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Central mediators involved in the febrile response induced by polyinosinic–polycytidylic acid: Lack of involvement of endothelins and substance P

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ABSTRACT

The present study evaluated the involvement of interleukin(IL)-1β, tumor necrosis factor-α (TNF-α), IL-6, interferon(IFN)-γ, prostaglandins of the E_2 series, endothelins, substance P and opioids within the central nervous system in polyinosinic:polycytidylic acid (Poly I:C)-induced fever in rats. Poly I:C injection induced a febrile response which was reduced by intracerebroventricular administration of the antibodies against TNF-α, IL-6, or IFN-γ, or by IL-1 or μ receptor antagonists. Intraperitoneal injection of indomethacin or oral administration of celecoxib also reduced Poly I:C-induced fever. Poly I:C increased prostaglandin E_2 levels in the cerebrospinal fluid of the animals which was also reduced by indomethacin. The intracerebroventricular injection of ET_B or NK_1 receptor antagonists did not alter Poly I:C-induced fever. These data suggest the involvement of IL-1β, TNF-α, IL-6, IFN-γ, prostaglandin E_2, and opioids but not endothelins and substance P on Poly I:C-induced fever.

1. Introduction

The double-stranded RNA (dsRNA) is a pathogen-associated molecular pattern (PAMP) and activates innate immunity (Alexopoulou et al., 2001). Most researchers use polyinosinic:polycytidylic acid (Poly I:C), a synthetic dsRNA analog, as a laboratory tool. Poly I:C is recognized by the cytosolic RNA helicase MDA-5 and by endosomal toll-like receptor-3 (Meylan and Tschopp, 2006). It is commonly used to stimulate toll-like receptor 3 because of its high molecular weight which makes it more potent than the smaller viral dsRNA fragments (Fang et al., 1999). Although there are other pathways through which the host responds to viral infection (Coccia et al., 2004; Edelmann et al., 2004; Li et al., 2005) Poly I:C has been extensively used to mimic the acute phase of a viral infection (Guha-Thakurta and Majde, 1997; Traynor et al., 2004). Stimulation of toll-like receptor-3 using dsRNA or Poly I:C induces type I interferon (IFN) α and β and type II IFN (IFN-γ) and many of the acute symptoms of viral infection have been attributed to these cytokines (for a review see Beetz et al., 2008; Majde, 2000). Besides, both Type I and Type II IFNs are important against virus infections (Muller et al., 1994). Signaling pathways that are triggered after toll-like receptor 3 activation differ from those that are triggered by most toll-like receptors, and particularly toll-like receptor-4, since they do not involve MyD88, an adaptor molecule that leads to nuclear factor κB (NF-κB) activation (Takeuchi et al., 2004). However, Poly I:C induces the activation of NF-κB independently of MyD88 (Alexopoulou et al., 2001). Additionally other transcription factors such as IFN-regulatory factor (IRF) 3 and IRF7 are important for the expression of IFN-β and other IFN-inducible genes in response to toll-like receptor 3 activation (Takeuchi et al., 2004).

Basal expression of toll-like receptor 3 in the central nervous system has been demonstrated in astrocytes and neurons (Farina et al., 2005; Lafon et al., 2006). It has been shown that Poly I:C, similarly to what happens with other toll-like receptor ligands, causes the reduction of body weight and sickness behavior characterized by a reduced activity and burrowing in mice (Cunningham et al., 2007; Matsumura et al., 2007; Konat et al., 2009). These changes were accompanied by a mild fever and an increased peripheral and central expression of cytokines such as interleukin(IL)-1β, tumor necrosis factor-α (TNF-α), IL-6 and IFN-β (Cunningham et al., 2007; Matsumura et al., 2007). Konat et al. also found an up-regulation of *Il1b*, *Tnfa*, *Il6* and *Ifnb* genes in the forebrain, including the hypothalamus, an area related to the febrile

Abbreviations: ANOVA, analysis of variance; dsRNA, double-stranded RNA; HLI, heat loss index; i.c.v, intracerebroventricular; IFN, interferon; IRF, interferon-regulatory factor; IL-1RA, interleukin-1 receptor antagonist; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; OVLT, *organum vasculosum* of the *lamina terminalis*; PAMP, pathogen-associated molecular pattern; Poly I:C, polyinosinic:polycytidylic acid; Tambient, ambient temperature; Tc, core temperature; TNF-α, tumor necrosis factor-α; Tskin, skin temperature.

