

# *N*-Methyl-D-aspartate Antagonists and WIN 55212-2 [4,5-Dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one], a Cannabinoid Agonist, Interact to Produce Synergistic Hypothermia

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Received April 11, 2002; accepted July 3, 2002

## ABSTRACT

CB<sub>1</sub> cannabinoid receptors mediate profound hypothermia when cannabinoid agonists are administered to rats. Glutamate, the principal excitatory neurotransmitter in the central nervous system (CNS), is thought to tonically increase body temperature by activating *N*-methyl-D-aspartate (NMDA) receptors. Because NMDA antagonists block cannabinoid-induced antinociception and catalepsy, intimate glutamatergic-cannabinoid interactions may exist in the CNS. The present study investigated the effect of two NMDA antagonists on the hypothermic response to WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one], a selective cannabinoid agonist, in rats. WIN 55212-2 (1–10 mg/kg i.m.) produced dose-dependent hypothermia that peaked 60 to 180 min postinjection. Dextromethorphan (5–75 mg/kg i.m.), a noncompetitive NMDA antagonist, or LY 235959 [(–)-6-[phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-isoquinoline-2-carboxylate]](1–4 mg/kg i.m.), a competitive and

highly selective NMDA antagonist, evoked hypothermia in a dose-sensitive manner, suggesting that endogenous glutamate exerts a hyperthermic tone on body temperature. A dose of dextromethorphan (10 mg/kg) that did not affect body temperature by itself potentiated the hypothermic response to WIN 55212-2 (1, 2.5, or 5 mg/kg). The enhancement was strongly synergistic, indicated by a 2.7-fold increase in the relative potency of WIN 55212-2. Similarly, a dose of LY 235959 (1 mg/kg) that did not affect body temperature augmented the hypothermia associated with a single dose of WIN 55212-2 (2.5 mg/kg), thus confirming that NMDA receptors mediated the synergy. We have demonstrated previously that CB<sub>1</sub> receptors mediate WIN 55212-2-evoked hypothermia in rats. The present data are the first evidence that NMDA antagonists exert a potentiating effect on cannabinoid-induced hypothermia. Taken together, these data suggest that interactions between NMDA and CB<sub>1</sub> receptors produce synergistic hypothermia.

Cannabinoid agonists produce four characteristic symptoms in rodents: hypothermia, analgesia, catalepsy, and hypoactivity. Two subtypes of cannabinoid receptors, CB<sub>1</sub> and CB<sub>2</sub>, mediate these cannabinoid-induced effects. CB<sub>1</sub> receptors are located centrally (Howlett, 1995), whereas CB<sub>2</sub> receptors are expressed almost exclusively by peripheral immune cells (Dragic et al., 1996).

Availability of the (+)-WIN 55212-2 [aminoalkylindole 4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one] has facilitated the characterization of cannabinoid receptor subtypes

and their pharmacological profiles. WIN 55212-2 is highly selective for cannabinoid receptors and interacts negligibly with other neurotransmitter systems and ion channels (Martin et al., 1991; Compton et al., 1992; Jansen et al., 1992). Several reports indicate that WIN 55212-2 elicits hypothermia in rodents via a CB<sub>1</sub> receptor mechanism (Compton et al., 1992; Fan et al., 1994; Fox et al., 2001). Our laboratory has recently confirmed these results and demonstrated that CB<sub>1</sub> receptors in the preoptic anterior nucleus of the hypothalamus (POAH), which is thought to be the central site of thermoregulation, play a critical role in the hypothermic response to WIN 55212-2 (Rawls et al., 2002). An involvement of the cannabinoid system in thermoregulation is supported further by the fact that CB<sub>1</sub> receptor immunoreactivity, binding, and mRNA are present in the POAH (Mailleux and Vanderhaeghen, 1992; Moldrich and Wenger, 2000).

This study was supported by National Institute on Drug Abuse Grants DA09793, DA00376, DA13429, and T32 DA07237.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.  
DOI: 10.1124/jpet.102.037473.

**ABBREVIATIONS:** CB, cannabinoid; WIN 55212-2, 4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one; POAH, preoptic anterior nucleus of the hypothalamus; NMDA, *N*-methyl-D-aspartate; LY 235959, (–)-6-[phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-isoquinoline-2-carboxylate]; AUC, area under the body temperature time curve; R, relative potency; CNS, central nervous system; SR141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide.

Besides cannabinoids, glutamate, the major excitatory amino acid neurotransmitter, participates in thermoregulation. Glutamate induces hyperthermia (Yakimova and Ovtcharov, 1989; Singh and Gupta, 1997; Huang et al., 2001), whereas NMDA antagonists have been reported to cause hypothermia (Corbett et al., 1990; Farfel and Seiden, 1995; Bhargava and Thorat, 1997) and hyperthermia (Pechnick et al., 1989). NMDA receptor immunoreactivity and high levels of glutamate have been detected in regions of the hypothalamus where cells express glutamate receptor mRNA, providing additional evidence that the glutamatergic system is involved in the regulation of body temperature (van den Pol, 1991; Paquet and Smith, 2000). Because NMDA receptors and cannabinoid systems mediate body temperature and interact closely in the hypothalamus, we hypothesized that NMDA receptors may play a modulatory role in cannabinoid-induced hypothermia. Such a role for NMDA receptors has been reported in regard to cannabinoid-induced antinociception and catalepsy (Kinoshita et al., 1994; Kelly and Chapman, 2001; Palazzo et al., 2001).

The present study, therefore, investigated whether dextromethorphan, a blocker of NMDA receptor-gated channels and voltage-sensitive calcium channels (Carpenter et al., 1988; Tortella et al., 1989), or LY 235959, a highly selective and competitive NMDA receptor antagonist, potentiates the hypothermic response to WIN 55212-2. In addition, we investigated further the effect of dextromethorphan or LY 235959 alone on body temperature.

## Materials and Methods

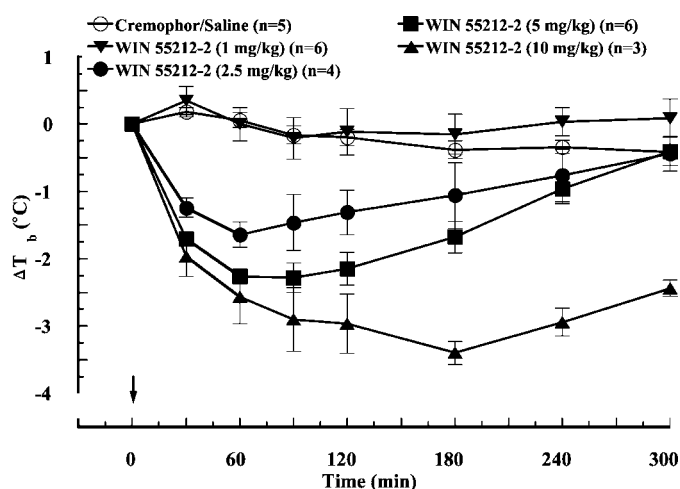
**Animals.** All animal use procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Zivic-Miller, Pittsburgh, PA) weighing 250 to 350 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 fed rat chow and water ad libitum.

