Respiratory Physiology & Neurobiology 230 (2016) 44–53

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Interleukin-1β and interleukin-6 enhance thermal prolongation of the LCR in decerebrate piglets

Luxi Xia, Donald Bartlett Jr, J.C. Leiter*

Department of Physiology & Neurobiology, Geisel School of Medicine at Dartmouth, Lebanon, NH, United States

ARTICLE INFO

Article history:
Received 29 December 2015
Received in revised form 9 May 2016
Accepted 10 May 2016
Available online 12 May 2016

Keywords:
SIDS
Laryngeal chemoreflex
Hyperthermia
Interleukin-1β
Interleukin-6
Nucleus of the solitary tract

ABSTRACT

Thermal stress and prior upper respiratory tract infection are risk factors for the Sudden Infant Death Syndrome. The adverse effects of prior infection are likely mediated by interleukin-1β (IL-1β). Therefore, we examined the single and combined effects of IL-1β and elevated body temperature on the duration of the Laryngeal Chemoreflex (LCR) in decerebrate neonatal piglets ranging in age from post-natal day (P) 3 to P7. We examined the effects of intraperitoneal (I.P.) injections of 0.3 mg/Kg IL-1β with or without I.P. 10 mg/Kg indomethacin pretreatment on the duration of the LCR, and in the same animals we also examined the duration of the LCR when body temperature was elevated approximately 2 °C. We found that IL-1β significantly increased the duration of the LCR even when body temperature was held constant. There was a significant multiplicative effect when elevated body temperature was combined with IL-1β treatment: prolongation of the LCR was significantly greater than the sum of independent thermal and IL-1β-induced prolongations of the LCR. The effects of IL-1β, but not elevated body temperature, were blocked by pretreatment with indomethacin alone. We also tested the interaction between IL-6 given directly into the nucleus of the solitary tract (NTS) bilaterally in 100 ngm microinjections of 50 μL and pretreatment with indomethacin. Here again, there was a multiplicative effect of IL-6 treatment and elevated body temperature, which significantly prolonged the LCR. The effect of IL-6 on the LCR, but not elevated body temperature, was blocked by pretreatment with indomethacin. We conclude that cytokines interact with elevated body temperature, probably through direct thermal effects on TRPV1 receptors expressed pre-synaptically in the NTS and through cytokine-dependent sensitization of the TRPV1 receptor. This sensitization is likely initiated by cyclo-oxygenase-2 dependent synthesis of prostaglandin E2, which is stimulated by elevated levels of IL-1β or IL-6. Inflammatory sensitization of the LCR coupled with thermal prolongation of the LCR may increase the propensity for apnea and Sudden Infant Death Syndrome.

1. Introduction

There is widespread recognition that apnea and subsequent hypoxia and bradycardia play an important role in the pathogenesis of SIDS (Hunt and Brouillette, 1987; Kahn et al., 1992; Kinney and Thach, 2009; Leiter and Böhm, 2007; Steinschneider, 1972). It seems likely that babies who die of SIDS experience an asphyxial stress associated with apnea during sleep to which they do not respond appropriately and from which they do not recover (Leiter and Böhm, 2007; Poets et al., 1999; Sridhar et al., 2003). Therefore, events or processes that initiate apnea or enhance apnea duration will promote the occurrence of SIDS, and events or processes that terminate apneas, restore eupnea and enhance arousal from sleep are likely to reduce the occurrence of SIDS. The laryngeal chemoreflex (LCR) is a protective response elicited when fluid enters the larynx, particularly fluid with a low chloride content or low pH (Boggs and Bartlett, 1982; Downing and Lee, 1975; Lee et al., 1977). The LCR is particularly prominent in neonatal animals and is frequently elicited in the normal course of neonatal life (Thach, 1997, 2001). The LCR consists of protective airway responses (laryngeal closure and apnea) that prevent aspiration of fluids into the upper airway, clear the fluids from the airway and preserve oxygen delivery to vital organs. To prevent aspiration, respiration is inhibited (Downing and Lee, 1975), and the glottis is closed (Haraguchi et al., 1983; Sasaki, 1979). To clear the airway, coughing and swallowing are activated (Thach, 2001; van der Velde et al., 2003). To preserve oxygen delivery, bradycardia occurs and blood flow may be redistributed to vital organs (Grogaard et al., 1982). These behaviors are elicited to varying degrees depending on the strength of the reflex

