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Short communication

Interleukin-1 induces c-Fos immunoreactivity in primary afferent neurons of the vagus nerve

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Abstract

Peripheral administration of bacterial endotoxin, an immune stimulant, induces evidence of activation in vagal primary afferent neurons. To determine whether interleukin-1β (IL-1β) is part of the molecular pathway leading to this activation, we assessed the expression of the neuronal activation marker c-Fos in vagal primary afferent neurons after intraperitoneal injections of IL-1β (2 μg/kg). IL-1β, but not vehicle, induced c-Fos expression, demonstrating that IL-1β is likely an important signal from the immune system to the vagus nerve, and thus the brain. © 1998 Published by Elsevier Science B.V. All rights reserved.

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When challenged by the presence of pathogens, the immune system signals the brain to activate and co-ordinate a number of responses that serves to facilitate host defense. These responses include hyperthermia, elevation of plasma corticosterone levels, increased sleep, as well as decreased social and food-motivated behaviors [5,15]. Although the precise mechanisms by which the immune system signals the brain are not entirely clear, one pathway that may be particularly important for signaling immune stimuli in internal tissues is likely to be the vagus nerve [25].

The vagus nerve innervates most viscera, including lung, alimentary canal, and liver, and thus is well situated to detect pathogens in these tissues. Sectioning the abdominal vagus nerve abrogates most brain-mediated illness responses induced by administration of bacterial endotoxin (LPS, a component of the cell wall of gram negative bacteria) [3,9,21,22], especially after intraperitoneal (i.p.) administration. In addition, peripheral endotoxin activates vagal primary afferent neurons [10], as indicated by induction of the immediate-early gene product, c-Fos, an activation marker for many neuronal populations. Taken together, these observations strongly support a role for the vagus nerve in signaling immune activation to the brain.

Although much evidence implicates the vagus as an important pathway for immune-to-brain communication, the mechanisms by which the immune system signals the vagus is less clear. Immune stimuli, such as endotoxin, induce the release of mediators, including the cytokine interleukin-1β (IL-1β), that are likely to be important in signaling the nervous system [5]. IL-1β administered exogenously induces similar illness responses as does endotoxin [5,16]. Pretreatment with interleukin-1 receptor antagonist (IL-1ra) prevents illness responses normally initiated by either endotoxin or IL-1β [16,20,23]. Thus, IL-1β likely constitutes a component of the signaling pathway from the immune system to the brain.

Evidence to date supports the hypothesis that i.p. IL-1β signals the brain via vagal sensory neurons. Sectioning the abdominal vagus abrogates the effects of i.p. administered IL-1β on several illness responses [3,8,11,13,14,24]. In addition, cells within sensory structures (paraganglia) innervated by vagal sensory neurons express binding sites for IL-1ra, which may represent IL-1 receptors [12]. Taken together, these findings suggest that IL-1β may indeed act via the vagus nerve. If so, then peripheral administration of IL-1β should result in detectable activation of vagal afferent neurons. To address this issue, we treated rats with either IL-1β or vehicle i.p., and assessed activation of vagal afferents using immunohistochemistry to detect expression of c-Fos.

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Adult male Sprague–Dawley rats ($n = 8$, Harlan) were received and maintained under specific-pathogen-free conditions. They were housed on a 12:12 light:dark cycle (lights on at 0700 h) with ad libitum food and water. Rats were injected i.p. with either 2 μg/kg IL-1β ($n = 6$) or equivolume vehicle (1 ml/kg pyrogen-free saline; $n = 2$) between 2 and 5 h after light onset. Ninety minutes following injection, they were anesthetized with 60 mg/kg Nembutal and perfused transcardially with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). The left and right glossopharyngeal–vagal ganglion complexes (referred to as vagal ganglia) were dissected, post-fixed overnight, and stored in 0.1 M PB containing 0.1% sodium azide, at 4°C. Prior to sectioning,

