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$GABA_A$ receptors modulate cannabinoid-evoked hypothermia

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Abstract

Cannabinoids evoke hypothermia by stimulating central CB_1 receptors. GABA induces hypothermia via $GABA_A$ or $GABA_B$ receptor activation. CB_1 receptor activation increases GABA release in the hypothalamus, a central locus for thermoregulation, suggesting that cannabinoid and GABA systems may be functionally linked in body temperature regulation. We investigated whether GABA receptors modulate the hypothermic actions of [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one] (WIN 55212-2), a selective cannabinoid agonist, in male Sprague–Dawley rats. WIN 55212-2 (2.5 mg/kg im) produced a rapid hypothermia that peaked 45–90 min postinjection. The hypothermia was attenuated by bicuculline (2 mg/kg ip), a $GABA_A$ antagonist. However, SCH 50911 (1–10 mg/kg ip), a $GABA_B$ blocker, did not antagonize the hypothermia. Neither bicuculline (2 mg/kg) nor SCH 50911 (10 mg/kg) by itself altered body temperature. We also investigated a possible role for CB_1 receptors in GABA-generated hypothermia. Muscimol (2.5 mg/kg ip), a $GABA_A$ agonist, or baclofen (5 mg/kg ip), a $GABA_B$ agonist, evoked a significant hypothermia. Blockade of CB_1 receptors with SR141716A (2.5 mg/kg im) did not antagonize muscimol- or baclofen-induced hypothermia, indicating that GABA-evoked hypothermia does not contain a CB_1-sensitive component. Our results implicate $GABA_A$ receptors in the hypothermic actions of cannabinoids and provide further evidence of a functional link between cannabinoid and GABA systems.

Keywords: Cannabinoid; GABA; WIN 55212-2; Bicuculline; Hypothermia; SCH 50911

1. Introduction

Cannabinoids modulate pain perception, motor behavior, cognition, immune responses, learning and memory, and body temperature by activating two subtypes of cannabinoid receptors, CB_1 and CB_2. CB_1 receptors are expressed primarily on neurons in the CNS and CB_2 receptors are located primarily on peripheral immune cells (Howlett, 1995; Dragic et al., 1996). Regarding body temperature, cannabinoids evoke hypothermia in rats and mice via a CB_1 mechanism (Compton et al., 1992, 1996; Rawls et al., 2002a,b; Wiley et al., 1995). A selective CB_1 receptor antagonist, SR141716A, abolishes the hypothermic actions of systemically administered cannabinoids (McGregor et al., 1996; Nava et al., 2000; Rawls et al., 2002a). Cannabinoids also fail to induce hypothermia in mice that lack CB_1 receptors (Ledent et al., 1999). Cannabinoid agonists injected directly into the preoptic anterior nucleus of the hypothalamus (POAH) evoke a significant hypothermia that is abolished by SR141716A, implicating hypothalamic CB_1 receptors in cannabinoid-induced hypothermia (Fitton and Pertwee, 1982; Rawls et al., 2002a). CB_2 receptors do not appear to play a critical role in the hypothermic actions of cannabinoids, because SR144528, a selective CB_2 antagonist, does not affect cannabinoid-evoked hypothermia (Rawls et al., 2002a).

The development of cannabinoid agonists and antagonists has facilitated the characterization of CB_1 and CB_2 receptors and their pharmacological profiles. One such agonist is the aminoalkylindole, (+)-WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one] (WIN 55212-2), which exhibits high selectivity for cannabinoid receptors and interacts negligibly with other neurotransmitter systems and ion channels (Martin et al., 1991). WIN 55212-2 injected systemically into rats causes a robust hypothermia, which is CB_1-sensitive, rapid in onset, and

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dose dependent (Compton et al., 1992; Rawls et al., 2002a,b). An emerging body of evidence suggests that other neurotransmitter systems play a modulatory role in cannabinoid-evoked hypothermia. Davies and Graham (1980) reported that inhibition of serotonin reuptake and blockade of dopamine receptors potentiate Δ^9-THC-evoked hypothermia in rats. Recent studies have shown that serotonin and dopamine modulate the hypothermic effects of Δ^9-THC (Malone and Taylor, 1998; Nava et al., 2000). Our laboratory demonstrated recently that NMDA antagonists potentiate the hypothermia produced by WIN 55212-2 (Rawls et al., 2002b).