**Drug Preparation and Administration.** Dextromethorphan hydrobromide, cremophor EL, WIN 55212-2, and WIN 55212-3 were purchased from Sigma-Aldrich (St. Louis, MO). LY 235959 was obtained from Tocris Cookson (St. Louis, MO). All drugs were dissolved in a 10% cremophor/saline solution and injected intramuscularly into the right thigh.

**Experimental Protocol.** Body temperature experiments were started between 9:00 and 10:00 AM. Rats were placed in an environmental room, which was maintained at a constant temperature of  $21 \pm 0.3^\circ\text{C}$  and relative humidity of  $52 \pm 2\%$ . After a 1-h acclimation interval, baseline temperature measurements were taken. A digital thermometer (model 49 TA; Yellow Springs Instrument Co.) 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 15, 45, 60, 90, 120, 180, 240, and 300 min postinjection.

**Effect of WIN 55212-2 on Body Temperature.** After a 60-min baseline interval, WIN 55212-2 (1–10 mg/kg) or 10% cremophor/saline was injected and body temperature was monitored for 300 min. These doses have been reported to produce hypothermia in rats (Fox et al., 2001; Rawls et al., 2002).

**Effect of Dextromethorphan on Body Temperature.** Dextromethorphan (5–75 mg/kg) or 10% cremophor/saline was injected after a 60-min interval, and body temperature was recorded for 300



**Fig. 1.** WIN 55212-2 (1–10 mg/kg i.m.) produces hypothermia in a dose-dependent manner. WIN 55212-2 or 10% cremophor/saline was injected at 0 min, as indicated by arrow. Data are expressed as the mean  $\pm$  S.E. of body temperature. *n*, number of rats.  $\Delta T_b$ , change in body temperature from baseline (time 0). Groups receiving 2.5, 5, and 10 mg/kg displayed significant hypothermia relative to the 10% cremophor/saline group,  $p < 0.05$ .

min. Farfel and Seiden (1995) have demonstrated previously that 75 mg/kg produces a modest hypothermia in rats.

**Effect of WIN 55212-2 and Dextromethorphan on Body Temperature.** We determined whether a dose of dextromethorphan (10 mg/kg) that did not alter body temperature by itself affected the hypothermia produced by WIN 55212-2. Dextromethorphan (10 mg/kg) alone, WIN 55212-2 (1, 2.5, or 5 mg/kg) alone, or a combination of dextromethorphan (10 mg/kg) and WIN 55212-2 (1, 2.5, or 5 mg/kg) was injected after a 60-min baseline interval. Body temperature was recorded for 300 min.

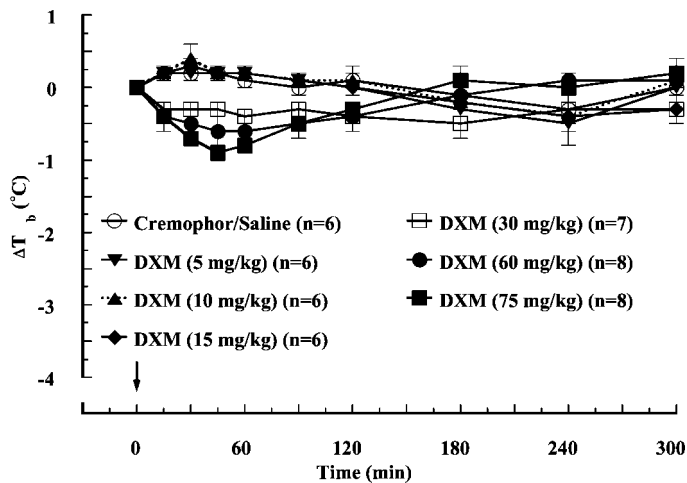
**Effect of WIN 55212-3 and Dextromethorphan on Body Temperature.** We determined whether WIN 55212-3, the inactive enantiomer of WIN 55212-2, alone or in combination with dextromethorphan affected body temperature. The effect on body temperature of WIN 55212-3, the inactive enantiomer of WIN 55212-2, alone and in combination with dextromethorphan was determined. WIN 55212-3 (5 mg/kg), dextromethorphan (10 mg/kg), 10% cremophor/saline, or a combination of WIN 55212-3 (5 mg/kg) and dextromethorphan (10 mg/kg) was injected after a 60-min baseline interval. Body temperature was recorded for 300 min.

**Effect of LY 235959 on Body Temperature.** After a 90-min baseline interval, LY 235959 (1, 2, or 4 mg/kg) or 10% cremophor/saline was injected, and body temperature was measured for 300 min. These doses have been shown to induce hypothermia in mice (Bhargava and Thorat, 1997).

**Effect of LY 235959 and WIN 55212-2 on Body Temperature.** We determined whether a dose of LY 235959 (1 mg/kg) that did not alter body temperature by itself affected the hypothermia caused by a single dose of WIN 55212-2. LY 235959 (1 mg/kg), WIN 55212-2 (2.5 mg/kg), or a combination of dextromethorphan (10 mg/kg) and WIN 55212-2 (2.5 mg/kg) was injected after a 60-min baseline interval. Body temperature was recorded for 300 min.

**Data Analysis.** To allow rats to adapt to the experimental technique, the first body temperature reading was discarded in all experiments. Then two consecutive body temperature readings were recorded and averaged to establish a baseline temperature before drug administration. In the temporal profiles, data were calculated as the mean  $\pm$  S.E. of body temperature. Statistical analysis of differences between groups was determined by a one-way analysis of variance followed by a Tukey's post hoc test. A value of  $p < 0.05$  was considered to be statistically significant.

