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Research Report

Serotonin 2C receptor signaling in a diffuse neuronal network is necessary for LPS anorexia

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ABSTRACT

LPS, a potent activator of the innate immune system, is commonly used to investigate the acute phase response to infection, including anorexia. Serotonin 2C-receptor signaling has been shown to be involved in the mediation of LPS anorexia. Here we used the selective, potent and brain-penetrant serotonin 2C-receptor antagonist SB 242084 to identify the brain sites involved in LPS anorexia. Male Long–Evans rats received 1 ml/kg intraperitoneal injections of 0 or 0.3 mg/kg SB 242084 and intraperitoneal injections of 0 or 100 μg/kg LPS 1 h later, at dark onset. Food intake was measured in one set of rats and c-Fos immunoreactivity in another, unfed, group 90 min after LPS injection. SB 242084 markedly attenuated the LPS-induced reduction in food intake, with no anorexia evident for the first 2 h after LPS. SB 242084 also completely blocked the LPS-induced increases in c-Fos expression in the paraventricular nucleus, central nucleus of the amygdala, nucleus tractus solitarii, median raphe nucleus and dorsal raphe nucleus and partially blocked it in the A1 noradrenergic area of the ventrolateral medulla and the raphe pallidus nucleus. SB 242084 did not significantly alter the c-Fos response in the arcuate nucleus or the raphe magnus nucleus. These data indicate that 2C receptor signaling activates a diffuse neural network, presumably mediating anorexia and other responses to LPS; they also suggest that the arcuate and the raphe magnus neurons that express c-Fos after LPS are not necessary for LPS anorexia.

1. Introduction

Anorexia is a prominent component of the innate immune system response that protects against bacterial infection, often modeled with the gram-negative bacterial toxin lipopolysaccharide (LPS), and other immune challenges. Peripheral infection initiates a complex cascade of immune events. A key initial element of this is the release of pro-inflammatory cytokines and chemokines from macrophages and other immune cells; these initiate both local and systemic responses, collectively called the acute phase response. The brain also reacts to this peripheral immune response, leading to anorexia, fever and further brain-mediated elements of the acute phase response. A principal mechanism through which peripheral immune signaling affects the brain is thought to be via the release of prostaglandins from blood brain barrier endothelial cells. Prostaglandins then act on receptors on neurons and glial cells in the brain. The sites where such

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Abbreviations: A1, A1noradrenergic area of the ventrolateral medulla; Arc, arcuate nucleus of the hypothalamus; CeA, central nucleus of the amygdale; DR, dorsal raphe nucleus; MnR, median raphe nucleus; α-MSH, α-melanocyte-stimulating hormone; NTS, nucleus tractus solitarii; PVN, hypothalamic paraventricular nucleus; RMg, raphe magnus nucleus; RPa, raphe pallidus nucleus

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responses are initiated, however, remain in doubt. It is thought that LPS anorexia arises from altered information processing within the same neural networks that control normal eating (Asarian and Langhans, 2005; Langhans, 2007).

The role of the brain neurotransmitter serotonin in the control of both normal eating (Blundell, 1984; Heisler et al., 2003; Simansky, 1996; Tecott, 2007) and LPS anorexia (Langhans, 2007) has been intensively investigated. In healthy animals, agonists of serotonin 1B and serotonin 2C receptors reduce food intake, and antagonists to these receptors attenuate the anorectic effect of the serotonin agonist *d*-fenfluramine (Vickers et al., 1996). Peripheral administration of 2C-receptor antagonists also attenuated LPS anorexia (von Meyenburg et al., 2003a), implicating serotonin, and in particular signaling via 2C receptors, in infection anorexia. LPS anorexia was also attenuated by direct injection of the serotonin 1A-receptor agonist 8-OH-DPAT into the midbrain raphe (Hrupka and Langhans, 2001). Because most serotonin 1A receptors are somatodendritic autoreceptors with a negative-feedback function, these findings suggest that increased activity of serotonin neurons in the midbrain raphe is involved in LPS anorexia.

