A Novel Role of Cannabinoids: Implication in the Fever Induced by Bacterial Lipopolysaccharide


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ABSTRACT

There is continuing interest in elucidating the actions of drugs of abuse on the immune system and on infection. The present study investigated the effects of the cannabinoid (CB) receptor agonist aminoalkylindole, (±)-WIN 55,212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i]quinolin-6-one], on fever produced after injection of lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, the best known and most frequently used experimental model. Intraperitoneal injection of LPS (50 μg/kg) induced a biphasic fever, with the first peak at 180 min and the second at 300 min postinjection. Pretreatment with a nonhypothermic dose of the cannabinoid receptor agonist WIN 55,212-2 (0.5–1.5 mg/kg i.p.) antagonized the LPS-induced fever. However, pretreatment with the inactive enantiomer WIN 55,212-3 [1.5 mg/kg i.p.; S-(+)-2,3-dihydro-5-methyl-3-(morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-1-naphthaneleny)methanone mesylate] did not. The inhibitory effect of WIN 55,212-2 on LPS-induced fever was reversed by SR141716 [N-(piperidin-1-y1)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride], a selective CB1 receptor antagonist, but not by SR144528 [N-(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-choro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide], a selective antagonist at the CB2 receptor. The present results show that cannabinoids interact with systemic bacterial LPS injection and indicate a role of the CB1 receptor subtype in the pathogenesis of LPS fever.

Fever is part of the acute-phase reaction to infection, characterized by a raised thermoregulatory set point, which leads to an elevation in body temperature (Tb). The systemic administration of LPS, a powerful activator of the innate immune system and the most commonly used experimental model for systemic infection, induces a variety of sickness-associated responses such as anorexia, increased slow-wave sleep (Hori et al., 1991; Elmqquist et al., 1997), change in nociceptive threshold, and fever (Benamar et al., 2000, 2005; Abe et al., 2001). The fever due to LPS and other exogenous pyrogens is believed to be caused by the synthesis and release from monocytes and macrophages of a number of well-characterized endogenous pyrogenic factors, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and macrophage inflammatory protein-1 (Myers et al., 1994; Blatteis, 2006). In addition, it has been shown that the opioid system is involved in LPS-induced fever (Benamar et al., 2000, 2005) and that pretreatment with capsaicin, an agonist at the vanilloid receptor, blocks the first phase of LPS-induced fever (Dogen et al., 2004).

Cannabis and its derivative compounds, collectively known as cannabinoids, produce an array of pharmacological symptoms in animals and humans (Ovadia et al., 1995; Chaperon and Thiebot, 1999). Two subtypes of receptors, CB1 and CB2, mediate cannabinoid-induced effects (Howlett, 1995). The development of synthetic cannabinoid agonists has provided remarkable advances in cannabis research. One such ligand is the aminoalkylindole, (±)-WIN 55,212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i]quinolin-6-one], which displays high selectivity for cannabinoid receptors. Previous studies have demonstrated that WIN 55,212-2 is highly potent and efficacious in the acute-phase response to infection, characterized by a raised thermoregulatory set point, which leads to an elevation in body temperature (Tb). The systemic administration of LPS, a powerful activator of the innate immune system and the most commonly used experimental model for systemic infection, induces a variety of sickness-associated responses such as anorexia, increased slow-wave sleep (Hori et al., 1991; Elmqquist et al., 1997), change in nociceptive threshold, and fever (Benamar et al., 2000, 2005; Abe et al., 2001). The fever due to LPS and other exogenous pyrogens is believed to be caused by the synthesis and release from monocytes and macrophages of a number of well-characterized endogenous pyrogenic factors, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and macrophage inflammatory protein-1 (Myers et al., 1994; Blatteis, 2006). In addition, it has been shown that the opioid system is involved in LPS-induced fever (Benamar et al., 2000, 2005) and that pretreatment with capsaicin, an agonist at the vanilloid receptor, blocks the first phase of LPS-induced fever (Dogen et al., 2004).
cious in vivo and in vitro. WIN 55,212-2 prevents i.v. cocaine self-administration, increases tail-flick reflexes, exerts anti-
hyperalgesic effects, and induces hypothermia in rats, indi-
cating that WIN 55,212-2 is pharmacologically active in vivo
(Fox et al., 2001). WIN 55,212-2 undergoes less nonspecific
binding than classical cannabinoids and interacts negligibly
with other neurotransmitter systems and ion channels
(Jansen et al., 1992). In contrast, ∆9-tetrahydrocannabinol
(∆9-THC) has been reported to produce hypothermia by in-
teracting with other neurotransmitters, including serotonin
(Davies and Graham, 1980). One of the major advances in CB
research has been the development of a potent and selective
antagonist of the CB1 receptor, SR141716A. This compound
was found to block the hypokinetin, hypothermic, cataleptic,
and antinociceptive effects of ∆9-THC and WIN 55,212-2 in
mice and rats (Rinaldi-Carmona et al., 1994; Reche et al.,
1996). In addition, studies with SR141716 have provided
evidence as for the presence of CB1 receptors in peripheral
tissues as well as in the central nervous system (Varga et al.,
1995; Lake et al., 1997).