The analysis of drug combinations to distinguish synergism from



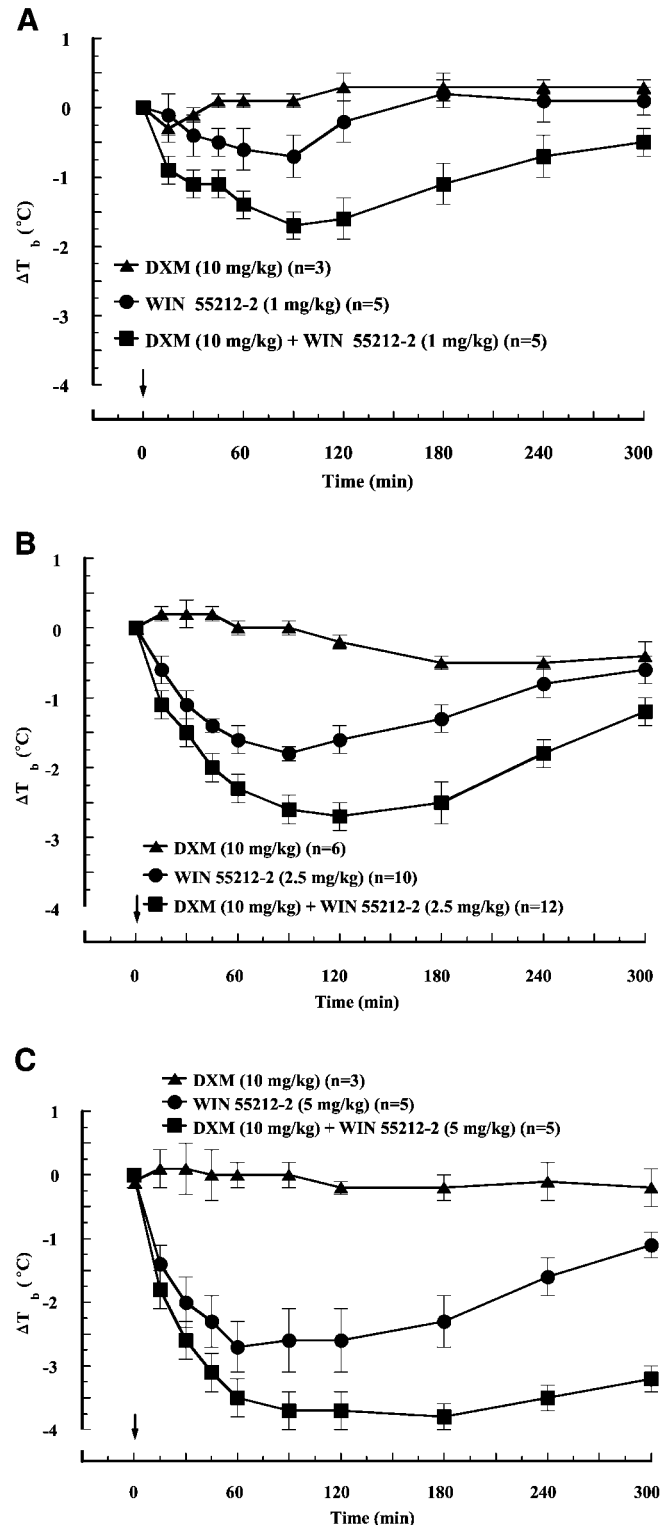
**Fig. 2.** Dextromethorphan (DXM) (5–75 mg/kg i.m.) produces dose-dependent hypothermia. DXM or 10% cremophor/saline was injected at 0 min, as indicated by arrow. Data are expressed as the mean  $\pm$  S.E. of body temperature. *n*, number of rats.  $\Delta T_b$ , change in body temperature from baseline (time 0). Groups receiving 30, 60, and 75 mg/kg displayed significant hypothermia relative to the 10% cremophor/saline group,  $p < 0.05$ .

simple additivity followed the procedure described previously (Tallarida, 2001). In that procedure, the graded dose-effect data of the individual drugs are first analyzed to determine an effect level that is reached by both. These equieffective doses (isoboles) are then used to determine the proportions of the combination for testing and to calculate the expected (additive) total dose of the combination needed to attain the specified effect level. This calculated quantity is then statistically compared with the total dose of the combination that produced the specified effect. If the combination total dose is less than the calculated additive total dose, the interaction is synergistic; equality means simple additivity. In cases in which one of the two drugs is inactive, its presence in a simply additive combination has no effect on the active drug's dose-effect curve. Therefore, the analysis becomes simply one in which the active drug's dose-effect curve is statistically compared before and after the addition of the inactive agent. In our experiments, the effect is either the peak reduction in body temperature (60 or 90 min) or area under the body temperature time curve (AUC) from 0 to 300 min.

## Results

**WIN 55212-2 Induces Hypothermia.** WIN 55212-2 (1–10 mg/kg) produced dose-dependent hypothermia (Fig. 1). Doses of 2.5, 5, and 10 mg/kg produced significant hypothermia relative to 10% cremophor/saline ( $p < 0.05$ ), but 1 mg/kg was ineffective. The onset of hypothermia was rapid, with a reduction in body temperature observed 15 min postinjection. The hypothermia peaked 60 to 90 min after the injection of WIN 55212-2. Thereafter, body temperature gradually returned to predrug levels, although the highest dose of WIN 55212-2 produced hypothermia that persisted for the entire 300-min recording interval. Because body temperature did not recover to baseline values, 10 mg/kg WIN 55212-2 was not used in subsequent experiments.

**Dextromethorphan Induces Hypothermia.** Dextromethorphan (5–75 mg/kg) produced hypothermia in a dose-sensitive manner (Fig. 2). Doses of 30, 60, and 75 mg/kg produced a modest but significant hypothermia relative to 10% cremophor/saline ( $p < 0.05$ ), but doses less than 30 mg/kg were ineffective. A reduction in body temperature occurred 15 min after dextromethorphan injection, indicating



**Fig. 3.** A to C, an ineffective dose of dextromethorphan (DXM) (10 mg/kg i.m.) potentiates the hypothermia produced by WIN 55212-2 (1, 2.5, or 5 mg/kg i.m.). Either DXM (10 mg/kg), WIN 55212-2 (1, 2.5, or 5 mg/kg), or a combination of DXM (10 mg/kg) and WIN 55212-2 (1, 2.5, or 5 mg/kg) was injected at 0 min, as indicated by arrow. Data are expressed as the mean  $\pm$  S.E. of body temperature. *n*, number of rats.  $\Delta T_b$ , change in body temperature from baseline (time 0). Groups receiving the drug combination of WIN 55212-2 (1, 2.5, or 5 mg/kg) and DXM displayed significant hypothermia relative to the WIN 55212-2 alone (1, 2.5, or 5 mg/kg) groups,  $p < 0.05$ .

a rapid onset of action. The hypothermia peaked 30 to 45 min postinjection, followed by a gradual return of body temperature to predrug values.

