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Research paper

Psychotropic drugs attenuate lipopolysaccharide-induced hypothermia by altering hypothalamic levels of inflammatory mediators in rats

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HIGHLIGHTS

- Inflammation may contribute to the pathophysiology of mental disorders and psychotropic drugs are known to exert various effects on brain inflammation.
- Systemic administration of lipopolysaccharide (LPS) to rats causes robust production of inflammatory mediators and pathological changes in body temperature.
- Four psychotropic drugs significantly attenuated LPS-induced hypothermia in rats.
- Lithium, carbamazepine, haloperidol and imipramine differently affected levels of prostaglandin E2, tumor necrosis factor-α and phosphorylated p65 levels in plasma and hypothalamus of LPS-treated rats.

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ABSTRACT

Recent evidence suggests that inflammation may contribute to the pathophysiology of mental disorders and that psychotropic drugs exert various effects on brain inflammation. The administration of bacterial endotoxin (lipopolysaccharide, LPS) to mammals is associated with robust production of inflammatory mediators and pathological changes in body temperature.

The objective of the present study was to examine the effects of four different psychotropic drugs on LPS-induced hypothermia and production of prostaglandin (PG) E_2, tumor necrosis factor (TNF)-α and phosphorylated-p65 (P-p65) levels in hypothalamus of LPS-treated rats.

Rats were treated once daily with lithium (100 mg/kg), carbamazepine (40 mg/kg), haloperidol (2 mg/kg), imipramine (20 mg/kg) or vehicle (NaCl 0.9%) for 29 days. On day 29, rats were injected with LPS (1 mg/kg) or saline. At 1.5 h post LPS injection body temperature was measured, rats were sacrificed, blood was collected and their hypothalami were excised, homogenized and centrifuged. PGE_2, TNF-α and nuclear P-p65 levels were determined by specific ELISA kits.

We found that lithium, carbamazepine, haloperidol and imipramine significantly attenuated LPS-induced hypothermia, resembling the effect of classic anti-inflammatory drugs. Moreover, lithium, carbamazepine, haloperidol and imipramine differently but significantly affected the levels of PGE_2, TNF-α and P-p65 in plasma and hypothalamus of LPS-treated rats.

The results suggest that psychotropic drugs attenuate LPS-induced hypothermia by reducing hypothalamic production of inflammatory constituents, particularly PGE_2. The effects of psychotropic drugs on brain inflammation may contribute to their therapeutic mechanism but also to their toxicological profile.

Abbreviations: BT, body temperature; CBZ, carbamazepine; HPL, haloperidol; HT, hypothalamus; IL, interleukin; IMP, imipramine; LIT, lithium; LPS, lipopolysaccharide; NFκB, nuclear factor κ B; P-p65, phosphorylated-p65; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α.

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1. Introduction

A large body of data has accumulated suggesting that inflammation may contribute to the pathophysiological mechanisms underlying mental illnesses such as depression [1,2] bipolar disorder [3,4] and schizophrenia [5,6]. Supporting evidence indicates that psychotropic drugs exert multiple anti-inflammatory effects [7–13]. These observations have laid down the foundation for the progressively acknowledged "inflammation hypothesis" of mental disorders. In support for this hypothesis, many studies demonstrated that regular anti-inflammatory drugs reduce the severity of symptoms among psychiatric patients [14–17]. For example, drugs that inhibit the enzyme cyclooxygenase (COX) and reduce prostaglandins (PGs) synthesis have been shown to exert beneficial effects as add-on therapy in patients with mental disorders [14–17].

Systemic inflammation occurs when infectious pathogens invade the body of mammals and circulate in the blood. For example, systemic administration of bacterial endotoxin (lipopolysaccharide, LPS) to mammals leads to a profound inflammatory response which includes robust production of inflammatory mediators, pathological changes in body temperature (BT), hypotension, among other pathological features [18–21]. In rats, LPS induces a biphasic change in BT—an initial decrease (hypothermia) followed by an elevation (fever) [20,21]. The mechanism of the hypothermic response to LPS is not fully understood. It seems that several inflammatory constituents contribute to this complex process [18,20,21], among which PGE_2 is known to play a pivotal role [20,21]. Pathological changes in BT (both hypothermia and fever) are associated with *increased* levels of PGE_2 in the hypothalamus. Consistently, anti-inflammatory drugs that decrease hypothalamic production of PGE_2 reduce LPS-induced hypothermia [20,22].