However, the CB2 receptor subtype has been defined as the
peripheral CB receptor, primarily because CB2 mRNA ex-
pression has been detected mainly in cells of the immune
system (Galieque et al., 1995). The CB2 is expressed induc-
ibly and is present at high levels compared with the CB1
when microglia are in responsive and primed states of acti-
vation (Cabral and Marciano-Cabral, 2005). Like the CB1
receptor subtype, the CB2 receptor is a member of the G
protein-coupled receptor family and on stimulation causes
inhibition of adenylyl cyclase. A potent, selective, and orally
active antagonist of the CB2 receptor, SR144528 was re-
cently identified and shown to have a 700-fold higher affinity
for the CB2 receptor than for the CB1 receptor (Rinaldi-
Carmona et al., 1998).

Because the cannabinoids and LPS both affect the immune
and thermoregulatory systems, an investigation was under-
taken to determine whether the cannabinoids affect the de-
velopment of fever after systemic injection by LPS. In this
present study, the in vivo effects of two cannabinoid agonists,
∆9-THC (main psychoactive constituent of marijuana) and
WIN 55,212-2 (synthetic cannabinoid agonist), were exam-
ined for effects on LPS-induced fever. Highly selective CB
receptor agonists SR141716 and SR144528 were used in an
attempt to identify the receptor subtype(s) through which
WIN 55,212-2 mediates its effects on LPS-induced fever.

Materials and Methods

Animals. All animal use procedures were conducted in strict
accordance with the National Institute of Health Guide for the Care
and Use of Laboratory Animals and were approved by the Institu-
tional Animal Care and Use Committee. Male Sprague-Dawley rats
(Zivic-Miller) weighing 250 to 300 g were used in this study. They
were housed three per cage for at least 1 week before surgery and
were fed laboratory chow and water ad libitum. Ambient tempera-
tion was 21 ± 0.3°C, and a 12-h light/dark cycle was used.

Surgery Procedures. Rats were anesthetized with an i.p. injec-
tion of a mixture of ketamine hydrochloride (80 mg/kg) and
acepromazine maleate (0.2 mg/kg). An incision 2 cm in length was
made along the linea alba, and the underlying tissue was dissected
and retracted. A transmitter (Mini-Mitter, Sunriver, OR) was then
inserted in the i.p. space. After the transmitter was passed through
the incision, the abdominal musculature and dermis were sutured
independently (Benamar et al., 2002). The animals were returned to
individual cages in the environmental room.

Body Temperature Measurement. One week after surgery, the
rats were tested in an environmental room (Hotpack), maintained at
21 ± 0.3°C ambient temperature and 52 ± 2% relative humidity.
After 1 h of adaptation, two readings at 15-min intervals were
averaged to determine the baseline. Either saline or drug was then
injected i.p. Tb was measured by a biotelemetry system (Mini-Mitter)
using calibrated transmitters implanted i.p. Signals from the trans-
mitter were delivered through a computer-linked receiver. This
method minimizes stress to animals during the Tb reading. Thus,
the Tb could be monitored continuously and recorded without re-
straint or any disturbance to the animal. All experiments were
started between 9:00 and 10:00 AM to minimize the effect of circa-
dian variation in Tb.