**Combination of WIN 55212-2 and Dextromethorphan Produces Synergistic Hypothermia.** Records of body temperature are shown for WIN 55212-2 (Fig. 1) and dextromethorphan (Fig. 2). A dose of 10 mg/kg dextromethorphan did not significantly alter body temperature (Fig. 2). Accordingly, this quantity of dextromethorphan (10 mg/kg) was used in combination experiments with three doses of WIN 55212-2 (1, 2.5, or 5 mg/kg). The experimental design, in which one of the two agents is used in a dose that is devoid of activity, allows a clear analysis of the combination. Temporal profiles for the WIN 55212-2/dextromethorphan combinations are shown in Fig. 3, A–C. Dextromethorphan (10 mg/kg) enhanced the magnitude and duration of the hypothermia associated with all three doses of WIN 55212-2 (1, 2.5, or 5 mg/kg). The WIN 55212-2/dextromethorphan drug combinations produced significant hypothermia relative to the WIN 55212-2 alone (1, 2.5, or 5 mg/kg) groups ( $p < 0.05$ ).

The time-course data reveal that dextromethorphan potentiates the hypothermia evoked by WIN 55212-2 (Fig. 3, A–C). Thus, we compared the dose-response relation of the active agent WIN 55212-2 and the dose-response relation of that agent (three doses) in combination with the inactive agent dextromethorphan. A simply additive interaction would lead to the same dose-response relation, whereas a significant shift in the combination curve means that an interaction has occurred (Tallarida, 2001). These two dose-response data sets, using the peak depression in body temperature (60 or 90 min) are shown in Table 1, and the regression lines (effect on log dose) are shown in Fig. 4A. It is seen that there is a pronounced leftward shift in the combination's regression line (Fig. 4A). Because these lines did not differ significantly in slope ( $p < 0.05$ ) it was possible to express this shift in terms of relative potency (R), a value computed with the assistance of Pharm Tools Pro (The McCary Group, Elkins Park, PA). R was found to have a mean of 2.77, with 95% confidence limits (2.17–3.53). This significant leftward shift in the regression line of WIN 55212-2 means that WIN 55212-2 was 2.77 times more potent in the presence of this nonhypothermic dose of dextromethorphan, a factor that quantitates the synergism. This value of R, significantly greater than unity, indicates enhanced potency and thus synergy for the interaction.

To confirm this synergy, we also determined AUC from 0 to 300 min using the trapezoidal rule. The combination dose-effect curve for WIN 55212-2 plus dextromethorphan was significantly elevated above the curve for WIN 55212-2 alone ( $F = 18.4, p < 0.05$ ) (Table 2; Fig. 4B). Again, it is seen that there is a pronounced leftward shift in the combination's regression line (Fig. 4B). R was determined to be 3.09 (95% confidence limits, 1.88–4.92), indicating that dextromethorphan increased the relative potency of WIN 55212-2 by 3.09-fold using AUC and confirming that the drug combination produces synergistic hypothermia.

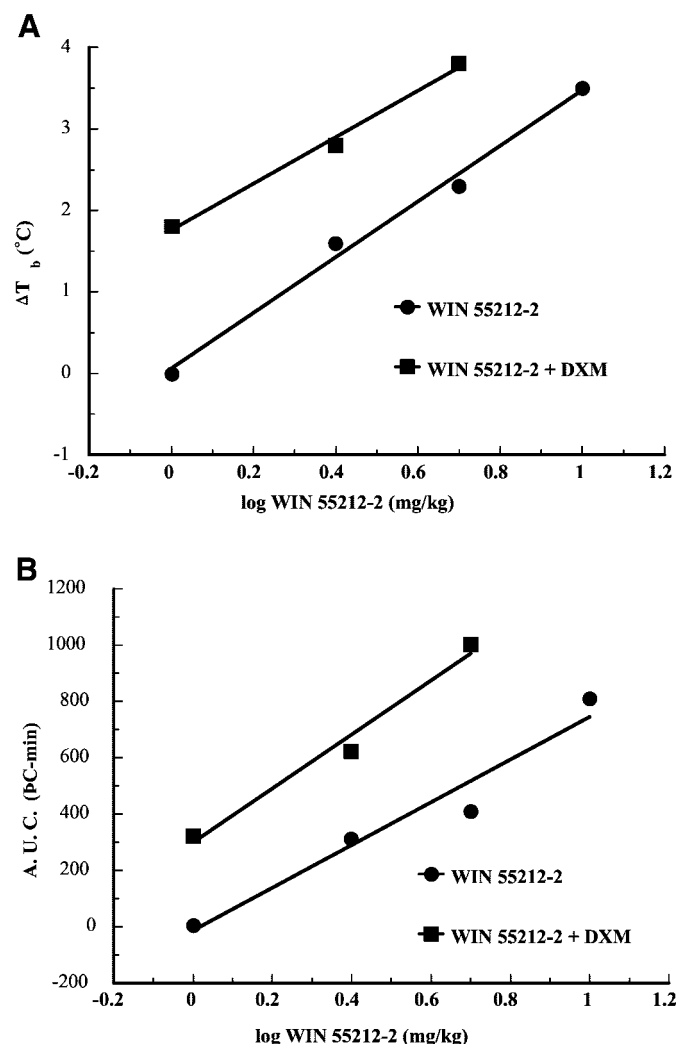
**WIN 55212-3 Does Not Produce Hypothermia by Itself or in Combination with Dextromethorphan.** WIN 55212-3 (5 mg/kg) did not cause hypothermia compared with 10% cremophor/saline (Fig. 5). Moreover, the combination of WIN 55212-3 (5 mg/kg) and dextromethorphan (10 mg/kg) was without effect on body temperature.

TABLE 1

Dose-response data for WIN 55212-2 and a combination of WIN 55212-2 + an inactive dose of dextromethorphan (10 mg/kg, i.m.) using the peak depression in body temperature (either 60 or 90 min)

WIN 55212-2 mg/kg	Effect* (Alone)	Effect (Combination)
1	0	1.8
2.5	1.6	2.8
5	2.3	3.8
10	3.5	

\* Value is the mean from at least 10 rats.



**Fig. 4.** A, regression analysis of WIN 55212-2 (1, 2.5, and 5 mg/kg i.m.) and WIN 55212-2 (1, 2.5, and 5 mg/kg i.m.) in combination with an inactive dose of dextromethorphan (10 mg/kg i.m.). The effect is the peak reduction in body temperature (°C) at 60 or 90 min and is determined from at least 10 animals. The log dose-response regression lines did not differ significantly from parallel ( $p < 0.05$ ) and revealed a shift measured as relative potency,  $r = 2.77$  (95% confidence limits, 2.17–3.53). B, regression analysis of WIN 55212-2 (1, 2.5, and 5 mg/kg i.m.) alone and in combination with an ineffective dose of dextromethorphan (10 mg/kg i.m.). The effect is AUC from 0 to 300 min and is determined from at least 10 animals. AUC was calculated using the trapezoidal rule. The combination dose-effect curve for WIN 55212-2 + DXM was significantly elevated ( $F = 18.4, p < 0.05$ ) above the curve for WIN 55212-2 alone and revealed a shift measured as relative potency,  $r = 3.09$  (95% confidence limits, 1.88–4.92).