The present study was undertaken to determine a possible role for GABA receptors in cannabinoid-evoked hypothermia. GABA, the major inhibitory neurotransmitter in the mammalian brain, is synthesized presynaptically from glutamate by the rate-limiting enzyme glutamic acid decarboxylase. GABA acts through at least three subtypes of GABA receptors, $GABA_A$, $GABA_B$, and $GABA_C$ (Chebib and Johnston, 1999). $GABA_A$ and $GABA_B$ receptors are expressed throughout the CNS and appear to be functionally unique. Ionotropic $GABA_A$ receptors exist as macromolecular complexes and are composed of binding sites for agonists and allosteric modulators, such as benzodiazepines and barbiturates (Sieghart et al., 1992). $GABA_A$ receptor activation produces an increased chloride conductance and membrane hyperpolarization while metabotropic $GABA_B$ receptors mediate intracellular effects through a G-protein-coupled mechanism following activation (Chebib and Johnston, 1999).

The injection of GABA or muscimol, a $GABA_A$ receptor agonist, induces hypothermia in rats (Zarrindast and Oveissi, 1988; Sancibrian et al., 1991). The effects of baclofen, a $GABA_B$ receptor agonist, are less clear, with previous studies reporting hypothermic and hyperthermic effects (Zarrindast and Oveissi, 1988; Sancibrian et al., 1991; Phillis et al., 2001). Evidence suggests that GABA interacts closely with cannabinoid systems in body temperature regulation. CB_1 receptor immunoreactivity is present in thermosensitive regions of the hypothalamus where GABA receptors are located (Moldrich and Wenger, 2000; Jha et al., 2001a,b; Okamura et al., 1990). GABAergic neurons throughout the CNS express high levels of CB_1 receptor immunoreactivity, advancing the notion that cannabinoid and GABA receptors interact to mediate a number of pharmacological endpoints (Marsicano and Lutz, 1999). Indeed, previous studies have reported that GABA agonists enhance cannabinoid-evoked hypothermia and catalepsy (Pertwee et al., 1991; Pertwee and Greentree, 1988; Pertwee et al., 1988).

Because hypothermic doses of Δ^9-THC increase GABA release in the hypothalamus by a CB_1-dependent mechanism (de Miguel et al., 1998), we investigated whether GABA receptors play a modulatory role in cannabinoid-induced hypothermia. Specifically, we determined the hypothermic effects of WIN 55212-2 in the absence and presence of bicuculline, a $GABA_A$ antagonist, or SCH 50911, a $GABA_B$ antagonist. In addition, we investigated a possible role for CB_1 receptors in the hypothermia evoked by GABA receptors.

2. Methods

2.1. Animals

All animal use procedures were conducted in strict accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Temple University Animal Care and Use Committee. Male Sprague–Dawley rats (Zivic–Miller, Pittsburgh, PA, USA) weighing 250–300 g were housed three per cage for a minimum of 5 days before experimental use. Rats were maintained on a 12-h light/dark cycle and were fed rat chow and water ad libitum.

2.2. Drug preparation and administration

WIN 55212-2, muscimol, baclofen, bicuculline, and SCH 50911 were purchased from Tocris–Cookson (St. Louis, MO, USA). SR141716A was obtained from the National Institute on Drug Abuse. WIN 55212-2 and SR141716A were dissolved in a 10% cremophor/saline solution, sonicated for 45 min, and injected intramuscularly into the right thigh. Bicuculline methobromide and SCH 50911 were dissolved in double-deionized water and injected intraperitoneally. Muscimol and baclofen were dissolved in pyrogen-free saline and injected intraperitoneally.

2.3. Experimental protocol

Body temperature experiments were started between 9 and 10 a.m. Rats were placed in an environmental room, which was maintained at a constant temperature of 21 ± 0.3 °C and relative humidity of $52 \pm 2\%$. Following a 1-h acclimation interval, baseline temperature measurements were taken. A thermistor probe (YSI series 400, Yellow Springs Instrument, Yellow Springs, OH, USA) was lubricated and inserted approximately 7 cm into the rectum. A digital thermometer (Model 49 TA, YSI) was used to record body temperature. Rats were unrestrained during the temperature readings, with only the tail being held gently between two fingers. Body temperature was recorded every 30 min during a 60-min baseline interval, followed by drug injection. Body temperature was recorded for at least 300 min postinjection.

2.4. Role of GABA receptors in muscimol- and baclofen-evoked hypothermia

To ascertain the effect of GABA antagonists on body temperature, either bicuculline (2 mg/kg) or SCH 50911 (10

mg/kg) alone was injected after a 60-min baseline interval, and body temperature was measured for 300 min. The doses were chosen on the basis of previous in vivo studies investigating thermoregulation (Zarrindast and Oveissi, 1988; Sancibrian et al., 1991; Phillis et al., 2001). Body temperature was not altered by bicuculline (2 mg/kg) or SCH 50911 (10 mg/kg). In separate experiments, bicuculline (0.5–2 mg/kg ip) or SCH 50911 (1–10 mg/kg ip) was injected after a 60-min baseline interval. Thirty minutes later, either muscimol (2.5 mg/kg) or baclofen (5 mg/kg) was administered and body temperature was measured for 300 min.

