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Lipopolysaccharide-induced hypothermia and hypotension are associated with inflammatory signaling that is triggered outside the brain

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ABSTRACT

Little is known about the neuroimmune mechanisms responsible for the switch from fever to hypothermia observed in severe forms of systemic inflammation. We evaluated whether bacterial lipopolysaccharide (LPS) acting directly on the brain could promote a fever-hypothermia switch as well as the hypotension that is often associated with hypothermia in models of systemic inflammation. At an ambient temperature of 22 °C, freely moving rats received intracerebroventricular (i.c.v.) injections of LPS at doses ranging from 0.5 to 25 μg. Despite the use of such high doses, the prevailing thermal response was fever. To investigate if a hypothermic response could be hidden within the prevailing febrile response, rats were pretreated with a cyclooxygenase-2 inhibitor (SC-236, 3.5 mg/kg i.v.) known to block fever, but this strategy also failed to reveal any consistent hypothermic response following i.c.v. LPS. At the doses tested, i.c.v. LPS was similarly ineffective at inducing hypotension. Additional doses of LPS did not need to be tested because the 25-μg dose was already sufficient to induce both hypothermia and hypotension when administered peripherally (intra-arterially). An empirical 3D model of the interplay among body temperature, arterial pressure and heart rate following intra-arterial LPS reinforced the strong association of hypothermia with hypotension and, at the same time, exposed a bell-shaped relationship between heart rate and body temperature. In summary, the present study demonstrates that hypothermia and hypotension are triggered exclusively by LPS acting outside the brain and provides an integrated model of the thermal and cardiovascular responses to peripheral LPS.

1. Introduction

The mechanisms by which pathogen-associated molecular patterns, such as bacterial lipopolysaccharide (LPS), trigger brain-driven responses in the host have been a matter of intense interest and debate. Considerable attention has been paid to the mechanisms of fever, a response that can be induced by LPS delivered peripherally or directly into the brain, and that is thought to be triggered by peripheral macrophages and brain endothelial cells, not necessarily at the same time (Engblom et al., 2003; Engstrom et al., 2012; Inoue et al., 2002; Li et al., 2006; Steiner et al., 2006). On the other hand, little attention has been given to the neuroimmune mechanisms responsible for the hypothermia that replaces fever in the most severe cases of systemic inflammatory syndromes such as sepsis. Naturally occurring hypothermia is observed in approximately 10% of septic patients (Arons et al., 1999; Clemmer et al., 1992), and its clinical implications are likely distinct from those of the forced hypothermia regimens that have been debated for use in septic patients (Crouser, 2012; Cunha, 2012). In animal models of systemic inflammation, naturally occurring hypothermia has been shown to be a regulated thermoregulatory response (Almeida et al., 2006a; Romanovsky et al., 1996b; Tsang et al., 2006) that, unlike general belief, may aid the host better than fever when the infectious and inflammatory insults are most severe (Liu et al., 2012). Although the brain is known to be ultimately involved in the hypothermic response to LPS (Almeida et al., 2006b; Juttler et al., 2007), it is currently unknown whether the upstream, LPS-triggered events responsible for hypothermia take place inside the brain, outside the brain, or in both compartments. The brain parenchyma may be particularly accessible to LPS of peripheral origin in severe forms of systemic inflammation given the pronounced alterations in the properties of the

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blood–brain barrier that accompany this disease state (Banks and Erickson, 2010).

The fact that neutralization of tumor necrosis factor (TNF)-α outside the brain attenuates the hypothermic response to peripherally administered LPS (Kozak et al., 1995; Tollner et al., 2000) does not rule out a possible contribution of inflammatory signaling initiated within the brain to this response. Accordingly, neuronal NF-κB has been shown to play a role in the mediation of LPS hypothermia (Juttler et al., 2007). Such role of neuronal NF-κB, however, does not necessarily reflect an upstream event triggered directly by LPS. To date, no study has been specifically designed to determine whether LPS acting inside the brain can trigger hypothermia, and the studies in which body temperature (T_b) was a tangential measurement in models of central nervous system inflammation yielded contradictory and inconclusive results when it comes to hypothermia. In more detail, there are reports of hypothermia occurring during bacterial meningitis (Koedel et al., 2001; Winkler et al., 2009) and one report of hypothermia occurring after intrathecal LPS (Sorge et al., 2011), but neither of these reports included any assessment as to whether the outflow of bacteria or LPS from the central nervous system to the periphery could have accounted for the observed hypothermia. On the other hand, there are studies in which hypothermia was not observed in response to high doses of LPS delivered to the brain ventricles (Walker et al., 1996; Zhang et al., 2010), but these studies are also limited for not verifying whether the experimental conditions would have allowed detection of hypothermia if this was present.