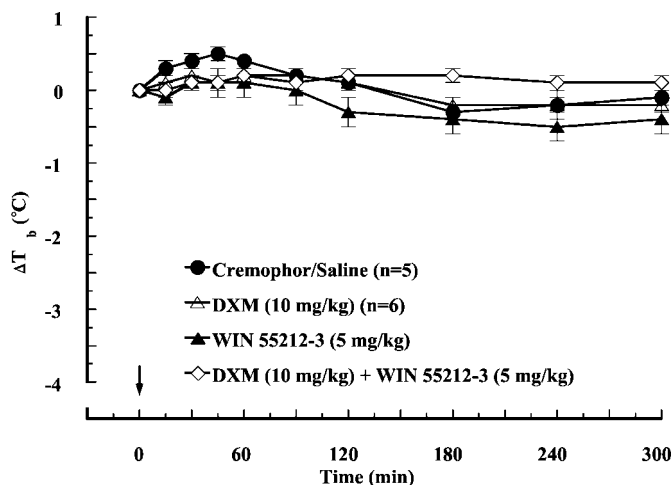
TABLE 2

Dose-response data for WIN 55212-2 and a combination of WIN 55212-2 + an inactive dose of dextromethorphan (10 mg/kg, i.m.)

The effect is the area under the body temperature time curve (AUC) from 0 to 300 min. The AUC was determined using the trapezoidal rule.

WIN 55212-2 mg/kg	Effect* (Alone)	Effect (Combination)
1	5	324
2.5	312	624
5	410	1003
10	810	

\* Value is the mean from at least 10 rats.

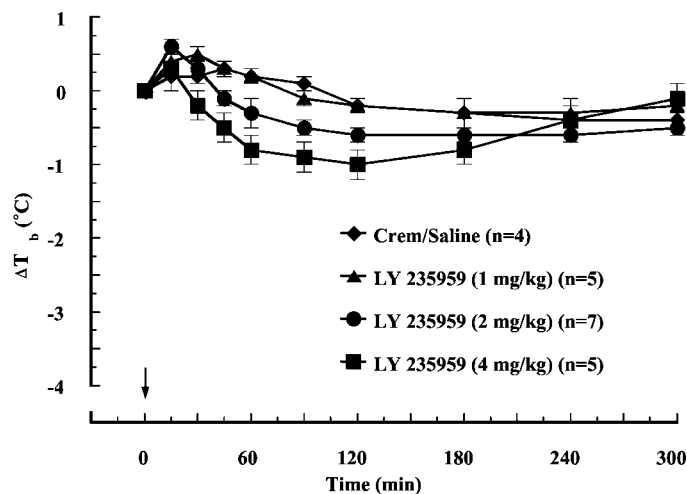


**Fig. 5.** WIN 55212-3 (5 mg/kg i.m.) by itself or in combination with dextromethorphan (DXM) (10 mg/kg i.m.) does not affect body temperature. Either 10% cremophor saline, DXM (10 mg/kg), WIN 55212-3 (5 mg/kg), or a combination of DXM (10 mg/kg) and WIN 55212-3 (5 mg/kg) was injected at 0 min, as indicated by arrow. Data are expressed as the mean  $\pm$  S.E. of body temperature. *n*, number of rats.  $\Delta T_b$ , change in body temperature from baseline (time 0). Tukey's post hoc analysis revealed that none of the groups differed significantly.

**LY 235959 Induces Hypothermia.** Doses of 2 and 4 mg/kg LY 235959 produced a slight, but significant, hypothermia relative to 10% cremophor/saline ( $p < 0.05$ ) (Fig. 6). The onset of hypothermia began 30 min postinjection, and the peak hypothermia occurred 120 min after the injection of LY 235959. Body temperature gradually recovered and approached predrug values 300 min postinjection.

**LY 235959 Augments WIN 55212-2-Evoked Hypothermia.** To validate the effects of dextromethorphan on WIN 55212-2-evoked hypothermia and confirm that NMDA receptors mediated the synergy, an inactive dose of LY 235959 (1 mg/kg; Fig. 6) was combined with a single dose of WIN 55212-2 (2.5 mg/kg). The combination of LY 235959 and WIN 55212-2 produced significant hypothermia relative to WIN 55212-2 alone (Fig. 7). On the basis of the temperature change at 90 min, there is a mean drop of 2.4°C for WIN 55212-2 and 3.3°C for the combination. On the basis of peak temperature drops, there is a mean reduction of 2.6°C for WIN 55212-2 and 3.3°C for the combination. Each of these determinations, based on  $n = 8$ , is significant ( $p < 0.05$ , Student's *t* test).

Two other aspects of the temporal profile are worth noting. First, LY 235959 prolonged the hypothermic response to WIN 55212-2, evident by the persistent hypothermia still present 300 min postinjection (Fig. 7). Second, WIN 55212-2



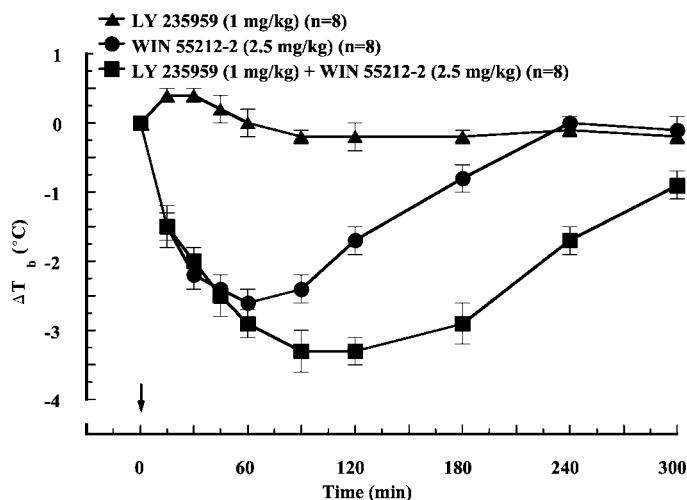
**Fig. 6.** LY 235959 (1–4 mg/kg i.m.) produces hypothermia in a dose-dependent manner. LY 235959 or 10% cremophor/saline was injected at 0 min, as indicated by arrow. Data are expressed as the mean  $\pm$  S.E. of body temperature. *n*, number of rats.  $\Delta T_b$ , change in body temperature from baseline (time 0). Groups receiving 2 or 4 mg/kg LY 235959 displayed significant hypothermia relative to the 10% cremophor/saline group,  $p < 0.05$ .