Enzyme-Linked Immunosorbent Assay. The concentration of
IL-6 in the plasma was determined by using an enzyme-linked im-
munosorbent assay kit from R&D Systems (Minneapolis, MN). The
assay was performed according to the manufacturer’s instructions.
At selected time points after i.p. injection of vehicle/LPS or WIN
55,212-2/LPS, rats were killed for collection of blood. Blood samples
were immediately centrifuged for measurement of IL-6 in the
plasma.

Results

The Effect of WIN 55,212-2 on Body Temperature. The
i.p. injection of WIN 55,212-2 (0.5–1.5 mg/kg) did not signif-
ically affect the Tb relative to vehicle (Table 1, p > 0.05).
However, a higher dose of WIN 55,212-2 (2 mg/kg) produced
significant hypothermia compared with control (p < 0.05).
Accordingly, we used the nonhypothermic doses of WIN
55,212-2 (0.5–1.5 mg/kg) to allow a clear analysis of the
effects of WIN 55,212-2 on LPS-induced fever.

The Effect of WIN 55,212-2 on LPS-Induced Fever. In
Fig. 1, LPS injected i.p. (50 µg/kg) induced an increase in Tb
that peaked at 180 min (1.25 ± 0.27°C) and again at 5 h
(1.52 ± 0.21°C), in agreement with our previous study
(Benamar et al., 2000). To determine whether a cannabinoid
receptor agonist would interfere with the LPS-induced fever,
WIN 55,212-2 (0.5–1.5 mg/kg) was injected 30 min before

<table>
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<th>TABLE 1</th>
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<tr>
<td>Maximal change (mean ± S.E.M.) in body temperature induced by 0.5 to 2 mg/kg WIN 55,212-2 i.p.</td>
</tr>
<tr>
<td>ΔTb, variation in body temperature, n, number of rats.</td>
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<td><strong>Dose</strong></td>
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* p < 0.05.
LPS (Fig. 1). WIN 55,212-2, at a dose of 0.5 mg/kg, did not affect the LPS-induced fever. The LPS-induced fever was partially attenuated by WIN 55,212-2 at a dose of 1 mg/kg (Fig. 1, \( p < 0.05 \)) and further reduced at a dose of 1.5 mg/kg WIN 55,212-2. Mean Tb before injection was 37.61 ± 0.15°C for the vehicle/LPS group, 37.64 ± 0.19°C for the WIN 55,212-2 (0.5 mg/kg)/LPS group, 37.60 ± 0.17°C for the WIN 55,212-2 (1 mg/kg)/LPS group, 37.87 ± 0.23°C for the WIN 55,212-2 (1.5 mg/kg) group, and 37 ± 14°C for vehicle/saline group.

Effect of WIN 55,212-3 (Inactive Form) on LPS-Induced Fever. To confirm that WIN55,212-2 functions through the cannabinoid receptor, we tested whether an inactive enantiomer of the aminoalkylindole could affect LPS-induced fever. WIN 55,212-3 (1.5 mg/kg) had no effect on Tb compared with vehicle (Fig. 2, \( p > 0.05 \)). Moreover, pretreatment with this inactive form did not alter the LPS-induced fever (Fig. 2, \( p > 0.05 \)). Mean Tb before injection was 37.77 ± 0.11°C for the vehicle/LPS group, 37.67 ± 0.14°C for the WIN 55,212-3/LPS group, 37.65 ± 0.09°C for the WIN 55,212-2/vehicle and group, and 37.62 ± 0.24°C for the vehicle/saline group.

Antagonism of WIN 55,212-2 Effect on LPS-Induced Fever by SR141716. To determine the contribution of CB1 receptors in the WIN 55,212-2 effect on LPS-induced fever, SR141716 was administered to rats 30 min before the LPS 50 \( \mu \)g/kg. SR141716 2.5 (mg/kg i.p.) was found to block the antagonistic effect of WIN 55,212-2 (1.5 mg/kg) on LPS-induced fever (Fig. 3, \( p < 0.05 \)). Neither the SR141716/vehicle/saline nor vehicle/vehicle/saline group showed an effect on Tb. The mean Tb before injection was 37.44 ± 0.18°C for the vehicle/vehicle/LPS group, 37.59 ± 0.11°C for the SR141716/WIN 55,212-2/LPS group, 37.62 ± 0.06°C for the vehicle/WIN 55,212-2/vehicle, 37.56 ± 0.14°C.