2.5. Role of GABA receptors in WIN 55212-2-evoked hypothermia

To determine a possible role for GABA receptors in cannabinoid-evoked hypothermia, we administered 2.5 mg/kg of WIN 55212-2, a dose that causes significant hypothermia, in the presence of bicuculline or SCH 50911. Following a 60-min baseline interval, either bicuculline (0.5–2 mg/kg ip) or SCH 50911 (1–10 mg/kg ip) was injected. WIN 55212-2 (2.5 mg/kg im) was administered 30 min later, and body temperature was measured for 300 min.

2.6. Role of CB_1 receptors in GABA-receptor-generated hypothermia

To determine a possible role for CB_1 receptors in the hypothermic effects of GABA agonists, we administered muscimol (2.5 mg/kg ip) or baclofen (5 mg/kg ip) in the absence and presence of SR141716A (2.5 mg/kg im). Following a 60-min baseline interval, SR141716A was administered. Muscimol or baclofen was injected 30 min later and body temperature was measured for 300 min. The dose of SR141716A was based on a previous study in our laboratory showing that 2.5 mg/kg blocks the hypothermia produced by 5 mg/kg of WIN 55212-2 (Rawls et al., 2002a).

2.7. Data and statistical analysis

The first body temperature reading was discarded in all experiments to allow rats to adapt to the experimental technique. Two consecutive body temperature readings were

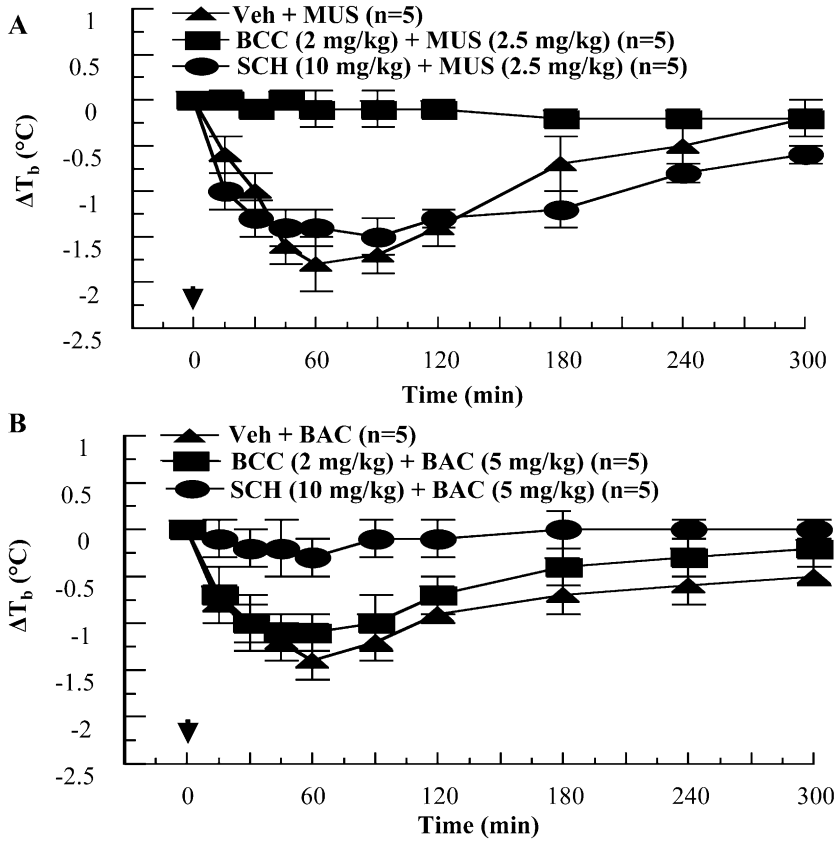

Fig. 1. (A) Effect of bicuculline (BCC; 2 mg/kg ip) or SCH 50911 (SCH; 10 mg/kg ip) on muscimol-evoked hypothermia [$F(2,27)=12.71$, $P=.0001$]. Muscimol (MUS; 2.5 mg/kg ip) was injected at 0 min, as indicated by arrow. BCC, SCH, or vehicle (Veh) was injected 30 min prior to muscimol. Data are expressed as the mean ± S.E. of body temperature. *n*, Number of rats. ΔT_b, change in body temperature from baseline (Time 0). Dunnett's post hoc analysis revealed that the group receiving BCC antagonized muscimol-evoked hypothermia, $P<.01$. (B) Effect of BCC (2 mg/kg ip) or SCH (10 mg/kg ip) on baclofen-evoked hypothermia [$F(2,27)=11.74$, $P=.0001$]. Baclofen (BAC; 5 mg/kg ip) was injected at 0 min, as indicated by arrow. BCC, SCH, or Veh was injected 30 min prior to muscimol. Dunnett's post hoc analysis revealed that the group receiving SCH 50911 antagonized baclofen-evoked hypothermia, $P<.05$.

then recorded and averaged to establish a baseline temperature prior to drug injection, according to standard procedures in our laboratory. Data were calculated as the mean ± S.E. of body temperature. Statistical analysis of differences between groups was determined by a one-way analysis of variance (ANOVA) followed by a Dunnett's or Tukey's post hoc test. A value of $P < .05$ was considered to be statistically significant.