(2.5 mg/kg) by itself produced maximal hypothermia 60 min postinjection. However, in the presence of LY 235959, WIN 55212-2 produced maximal hypothermia 90 to 120 min postinjection.

## Discussion

The present experiments demonstrate that nonhypothermic doses of NMDA antagonists potentiate WIN 55212-2-evoked hypothermia. This enhancement was strongly synergistic, indicating that administration of these drug combinations leads to a greater-than-additive effect, most likely via interactions between NMDA and CB<sub>1</sub> receptors.

WIN 55212-2 elicited dose-dependent hypothermia, which



**Fig. 7.** An ineffective dose of LY 235959 (1 mg/kg i.m.) potentiates the hypothermia produced by a single dose of WIN 55212-2 (2.5 mg/kg i.m.). Either LY 235959 (1 mg/kg), WIN 55212-2 (2.5 mg/kg), or a combination of LY 235959 (1 mg/kg) and WIN 55212-2 (2.5 mg/kg) was injected at 0 min, as indicated by arrow. Data are expressed as the mean  $\pm$  S.E. of body temperature. *n*, number of rats.  $\Delta T_b$ , change in body temperature from baseline (time 0). The group receiving the drug combination of WIN 55212-2 (2.5 mg/kg) and DXM (10 mg/kg) displayed significant hypothermia relative to the WIN 55212-2 alone (2.5 mg/kg) group,  $p < 0.05$ .

was rapid in onset and persisted for at least 180 min. WIN 55212-3, the inactive enantiomer of WIN 55212-2, did not alter body temperature, suggesting a cannabinoid receptor mechanism. Indeed, previous studies demonstrating that SR141716A, a selective CB<sub>1</sub> antagonist, blocks cannabinoid-induced hypothermia and that WIN 55212-2 fails to evoke hypothermia in mice devoid of CB<sub>1</sub> receptors have established a role for CB<sub>1</sub> receptors in the hypothermic effects of cannabinoids (Compton et al., 1996; Costa et al., 1999; Lédent et al., 1999; Fox et al., 2001).

Dextromethorphan or LY 235959 alone produced a slight, but significant, hypothermia, supporting the existence of an endogenous glutamate system that exerts a hyperthermic tone via NMDA receptor activation. These data concur with previous studies, which have reported that 75 mg/kg dextromethorphan or 2 mg/kg LY 235959 cause hypothermia in rats (Farfel and Seiden, 1995; Bhargava and Thorat, 1997). Although this hypothermia is modest compared with other drugs, such as cannabinoid and  $\kappa$ -opioid agonists (Adler and Geller, 1987; Compton et al., 1992; Fan et al., 1994), it nonetheless implicates NMDA receptors in the tonic modulation of body temperature. A role for NMDA receptors in thermoregulation is underscored further by the fact that a NMDA receptor mechanism mediates the fever caused by injections of glutamate into the ventricles or organum vasculosum laminae terminalis (Singh and Gupta, 1997; Huang et al., 2001). Cells expressing NMDA receptor mRNA have been detected in thermosensitive regions of the hypothalamus (van den Pol, 1991) where NMDA receptor immunoreactivity and NMDA receptor binding sites are present (Bhat et al., 1995; Paquet and Smith, 2000). Although it is beyond the scope of the present investigation to determine the site of action of dextromethorphan- and LY 235959-induced hypothermia, the presence of NMDA receptor protein in the preoptic area implicates the POAH.

The remarkable similarities between dextromethorphan- and LY 235959-induced hypothermia suggest that a common mechanism mediates the effects. LY 235959 is a highly selective NMDA antagonist that competitively blocks the glutamate recognition site at the NMDA receptor complex. This site is distinct from that of the well known NMDA antagonist MK-801 (dizocilpine maleate), which produces a noncompetitive block of the NMDA receptor-operated ion channel (Rasmussen et al., 1991). The site of action of LY 235959 is also distinct from that of dextromethorphan, which produces an allosteric, noncompetitive block of NMDA receptor-gated channels (Tortella et al., 1989). Unlike LY 235959, dextromethorphan interacts with a number of neurotransmitter systems, raising the possibility that receptors other than NMDA mediated the hypothermia associated with dextromethorphan. Indeed, dextromethorphan blocks voltage-sensitive calcium channels, and calcium channel antagonists produce hypothermia in rodents (Gordon and Stead, 1986; Carpenter et al., 1988; Tortella et al., 1989). Dextromethorphan also inhibits serotonin uptake transporters and interacts with sigma sites (Tortella et al., 1989; Henderson and Fuller, 1992). Because serotonin and sigma sites have been implicated in thermoregulation (Bejanian et al., 1991; Malone and Taylor, 2001), an involvement of these systems in dextromethorphan-induced hypothermia cannot be discounted.

The major finding in the present study is that drug com-

binations of nonhypothermic doses of NMDA antagonists and WIN 55212-2 produce synergistic effects on hypothermia. In particular, dextromethorphan increased the relative potency of WIN 55212-2 by approximately 3-fold, indicating that the drug interaction was strongly synergistic. To confirm that NMDA receptors mediated the synergy, a dose of LY 235959 that did not alter body temperature by itself was coadministered with WIN 55212-2. LY 235959 also potentiated WIN 55212-2-evoked hypothermia, with the most pronounced augmentation occurring 90 to 300 min postinjection. The enhancement by LY 235959 of WIN 55212-2-evoked hypothermia validates the synergy caused by the combination of dextromethorphan and WIN 55212-2 and strengthens our observation that blocking NMDA receptors augments cannabinoid-evoked hypothermia.

Little has been reported on the effect of NMDA antagonists on cannabinoid-induced responses. A recent study demonstrated that pretreatment with NMDA receptor antagonists blocked WIN 55212-2-induced analgesia, leading the authors to suggest that CB<sub>1</sub> receptors may modulate cannabinoid-mediated antinociception in the periaqueductal gray matter of rats (Palazzo et al., 2001). Other studies have demonstrated that competitive, but not noncompetitive, NMDA antagonists enhance the catalepsy induced by  $\Delta$ -9-tetrahydrocannabinol in mice (Kinoshita et al., 1994). LY 235959 has been shown to potentiate  $\kappa$ -agonist-induced hypothermia in rats (Bhargava and Thorat, 1997).