* Corresponding author at: Department of Physiology & Neurobiology, Geisel School of Medicine at Dartmouth, One Medical Center Drive, Lebanon, NH 03756, United States.
E-mail address: james.c.leiter@dartmouth.EDU (J.C. Leiter).

http://dx.doi.org/10.1016/j.resp.2016.05.006
1569-9048/

response. Weak reflex responses may consist of brief respiratory disruption, coughing and swallowing; whereas the entire range of behaviors may be elicited when the LCR is strongly activated (Thach, 2001; van der Velde et al., 2003). Despite the protective nature of the LCR, many investigators have suggested that the LCR may be an important initiator of apneas that begin a chain of events leading to SIDS (Downing and Lee, 1975; Lanier et al., 1983; Leiter and Böhm, 2007; Page and Jeffery, 2000; Page et al., 1996; Thach, 1997, 2001, 2005). Infants who are susceptible to SIDS may have stronger LCR responses and may have weaker arousal mechanisms and/or difficulty restoring eupnea after reflex apneas—they may fail to autoresuscitate (Cummings et al., 2011; Kinney and Thach, 2009; Leiter, 2009; Leiter and Böhm, 2007; Poets et al., 1999; Sridhar et al., 2003).

A variety of airway receptors contribute to the LCR, and the behavioral diversity of the reflex response may reflect the diversity of receptors activated within the larynx when the LCR is elicited. The LCR can be mediated by 'water' receptors within the mucosa of the larynx (Boggs and Bartlett, 1982). More than half of these water sensitive receptors are mechanoreceptive, and the afferent nerves derived from these receptors have rapid conduction velocities, typical of myelinated A-fibers (Anderson et al., 1990; Harding et al., 1978). On the other hand, capsaicin-sensitive laryngeal afferents also elicit apnea, bradycardia, swallowing and other behaviors typical of the LCR. These capsaicin-sensitive fibers are unmyelinated C-fibers and clearly separable from water receptors (Mutoh et al., 2000; Roulier et al., 2003). A-fibers and C-fibers express a different constellation of presynaptic receptors in the NTS (Jin et al., 2004), and transient receptor potential vanilloid 1 (TRPV1) receptors are functionally associated with C-fibers. Both A-fibers and C-fibers run in the internal branch of the superior laryngeal nerve and transmit sensory information from the larynx to the brainstem, where the afferents innervate second order neurons within the caudal NTS (Hayakawa et al., 2001; Patrickson et al., 1991). These second order neurons express non-NMDA glutamate receptors, and when visceral afferents are stimulated, glutamatergic excitatory post-synaptic currents (EPSCs) are elicited in the second order neurons (Doyle et al., 2002; Jin et al., 2004; Peters et al., 2010), and elicit apnea. In previous studies, we have focused on laryngeal stimuli and central regulatory pathways that emphasize the role of C-fiber modulation of the LCR (Xia et al., 2011).

