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Journal of Neuroimmunology 203 (2008) 39–49

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

Lipopolysaccharide-induced hypotension is mediated by a neural pathway involving the vagus nerve, the nucleus tractus solitarius and alpha-adrenergic receptors in the preoptic anterior hypothalamic area

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Received 1 February 2008; received in revised form 16 June 2008; accepted 17 June 2008

Abstract

We recently reported that the preoptic anterior hypothalamic area (POA) mediates the hypotensive response evoked by lipopolysaccharide (LPS). In this study, we investigated how the inflammatory signal induced by LPS reaches the POA. Subdiaphragmatic vagotomy and abdominal perivagal lidocaine administration, or lidocaine injection into the nucleus tractus solitarius (NTS) prevented LPS hypotension. Microinjection of the alpha-adrenergic receptor antagonist phentolamine into the POA, blocked initiation of the hypotensive response and prevented the late decompensatory phase. These data suggest that LPS hypotension is mediated by the vagus nerve which conveys the signal to the NTS and, in turn, stimulates norepinephrine release within the POA.

Keywords: Endotoxic shock; Hypotension; Vagus nerve; Nucleus tractus solitarius; Alpha-adrenergic receptors

1. Introduction

Despite rapid advances in our ability to understand the molecular mechanisms that cause septic shock, mortality remains unacceptably high. Recent clinical trials with agents designed to block the effects of tumor necrosis factor-α (TNF) and other presumed mediators of sepsis have failed to significantly increase survival (Kirkeboen and Strand, 1999). It is therefore essential to better understand the mechanisms that underlie septic shock in order to develop new therapeutic strategies for precluding its harmful effects.

In experimental animals, lipopolysaccharide (LPS) administration produces a biphasic fall in arterial pressure. The late phase is thought to be mediated by nitric oxide (NO) (Kirkeboen and Strand, 1999) but the mechanism responsible for the initial fall in arterial pressure has not been systematically investigated. Tumor necrosis factor-α is often implicated as a critical mediator of endotoxic shock (Ulloa and Tracey, 2005). However, LPS causes arterial pressure to fall within minutes, long before TNF is detectable in the circulation (Li et al., 2006; Yilmaz et al., 2008). This discrepancy suggests that an alternative mechanism may be responsible for initiation of endotoxic hypotension.

The mechanism by which LPS and peripheral cytokines signal the brain during systemic inflammation is still controversial (Blatteis and Sehic, 1998; Dunn, 2002). Both neural and humoral pathways of communication have been proposed (Blatteis and Sehic, 1998; Dunn, 2002; Blatteis, 1992; Blatteis et al., 2000; Blatteis et al., 2004). Because of the rapidity of the response, we have suggested that the initial fall in arterial pressure triggered by LPS is mediated by a neural mechanism

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doi:10.1016/j.jneuroim.2008.06.023

(Yilmaz et al., 2008). This hypothesis is supported by evidence that LPS causes fever through a central mechanism in which the preoptic area/anterior hypothalamus (POA) plays a pivotal role. We recently reported evidence that the POA may also participate in endotoxic hypotension. Specifically, we found that the initial fall in arterial pressure evoked by LPS could be prevented completely by inhibiting neuronal activity in the POA with the local anesthetic lidocaine. Lidocaine injection into the POA was effective in both conscious and anesthetized rats but lidocaine did not affect LPS-induced hypotension when injected into the lateral hypothalamus (Yilmaz et al., 2008). Unexpectedly, lidocaine pretreatment also inhibited the second, delayed fall in arterial pressure induced by LPS. Together, these data suggest that the initial phase of endotoxic hypotension is mediated by the POA and that the POA may also play a role in the development of the late fall in blood pressure (Yilmaz et al., 2008).

