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Attenuation of morphine antinociceptive tolerance by a CB_1 receptor agonist and an NMDA receptor antagonist: Interactive effects

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ABSTRACT

CB_1 cannabinoid (CB_1) receptor agonists and *N*-Methyl-D-Aspartate (NMDA) receptor antagonists attenuate the development of morphine antinociceptive tolerance. The present study used dose-addition analysis to evaluate CB_1/NMDA receptor interactions on this endpoint. Chronic morphine administration (5 days, 100 mg/kg, twice daily) resulted in a 2.8-fold rightward shift in the morphine dose-effect curve. Co-administration of either the CB_1 receptor agonist CP-55940 (5-(1,1-Dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol; 0.32–1.0 mg/kg) or the NMDA receptor antagonist (−)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959; 1.0–3.2 mg/kg) with morphine dose-dependently attenuated morphine tolerance. The relative potency of each drug alone was quantified using a defined level of effect (one-quarter log shift in the morphine dose-effect curve), resulting in equieffective doses of 0.42 mg/kg and 1.1 mg/kg for CP-55940 and LY235959, respectively. Subsequent experiments assessed CP-55940/LY235959 interactions using a fixed-proportion design. Co-administration of CP-55940/LY235959 mixtures (1:1, 1:3.2, or 1:10 CP-55940/LY235959) with morphine dose-dependently attenuated morphine tolerance. Isobolographic and dose-addition analysis were used to statistically compare the experimentally determined potency for each mixture (z_{mix}) with predicted additive potency (z_{add}). Mixtures of 1:1 and 1:3.2 CP-55940/LY235959 produced additive effects ($z_{add} = z_{mix}$), while the mixture of 1:10 CP-55940/LY235959 produced a supra-additive effect ($z_{add} > z_{mix}$). These results suggest that CP-55940 and LY235959 produce additive or supra-additive attenuation of morphine antinociceptive tolerance after repeated morphine administration, depending on their relative concentrations.

1. Introduction

Repeated administration of opioids such as morphine results in the development of tolerance and physical dependence. Drugs that modulate the CB_1 cannabinoid or *N*-Methyl-D-Aspartate (NMDA) glutamate receptor systems can modulate the development of morphine tolerance and dependence. For example, chronic cannabinoid administration attenuates morphine antinociceptive tolerance and reduces naloxone-precipitated withdrawal in morphine dependent rodents (Vela et al., 1995; Cichewicz and Welch, 2003; Smith et al., 2007). Similarly, drugs with antagonist activity at NMDA receptors reduce tolerance and physical dependence after repeated morphine administration (for review, see Trujillo, 2000). Together with data from CB_1 receptor knockout mice (Ledent et al., 1999; Lichtman et al., 2001), these findings suggest that both the CB_1 and NMDA receptor systems play a key role in the behavioral plasticity associated with chronic morphine administration.

In addition to the common behavioral effect of attenuating morphine tolerance, CB_1 and NMDA receptors have been linked neurochemically, vis a vis their influence over and dependence on glutamatergic synaptic transmission, respectively. Through retrograde neurotransmission, cannabinoid systems modify the activity of pre-synaptic neurons, including those that contain excitatory glutamate receptor systems (Ohno-Shosaku et al., 2001; Alger, 2002; Freund et al., 2003). Specifically, activation of CB_1 cannabinoid receptors inhibits excitatory neurotransmission by decreasing glutamate release, thereby reducing activity at glutamatergic NMDA receptors (Auclair et al., 2000; Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2002; Brown et al., 2004; Godino et al., 2007).

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Therefore, it is possible that pharmacological stimulation of CB_1 cannabinoid receptors may augment the neurochemical and behavioral effects of an NMDA receptor antagonist.

The purpose of the current study was to assess the interactive effects of a CB_1 receptor agonist and an NMDA receptor antagonist on the attenuation of morphine antinociceptive tolerance. Interactions between CB_1 and NMDA receptor systems were first assessed graphically with the use of isobolograms to distinguish effects that are additive from effects that are infra-additive or supra-additive. Statistical analysis of dose-addition was also used for the quantitative assessment of drug interactions (Tallarida et al., 1997; Tallarida, 2000). Both isobolographic analysis and dose-addition analysis are based on the theory of dose equivalence, and are used to make predictions on the effects of two drugs administered concurrently based on the relative potency of each drug administered alone. If the mechanisms of action of two drugs are mediated through different receptors, deviation from additivity suggests an interaction between their receptor-mediated signals.