Many risk factors for SIDS seem to enhance the LCR. For example, thermal stress (elevated room temperature, increased covering with bed clothes, etc.) is a risk factor for SIDS, and elevated body temperature enhanced laryngeal adduction induced by superior laryngeal nerve stimulation in dogs (Haraguchi et al., 1983) and prolonged the LCR elicited by injection small volumes of water into the larynx in decerebrate piglets (Curran et al., 2005). The effect of elevated body temperature on the LCR depends on activation of TRPV1 receptors within the nucleus of the tractus solitarius (NTS) (Xia et al., 2011). A recent history of an upper respiratory tract infection (URI) is also a risk factor for SIDS (Steinschneider, 1972), and viral infections, particularly respiratory syncytial virus infections, may prolong reflex apneas in newborn lambs (Lindgren and Grogaard, 1996; Lindgren et al., 1992). The effect of recent URIs depends on the synthesis and release of inflammatory cytokines (Frøen et al., 2000, 2002). In anesthetized piglets, endotoxin administration, which initiates a cytokine cascade through a toll-like receptor, prolonged apneas elicited by insufflation of acidic water into the larynx, and systemic administration of interleukin-1β (IL-1β) prolonged apnea induced by ammonia saturated air insufflated into the larynx (Stoltenberg et al., 1994). Infections that increase IL-1β or tumor necrosis factor-alpha (TNFα) may, therefore, sensitize the LCR. In neither of the foregoing studies was body temperature allowed to rise, but both endotoxin and IL-1β are pyrogenic. Elevated levels of IL-1β and TNFα generate a cascade of cytokines, including IL-6, which in turn stimulate cyclooxygenase-2 (COX-2) activity, and generate prostaglandins, especially prostaglandin E_2 (PGE_2), which is the ultimate mediator of altered neuronal activity in temperature sensitive cells in the hypothalamus that causes fever (Dinarello, 2004). Therefore, we investigated the interaction between inflammation mediated by IL-1β and the direct effects of temperature on the duration of the LCR. We tested the hypothesis that IL-1β and elevated body temperature would independently increase the duration of the LCR. In addition, we tested the hypothesis that IL-1β and elevated body temperature would demonstrate a significant interaction—that the combined stimuli would have a greater effect than the sum of the two individual stimuli. We also tested the effect of IL-1β in the presence of the mixed cyclooxygenase-1 and -2 inhibitor, indomethacin, to determine whether IL-1β-dependent effects on the LCR are mediated by IL-1β itself or by downstream mediators such as PGE_2. Moreover, we tested the hypothesis that the IL-1β-dependent effects on the LCR are mediated centrally within the brainstem by IL-6, the synthesis of which may be increased when IL-1β levels increase.

2. Methods

Experiments were performed on 43 piglets (22 male and 21 female) ranging in age from 3 to 7 days (4.9 ± 1.0 days; mean ± SEM) with an average weight of 2.3 ± 0.1 kg. The Institutional Animal Care and Use Committee of Dartmouth College approved all surgery and experimental protocols.

2.1. Surgical preparation

Animals were anesthetized with 2% halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; SigmaAldrich) in O_2. A rectal probe was inserted, and rectal temperature ('body temperature') was maintained between 38 and 39 °C using a servo-controlled heating pad. Femoral arterial and venous catheters were inserted to measure blood pressure and administer drugs, respectively. Each animal was tracheostomized and artificially ventilated (Harvard Apparatus Dual Phase Respirator, South Natick, MA) to maintain the end-tidal CO_2 concentration at approximately 5%, which was also servo-controlled so that end-tidal CO_2 did not vary as a function of any of the experimental treatments. After exposing the carotid sinus regions bilaterally, the internal and external carotid arteries were ligated to facilitate decerebration. The vagus nerves were sectioned bilaterally to prevent entrainment of the phrenic rhythm to the mechanical ventilator (Graves et al., 1986; Petrillo et al., 1983). Each animal was placed prone with its head in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The skull was opened, the animal was decerebrated at the level of the superior colliculi and all brain tissue rostral to the section was removed by suction. Following decerebration, isoflurane anesthesia was discontinued. Each animal was paralyzed using pancuronium bromide (1 mg/kg, iv; Elkins-Sinn Inc., Cherry Hill, NJ), and supplemental doses of pancuronium were given as required, usually at a rate of 0.5 mg/Kg/h. A phrenic nerve was exposed and sectioned, and the central cut end was placed on a bipolar recording electrode to monitor respiratory output. Phrenic activity was amplified (Gould Universal Amplifier, Cleveland, OH), and the moving time average ("integrated activity") was calculated electronically (100 ms time constant; CWE, Ardmore, PA). Integrated phrenic nerve activity, body temperature, end-tidal CO_2 and blood pressure were recorded on a computer (PowerLab, ADI, Australia) for later analysis.

Intravenous drugs were delivered through the femoral venous catheter. We made microinjections of drugs into the dorsal area of the brainstem using a 0.5 μl syringe (SGE Analytical Sciences, Austin, TX) with a 0.47 mm O.D. The needle of the injection syringe