Because of a lack of information regarding interactions between cannabinoid and glutamate systems, elucidation of a mechanism is difficult. Although conjectural, glutamate release may increase in response to cannabinoid-induced hypothermia, possibly as a compensatory reaction to the marked reduction in body temperature. In this model, WIN 55212-2 evokes hypothermia by activating CB<sub>1</sub> receptors. In turn, glutamate release, and eventually extracellular glutamate levels, increase in regions of the CNS that mediate body temperature. The rise in glutamate levels stimulates NMDA receptors, generating a hyperthermia that counteracts the CB<sub>1</sub>-mediated hypothermia. This model is supported by a recent *in vivo* microdialysis study demonstrating that 1 mg/kg WIN 55212-2 increased extracellular glutamate levels in the prefrontal cortex, although higher doses were ineffective (Ferraro et al., 2001). Our data suggest that NMDA receptor blockade may potentiate cannabinoid-induced hypothermia by diminishing the compensatory effects of glutamate-induced hyperthermia.

Although Ferraro et al. (2001) reported that WIN 55212-2 increased *in vivo* extracellular glutamate levels, it is possible that WIN 55212-2 produced hypothermia by decreasing glutamate release. One explanation is that activation of CB<sub>1</sub> receptors by WIN 55212-2 reduces glutamate release in regions of the CNS that regulate body temperature, leading to a decline in cumulative glutamate levels and removal of the hyperthermic tone mediated by endogenous glutamate. This effect of WIN 55212-2 may be enhanced further in the presence of NMDA antagonists, which disrupt NMDA transmission by blocking NMDA receptors. Another study investigating cannabinoid-glutamate interactions reported that SR141716A produced nociception in the spinal cord, presumably by abolishing an endogenous cannabinoid tone mediated via CB<sub>1</sub> receptors (Richardson et al., 1998). In the same study, NMDA antagonists blocked SR 141716A-induced hy-

peralgesia, leading Richardson and colleagues to conclude that hypoactivity of the spinal cannabinoid system results in NMDA-dependent nociception.

Indeed, most *in vitro* studies indicate that cannabinoids inhibit glutamate release. WIN 55212-2 has been reported to diminish glutamatergic transmission in the striatum and hippocampus via a CB<sub>1</sub> mechanism (Gerdeman and Lovinger, 2001; Hajos et al., 2001). Cannabinoids also inhibit electrically evoked glutamate release in cultured hippocampal neurons (Shen et al., 1996). Furthermore, activation of CB<sub>1</sub> receptors inhibits adenylyl cyclase, which has been implicated in the regulation of exocytosis (Howlett, 1984; Chavez-Noriega and Stevens, 1994) and closes certain calcium channels that are necessary for neurotransmitter release (Mackie and Hille, 1992). It is also worth noting that anandamide, an endogenous cannabinoid ligand, has been reported to modulate NMDA receptor activity in a cannabinoid-dependent and -independent manner (Hampson et al., 1998). Those *in vitro* data suggest that WIN 55212-2 suppresses glutamatergic transmission, an effect that would presumably be enhanced in the presence of NMDA antagonists, leading to an overall potentiation in WIN 55212-2-induced hypothermia. Unfortunately, lack of information concerning the effect of cannabinoid agonists on *in vivo* glutamate levels and the cellular localization of NMDA and CB<sub>1</sub> receptors in thermosensitive substrates precludes determining the mechanism of the cannabinoid-glutamatergic interaction.

In conclusion, we have shown that drug combinations of a NMDA antagonist and cannabinoid agonist generate synergistic hypothermia. Our data indicate that intimate interactions between NMDA and CB<sub>1</sub> receptors exist within the CNS and that the glutamate system plays a crucial role in cannabinoid-mediated effects. It is possible that the interactive synergy between dextromethorphan and WIN 55212-2 may have clinical relevance. Dextromethorphan, the active ingredient in several over-the-counter antitussive formulations, is readily available to users of  $\Delta^9$ -THC-like drugs. Furthermore, we have shown a potential use for low doses of dextromethorphan to enhance the potency of cannabinoid drugs, thereby reducing the need to escalate doses of cannabinoids. This possibility could be especially meaningful, because dextromethorphan is as potent as codeine in suppressing cough, yet lacks the central side effects of opiates in humans, such as respiratory depression, analgesia, and abuse liability. The use of body temperature, a precise metric, provides an analysis that demonstrates synergism and its statistical confirmation. Thus, the present data could provide an initial step in illuminating a mechanism because the same drug combination may also apply to endpoints other than body temperature.

#### Acknowledgments

We thank David Kon and Andy Baron for assistance in the recording of body temperature data.