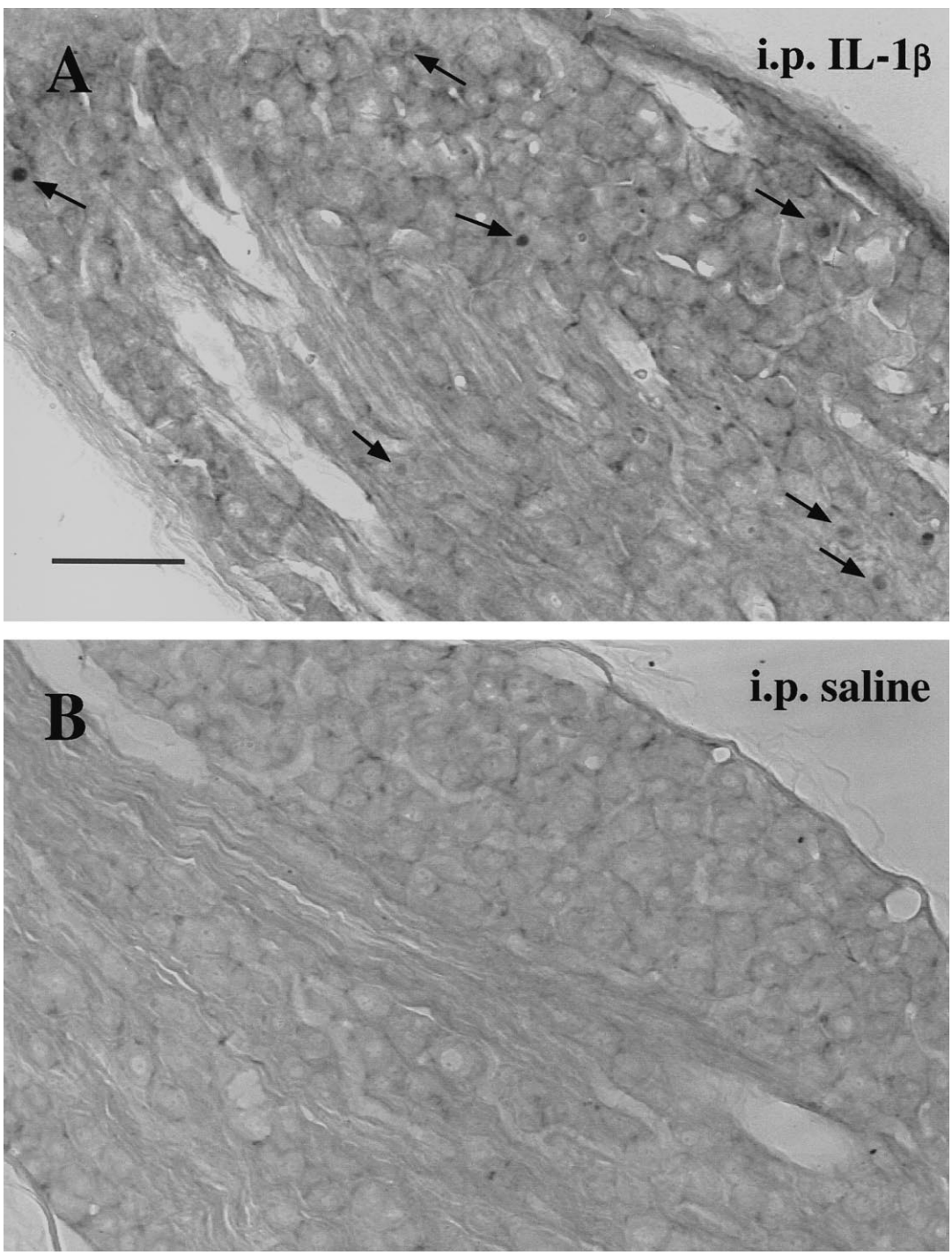

Fig. 1. Photomicrograph images of nodose ganglia of a IL-1b-injected rat (A) and a saline-treated rat (B) showing the induction of c-Fos immunoreactivity in vagal sensory neurons following IL-1b administration (A). Saline injection does not lead to c-Fos expression (B). Scale bar in (A) = 100 μm (also applies to (B)).

the tissue was cryoprotected in 22% sucrose in 0.1 M PB. The vagal ganglia were sectioned at 16 μm thickness on a precision cryostat (Leica) and thaw-mounted on gelatin–chromalum-coated slides. Vagal ganglia sections were processed for c-Fos immunohistochemistry using a polyclonal antiserum raised in rabbits against the N-terminal region of the c-Fos protein (Ab5, Oncogene Research Products; Lot #60950101), as previously described [10]. Specificity of the immunostaining was assessed by omitting the primary antiserum. Numbers of c-Fos immunoreactive (c-Fos-IR) cells in the vagal ganglia were counted in serial sections (64 μm apart) through the ganglion complex under 200 × magnification, using an Olympus Vanox II brightfield microscope. Both lightly and darkly stained nuclei were counted for all cases. Lightly stained nuclei were distinguished from non-labeled nuclei by their darker color relative to the surrounding cytoplasm, in contrast to non-labeled nuclei which are lighter than the surrounding cytoplasm. A representative cumulative number of Fos-IR cells was obtained from complete series of sections from both the left and right vagal ganglia of each animal. The distribution of c-Fos-IR sensory cells in the left vagal

