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Airway nociceptors activated by pro-inflammatory cytokines

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Abstract

The present studies evaluate whether the vagus nerves link the lungs' immune and neural systems by transmitting information through pulmonary nociceptors. Single unit activities from pulmonary nociceptors [C fiber receptors (CFRs) and high threshold Aδ fiber receptors (HTARs)] were recorded from the cervical vagus nerve in anesthetized, open-chest, and mechanically ventilated rabbits. Interleukin1β was then injected into the nociceptor field (IL-1β, 10 μg/ml, 20 μl). Both CFRs and HTARs were stimulated by the local injection; their activities increased from 0.2 ± 0.1 to 1.8 ± 0.5 imp/s ($n = 10$; $p < 0.01$), and from 0.2 ± 0.1 to 1.1 ± 0.1 imp/s, respectively ($n = 6$; $p < 0.01$). These increases were greatly attenuated by simultaneous administration of IL-1β with IL-1ra, a natural IL-1 receptor antagonist. The nociceptors were not stimulated by local injection of normal saline. Our data demonstrate that nociceptors can be activated by pro-inflammatory cytokines and support the hypothesis that airway nociceptors transmit immune signals from the lung to the brain.

Keywords: Interleukin; Vagal afferents; Pulmonary receptors; Inflammation; Neural immune interaction

1. Introduction

In face of pathogen insult, the immune system signals the body, including the brain, to evoke an array of host defense responses. These brain-mediated responses include hyperthermia, corticosteroid secretion, lethargy, and anorexia (Goehler et al., 2000). Each is associated with the vagus nerves. Inflammation is a normal bodily reaction to foreign invaders. Our understanding of the molecular, cellular and therapeutic aspects of cytokines and their receptors during inflammation is growing, but regarding the role played by the visceral nervous system during inflammation is still limited. It is increasingly clear that there is interaction between the neural and immune systems and that the vagus nerve is a potential carrier for transmitting such information (Czura and Tracey, 2005; Goehler et al., 2000). There are two major types of sensors in the lung: mechanosensitive receptors and chemosensitive receptors (Lee and Pisarri, 2001; Yu, 2005). Both are connected with the vagus nerves and monitor environmental changes. The chemosensitive receptors, or nociceptors, include high-threshold Aδ receptors (HTARs) and non-myelinated C-fibers receptors (CFRs) (Lee and Pisarri, 2001; Yu, 2002). During lung inflammation, numerous mediators are released, including: histamine, 5-HT, bradykinin, prostanoids, and cytokines. The first four are known to stimulate airway nociceptors (Lee and Pisarri, 2001), but whether the cytokines can is unknown. IL-1β has been reported to activate vagal sensory endings evidenced by increased *c-Fos* mRNA expression in the nodose ganglia, and IL-1 receptors are also found in the nodose ganglion (Ek et al., 1998). IL-1β injected into portal vein stimulates hepatic vagal afferents (Niijima, 1996). The vagus nerve may provide a communication link between the peripheral organs and the brain. Thus, it is likely that the pulmonary sensory receptors, especially the nociceptors, can also be activated by pro-inflammatory cytokines, such as IL-1β, providing information to regulate the immune response. The present studies test the hypothesis that vagal nerves play a role in immune response through a reflex mechanism to control inflammatory intensity (Czura and Tracey, 2005). More specifically, we sought to determine whether the airway nociceptors act as biosensors in monitoring the inflammatory process, including the level of cytokines. Single unit activity from nociceptors was recorded before and after IL-1β delivery to the receptive field. Increased nociceptor activity was observed; this demonstrates that the airway nociceptors serve as biosensors for pro-inflammatory cytokines.

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2. Methods

Experiments were carried out in 35 male New Zealand white rabbits anesthetized with 20% urethane (1 g/kg, iv). Study procedures were in accordance with ethics codes set by the NIH and approved by the IACUC of the University of Louisville. A midline incision was made to expose the trachea and vagus nerve. The trachea was cannulated low in the neck and the lungs were mechanically ventilated with a Harvard ventilator (Model 683, South Natick, MA). Positive-end-expiratory pressure (PEEP) was maintained by placing the expiratory outlet under 3–4 cm H_2O. Airway pressure was monitored at the tracheal tube with a pressure transducer (Statham P23). The chest was opened widely in the midline to allow locating the receptive field. Airway pressure and afferent activities were recorded by a thermorecorder (Astro-Med Dash IV).