Failure of CB2-Selective Antagonist SR144528 to Reverse the WIN 55,212-2 Effect on LPS-Induced Fever. To determine whether SR14428 antagonizes the effects of WIN 55,212-2 on LPS-induced fever, SR144528 (2.5 mg/kg i.p.) was administered to rats 30 min before treatment with WIN 55,212-2 (1.5 mg/kg i.p.) and 1 h before LPS challenge (Fig. 4). There was no evidence of antagonism by SR144528 of the inhibitory effect of WIN 55,212-2 on LPS-induced fever, even at a high dose of 5 mg/kg (data not shown), nor was there any change in Tb after the administration of SR 144528/vehicle/saline. The mean Tb before injection was 37.76 ± 0.08°C for the vehicle/vehicle/LPS group, 37.71 ± 0.13°C for the SR144528/WIN 55,212-2/LPS group, and 37.63 ± 0.09°C for the SR144528/vehicle/vehicle.

Fig. 1. Effect of i.p. pretreatment with WIN 55,212-2 (0.5–1.5 mg/kg, −30 min) on LPS-induced fever. LPS was injected at time 0. Data are expressed as the mean ± S.E.M. from baseline. N, number of rats. \( \Delta \text{Tb} \), variation in body temperature. *, \( p < 0.05 \); **, \( p < 0.01 \). Vehicle + LPS versus WIN 55,212-2 + LPS at various concentrations.

LPS (Fig. 1). WIN 55,212-2, at a dose of 0.5 mg/kg, did not affect the LPS-induced fever. The LPS-induced fever was partially attenuated by WIN 55,212-2 at a dose of 1 mg/kg (Fig. 1, \( p < 0.05 \)) and further reduced at a dose of 1.5 mg/kg WIN 55,212-2. Mean Tb before injection was 37.61 ± 0.15°C for the vehicle/LPS group, 37.64 ± 0.19°C for the WIN 55,212-2 (0.5 mg/kg)/LPS group, 37.60 ± 0.17°C for the WIN 55,212-2 (1 mg/kg)/LPS group, 37.87 ± 0.23°C for the WIN 55,212-2 (1.5 mg/kg) group, and 37 ± 14°C for vehicle/saline group.

Effect of WIN 55,212-3 (Inactive Form) on LPS-Induced Fever. To confirm that WIN55,212-2 functions through the cannabinoid receptor, we tested whether an inactive enantiomer of the aminoalkylindole could affect LPS-induced fever. WIN 55,212-3 (1.5 mg/kg) had no effect on Tb compared with vehicle (Fig. 2, \( p > 0.05 \)). Moreover, pretreatment with this inactive form did not alter the LPS-induced fever (Fig. 2, \( p > 0.05 \)). Mean Tb before injection was 37.77 ± 0.11°C for the vehicle/LPS group, 37.67 ± 0.14°C for the WIN 55,212-3/LPS group, 37.60 ± 0.09°C for the WIN 55,212-2/vehicle and group, and 37.62 ± 0.24°C for the vehicle/saline group.

Antagonism of WIN 55,212-2 Effect on LPS-Induced Fever by SR141716. To determine the contribution of CB1 receptors in the WIN 55,212-2 effect on LPS-induced fever, SR141716 was administered to rats 30 min before the WIN 55,212-2 and 1 h before LPS (50 \( \mu \)g/kg). SR141716 2.5 (mg/kg i.p.) was found to block the antagonistic effect of WIN 55,212-2 (1.5 mg/kg) on LPS-induced fever (Fig. 3, \( p < 0.05 \)). Neither the SR141716/vehicle/saline nor vehicle/vehicle/saline group showed an effect on Tb. The mean Tb before injection was 37.61 ± 0.15°C for the vehicle/LPS group, 37.64 ± 0.19°C for the WIN 55,212-2 (0.5 mg/kg)/LPS group, 37.60 ± 0.17°C for the WIN 55,212-2 (1 mg/kg)/LPS group, 37.87 ± 0.23°C for the WIN 55,212-2 (1.5 mg/kg) group, and 37 ± 14°C for vehicle/saline group.