The present studies were undertaken to further investigate the role of serotonin 2C receptor signaling in LPS anorexia in rats using the specific 2C-receptor antagonist SB 242084. In particular, we wished to focus on the role of serotonin 2C receptors in the initial stage of LPS anorexia. This is important because LPS appears to elicit a cascade of immune (Asarian and Langhans, 2005; Langhans, 2007) and neural responses, such that more brain areas are involved as the acute phase response progresses (Elmquist et al., 1996, Rivest and Laflamme, 1995). In previous studies of the influence of SB 242084 on LPS anorexia (von Meyenburg et al., 2003a,b), SB 242084 first attenuated anorexia starting 4 h after LPS injection, perhaps because the antagonist was injected simultaneously with LPS. Therefore, in order to determine if serotonin 2C receptors contribute to the initial phase of LPS anorexia, we injected SB 242084 prior to LPS. First, we characterized the effects of SB 242084 pretreatment on LPS anorexia. Second, we used immunocytochemical techniques to assay the numbers of cells expressing c-Fos, the product of the immediate early gene c-*fos*, in brain sites where altered neuronal activity, either in serotonin neurons or in their projection sites, might cause anorexia.

To identify sites potentially involved in the development of LPS anorexia and perhaps necessary for its subsequent maintenance, we collected brain tissue 90 min after LPS injection. We examined c-Fos expression in two midbrain sites rich in rostrally projecting serotonin neurons, the dorsal (DR) and median (MnR) raphe nuclei, and three forebrain sites that are rich in serotonin 2C receptors, the hypothalamic paraventricular (PVN) and arcuate (Arc) nuclei and the central nucleus of the amygdala (CeA). We also examined c-Fos expression in two hindbrain sites rich in serotonin neurons, the raphe magnus (RMg) and raphe pallidus (RPa) nuclei. All these raphe nuclei are interconnected, and each expresses serotonin 2C receptors (Hoffman and Mezey, 1989; Mengod et al., 1990; Molineaux et al., 1989; Wright et al., 1995). Finally, we examined the nucleus tractus solitarii (NTS) and the A1 noradrenergic area of the ventrolateral medulla (A1) because several studies implicate these areas in LPS anorexia (Laflamme et al., 1999, Lacroix and Rivest, 1997, Rivest and Laflamme, 1995, Valles et al., 2005).

2. Results

2.1. *Food intake*

LPS significantly reduced cumulative food intake at 2, 4 and 23 h after intraperitoneal injection (Fig. 1). SB 242084 by itself had no detectable effect on food intake at any of these times but completely reversed LPS anorexia at each time. The statistical criteria for complete reversal were, first, that LPS alone reduce food intake (i.e., a significant difference between control-injected rats and LPS-injected rats); second, that the reversal of LPS anorexia by SB 242084 be significant (i.e., a significant difference between the effects of LPS without SB 242084 and with SB 242084); and third, that there be no detectable LPS effect after SB 242084 treatment (i.e., between SB 242084 alone and SB 242084 together with LPS).

2.2. *C-Fos expression*

LPS increased c-Fos expression significantly in all nine brain regions examined. In five of these areas (DR, MnR, PVN, CeA, NTS), SB 242084 reversed LPS-induced c-Fos expression completely, according to the criteria described above. In two, the RPa and A1, SB 242084 partially reversed LPS-induced c-Fos expression (i.e., the first two criteria were met, but there was a significant residual effect of LPS). Finally, in the Arc and RMg, no significant differences between the effects of LPS with versus without SB 242084 were detected (nevertheless, in

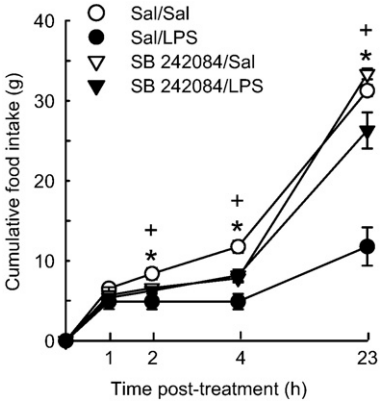

Fig. 1 – Reversal of LPS anorexia by SB 242084. Rats were ip injected with saline or 0.3 mg/kg SB 242084 1 h before dark onset and saline or 100 μg/kg LPS at dark onset. Data are cumulative food intakes (mean ± SEM), 4–5 rats/group. Friedman $H(3)=7.94$, $P<0.05$; $F(3,17)=12.44$, $P<0.001$, SED = 1.2 g; and $H(3)=13.54$, $P<0.05$; for 2, 4 and 23 h, respectively. *Saline/LPS significantly different from saline/saline, Bonferroni–Holm test after significant ANOVA, $P<0.05$. +(saline/saline–saline/LPS) significantly different from (SB 242084/saline–SB 242084/LPS), Bonferroni–Holm test after significant ANOVA, $P<0.05$.