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BRAIN RESEARCH

Research Report

Immune challenge and satiety-related activation of both distinct and overlapping neuronal populations in the brainstem indicate parallel pathways for viscerosensory signaling

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ARTICLE INFO

Article history:
Accepted 22 July 2009
Available online 30 July 2009

Keywords:
Catecholamine neurons
Lipopolysaccharide
Ventrolateral medulla
Nucleus of the solitary tract
Parabrachial nucleus
Danger pathway

ABSTRACT

Caudal brainstem viscerosensory nuclei convey information about the body's internal state to forebrain regions implicated in feeding behavior and responses to immune challenge, and may modulate ingestive behavior following immune activation. Illness-induced appetite loss might be attributed to accentuated "satiety" pathways, activation of a distinct "danger channel" separate from satiety pathways, or both. To evaluate neural substrates that could mediate the effects of illness on ingestive behavior, we analyzed the pattern and phenotypes of medullary neurons responsive to consumption of a preferred food, sweetened milk, and to intraperitoneal lipopolysaccharide challenge that reduced sweetened milk intake. Brainstem sections were stained for c-Fos, dopamine β-hydroxylase, phenylethanolamine-*N*-methyltransferase, and glucagon-like peptide-1 (GLP-1) immunoreactivity. Sweetened milk intake activated many neurons throughout the nucleus of the solitary tract (NTS), including A2 noradrenergic neurons in the caudal half of the NTS. LPS challenge activated a similar population of neurons in the NTS, in addition to rostral C2 adrenergic and mid-level A2 noradrenergic neurons in the NTS, many C1 and A1 neurons in the ventrolateral medulla, and in GLP-1 neurons in the dorsal medullary reticular nucleus. Increased numbers of activated GLP-1 neurons in the NTS were only associated with sweetened milk ingestion. Evidence for parallel processing was reflected in the parabrachial nucleus, where sweetened milk intake resulted in activation of the inner external lateral, ventrolateral and central medial portions, whereas LPS challenge induced c-Fos expression in the outer external lateral portions. Thus, signals generated in response to potentially dangerous physiological conditions seem to be propagated via specific populations of catecholaminergic neurons in the NTS and VLM, and likely include a pathway through the external lateral PBN. The data indicate that immune challenge engages multiple ascending neural pathways including both a distinct catecholaminergic "danger" pathway, and a possibly multimodal pathway derived from the NTS.

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doi:10.1016/j.brainres.2009.07.076

1. Introduction

One of the most common consequences of immune activation is alteration in ingestive behavior. Reduction in drinking or eating (anorexia) are observed in many clinical syndromes and are found in animals treated with experimental immune challenge as well. Because reduced food intake is associated with poorer outcomes of chronic illness (Hauser et al., 2006; Strassburg and Anker, 2006), understanding of the neurobiological substrates impacted by immune challenges and inflammation could contribute to clinically important strategies for intervention.

Ingestive behavior is controlled by interactions between peripheral "bottom up" signals related to physiological states (including immune challenge) and "top-down" cognitive and affective influences related to learning, arousal and hedonics (Berthoud, 2004). We have previously shown (Park et al., 2008) that an intraperitoneal challenge with lipopolysaccharide (LPS) that inhibited ingestion of a palatable sweetened milk solution influenced activity of a network of brain regions that included the paraventricular thalamus, the rostral nucleus accumbens (NAc) (which has been implicated in negative hedonics and the inhibition of food intake; Reynolds and Berridge, 2002, 2003; Zheng et al., 2003) and the orexin neurons of the lateral hypothalamus (LH). Neurons in the LH act as a nodal point where bottom-up and top-down regulatory signals converge to modulate hypothalamic drive on the brainstem neurons that control motor aspects of ingestive behavior. The findings from Park et al. (2008) support the idea that one way illness inhibits food intake is by influencing "top-down" basal forebrain and diencephalic neurocircuitry that integrate noxious or aversive stimuli with control of ingestive behavior.