3. Results

3.1. Endogenous GABA does not tonically regulate body temperature

We injected bicuculline (10 mg/kg ip) or SCH 50911 (10 mg/kg ip) by itself to determine whether the endogenous GABA system is involved in the tonic regulation of body temperature (data not shown). Neither bicuculline (2 mg/kg ip) nor SCH 50911 (10 mg/kg ip) significantly altered body temperature as compared to vehicle [$F(2,27) = 0.1757$, $P = .8401$; $P > .05$]. Moreover, the injection of bicuculline or SCH 50911 did not elicit any visible behavioral effects.

3.2. $GABA_A$ receptors mediate muscimol-evoked hypothermia whereas $GABA_B$ receptors mediate baclofen-evoked hypothermia

We injected bicuculline (10 mg/kg ip) or SCH 50911 (10 mg/kg ip) 30 min prior to a dose of muscimol (2.5 mg/kg) that produces marked hypothermia [$F(2,27) = 12.71$, $P = .0001$; Fig. 1A]. Bicuculline, but not SCH 50911, antagonized the hypothermic actions of muscimol, indicating that $GABA_A$ receptors mediate muscimol-evoked hypothermia ($P < .01$). On the basis of those data and the fact that higher doses of bicuculline have been reported to produce seizures (Matejovska et al., 1998), a dose range of 0.5–2 mg/kg of bicuculline was chosen for experiments with WIN 55212-2.

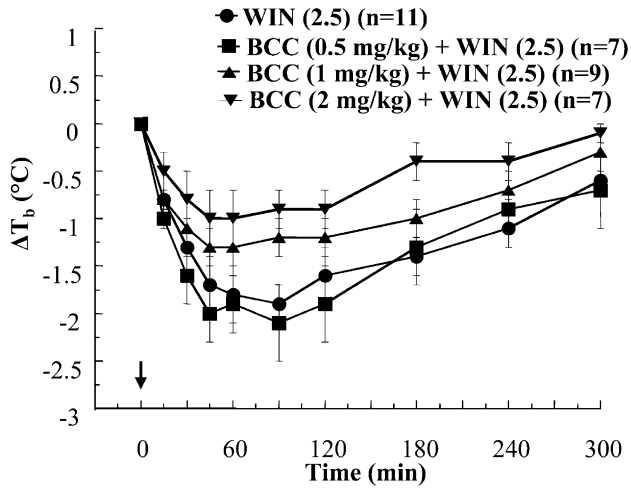

Fig. 2. Effect of bicuculline (BCC; 0.5–2 mg/kg ip) on the hypothermia caused by WIN 55212-2 (2.5 mg/kg im) [$F(3,36) = 3.831$, $P = .0177$]. WIN 55212-2 was injected at 0 min, as indicated by arrow. BCC was injected 30 min prior to WIN 55212-2. Data are expressed as the mean ± S.E. of body temperature. n, Number of rats. ΔT_b, change in body temperature from baseline (Time 0). Dunnett's post hoc analysis revealed that the group pretreated with 2 mg/kg BCC displayed significant hypothermia as compared to the WIN 55212-2 alone group, $P < .05$.

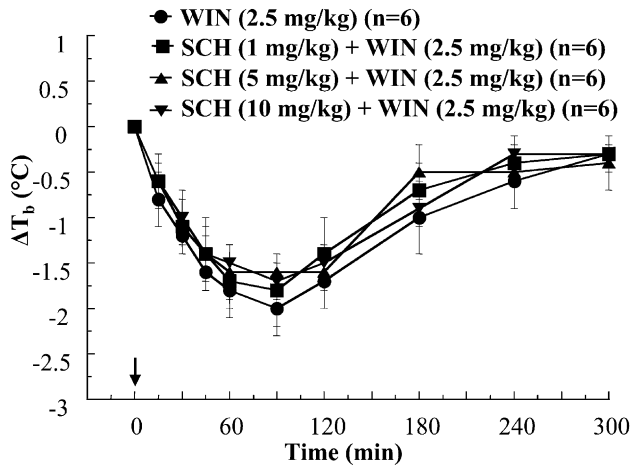

Fig. 3. Effect of SCH 50911 (SCH; 1–10 mg/kg ip) on the hypothermia caused by WIN 55212-2 (2.5 mg/kg im) [$F(3,36) = 0.1862$, $P = .9051$]. WIN 55212-2 was injected at 0 min, as indicated by arrow. SCH was injected 30 min prior to WIN 55212-2. Data are expressed as the mean ± S.E. of body temperature. n, Number of rats. ΔT_b, change in body temperature from baseline (Time 0). Dunnett's post hoc analysis revealed that the groups pretreated with SCH (1–10 mg/kg) did not differ significantly relative to the WIN 55212-2 alone group, $P > .05$.