Brain inflammation occurs in response to pathological processes such as invasion of infectious microorganisms, traumatic injury, ischemia and degeneration [23–25]. Glia cells play a central role in most types of brain inflammation as they secrete pro-inflammatory substances such as interleukin (IL) 1-β, IL-6, PGE_2 and tumor necrosis factor (TNF)-α [26,27]. On the other hand, glia cells can suppress the inflammatory response by producing anti-inflammatory elements such as IL-10 [26,27]. However, *not* all brain inflammatory processes are detrimental as they may reflect tissue homeostasis [28]. Thus, inhibition of inflammation does not always necessarily benefit the brain.

Inflammatory mediators modulate a number of crucial processes in the brain. For example, PGE_2 regulates synaptic transmission, hypothalamus-pituitary-adrenal axis function, neurotransmitter release, thermoregulation and appetite [29]. TNF-α regulates the expression of many genes that are important for neuron function and survival [30]. Nuclear factor κ B (NF-κB) is another cellular pathway that is activated during brain inflammation. Mammalian NF-κB family consists of several members such as p50 and p65 which are involved in healthy as well as pathologic processes [31]. At resting conditions, NF-κB interacts with inhibitor of κB (IκB) proteins which inhibit its activity by preventing its translocation to the nucleus [31]. Upon activation, phosphorylation of IκB leads to its dissociation from NF-κB and translocation of the latter to the nucleus for target gene transcription [31].

As mentioned above, psychotropic drugs exert various anti-inflammatory effects [7–13], however, they have also been shown to have pro-inflammatory properties [6,11,32]. Lithium in particular exerts multiple effects on inflammation [12]. We have shown that *acute* treatment with lithium attenuated the hypothermic response to a *high* dose of LPS in rats, which was accompanied by a reduction in hypothalamic PGE_2 levels [21].

The present study was undertaken to examine the effects of *chronic* treatment with lithium (LIT), carbamazepine (CBZ), haloperidol (HPL) and imipramine (IMP) on LPS-induced hypothermia and hypothalamic production of inflammatory mediators in rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 200–220 g at the beginning of the experiments were used throughout the studies. Animals were housed 3 per cage and maintained under controlled environmental conditions (ambient temperature 22 ± 1 °C, humidity 55–60%, photoperiod cycle 12 h light: 12 h dark), fed Purina Lab Chow and water *ad libitum*. Only animals with no overt pathology have been included in the studies. The procedures of the study were in accordance with the guidelines of the Committee for the Use and Care of Laboratory Animals in Ben-Gurion University of the Negev (authorization # IL-61-11-2010).

2.2. Drug treatment

LIT, CBZ, HPL and IMP were administered for 4 weeks through a single daily intraperitoneal (ip) injection. Control rats were injected with vehicle. The drugs were given at the following doses: LIT 100 mg/kg; CBZ 40 mg/kg; HPL 2 mg/kg; and, IMP 20 mg/kg, similar to previous studies which used similar protocols [33–36].

2.3. Measurement of body temperature (BT)

BT was measured with a thermocouple probe (HL 600 Thermometer, Anristu Meter Co., Japan) inserted into the rectum. Rats were acclimated to this procedure during 3 days before experiments were initiated. Chronic treatment with LIT, CBZ, HPL and IMP did not significantly alter BT in the very most of measurement points during the initial 4 weeks of drug treatment (data not shown). It is important to mention that rats' BT is highly affected by various environmental factors such as ambient temperature, light, and surrounding noise. Short-term changes in BT do not necessarily reflect a *pathologic* process but may be a response to an environmental stimulus. In the present study, rats with persistent alterations in BT (in 2 successive measurements) were excluded from the studies.

2.4. Induction of inflammation by LPS

LPS from *Escherichia coli* was dissolved in sterile NaCl 0.9%. On day 29 of the treatment protocol, LPS 1 mg/kg was given *ip* at 2 h after drug/saline injection. Control rats were injected *ip* with sterile NaCl 0.9%. The dose of LPS (1 mg/kg) was chosen in order to induce an inflammatory response of a mild to moderate magnitude [10], as we wished to test the drugs under condition that resemble a possibly-occurring chronic low-grade inflammation that takes place in the brain of mentally affected patients. A high dose of LPS would have led to a severe inflammatory response [10] which would be difficult to influence by pharmacological interventions that are *not* potent anti-inflammatory drugs.

2.5. Blood collection and preparation of hypothalamic samples

BT was measured before and at ~1.5 h after LPS injection. Then, rats were briefly anesthetized with a mixture of 4% isoflurane in 100% oxygen and immediately sacrificed by decapitation. Blood was collected (in heparin-containing tubes) for plasma separation