A missing part of the known neurocircuitry involved in immune effects on behavior involves the identity of the "bottom-up" neural pathways that relay incoming sensory information signaling immune challenges and that impinge upon the forebrain neural networks that control ingestive behavior. Candidates include the immune-sensitive caudal brainstem neurons in the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM) that project to the paraventricular (Gaykema et al., 2007; Rinaman, 1999) and possibly adjacent hypothalamic areas involved in ingestive behavior. These neurons receive immune-related signals from sensory terminations of the vagus nerve, and from the area postrema (a circumventricular organ in which the blood barrier is weak). Caudal brainstem catecholamine neurons drive the HPA axis in the context of immune challenge (Dayas et al., 2001; Ericsson et al., 1994; Rinaman, 2007), and have been proposed to signal "physiological" or interoceptive stress. In addition, glucagon-like peptide-1 (GLP-1) containing neurons in the NTS have been reported to respond to peripheral LPS challenge (Rinaman, 1999) and may contribute to LPS-related anorexia (Grill et al., 2004). Thus, caudal brainstem-derived neuronal projections make plausible candidates as conduits from immunosensory interfaces to brain regions controlling ingestive behavior.

Ascending projections from the caudal brainstem carry a diverse range of signals related to normal viscerosensory stimuli (e.g. presence of food in stomach) as well as information indicating physiological challenges (e.g. pathogenic bacteria in the gastrointestinal tract). The extent to which these different types of information, which likely induce somewhat different physiological or behavioral responses, are coded differently is not known. For instance, the nucleus of the solitary tract (NTS) is responsive to both satiety (Emond et al., 2001; Faipoux et al., 2008; Rinaman et al., 1998) and immune-related signals (Bienkowski and Rinaman, 2008; Elmquist et al., 1996; Gaykema et al., 2004; Goehler et al., 2005; Rinaman and Dzmura, 2007; Wan et al., 1994), and both types of stimuli serve to reduce further food intake. An immune challenge could activate a selective sickness-mediating ("danger") channel, or it could act via enhancement of ascending projections that carry signals related to satiety, which would also lead to a reduction in ingestive behavior, or activate both types of pathways.

If information related to immune challenge is conveyed to the forebrain by a selective "danger-related" pathway, a discernable difference in the pattern of activated neurons in the caudal brainstem should be evident following immune challenge compared to nonthreatening viscerosensory stimuli such as a satiating meal. On the other hand, if immune challenge utilizes the same pathway as do, for instance, satiety-related signals, the same neurons should be active irrespective of type of stimulus. Thus, to determine whether sickness and satiety pathways can be distinguished in the brainstem, we compared the pattern of neuronal activation in brainstem regions between animals that were offered either sweetened milk or water, and received either LPS or saline injections. The animals used were the same as those used for the description of activation patterns in the forebrain associated with LPS-induced reduction of sweetened milk ingestion (Park et al., 2008). We analyzed c-Fos expression throughout the rostrocaudal extent of the NTS (the first order gustatory and visceral sensory relay nucleus) and the VLM. To identify specific populations of projection neurons, we assessed c-Fos protein induction in specific populations, i.e., adrenergic, noradrenergic, and glucagon-like peptide (GLP)-containing neurons. Animals challenged with LPS consume much less sweetened milk than saline-treated controls, which could be reflected in differences in neuronal activation patterns independent of immune challenge (especially due to differences in gastric distension, see Rinaman et al., 1998). To distinguish between possible contributions of ingestion of sweetened milk *per se* from c-Fos activation due to immune challenge (in LPS-treated rats that had sweetened milk access) we included two groups of "yoked controls". These animals were allowed access to a rationed amount of sweetened milk, corresponding to the average amount consumed by the LPS-treated rats given *ad libitum* access.

One of the primary targets for ascending viscerosensory projections is the pontine parabrachial nucleus (PBN). The PBN constitutes a major of node of immune-responsive neural networks (Buller et al., 2004; Gaykema et al., 2007), although its functional contribution to sickness responses is not clear. Neurons in this nucleus seem to be organized into regions that respond selectively to functional aspects of the stimulus (Yamamoto et al., 1994). Thus, if LPS activates a unique pathway, distinct patterns of immune challenge-related and sweetened milk-induced c-Fos induction should be observable in the PBN. To acquire further evidence regarding the organization of ascending neural projections carrying categorically different