Failure of CB2-Selective Antagonist SR144528 to Reverse the WIN 55,212-2 Effect on LPS-Induced Fever. To determine whether SR14428 antagonizes the effects of WIN 55,212-2 on LPS-induced fever, SR144528 (2.5 mg/kg i.p.) was administered to rats 30 min before treatment with WIN 55,212-2 (1.5 mg/kg i.p.) and 1 h before LPS challenge (Fig. 4). There was no evidence of antagonism by SR144528 of the inhibitory effect of WIN 55,212-2 on LPS-induced fever, even at a high dose of 5 mg/kg (data not shown), nor was there any change in Tb after the administration of SR 144528/vehicle/saline. The mean Tb before injection was 37.76 ± 0.08°C for the vehicle/vehicle/LPS group, 37.71 ± 0.13°C for the SR144528/WIN 55,212-2/LPS group, and 37.63 ± 0.09°C for the SR144528/vehicle/vehicle.

Fig. 2. Effect of i.p. pretreatment with WIN 55,212-3 (1.5 mg/kg, –30 min) on LPS-induced fever. LPS was injected at time 0. Data are expressed as the mean ± S.E.M. from baseline. N, number of rats. \( \Delta \text{Tb} \), variation in body temperature.

Fig. 3. Antagonism of WIN 55,212-2 effect on LPS-induced fever by SR141716. Rats were treated i.p. with WIN 55,212-2 (1.5 mg/kg) 30 min before LPS. SR141716 was administered to rats at a dose of 2.5 mg/kg 1 h before LPS (at 0 min). Data are expressed as the mean ± S.E.M. from baseline. N, number of rats. \( \Delta \text{Tb} \), variation in body temperature. *, \( p < 0.05 \).
Effects of SR141716 or SR144528 on LPS-Induced Fever. In separate experiments, we also determined whether CB1 or CB2 receptor antagonists themselves affected the LPS-induced fever. SR141716 (2.5 mg/kg i.p.) completely abolished the fever produced by LPS (Fig. 5, $p < 0.05$). In contrast, SR144528 2.5 mg/kg did not alter LPS-evoked fever (Fig. 6, $p > 0.05$). Neither SR141716 nor SR144528 significantly affected baseline temperatures (Figs. 5–7), suggesting that the endocannabinoid system does not tonically regulate $T_b$. Mean $T_b$ before injection was $37.76 \pm 0.08^\circ C$ for the vehicle/LPS group, $37.74 \pm 0.15^\circ C$ for the SR141716A/LPS group, $37.67 \pm 0.23^\circ C$ for the SR141716/vehicle group, $37.69 \pm 0.15^\circ C$ for the vehicle/saline group, $37.63 \pm 0.13^\circ C$ for the SR144528/LPS group, $37.66 \pm 0.2^\circ C$ for the SR144528 (2.5 mg/kg) group, $37.59 \pm 0.17^\circ C$ for the SR144528 (1 mg/kg) group, and $37.71 \pm 0.21^\circ C$ for the SR144528 (5 mg/kg) group. The doses of SR141716 and SR144528 were chosen for these studies based on our previous data (Rawls et al., 2002).

WIN 55,212-2 Blocks LPS-Induced Increases in IL-6 Levels in Plasma. To test whether inflammatory cytokine levels were affected by this cannabinoid treatment, we determined the levels of plasma IL-6 following cannabinoid addition. LPS induced a significant increase in plasma IL-6 at levels 2 and 4 h postinjection of LPS (Fig. 8). WIN 55,212-2 given 30 min before LPS significantly attenuated the increase in the levels of IL-6 at the 3- and 5-h time points. Neither WIN 55,212-2 by itself nor vehicle affected significantly the levels of IL-6 (data not shown).
The Effect of Δ9-THC on Body Temperature and on LPS-Induced Fever. To ensure that the ability of cannabinoids to modulate LPS-induced fever was not limited to the aminoalkylindole WIN 55,212-2, we carried out similar experiments using the chemically unique Δ9-THC. The i.p. injection of Δ9-THC at doses of 0.5 or 1 mg/kg did not significantly affect the Tb relative to vehicle (Table 2, \( p > 0.05 \)). However, a higher dose of Δ9-THC (1.5 mg/kg) produced significant hypothermia compared with control (\( p < 0.05 \)). The LPS-induced fever showed a trend toward reduction by Δ9-THC at a dose of 0.5 mg/kg (Fig. 9) and significantly reduced at a dose of 1 mg/kg (Fig. 9, \( p < 0.05 \)). Mean Tb before injection was 37.64 ± 0.12°C for the vehicle/LPS group, 37.71 ± 0.21°C for the Δ9-THC (0.5 mg/kg)/LPS group, and 37.55 ± 0.23°C for the Δ9-THC (1 mg/kg)/LPS group.