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response after the peripheral injection of Poly I:C (Konat et al., 2009). In addition Poly I:C has been shown to induce a fever in rats that is at least partially dependent on IL-1β and transforming-growth factor-β (Fortier et al., 2004; Matsumura et al., 2007; Hopwood et al., 2009). However, compared with Gram-negative bacteria lipopolysaccharide (LPS), the classical model for fever induction, little is known about the involvement of these cytokines in the febrile response induced by Poly I:C.

LPS, acting on the transmembrane toll-like receptor 4, triggers the activation of NF-κB which activates the synthesis and release of cytokine interleukin(IL)-1β, IL-1α, tumor necrosis factor-α (TNF-α), IL-6, IL-8, cytokine-induced neutrophil chemoattractant 1, macrophage inflammatory protein-1α and β, IFN–α, IFN-γ, and receptor activator of nuclear factor κB ligand (Kluger, 1991; Zampronio et al., 1994; Minano et al., 1996; Soares et al., 2008; Hanada et al., 2009). Among those, IL-8, cytokine-induced neutrophil chemoattractant 1, macrophage inflammatory protein-1α, and the receptor activator of nuclear factor κB ligand are generated directly within the central nervous system (Zampronio et al., 1994; Minano et al., 1996; Hanada et al., 2009). Conversely, IL-1β, TNF-α, and IL-6 can be produced at peripheral and central sites (Rothwell et al., 1991; Gatti and Bartfai, 1993; Luheshi et al., 1997; Cartmell et al., 2000; Rummel et al., 2006; Harden et al., 2011). Once these cytokines reach the central nervous system they induce the synthesis/release of central mediators such as prostaglandin E_2 and $F_{2\alpha}$ (Milton, 1989; Coelho et al., 1993), corticotrophin-releasing hormone (Rothwell, 1989), endogenous opioids (Blatteis et al., 1991; Fraga et al., 2008), substance P (Blatteis et al., 1994; Reis et al., 2011), and endothelin-1 (Fabricio et al., 1998), among others. These mediators are the final mediators that induce the change in the hypothalamic set point to induce fever.

Davidson et al. showed that an increase in plasma and cerebrospinal fluid levels of prostaglandin E_2 occurred simultaneously with the onset of fever suggesting that this prostanoid is involved in the febrile response induced by Poly I:C (Davidson et al., 2001). However, the involvement of other central mediators in the febrile response induced by Poly I:C is unknown.

Therefore, the aim of this study was to evaluate the participation of cytokines such as IL-1β, TNF-α, IL-6 and IFN-γ and of central mediators such as prostaglandins, endothelins, opioids and substance P in the febrile response induced by Poly I:C.

2. Experimental procedures

2.1. Animals

Experiments were conducted in male Wistar rats (180–220 g), housed four per cage at 22 ± 1 °C, under a 12:12-h light–dark cycle (lights on at 07:00 AM) and with free access to food chow and tap water. Animals were used only once. All experiments were approved by the institution's Ethical Committee in Animal Use and were in accordance with Brazilian and International Guidelines for Animal Care.

2.2. Drugs

Poly I:C, the non-selective cyclooxygenase inhibitor indomethacin, the ET_B receptor antagonist BQ788, and the μ-opioid receptor antagonist CTAP were purchased from Sigma (St Louis, MO, USA). The NK_1 receptor antagonist SR140333B ((s) 1 {2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl acetyl)]-piperidine-3-yl}-4-phenyl-1 azonialicyclo [2,2,2] octane benzensulfonate) was a kind gift from Sanofi-Aventis, France. The antibodies against TNF-α, IL-6, IFN-γ as well as IL-1 receptor antagonist (IL-1RA) were obtained from R&D Systems (Minneapolis, PA, USA). Ketamine was purchased from Vetnil (Louveira, SP, Brazil). Xylazine was purchased from Syntec (Cotia, SP, Brazil). Oxytetracycline hydrochloride and the cyclooxygenase-2 selective inhibitor celecoxib were obtained from Pfizer (São Paulo, SP, Brazil). The non-selective cyclooxygenase inhibitor ketoprofen was purchased from Medley (São Paulo, SP, Brazil).

