., Cunningham et al., 1990; Ericsson et al., 1994; Bienkowski and Rinaman, 2008;) including immune-related stimuli (Ericsson et al., 1997; Elmquist and Saper, 1996). Its contribution to other brain mediated sickness responses is less clear. In contrast, the non-catecholamine projections – which mainly arise from the DVC – preferentially target the external lateral parabrachial nucleus (PBel) in the pons, which then drives immune-related responses of the amygdala and bed nucleus of the stria terminalis (Tkacs and Li, 1999; Richard et al., 2005). This arrangement suggests that separate neurocircuitries, or parallel pathways, could mediate different aspects of sickness behavior.

As noted previously, immune challenge with LPS can lead to marked reductions in motor activity and motivated behavior that could relate to the psychological experience of "fatigue". Fatigue is common in acute and chronic illness, but the neurological substrates involved have yet to be established. Our previous studies have indicated that suppression of hypothalamic arousal systems may contribute to reductions in behavior associated with immune challenge (Gaykema et al., 2008; Gaykema and Goehler, 2009; Park et al., 2008). For the experiments reported here, we assessed exploratory motor behavior to provide an index of motivation and fatigue (Stone et al., 2006). To determine functional relationships between the caudal brainstem and the forebrain structures involved in motivation and movement that mediate sickness-induced reductions in exploratory behavior, we addressed three questions. First, can a targeted lesion of the immune-responsive catecholaminergic pathway originating in the caudal medulla prevent LPS-induced deficits in exploratory behavior, as does DVC inactivation? The answer to this question clarifies which of the ascending projections are most relevant for the effects of LPS on behavior. Second, how does LPS change the brain activation patterns normally induced during exploratory behavior, and how does catecholamine lesion or DVC inactivation modify the pattern? The answer to this question establishes potential target brain regions relevant to behavioral deficits during sickness. Finally, if catecholamine lesion is sufficient to ameliorate deficits in exploratory behavior, what are the possible targets of these neurons (i.e., brain regions depleted from catecholaminergic input)? Those brain regions could provide the substrates that link immune challenge and behavior.

2. Materials and methods

2.1. Animals

The experiments involved 41 male Sprague–Dawley rats (Taconic Laboratories, Germantown, NY, USA) with initial body weights of 250–270 g. The rats were housed in pairs in propylene boxes on a barrier cage rack (Allentown, Caging Smart Bio-Pak, Allentown, NJ, USA) in a temperature and humidity controlled room. The rats were maintained on a 12-h light–dark cycle (lights on at 7:00 AM) with free access to Purina Rat Chow #R001 and water. Rats were acclimatized for at least a week before they received surgery (guide cannulae aimed at the DVC or DSAP microinjection targeted to hypothalamus). Directly after surgery, all rats were singly housed during one week. DSAP-injected animals and controls were re-housed with their cage mate thereafter. Animals that received guide cannulae for DVC inactivation remained singly housed, to reduce likelihood the cannulae would be damaged. All procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996) and were in accordance with protocols approved by the University of Virginia Animal Care and Use Committee. Every attempt was made to minimize the number of animals used in these studies and to limit their distress and potential suffering.

2.2. Surgical procedures

2.2.1. Lesion of the ascending noradrenergic/adrenergic projections

2.2.1.1. Rationale. To investigate the role of the catecholaminergic component of the immune-responsive brainstem-derived pathway, we applied a targeted lesion approach using an anti-dopamine beta hydroxylase antibody conjugated to saporin (DSAP). When injected into a target region, DSAP is taken up selectively by noradrenergic/adrenergic neurons that innervate the target (Fig. 1A and B) The toxin is retrogradely transported to the soma, which it destroys. DSAP was micro-injected bilaterally into the hypothalamic paraventricular nucleus (PVN) because this structure receives particularly dense innervation from the medullary catecholamine projections, and because pilot experiments showed that DSAP injection into the PVN produced a dramatic reduction in the