In separate experiments, bicuculline (10 mg/kg ip) or SCH 50911 (10 mg/kg ip) was injected 30 min prior to a dose of baclofen (5 mg/kg) that produces hypothermia [$F(2,27) = 11.74$, $P = .0001$; Fig. 1B]. SCH 50911 antagonized the hypothermic actions of baclofen, indicating that $GABA_B$ receptors mediate baclofen-evoked hypothermia ($P < .01$). Bicuculline did not significantly alter the hypothermic effects of baclofen ($P > .05$). Thus, a dose range of 1–10 mg/kg of SCH 50911 was chosen to investigate a possible role for $GABA_B$ receptors in the hypothermic actions of WIN 55212-2.

3.3. $GABA_A$ receptors modulate WIN 55212-2-evoked hypothermia

As shown previously (Rawls et al., 2002a), the intramuscular injection of 2.5 mg/kg WIN 55212-2 evoked a rapid hypothermia that persisted for several hours (Figs. 2 and 3). The decline in body temperature began 15 min after the injection of WIN 55212-2. A peak hypothermia of -1.7 ± 0.3 °C was recorded 90 min postinjection, and body temperature returned gradually toward predrug levels thereafter (Fig. 2).

We administered bicuculline (0.5–2 mg/kg ip) 30 min prior to WIN 55212-2 to determine a possible role for $GABA_A$ receptors in cannabinoid-evoked hypothermia (Fig. 2). The highest dose of bicuculline, 2 mg/kg, signi-

ficantly attenuated the hypothermia caused by WIN 55212-2 [$F(3,36) = 3.831$, $P = .0177$; $P < .05$]. A dose of 1 mg/kg bicuculline decreased the WIN 55212-2-induced hypothermia, but Dunnett's post hoc analysis revealed that the effect was not statistically significant ($P > .05$). The WIN 55212-2-precipitated decline in body temperature was not altered by the lowest dose of bicuculline, 0.5 mg/kg ($P > .05$).

The effect of a $GABA_B$ receptor antagonist, SCH 50911, on cannabinoid-evoked hypothermia is shown in Fig. 3. We injected SCH 50911 (1–10 mg/kg ip) 30 min prior to WIN 55212-2 (2.5 mg/kg). None of the doses of SCH 50911 significantly altered the hypothermic response to WIN 55212-2 [$F(3,36) = 0.1862$, $P = .9501$; $P > .05$].

3.4. CB_1 receptors do not modulate GABA receptor-evoked hypothermia

Fig. 4A illustrates the role of CB_1 receptors in the hypothermia produced by muscimol. The injection of muscimol (2.5 mg/kg ip) by itself produced a hypothermia that was rapid in onset and significant relative to vehicle [$F(3,36) = 13.56$, $P < .0001$; $P < .001$]. A peak hypothermia of -1.7 ± 0.2 °C was recorded 60 min postinjection. Thirty-minute pretreatment with SR141716A (2.5 mg/kg im) did not significantly affect muscimol-evoked hypothermia, suggesting that the hypothermia generated by $GABA_A$ receptor activation occurs independently of CB_1 receptors ($P > .05$). Moreover, Tukey's post hoc analysis revealed that the injection of SR141716A by itself did not alter body temperature significantly ($P > .05$).

Similar experiments were conducted with baclofen to determine whether CB_1 receptors contribute to $GABA_B$-receptor-induced hypothermia (Fig. 4B). Baclofen (5 mg/kg ip) produced a rapid hypothermia, with a fall in body temperature occurring 15 min postinjection [$F(3,36) = 14.02$, $P < .0001$]. The hypothermia was significant relative to vehicle, and a peak hypothermia of -1.8 ± 0.4 °C was recorded 60 min postinjection ($P < .001$). Thirty-minute pretreatment with SR141716A did not significantly affect the hypothermic response to baclofen, indicating that $GABA_B$-receptor-generated hypothermia is insensitive to CB_1 receptors ($P > .05$). SR141716A by itself did not alter body temperature as compared to vehicle ($P > .05$).

