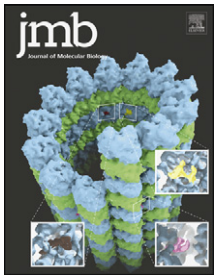
Review



Bacterial Signaling to the Nervous System through Toxins and Metabolites

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Abstract

Mammalian hosts interface intimately with commensal and pathogenic bacteria. It is increasingly clear that molecular interactions between the nervous system and microbes contribute to health and disease. Both commensal and pathogenic bacteria are capable of producing molecules that act on neurons and affect essential aspects of host physiology. Here we highlight several classes of physiologically important molecular interactions that occur between bacteria and the nervous system. First, clostridial neurotoxins block neurotransmission to or from neurons by targeting the SNARE complex, causing the characteristic paralyses of botulism and tetanus during bacterial infection. Second, peripheral sensory neurons—olfactory chemosensory neurons and nociceptor sensory neurons—detect bacterial toxins, formyl peptides, and lipopolysaccharides through distinct molecular mechanisms to elicit smell and pain. Bacteria also damage the central nervous system through toxins that target the brain during infection. Finally, the gut microbiota produces molecules that act on enteric neurons to influence gastrointestinal motility, and metabolites that stimulate the "gut–brain axis" to alter neural circuits, autonomic function, and higher-order brain function and behavior. Furthering the mechanistic and molecular understanding of how bacteria affect the nervous system may uncover potential strategies for modulating neural function and treating neurological diseases.

Introduction

Mammals host an incredibly complex community of commensal bacteria, with an estimated 10 trillion organism residents in an adult human gut [1]. Increasing evidence suggests that microbes residing in the gut, respiratory tract, genitourinary tract, and other barrier tissues actively participate in shaping and maintaining our physiology during development and homeostasis—almost as an extra "organ" [2]. In contrast, pathogenic bacteria have developed molecular strategies to survive within hosts, damaging physiological function and fitness through secreted toxins and metabolites. However, despite these differences, commensal and pathogenic bacteria share a common incentive to influence host physiology for their benefit. In this aspect, the nervous system is a desirable target as a master regulator of host function. By signaling to the nervous system, bacteria are granted a handle to influence a broad range of complex physiology, including motor coordination, sensation, metabolism, temperature control, mood, behavior, and cognition. In this review, we focus on two molecular classes of microbial signals that regulate the nervous system: bacterial toxins and metabolites.

From the perspective of the host, the nervous system provides a rapidly responsive and robust mechanism to detect bacterial cues and coordinate the appropriate defensive response. For example, peripheral sensory neurons densely innervate host barrier tissues, and are thus well positioned to detect unwanted microbes and microbial products when infection occurs [3,4]. Alternatively, in health, the gastrointestinal tract is densely inhabited by commensal bacteria, which easily outnumber local host cells by orders of magnitude [1]. As the gut microbiota is also an active producer of metabolites, its chemical signature allows the host nervous system to sample the status of gut bacterial communities and health.

An improved mechanistic understanding of how bacterial molecules act on the nervous system could yield improved therapeutics for treating neurological

diseases, as well as research tools for perturbing and analyzing the nervous system. Botulinum neurotoxin (BoNT) is a prime example where the toxin's ability to silence neurotransmission is currently utilized for the treatment of migraine and muscle spasticity [5]. The receptor-binding subunit of cholera toxin (CT) is widely used for retrograde tracing of neuronal connections as an experimental tool [6]. Identifying novel molecular interactions through which bacteria act on the nervous system, and characterizing known interactions in greater detail could highlight additional molecular pathways to be targeted or utilized. In the case of infectious disease, a better understanding of pathogenic mechanisms involving neuron–microbe interactions could also lead to novel antimicrobial approaches.