**Discussion**

The major finding in the present study is that nonhypothermic doses of WIN 55,212-2 significantly reduced LPS-induced fever. This inhibitory effect is not due to a nonspecific interaction with hydrophobic regions of functional proteins or their lipid surroundings in the cell membrane since WIN 55,212-3, an enantiomer of WIN 55,212-2, did not affect the LPS-induced fever, indicating that the effect of WIN 55,212-2 on LPS-induced fever is stereoselective. To further characterize the participation of cannabinoids in the inhibitory effect on LPS-induced fever, we evaluated another agonist at cannabinoid receptors, Δ9-THC. This agonist at doses of 0.5 or 1 mg/kg also attenuated LPS-induced fever.

It has been demonstrated that circulating levels of IL-6 rise dramatically following LPS injection with a profile that correlates closely with the development of fever (Harre et al., 2002) and that the neutralization of endogenous IL-6 (Cartmell et al., 2000) or absence of IL-6 in knockout mice (Chai et al., 1996) results in an almost total inhibition of the LPS-induced fever, suggesting that IL-6 is an essential circulating mediator of the brain-derived fever response. In an attempt to investigate the involvement of this inflammatory cytokine in the inhibitory effect of WIN 55,212-2 on LPS-induced fever, we determined the effect of WIN 55,212-2 on plasma levels of IL-6 induced by LPS, examined concurrently at two different time points that coincide with the first (3 h) and second (5 h) peaks of LPS-induced fever. In parallel with the inhibitory effect of WIN 55,212-2 on LPS-induced fever, the plasma level of IL-6 was also attenuated at both time points.

Several studies have reported the immunosuppressive effect of cannabinoids on peripheral circulating cytokines, including TNF-α, IL-10, IL-12, IL-6, and IL-1β (Galily et al., 1997; Smith et al., 2000; Roche et al., 2006). HU210 fully attenuated the LPS-induced increase in the levels of IL-1β, TNF-α, and IL-6, and changes in cytokine levels were accompanied by reduced circulating lymphocyte numbers and increased plasma corticosterone levels in response to acute administration of LPS and/or cannabinoid drugs (Roche et al., 2006). Δ9-THC and WIN 55,212-2 were investigated for their effects on LPS-induced bronchopulmonary inflammation in mice (Berdyhev et al., 1998). Both drugs were found to cause a dose-related decrease in TNF-α in bronchoalveolar lavage fluids. The effect of WIN 55,212-2 on LPS-induced serum cytokine responses has been also investigated in mice. The levels of TNF-α, IL-12, IL-1, and IL-6 were reduced in mice pretreated with WIN 55,212-2 (Smith et al., 2000). The mechanism by which WIN 55,212-2 produces its effect on LPS-induced fever is unknown. The fact that cytokines, such as

#### Table 2

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<thead>
<tr>
<th>Dose (mg/kg i.p.)</th>
<th>Baseline Tb (°C)</th>
<th>Maximal Decrease Tb (°C)</th>
<th>( n )</th>
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<tbody>
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<td>Vehicle</td>
<td>37.56 ± 0.15</td>
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<tr>
<td>0.5</td>
<td>37.51 ± 0.10</td>
<td>-0.20 ± 0.09</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>37.61 ± 0.15</td>
<td>-0.37 ± 0.10</td>
<td>6</td>
</tr>
<tr>
<td>1.5</td>
<td>37.68 ± 0.13</td>
<td>-0.70 ± 0.15*</td>
<td>6</td>
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</table>

* \( p < 0.05 \).
IL-6, act as endogenous pyrogens and play an important role in the mechanisms responsible for the development of the febrile response during infection and inflammation (Kluger et al., 1995) and that cannabinoids have immunosuppressive effects may provide an explanation for our results. WIN 55,212-2, by diminishing the IL-6 production by LPS, may cause a reduction in LPS-induced fever. Of course, this explanation does not dismiss the possible implication of other mediators of fever, other cytokines (e.g., interleukin-1β), and/or chemokines.