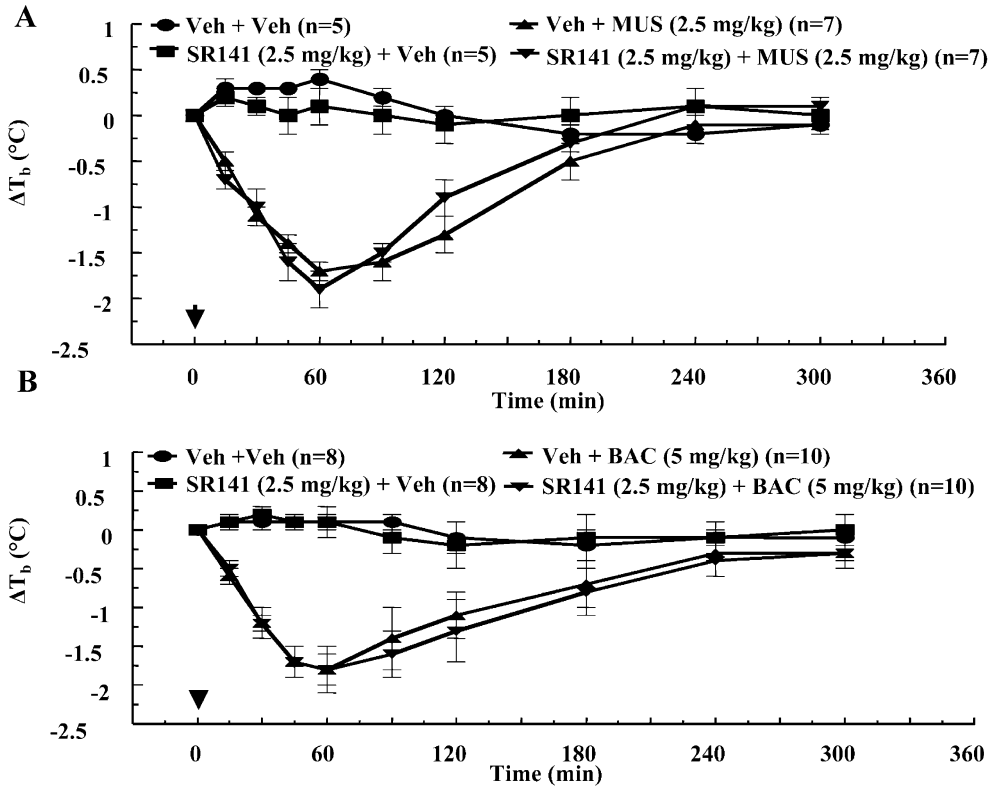

Fig. 4. (A) Effect of SR141716A (2.5 mg/kg im) on the hypothermia caused by muscimol (MUS; 2.5 mg/kg ip) [$F(3,36) = 13.56$, $P < .0001$]. MUS was injected at 0 min, as indicated by arrow. SR141716A was injected 30 min prior to MUS. Data are expressed as the mean ± S.E. of body temperature. *n*, Number of rats. ΔT_b, change in body temperature from baseline (Time 0). Tukey's post hoc analysis revealed the following. The vehicle + muscimol alone group differed significantly relative to the vehicle + vehicle group ($P < .001$). The vehicle + muscimol group did not differ significantly relative to the SR141716A + muscimol group ($P > .05$). The SR141716A + vehicle group did not differ significantly relative to the vehicle + vehicle group ($P > .05$). (B) Effect of SR141716A (2.5 mg/kg im) on the hypothermia caused by baclofen (BAC; 5 mg/kg ip) [$F(3,36) = 14.02$, $P < .0001$]. BAC was injected at 0 min, as indicated by arrow. SR141716A was injected 30 min prior to muscimol. Tukey's post hoc analysis revealed the following. The vehicle + baclofen alone group differed significantly relative to the vehicle + vehicle group ($P < .001$). The vehicle + baclofen group did not differ significantly relative to the SR141716A + muscimol group ($P > .05$). The SR141716A + vehicle group did not differ significantly relative to the vehicle + vehicle group ($P > .05$).

4. Discussion

The present study demonstrated that GABA receptors modulate the hypothermia generated by cannabinoids. Bicuculline, a $GABA_A$ receptor blocker, attenuated WIN 55212-2-induced hypothermia whereas a $GABA_B$ antagonist, SCH 50911, was ineffective, thus implicating specifically $GABA_A$ receptors in cannabinoid-induced hypothermia. SR141716A, a CB_1 antagonist, did not affect the ability of muscimol, a $GABA_A$ agonist, or baclofen, a $GABA_B$ agonist, to decrease body temperature, suggesting that GABA-evoked hypothermia lacks a CB_1-sensitive component.