Single-unit activities were recorded from the vagal afferent according to conventional methods. The vagus nerve (either right or left) was separated from the carotid sheath, placed on a dissecting platform, and covered with mineral oil. A small afferent bundle was cut from the vagus nerve. This bundle was dissected into thin filaments with two pairs of fine forceps. The filaments were further divided and placed on electrodes to record action potentials. The electrodes were connected to a high impedance probe (Grass Model HIP 511), from which the output was fed into an amplifier (Grass P 511). After suitable amplification, action potentials from a single-unit of the vagal sensory receptors were displayed on an oscilloscope and monitored by a loudspeaker. In addition, a voltage analogue of impulse frequency was produced by a rate meter (Frederick Haer, Brunswick, ME) at a band width of 0.1 s. The receptive field was located by identifying the most sensitive point on the lung surface with a glass rod having a 0.5 mm round tip. A total of 24 CFRs and 16 HTARs were identified. They were identified by their discharge pattern, and confirmed by verifying their receptive fields in the lung and by their conduction velocities (above 1.6 m/s for HTARs and less than 1.5 m/s for CFRs). HTARs do not behave like SARs or RARs, but more similarly to CFRs, with a conduction velocity in Aδ fiber range (Yu, 2002). More specifically, these sensors do not respond to lung deflation and have very high activation threshold to lung inflation (Fig. 1C–E).

There are two types of IL-1 receptors: IL-1RI and IL-1RII (Arend, 2002). IL-1β exerts its biological effects by binding to IL-1RI, with no effects on IL-1RII. Thus, IL-1RII functions as a decoy. IL-1ra is a natural IL-1 receptor antagonist, with equally potent binding to IL-1RI; it serves as a competitive receptor blocker. In the current studies, receptor responses to pro-inflammatory mediators (IL-1β) were examined. IL-1β (10 μg/ml) or a mixture containing IL-1β (10 μg/ml) with IL-1ra (50 μg/ml) was directly injected into the receptive field through a needle (30 GD) in a volume of 20 μl. Receptor response to the injection was observed for 20 min.

3. Results

Airway nociceptors, HTARs and CFRs, behaved similarly although they have different conduction velocities, which ranged from 1.6 to 8.9 m/s with a mean of 4.3 ± 0.4 m/s for HTARs ($n = 16$) and from 0.3 to 1.3 m/s with a mean of 0.8 ± 0.1 m/s for CFRs ($n = 24$). These sensory units had low background activities and were not very sensitive to changes in lung mechanics, but they were activated by the major pro-inflammatory mediator IL-1β. They discharged sporadically and irregularly [0.2 ± 0.1 and 0.2 ± 0.1 imp/s for HTARs ($n = 16$) and CFRs ($n = 24$), respectively] under resting conditions and were stimulated after about 20 s following injection of IL-1β, peaking within the first few minutes. HTARs and CFRs discharges increased from 0.2 ± 0.1 to 1.1 ± 0.1 imp/s ($n = 6$), and from 0.2 ± 0.1 to 1.8 ± 0.5 imp/s ($n = 10$), respectively at the peak response. Fig. 1 illustrates an individual HTAR. Since these receptors behaved similarly, we pooled the data together. On the whole, the nociceptor activities peaked within a few minutes, and then subsided but were still elevated 10 min after the injection (Fig. 2). These stimulatory effects were significantly attenuated after using the natural receptor antagonist (IL-1ra) to block IL-1RI (Fig. 2). The activities during peak responses were increased by 0.5 ± 0.2 ($n = 17$) and 1.3 ± 0.3 ($n = 16$) imp/s, respectively for IL-1β with and without IL-1ra. The difference between the two groups was statistically significant ($p < 0.05$).

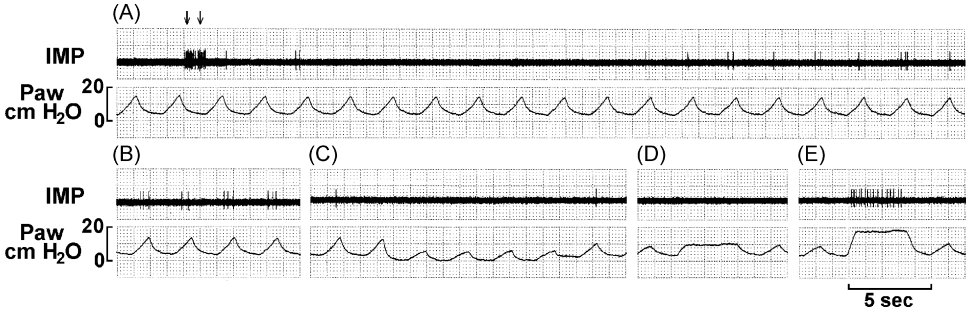

Fig. 1. Activation of a high threshold Aδ receptor (HTAR) by IL-1β, a potent inflammatory mediator. This HTAR was recorded from the left cervical vagus nerve in an anesthetized, open-chest, and mechanically ventilated rabbit. The receptive field was located close to the left hilum. Traces are: IMP, afferent activity (impulses); Paw, airway pressure. A and B are consecutive recordings with 94 s apart. Two arrows in A denote insertion of a needle into the receptive field and injection of IL-1β (10 μg/ml, 20 μl). This receptor was virtually inactive during its resting condition and was stimulated 26 s after IL-1β injection. The stimulation reached a peak about 120 s after the injection (B). The receptor is insensitive to lung deflation (C) and has a high activation threshold to lung inflation (D and E), with a conduction velocity of 6.9 m/s.