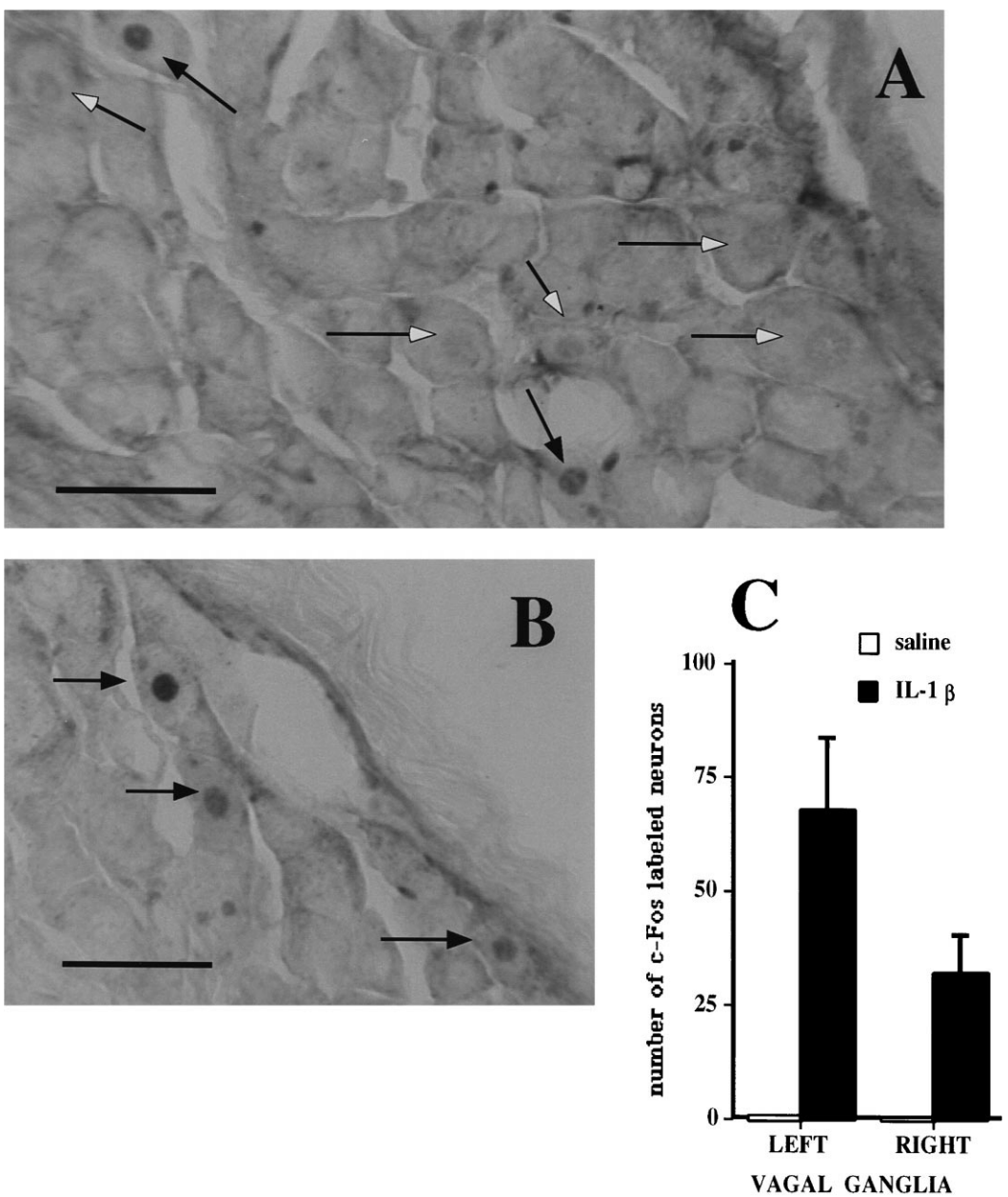

Fig. 2. (A) and (B) IL-1b-induced c-Fos immunoreactivity in vagal sensory cells varies from light (white arrows in (A)) to very strong (black arrows in (A) and (B)). Neurons showing c-Fos staining are often clustered (A) and (B). Small (< 4 μm) immunoreactive profiles ((A) and (B)) likely represent nuclei of satellite cells, which can also be observed in saline treated animals (Fig. 1B). (C) Vagal sensory cells expressing IL-1b-induced c-Fos-IR are more numerous in the left vagal ganglia than in the right. An i.p. saline does not induce c-Fos-IR in either ganglion. Scale bars in A and B = 50 μm.