The WIN 55,212-2 effects on LPS-induced fever could occur through the CB1 and/or CB2 receptors. CB1 receptors are located primarily in the central nervous system and are thought to mediate the central effects of cannabinoids (Howlett, 1995). CB1 receptor immunoreactivity has been detected in the hypothalamus (Tsou et al., 1998), including the lateral hypothalamic area and the preoptic anterior hypothalamus, the primary area implicated in body temperature regulation (Moldrich and Wenger, 2000). In contrast, CB2 receptors are expressed mainly in the peripheral immune cells (Dragic et al., 1996). CB2 receptor mRNA has been found in spleen, tonsils, and thymus, which are the major tissues of immune cell production and regulation (Cabral and Pettit, 1998). However, recent evidence shows the expression of CB2 receptor mRNA and protein localization on brainstem neurons and microglia (Cabral and Marciano-Cabral, 2005; Van Sickle et al., 2005). The present study shows that SR141716 prevented the WIN 55,212-2 effects on LPS-induced fever, indicating that a CB1 receptor mechanism mediated the response. The blockade by SR141716 of WIN 55,212-2 effects in the present study is consistent with previous reports. For example, SR141716 blocked cannabinoid agonist-induced hypothermia in rats (Rawls et al., 2002). Several lines of evidence implicate the involvement of cannabinoids, acting via cannabinoid CB1 receptors, in the action of LPS in the rat (Varga et al., 1998). The systemic administration of a selective CB1 receptor antagonist SR141716 protects rats against hypotension induced by bacterial LPS (Varga et al., 1998), and in the initial phase of septic shock induced by LPS, the activation of CB1 receptors by endogenously formed cannabinoids contributes to the inhibition of the neurogenic vasopressor response (Godlewski et al., 2004). CB1 receptors contribute to the immunosuppressive effects of HU210, both centrally and peripherally, since SR141716 attenuated, albeit partially, the decrease in LPS-induced cytokine release induced by this cannabinoid receptor agonist (Roche et al., 2006). Because cytokines are released in response to LPS, and the CB2 receptor has a modulatory role in the immune system, including the cytokine network (Klein et al., 1998), it was tempting to assume that CB2 receptors might contribute to the LPS-induced fever as well. In contrast to SR141716, the present study shows that SR144528 did not affect the inhibitory effects of WIN 55,212-2 on LPS-induced fever, indicating that the thermo-regulatory interaction between WIN 55,212-2 and LPS is insensitive to CB2 receptor activation.

An unexpected finding in the present study was the ability of SR141716 itself to attenuate LPS-induced fever. Its effect on LPS-induced fever was similar to that of WIN 55,212-2 and LPS. Interestingly, the LPS-induced cytokines can be modulated by CB agonists through activation of the CB1 receptors in mice (Smith et al., 2000). The decreases in serum TNF-α and IL-12 that occurred in cannabinoid-agonist-treated mice could be blocked by SR141716 but not by SR144528 (Smith et al., 2000). SR141716 itself modulated LPS-induced cytokine responses, and its effects on immunosuppressive cytokine responses and anti-inflammatory IL-10 were qualitatively similar to those of the CB agonist WIN 55,212-2, suggesting that cytokine modulation by SR141716 seems to be a result of partial agonism (Smith et al., 2000).

Several lines of evidence suggest that SR141716, initially characterized as the first potent and selective cannabinoid CB1 receptor antagonist (Rinaldi-Carmona et al., 1994), also has inverse agonist properties. The evidence mainly comes from biochemical studies regarding adenylyl cyclase and mitogen-activated protein kinase activity in heterologous expression systems (Bouaboula et al., 1997). The administration of SR141716 alone was sufficient to suppress central and peripheral cytokine responses, in a manner qualitatively and quantitatively similar to the immunosuppressive effects of HU210 (Roche et al., 2006). It is possible that such an effect may occur with SR141716 and LPS-induced fever. In these studies, we also determined whether CB2 receptors are implicated directly in LPS-induced fever. In contrast to SR141716, SR144528 did not influence the development of fever evoked by LPS. In agreement with our previous studies (Rawls et al., 2002), neither SR141716 nor SR144528 by itself altered Tb, suggesting that the endocannabinoid system does not tonically modulate Tb. The present report represents a novel role for cannabinoids by demonstrating a thermoregulatory interaction between cannabinoids and LPS and showing that cannabinoid ligands can prevent the development of fever induced by systemic LPS administration.

References


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