2.3. Intracerebral cannula implantation and microinjection

For intracerebroventricular (i.c.v.) administration of antibodies and antagonists, a 22-gauge stainless steel guide cannula (0.8 mm outer diameter, 12 mm long) was stereotaxically implanted into the right lateral ventricle under ketamine (90 mg/kg) and xylazine (10 mg/kg) anesthesia in aseptic conditions. The stereotaxic coordinates were as follows: 0.8 mm lateral to the midline, 1.5 mm posterior to the bregma, and 2.5 mm below the brain surface, with the incisor bar lowered by 3.3 mm below the horizontal zero (Paxinos and Watson, 1998). Cannulas were fixed to the skull with jeweler's screws that were embedded in dental acrylic cement. Animals were treated with oxytetracycline hydrochloride (400 mg/kg, by intramuscular route) and ketoprofen (10 mg/kg, by oral route, for 2 days) after surgery and allowed to recover for at least 5 days before experimental use. All i.c.v. injections were administered under aseptic conditions, using a 30-gauge needle that was connected to a polyethylene tube (PE-10). The needle protruded by 2 mm beyond the cannula tip, and a volume of 2 μl was slowly injected (over 1 min) using a 25-μL Hamilton syringe. After the experiment, each rat was microinjected into the lateral ventricle with Evans blue (2.5% in saline). Brains were removed and the animals showing cannula misplacement, blockage on injection, or abnormal body weight gain patterns after surgery were excluded from the study.

2.4. Abdominal and tail skin temperature measurement

Abdominal core temperature (Tc) was measured in conscious unrestrained rats using data loggers (Subcue, Calgary, AB, Canada). Briefly, data loggers were implanted in the peritoneal cavity, at least 5 days before the experiment, under ketamine (90 mg/kg) and xylazine (10 mg/kg) anesthesia. When appropriate, data loggers were implanted immediately after the intracerebroventricular cannula implantation under the same anesthesia. Animals received the same post-surgical care described above. On the day of the experiment, Tc was continuously monitored at 15-min intervals from 2 h before any injection until 6 h after the injection of the pyrogenic stimulus. Animals showing basal (before any injection) body temperature above 37.4 or below 36.7 °C were excluded from the study. During the experiment, room temperature was maintained at 28 ± 1 °C (within the thermoneutral zone for rats) (Poole and Stephenson, 1977; Romanovsky et al., 2002).

For skin temperature (Tskin) measurement, the same type of data logger was inserted into a protective covering device (Insight, Ribeirão Preto, SP, Brazil). On the day before the experiment, rats that had received i.p. data loggers 5 days earlier were briefly anesthetized with halothane, and the device containing another data logger was attached at 4.0 cm from the base of the tail (on the ventral surface) using double-sided tape (Williams et al., 2010). These data loggers were programmed to measure Tskin at time intervals corresponding to those of the intraperitoneally-implanted ones. An extra data logger was programmed and left inside an empty animal cage to measure ambient temperature (Tambient) also at the same time intervals. Heat loss index (HLI) was calculated using Tc, Tskin, and Tambient according to the following formula: HLI = (Tskin − Tambient) / (Tc − Tambient). The value of HLI varied from 0 (full vasoconstriction) to 1 (full vasodilation) (Romanovsky et al., 2002).

2.5. In vivo experimental protocols

All pyrogenic stimuli were injected between 09:00 and 11:00 AM. In the first series of experiments, we aimed at determining the dose of Poly I:C to induce fever. Poly I:C at doses of 3, 30 and 300 μg/kg was injected