As such, here we highlight the molecular mechanisms through which commensal and pathogenic bacteria signal to the host central and peripheral nervous systems. Here, we focus mainly on recent work showing direct microbe–neuron molecular interactions, where bacterial molecules bind specifically or non-specifically to neurons to alter their biology and subsequent host physiology. We also introduce examples of indirect interactions where bacterial molecules act on an intermediary cell type such as endocrine or immune cells, which in turn produce neurochemicals and immune mediators that affect neurons. First, we discuss neurotoxins that specifically inhibit the vesicular release of neurotransmitters. Next, we introduce examples of bacterial molecules that affect smell and pain through their action on sensory neurons. In primitive organisms such as *Caenorhabditis elegans*, pathogenic bacteria are detected by chemosensation, which leads to avoidance behavior [7]. Recent work shows that similar mechanisms are also present in mammals, whose vomeronasal olfactory neurons and pain-sensing nociceptor neurons respond to bacterial effectors such as pore-forming toxins, N-formyl peptides, and various pathogen-associated molecular patterns (PAMPs). Bacteria also act on the intrinsic neurons of the enteric nervous system (ENS) to regulate gut motility through structural molecules such as polysaccharide A (PSA) or metabolites such as short-chain fatty acids (SCFAs). Bacteria-induced changes in gut motility could facilitate their colonization or maintenance of gut microflora composition. Lastly, we explore the increasingly complex area of research of the gut–brain axis, where neurotransmitters, toxins, and metabolites produced by the gut microbiota affect neural circuits, behavior, and central nervous system (CNS) function, with implications in neurological disorders such as autism.

Bacterial blockade of neurotransmission

Neurons transmit signals to each other at synapses through their release of neurotransmitters such as glutamate and γ-aminobutyric acid (GABA) stored within synaptic vesicles. Certain bacterial pathogens have evolved toxins that block this vesicular release. Depending on the type of neuron affected (e.g., motor or sensory neuron), inhibiting neurotransmitter release can cause diverging physiological consequences, such as flaccid paralysis observed with BoNT or spastic paralysis observed with tetanus neurotoxin (TeNT). Here we discuss the specific molecular mechanisms through which these bacterial toxins block neurotransmission (Fig. 1).

Botulinum neurotoxins

BoNTs and TeNT together compose the clostridial family of neurotoxins. BoNTs are the causative toxin for botulism in humans, which initially presents with blurred vision, difficulty swallowing and speaking, and muscle weakening, progressing to descending flaccid paralysis [8]. Among the known clostridial toxins, BoNTs are the most potent [9], and in the wild, amounts below the lethal dose are sufficient to paralyze a host and effectively terminate its survival [10]. Consequently, BoNTs play a central role in facilitating bacterial spread as cadavers provide an organic-rich, anaerobic environment that allows toxigenic clostridial species to proliferate [10].

BoNTs are produced by multiple strains of clostridia, including *Clostridium botulinum*, *Clostridium butyricum*, and *Clostridium baratii*. BoNTs are a genetically diverse group that can be classified into seven serotypes (BoNT/A–BoNT/G) based on immunoreactivity. An additional serotype (BoNT/H) was proposed, but recent studies suggest that it is a hybrid of BoNT serotypes A and F [11].

BoNTs are synthesized as a single-chain precursor, which is cleaved by clostridial or host proteases to yield a heavy chain (HC) and light chain (LC) that compose the mature toxin. The two chains are linked by a disulfide bridge and held together by a "belt region," a loop that extends from the HC and wraps around the LC [12–14]. In instances of food-borne or inhalation botulism where the toxin is first present in the lumen of the gut or respiratory tract, the HC mediates the transcytosis of the toxin across epithelial cells [15–17]. Subsequently, the intact holotoxin can enter the general circulation and accumulate at nerve terminals [18].

The selective binding of BoNTs to nerve terminals is possible by their recognition of two independent host receptors, which synergistically increase apparent affinity [19]. Initial BoNT binding is to membrane polysialogangliosides (PSGs), mediated by the C terminal domain of the HC [20–22]. Subsequent binding occurs through proteinaceous receptors, mediated by a separate site within the C terminal domain of the HC. The synaptic vesicle protein SV2 serves as the proteinaceous receptor for BoNT/A [23,24], BoNT/D [25], BoNT/E [26], and BoNT/F [27]. BoNT/A binds