#### References

- Adler MW and Geller EB (1987) Hypothermia and poikilothermia induced by a  $\kappa$ -agonist opioid and a neuroleptic. *Eur J Pharmacol* **140**:233–237.
- Bejanian M, Pechnick RN, Bova MP, and George R (1991) Effects of subcutaneous and intracerebroventricular administration of the sigma receptor ligand 1,3-di-O-tolylguanidine on body temperature in the rat: interactions with BMY 14802 and rimcazole. *J Pharmacol Exp Ther* **258**:88–93.
- Bhargava HN and Thorat SN (1997) Differential effects of LY235959, a competitive antagonist of the NMDA receptor on kappa-opioid receptor agonist induced responses in mice and rats. *Brain Res* **747**:246–251.
- Bhat GK, Mahesh VB, Lamar CA, Ping L, Aguan K, and Brann DW (1995) Histochemical localization of nitric oxide neurons in the hypothalamus: association with gonadotropin-releasing hormone neurons and co-localization with N-methyl-D-aspartate receptors. *Neuroendocrinology* **62**:187–197.
- Carpenter CL, Marks SS, Watson DL, and Greenberg DA (1988) Dextromethorphan and dextrorphan as calcium channel antagonists. *Brain Res* **439**:372–375.
- Chavez-Noriega LE and Stevens CF (1994) Increased transmitter release at excitatory synapses produced by direct activation of adenylyl cyclase in rat hippocampal slices. *J Neurosci* **14**:310–317.
- Compton DR, Aceto MD, Lowe J, and Martin BR (1996) *In vivo* characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. *J Pharmacol Exp Ther* **277**:586–594.
- Compton DR, Gold LH, Ward SJ, Balster RL, and Martin BR (1992) Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. *J Pharmacol Exp Ther* **263**:1118–1126.
- Corbett D, Evans S, Thomas C, Wang D, and Jonas RA (1990) MK-801 reduced cerebral ischemic injury by inducing hypothermia. *Brain Res* **514**:300–304.
- Costa B, Vailati S, and Colleoni M (1999) SR 141716A, a cannabinoid receptor antagonist, reverses the behavioural effects of anandamide-treated rats. *Behav Pharmacol* **10**:327–331.
- Dragic T, Litwin V, Allaway GP, Martin SR, Huang YX, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, et al. (1996) HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature (Lond)* **381**:667–673.
- Fan F, Compton DR, Ward S, Melvin L, and Martin BR (1994) Development of cross-tolerance between delta 9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. *J Pharmacol Exp Ther* **271**:1383–1390.
- Farfel GM and Seiden LS (1995) Role of hypothermia in the mechanism of protection against serotonergic toxicity. II. Experiments with methamphetamine, p-chloroamphetamine, fenfluramine, dizocipine and dextromethorphan. *J Pharmacol Exp Ther* **272**:868–875.
- Ferraro L, Tomasini MC, Gessa GL, Bebe BW, Tanganelli S, and Antonelli T (2001) The cannabinoid receptor agonist WIN 55,212[hyphen 2 regulates glutamate transmission in rat cerebral cortex: an *in vivo* and *in vitro* study. *Cereb Cortex* **11**:728–733.
- Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L, and James I (2001) The role of central and peripheral cannabinoid<sub>1</sub> receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain* **92**:91–100.
- Gerdeman G and Lovinger DM (2001) CB<sub>1</sub> cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J Neurophysiol* **85**:468–471.
- Gordon CJ and Stead AG (1986) Effect of nickel and cadmium chloride on autonomic and behavioral thermoregulation in mice. *Neurotoxicology* **7**:97–106.
- Hajos N, Ledent C, and Freund TF (2001) Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* **106**:1–4.
- Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM, Leonoudakis DJ, and Bickler PE (1998) Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J Neurochem* **70**:671–676.
- Henderson MG and Fuller RW (1992) Dextromethorphan antagonizes the acute depletion of brain serotonin by p-chloroamphetamine and H75/12 in rats. *Brain Res* **594**:323–326.
- Howlett AC (1984) Inhibition of neuroblastoma adenylylase by cannabinoid and nandradol compounds. *Life Sci* **35**:1803–1810.
- Howlett AC (1995) Pharmacology of cannabinoid receptors. *Annu Rev Pharmacol Toxicol* **35**:607–634.
- Huang WT, Tsai SM, and Lin MT (2001) Involvement of brain glutamate release in pyrogenic fever. *Neuropharmacology* **41**:811–818.
- Jansen EM, Haycock DA, Ward SJ, and Seybold VS (1992) Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. *Brain Res* **575**:93–102.
- Kelly S and Chapman V (2001) Selective cannabinoid CB<sub>1</sub> receptor activation inhibits spinal nociceptive transmission *in vivo*. *J Neurophysiol* **86**:3061–3064.
- Kinoshita H, Hasegawa T, Kameyama T, Yamamoto I, and Nabeshima T (1994) Competitive NMDA antagonists enhance the catalepsy induced by delta 9-tetrahydrocannabinol in mice. *Neurosci Lett* **174**:101–104.
- Ledent C, Valverde O, Cossu G, Petit F, Aubert J-F, Beslot F, Böhme GA, Imperato A, Pedrazzini T, Roques BP, et al. (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB<sub>1</sub> receptor knockout mice. *Science (Wash DC)* **283**:401–404.
- Mackie K and Hille B (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* **89**:3825–3829.
- Mailleux P and Vanderhaeghen JJ (1992) Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and *in situ* hybridization histochemistry. *Neuroscience* **48**:655–668.
- Malone DT and Taylor DA (2001) Involvement of somatodendritic 5-HT<sub>1A</sub> receptors in Delta(9)-tetrahydrocannabinol-induced hypothermia in the rat. *Pharmacol Biochem Behav* **69**:595–601.
- Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, and Ward SJ (1991) Behavioral, biochemical and molecular modeling evaluations of cannabinoid analogs. *Pharmacol Biochem Behav* **40**:471–478.
- Moldrich G and Wenger T (2000) Localization of the CB<sub>1</sub> cannabinoid receptor in the rat brain. An immunohistochemical study. *Peptides* **21**:1735–1742.
- Palazzo E, Marabese I, de Novellis V, Oliva P, Rossi F, Berrino L, Rossi F, and Maione S (2001) Metabotropic and NMDA glutamate receptors participate in the cannabinoid-induced antinociception. *Neuropharmacology* **40**:319–326.
- Paquet M and Smith Y (2000) Presynaptic NMDA receptor subunit immunoreactivity in GABAergic terminals in rat brain. *J Comp Neurol* **423**:330–347.

- Pechnick RN, Wong CA, George R, Thurkauf A, Jacobson AE, and Rice KC (1989) Comparison of the effects of the acute administration of dexoxadrol, levoxadrol, MK-801 and phencyclidine on body temperature in the rat. *Neuropharmacology* **28**:829–835.
- Rasmussen K, Fuller RW, Stockton ME, Perry KW, Swinford RM, and Ornstein PL (1991) NMDA receptor antagonists suppress behaviors but not norepinephrine turnover or locus coeruleus unit activity induced by opiate withdrawal. *Eur J Pharmacol* **197**:9–16.
- Rawls SM, Cabassa J, Geller EB, and Adler MW (2002) CB<sub>1</sub> receptors in the preoptic anterior hypothalamic nucleus regulate WIN 55212-2-induced hypothermia. *J Pharmacol Exp Ther* **301**:963–968.
- Richardson JD, Aanonsen L, and Hargreaves KM (1998) Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. *J Neurosci* **18**:451–457.
- Shen M, Piser TM, Seybold VS, and Thayer SA (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci* **16**:4322–4334.
- Singh J and Gupta MC (1997) Effect of aspartate and glutamate on nociception, catalepsy and core temperature in rats. *Indian J Physiol Pharmacol* **41**:123–128.
- Tallarida RJ (2001) Drug synergism: its detection and applications. *J Pharmacol Exp Ther* **298**:865–872.
- Tortella FC, Pellicano M, and Bowery NG (1989) Dextromethorphan and neuro-modulation: old drug coughs up new activities. *Trends Pharmacol Sci* **10**:501–507.
- van den Pol AN (1991) Glutamate and aspartate immunoreactivity in hypothalamic presynaptic axons. *J Neurosci* **11**:2087–2101.
- Yakimova K and Ovtcharov R (1989) Central temperature effects of the transmitter amino acids. *Acta Physiol Pharmacol Bulg* **15**:50–54.

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