Consistent with previous studies, the intramuscular injection of WIN 55212-2 evoked a significant hypothermia that was rapid in onset and lasted for several hours. While it is generally accepted that WIN 55212-2 and other cannabinoids suppress body temperature by activating CB_1 receptors in the brain, the involvement of other neurotransmitter systems in cannabinoid-evoked hypothermia is not as well defined. Nava et al. (2000) demonstrated that Δ^9-THC-evoked hypothermia is blocked by dopamine D_2 receptor antagonists and potentiated by D_2 agonists. The drug combination of dextromethorphan, a clinically available NMDA antagonist, or LY 235959, a selective and competitive NMDA antagonist, and WIN 55212-2 produces synergistic hypothermia, suggesting that NMDA receptors partially mediate cannabinoid-induced hypothermia (Rawls et al., 2002b). Malone and Taylor (1998) demonstrated that pretreatment with fluoxetine, a serotonin reuptake inhibitor, blocks Δ^9-THC-induced hypothermia, but the administration of fluoxetine after Δ^9-THC enhances the hypothermia. An involvement of nitric oxide, a soluble second messenger in the CNS and PNS, and the opioid system in the hypothermic actions of cannabinoids have also been reported (Azad et al., 2001; Rawls et al., 2002c, 2004).

The present results support a role for GABA in the hypothermic actions of cannabinoids, too. Bicuculline attenuated the WIN 55212-2-evoked decline in body temperature, suggesting that a $GABA_A$-sensitive component contributes to cannabinoid-induced hypothermia. The hypothermia does not seem to involve $GABA_B$ receptors, however, because SCH 50911 did not alter WIN 55212-2-generated hypothermia. A role for $GABA_A$ receptors in the hypothermic effects of cannabinoids has been proposed previously, because flurazepam, a benzodiazepine that facilitates $GABA_A$ responses, enhances Δ^9-THC-induced hypothermia in mice (Pertwee et al., 1991).

Although speculative, an increase in extracellular GABA levels in the CNS following the injection of WIN 55212-2 may partially mediate cannabinoid-evoked hypothermia. However, a direct elevation of GABA release by cannabinoids in the hypothalamus and other brain regions is unlikely. Numerous studies indicate that cannabinoid agonists suppress the release of GABA and other neurotransmitters in the CNS (Schlicker and Kathmann, 2001; Davies et al., 2002). In vivo studies, particularly those using the technique of microdialysis, also indicate that the activation of CB_1 receptors by cannabinoid agonists decreases extracellular GABA levels in the cortex of rats (Ferraro et al., 2001; Pistis et al., 2002). Thus, the most likely explanation of a CB_1-mediated elevation in brain GABA levels would be via an indirect mechanism, probably by inhibition of another neurotransmitter which exerts an endogenous inhibitory tone on GABA release.

It has been suggested that the hypothermic activity of cannabinoids is mediated through central catecholamines, and that noradrenalin is involved (Singh and Das, 1976). Cannabinoids inhibit norepinephrine release in vitro and in vivo (Ishac et al., 1996; Niederhoffer and Szabo, 1999; Kathmann et al., 1999; Vizi et al., 2001; Tzvara et al., 2001). Δ^9-THC also reduces norepinephrine metabolism in the hypothalamus (Steger et al., 1990), a major thermoregulatory center in the brain. Norepinephrine pathways in the hypothalamus are known to produce hyperthermia in rats by increasing heat production and inhibiting heat loss (Lin et al., 1984). Administration of noradrenalin into the POAH evokes a dose-dependent rise in rectal temperature in conscious rats (Lin et al., 1985). Although the effects of the adrenergic system on GABA release in the hypothalamus are contradictory (Wang et al., 1998; Herbison et al., 1990), some GABAergic neurons in the hypothalamus are inhibited by noradrenergic modulation (Han et al., 2002). Those data raise the possibility that norepinephrine exerts an endogenous inhibitory tone on GABA release in the hypothalamus. If so, a possible interpretation of our data is that WIN 55212-2 inhibits norepinephrine release, thus diminishing the tonic inhibitory tone on GABAergic neurons and producing an increase in GABA release. The disinhibition of GABA systems by WIN 55212-2 leads to an overall increase in $GABA_A$ transmission, which mediates a portion of the cannabinoid-induced hypothermia. In the presence of bicuculline, the enhanced $GABA_A$ transmission is blocked and the hypothermic response to cannabinoids is decreased. This notion is supported by data from de Miguel et al. (1998), which demonstrated that the intraperitoneal injection of 5 mg/kg of Δ^9-THC increases the GABA content in the hypothalamus 60 min following Δ^9-THC administration. Because SR141716A abolished the rise in hypothalamic GABA, the authors surmised that CB_1 receptor activation mediated the increase in GABA release. A dose of 5 mg/kg of Δ^9-THC spawns a substantial hypothermia 60 min postinjection, and this hypothermia is blocked by SR141716A (Compton et al., 1996; Nava et al. 2000). The parallel decline in body temperature and augmentation in hypothalamic GABA following Δ^9-THC administration supports our idea that increased GABA transmission in the hypothalamus contributes to CB_1-dependent hypothermia.