To shed some light on this matter, we have conducted a well-controlled series of experiments specifically designed to determine whether LPS acting on the brain itself is able to trigger a hypothermic response or, alternatively, whether LPS signaling in peripheral tissues is the sole trigger of the hypothermic response. As a secondary goal, we evaluated whether LPS delivery to the brain could also trigger the fall in arterial pressure (hypotension) that occurs alongside hypothermia in models of systemic inflammation (Liu et al., 2012; Romanovsky et al., 1996b). Although it is generally accepted that this hypotensive response is triggered outside the central nervous system (Landry and Oliver, 2001; Yilmaz et al., 2008a), the possibility that centrally acting LPS may be able to induce hypotension has not been ruled out (Yilmaz et al., 2008a).

2. Materials and methods

2.1. Animals

The study was conducted in male Wistar rats obtained from Charles River Laboratories (Wilmington, MA). The rats were caged with corn-cob bedding and had free access to tap water and standard chow. The animal colony room was maintained under a 12:12 h light–dark cycle (lights on at 07:00 am) and at a temperature of 23–26 °C. The rats were housed in groups of 2–3 prior to surgery; single housing was necessary after surgery. The rats weighed 240–340 g at the time of the experiments. Each rat was used in an experiment once and euthanized with sodium pentobarbital (100 mg/kg IP) immediately thereafter. All protocols were approved by the animal care and use committee of the Albany College of Pharmacy and Health Sciences.

2.2. Surgical preparation

Surgical procedures were performed aseptically under general anesthesia (ketamine–xylazine–acepromazine, 80:8:1 mg/kg i.p.) and antibiotic protection (enrofloxacin, 5 mg/kg s.c.). The operating board was maintained at 37 °C by a Deltaphase isothermal pad (Braintree Scientific, Braintree, MA). A single dose of acetaminophen (150 mg/kg s.c.) was given for pain management immediately after a surgical intervention.

Twelve days prior to an experiment, rats designated to receive an intracerebroventricular (i.c.v.) injection of LPS were implanted with a guide cannula directed at the lateral cerebral ventricle. To this end, the rat's head was fixed to a stereotaxic apparatus (David Kopf, Tujunga, CA) with the incisor bar set at −3.3 mm. The skin was incised over the sagittal suture, the periosteum was excised, supporting microscrews were driven into the skull, and a steel guide cannula (Plastics One, Roanoke, VA) was implanted. The tip of the cannula was directed at the right ventricle using the following stereotaxic coordinates: −0.5 mm from Bregma, −1.5 mm from midline, and 3.5–4.0 mm from the skull surface. The implanted cannula was attached to the supporting microscrews with acrylic cement.

Under a separate surgical intervention (5 days prior to an experiment), rats were implanted with an abdominal telemetry transponder and with a catheter, either arterial or venous. The transponder (G2 E-Mitter; Mini Mitter, Bend, OR) was implanted via a midline laparotomy and sutured to the dorsolateral abdominal wall. It allowed the telemetric recording of T_b and gross locomotor activity. The arterial catheter was implanted via the left carotid artery in rats designated to have their arterial pressure recorded, to receive an intra-arterial (i.a.) injection of LPS, or both. The venous catheter was implanted via the left jugular vein in rats designated to receive an intravenous (i.v.) infusion of SC-236 (for details, see Section 2.4). All catheters—made of 3-Fr polyurethane tubing (Instech Laboratories, Plymouth Meeting, PA)—were filled with heparinized (50 U/mL) saline, secured in place with ligatures, and exteriorized at the nape. They were flushed daily with heparinized saline.

2.3. Experimental setup

At ~7 am on the day of the experiment, the individually caged, freely moving rats were transferred to an environmental chamber (model NQ1; Environmental Growth Chambers, Chagrin Falls, OH). The ambient temperature in the chamber was maintained at 22.0 ± 0.1 °C with relative air humidity at 50 ± 10%, a condition that is subthermoneutral for rats in our experimental setup and allows development of hypothermic responses (Krall et al., 2010). Under each cage was an ER-4000 receiver (Mini Mitter), which captured the radio frequency of the pre-implanted telemetry transponder and conveyed it to a computer, where the T_b and locomotor activity signals were processed and recorded by the Vital View software. The arterial catheter (if present) was extended with PE50 tubing filled with saline, and the extension was passed via a swivel system (Instech Laboratories) to the outside of the environmental chamber, where it was connected to a differential pressure transducer. The transducer was, in turn, connected to the Datamax analog-to-digital converter (Columbus Instruments, Columbus, OH), which conveyed the pulsatile arterial pressure data to the corresponding computer software. The brain cannula (if present) was connected to an i.c.v. injection system, which was prefilled with the solution to be delivered. The system consisted of the following elements: an injection needle that was 1 mm longer than the guide cannula (Plastics One); a threaded collar that secured the injection needle in place (Plastics One); and a PE50 tubing supply line that was connected sequentially to the injection needle, to the swivel system, and to a 10-μL Hamilton syringe located outside the chamber. If the rat had a venous catheter, this was connected to a 1-mL syringe located outside the chamber via another PE50 extension line passed through the swivel system. A multiple-channel swivel was used as necessary. An infusion harness worn by the rat and a spring coil protected the arterial and venous extensions from bites and scratches. Another spring coil protected the i.c.v. supply