It remains to be determined how a peripheral signal from LPS is transmitted to the POA. Recent data indicates that the vagus nerve may play an important role in some of the effects of LPS and inflammatory cytokines. Subdiaphragmatic vagotomy prevents the sickness behavior evoked by LPS in both rats and mice, for example, and inhibits LPS-induced ACTH secretion (Laye et al., 1995; Gaykema et al., 1995; Ericsson et al., 1994). Gaykema et al. (1995) also reported that subdiaphragmatic vagal transection attenuates the febrile response of rats to interleukin-1β (IL-1β) (Gaykema et al., 1995), consistent with a previous report that IL-1β injection into the hepatic portal vein increased electrical activity in the vagus nerve (Nijima, 1996). Both LPS and IL-1β induce c-*fos* expression in the nucleus of the solitary tract (NTS) (the primary projection area of the vagus nerve), again suggesting that the vagus nerve conveys the inflammatory signal from the peripheral circulation to the brain (Wan et al., 1994). Moreover, Mailman et al. recently reported that application of lidocaine or resinferatoxin (which inhibits afferent, but not efferent impulse flow in the vagus) to the abdominal vagus inhibits LPS-induced hypotension (Mailman, 2002). Together these findings support the hypothesis that LPS initially lowers arterial pressure through a neural pathway that involves the vagus nerve and POA.

The next question is, how is the signal from LPS communicated from the NTS, where vagal afferents synapse, to the POA? At least two routes are possible: 1) by way of intermediate relays in the brainstem reticular formation (Ramon-Moliner and Nauta, 1966) or 2) by way of noradrenergic projections originating in the A1 and A2 regions of the medulla (Fernandez-Galaz et al., 1994) and arriving in the POA via the ventral noradrenergic bundle (Palkovits et al., 1980). Excitatory abdominal vagal inputs to A1 neurons have been reported (Gieroba et al., 1995) and projections from the A2 cell group have been shown to convey LPS and cytokine information to the anterior hypothalamus (Blatteis and Sehic, 1998; Dunn, 2002; Blatteis, 1992; Blatteis et al., 2000; Blatteis et al., 2004). Numerous studies have shown that systemic administration of LPS or cytokines provokes norepinephrine (NE) release in the POA (reviewed in Linthorst et al., 1995; Dunn and Wang, 1995; Dunn and Hall, 1999; Dunn, 2001). In an earlier study, we showed, by using microdialysis, that extracellular NE concentrations increase rapidly in the POA following intravenous (i.v.) LPS administration, and that changes in NE levels were temporally correlated with the early phase of the acute phase response (Feleder et al., 2007). These results are in agreement with previous evidence that LPS causes parallel elevations in POA NE levels and core body temperature (Dunn and Wang, 1995; Dunn and Hall, 1999; Dunn, 2001). Hence, there is substantive evidence for involvement of NE neurons in the activation of the POA during the host response against pathogens. Taking into account all these data, we hypothesize that intravenous LPS administration causes blood pressure to fall, at least initially, by stimulating NE release in the POA.

Here we report that acute subdiaphragmatic vagotomy as well as subdiaphragmatic perivagal administration of lidocaine, prevent the fall in arterial pressure evoked by LPS. Lipopolysaccharide-induced hypotension was also attenuated by injecting lidocaine into the NTS, consistent with the idea that vagal nerve afferents convey the immune signal to the brain. Microinjection of the alpha-adrenergic receptor antagonist phentolamine into the POA blocked the initial and the late decompensatory fall in blood pressure induced by LPS completely; the beta-adrenergic receptor antagonist propranolol enhanced the response. These data support the conclusion that a neural pathway initiates the hypotensive response during septic shock.

2. Materials and methods

Male Sprague-Dawley rats (250–300 g; Charles River Laboratories, Wilmington, MA) were anesthetized with 4% isoflurane and maintained with 1.5% isoflurane in 100% O_2. The left femoral artery and left jugular vein were cannulated with PE-50 tubing filled with heparinized saline (100 U/ml) to record arterial pressure and administer drugs. At the beginning of each experiment, the arterial cannula was connected to a volumetric pressure transducer. Blood pressure and heart rate were monitored and recorded using a MicroMed BPA-200 blood pressure analyzer (MicroMed, Louisville, KY). The animal protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.1. Acute subdiaphragmatic vagotomy

The vagus nerve was dissected bilaterally as described previously by Watkins et al. (1994). After a midline incision of the abdominal wall, the esophagus was exposed below the diaphragm, the dorsal and ventral trunks of the vagus nerve around the esophagus were carefully dissected and cut with microsurgery scissors immediately below the diaphragm. Control animals underwent the same procedure except no nerves were severed. Fifteen minutes later animals were administered with LPS (1 mg/kg) or saline (1 ml/kg) i.v. and blood pressure and heart rate were monitored for 60 min.

2.2. Bilateral subdiaphragmatic perivagal lidocaine administration

The subdiaphragmatic vagus was isolated through a laparotomy. Care was taken not to disturb the nerves which