Another explanation involves the effect of cannabinoids on glutamate and GABA systems. Microdialysis studies have established that extracellular glutamate and GABA levels are abundant in the hypothalamus (Anderson and DiMicco, 1992). Extracellular levels of glutamate and

GABA maintain a balance between excitatory and inhibitory systems, and alterations in their levels can disrupt the homeostasis, causing an exaggeration of excitatory or inhibitory influences (Petroff, 2002). GABA produces hypothermia whereas glutamate produces primarily hyperthermia (Yakimova and Ovtcharov, 1989). Moreover, cannabinoids inhibit glutamate release in vitro (Davies et al., 2002; Huang et al., 2001). Therefore, it is possible that the direct suppression by cannabinoids of glutamate release in brain regions that regulate body temperature disrupt the balance between excitatory and inhibitory systems, thus leading to increased GABAergic transmission. The indirect potentiation of GABAergic transmission mediates a portion of the cannabinoid-induced hypothermia. An involvement of other transmitters, such as acetylcholine, cannot be discounted (Gessa et al., 1998). In addition, cannabinoid agonists inhibit N-type, and possibly other, calcium channels that modulate synaptic transmitter release (Caulfield and Brown, 1992; Mackie and Hille, 1992).

Mechanisms other than increased GABA transmission in the hypothalamus may underlie the WIN 55212-2-generated hypothermia. In vivo microdialysis studies have demonstrated that systemically administered cannabinoids decrease extracellular GABA levels in the cortex (Pistis et al., 2002; Ferraro et al., 2001). Moreover, the effect of cannabinoids on GABA uptake in vitro is inconclusive, with studies reporting that synaptosomal GABA accumulation following cannabinoid treatment can be inhibited or enhanced (Banerjee et al., 1975; Romero et al., 1998). In contrast to flurazepam, nipecotic acid hydrochloride, a GABA uptake blocker, did not potentiate Δ^9-THC-evoked hypothermia in rats (Pertwee et al., 1991). Those results prompted the authors to assert that Δ^9-THC decreased body temperature by increasing the response of GABA receptors to neuronally released GABA via alteration of the receptor's recognition sites or signal transduction pathways (Pertwee et al., 1991). Although the dose, 5 mg/kg, of nipecotic acid hydrochloride used by Pertwee et al. (1991) enhances Δ^9-THC-evoked catalepsy (Pertwee et al., 1988b), it is possible that this dose may not have been high enough to inhibit GABA uptake in the CNS. Most in vivo microdialysis studies, in fact, have shown that doses of at least 20 mg/kg of nipecotic acid are required to elevate extracellular GABA levels in various brain compartments (Ipponi et al., 1999; Richards and Bowery, 1996). Because 10 mg/kg of nipecotic acid did not significantly alter GABA levels in either study, it seems unlikely that an even lower dose, 5 mg/kg, would effectively block central GABA uptake. While our data and the Pertwee et al. (1991) study both implicate the GABA system in cannabinoid-evoked hypothermia, it is unclear whether elevated extracellular GABA levels in the brain, possibly in the hypothalamus, are partly responsible for the hypothermic response to cannabinoids.

The hypothalamus is considered to be the central thermoregulatory locus, with extrahypothalamic compartments, such as the pons, medulla, midbrain, and spinal cord, also playing a role (Nakayama, 1985). The injection of Δ^9-THC or cannabinoid agonists causes dose-dependent hypothermia via CB_1 receptors (Fitton and Pertwee, 1982; Rawls et al., 2002a). Injections of GABA or GABA ligands directly into the hypothalamus have been shown to alter body temperature (Jha et al., 2001b; Drummer and Woolley, 1991). The mechanism of action of GABA in temperature regulation at the cellular level has been investigated by applying picrotoxin, a $GABA_A$ antagonist, onto individual hypothalamic neurons in anaesthetized rats (Jha et al., 2001a). Picrotoxin excited the majority of cold-sensitive neurons but inhibited most of the warm-sensitive neurons, prompting Jha et al. to hypothesize that GABA modulates spontaneous activity in thermosensitive neurons via a $GABA_A$ receptor mechanism and exerts a direct inhibitory action on the cold-sensitive neurons. The intimate association of CB_1 and $GABA_A$ receptors in thermosensitive regions of the hypothalamus strengthens our contention that cannabinoid and GABA systems are linked in body temperature control.

In conclusion, we have shown that bicuculline attenuates WIN 55212-2-induced hypothermia. Our data implicate $GABA_A$ receptors in the hypothermia induced by cannabinoids. In contrast, CB_1 receptor blockade did not alter the hypothermic actions of muscimol or baclofen, suggesting that GABA receptor activation causes a hypothermia that is independent of the cannabinoid system. The present study advances the belief that $GABA_A$ receptors play a modulatory role in cannabinoid pharmacology.

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