ganglia compared to the right ganglia was analyzed using a paired *t*-test (Statview, Abacus Concepts, Berkeley, CA). Brightfield images were acquired with a COHU CCD camera coupled to an Apple PowerMac 7200 equipped with NIH Image software (version 1.60) and stored on a Syquest EZ Drive cartridge. The obtained micrographs were exported to and labeled using Canvas. Except for small adjustments of brightness and contrast, the images were not altered.

Treatment with i.p. IL-1β induces the expression of c-Fos in the nuclei of vagal afferent neurons within the vagal sensory ganglia (Fig. 1A). In contrast, and in agreement with our earlier work [10], vagal primary afferent neurons of saline-treated rats show virtually no c-Fos-IR (Fig. 1B, Fig. 2C). C-Fos-IR occurs primarily in cells located in the caudal regions of the ganglia. The intensity of c-Fos-IR varies from light to very strong (Fig. 2A). Cells expressing c-Fos-IR are sometimes clustered together (Fig. 2A,B) but many are scattered among cells not positive for c-Fos-IR (Fig. 1A). Cell counts of c-Fos expressing primary afferent neurons from IL-1β and saline treated animals are depicted in Fig. 2C. About twice as many sensory neurons in the left vagal ganglion (mean = 68.0) as in the right (mean = 32.3) express c-Fos-IR after i.p. IL-1β. This difference is statistically significant ($t(5) = 2.85$, $p < 0.04$).

The results from this study demonstrate that i.p. injected IL-1β activates primary afferent neurons of the vagus. These findings are in accord with those from our earlier study [10], showing the induction of nuclear c-Fos immunoreactivity in vagal primary afferent neurons after peripheral administration of bacterial endotoxin. They are also consistent with those reported by Ek et al. [6], showing the induction of c-*fos* mRNA after intravenous administration of IL-1β. These data strongly implicate vagal afferents as a pathway by which the immune system, via the release of IL-1β, signals the nervous system.

Administration of LPS induces IL-1β expression [5], and i.p. administration of either LPS [10], or IL-1β induces c-Fos-IR in vagal sensory neurons. These observations imply that IL-1β may be mediating the actions of LPS on vagal afferent neurons. However, other mediators can be induced by LPS, in addition to (and possibly in concert with) IL-1β, that contribute to brain-mediated illness consequences. These substances include the pro-inflammatory cytokines tumor necrosis factor-alpha and interleukin-6, prostaglandins, as well as other potential mediators such as the mast cell products serotonin, substance P, and histamine. Some or all of these substances may influence illness responses through a vagal afferent pathway.

At least two-thirds of all vagal sensory neurons expressing c-Fos-IR after i.p. IL-1β reside in the left vagal ganglia. This difference may reflect the fact that the hepatic branch of the vagus, which has been implicated in immune to brain communication [18,22], forms a component of the left trunk of the vagus nerve, and thus the cell bodies of hepatic vagal afferents are likely to reside in the left vagal ganglion. Section of the hepatic vagal branch alone blocks endotoxin-induced hyperalgesia [22], and intrahepatic portal injections of IL-1β increase the firing rate of hepatic vagal fibers [18]. However, our observation that vagal sensory neurons of both left and right ganglia respond to i.p. IL-1β, and the observation that selective hepatic branch vagotomy does not inhibit IL-1β-induced illness responses [8], indicates that vagal afferents traversing the other branches of both left and right vagal nerves also contribute to IL-1β-induced responses.

Intraperitoneal IL-1β rapidly induces the release of glutamate (the neurotransmitter for a majority of vagal afferents [19]), in vagal afferent terminal fields within the nucleus of the solitary tract (nTS) [17]. The observation that IL-1β activates vagal afferents provides an essential link between immune stimulation in the peritoneum and activation of brainstem regions such as the nTS [2,4,7]. Taken together, these findings support a model for immune-to-brain communication whereby pathogens, such as bacteria, stimulate immune cells to release cytokines such as IL-1β. IL-1β then activates vagal afferent nerve fibers, either directly or via chemoreceptive cells of the vagal paraganglia [12]. Immune-responsive vagal afferents activate the dorsal vagal complex, which then co-ordinates local protective reflexes [1], and relays immune-related information to rostral brain regions involved in host defense mechanisms.

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