The effects of 5-(1,1-Dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol (CP-55940), a cannabinoid agonist whose central actions are mediated via CB_1 receptors, and (−)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959), an NMDA receptor antagonist with high selectivity for the competitive site on the NMDA receptor complex, were initially assessed for their ability to attenuate morphine antinociceptive tolerance. The relative potency of each drug on this endpoint was determined using a defined level of effect (one-quarter log rightward shift in the morphine dose-effect curve). Subsequently, fixed-ratio mixtures containing both CP-55940 and LY235959 were administered during the chronic morphine regimen, and the experimentally determined effects of these drug mixtures were compared to their predicted additive effects.

2. Methods

2.1. Animals

Adult male C57BL/6 mice were purchased from Jackson Labs (Raleigh, NC). Upon arrival, mice were group housed in standard plexiglas cages in a colony room maintained on a 12-h light/dark cycle (lights on at 7:00 PM). All mice had continuous access to food and water throughout the study and were habituated to the colony room environment for 2 weeks prior to any experimental manipulation. Throughout all testing the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) was adhered to. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Drugs

Morphine sulfate was provided by NIDA (Bethesda, MD) and LY235959 by Lilly Research Laboratories (Indianapolis, IN). CP-55940 was purchased from Sigma (St. Louis, MO). Morphine and LY235959 were dissolved in 0.9% phosphate buffered saline. CP-55940 was dissolved in a vehicle of 100% ethanol, Alkamuls EL-620 (Rhodia, Cranbury NJ) and saline in a 1:1:18 ratio. All drugs were injected s.c. at a volume of 0.1 ml/10 g.

2.3. Hot plate procedure

Nociception and morphine-induced antinociception were assessed with a hot plate analgesia meter (Columbus Instruments, Columbus, OH) maintained at 56 ± 0.1 °C. The hot plate procedure was chosen as the behavior required to respond to the stimulus is thought to be associated with both spinal and supraspinal involvement in nociception (Chapman et al., 1985; Dubner and Ren, 1999). During this procedure, the nociceptive response was evaluated by recording the latency to lick or shuffle the hind paw(s), and/or to jump from the hot plate surface. A stopwatch measured responses to the nearest 0.1 s. A predetermined cutoff time of 20 s was defined as a maximal response and was employed to prevent tissue damage. Immediately following the termination of a trial, mice were removed from the hot plate surface and returned to the homecage. The latency to respond at 56 °C was measured twice at 30 and 15 min prior to the beginning of drug administration, and these data were averaged to yield one baseline value. Following baseline latency measurements, multiple 30 min cycles were run and drug mixtures were administered cumulatively. During this procedure, cumulative doses of morphine were administered during the first min of each cycle (i.e., 30-min inter injection interval), increasing in one-half log unit increments, and antinociceptive measurements were determined during the last minute of each cycle. Latencies obtained following morphine administration are expressed as a percentage of the maximal possible effect (%MPE) using the following formula: %MPE = [postdrug latency (sec) − baseline latency (s)]/[cutoff time (20 s) − baseline latency (s)].

2.4. Tolerance induction

Morphine dose-effect curves (1.0–32.0 mg/kg) were first determined at 13:00 on day 1. Morphine injections (100 mg/kg) were then administered twice daily at 08:00 and 18:00 for 5 days (days 2–6). A morphine dose-effect curve (3.2–100 mg/kg) was then re-determined at 08:00 on day 7. Initial experiments assessed the effects of chronic morphine alone, and in combination with CP-55940 (0.32–1.0) and LY235959 (1.0–3.2). Subsequent experiments assessed the effects of chronic morphine in combination with mixtures containing both CP-55940 and LY235959. Mixtures of CP-55940 and LY235959 were assessed at fixed ratios of 1:1 (0.32–3.2 mg/kg), 1:3.2 (0.1–3.2 mg/kg), and 1:10 (0.1–3.2 mg/kg) CP-55940/LY235959.

2.5. Data analysis

The dose of morphine required to produce 50% maximum antinociceptive effect (ED_{50}) during the pre-chronic and post-chronic hot plate tests was derived using linear regression when at least three data points were available on the linear portion of the dose-effect curve or by interpolation when only two data points (one above and one below 50%) were available. An index of relative potency was determined for each mouse by subtracting the log ED_{50} dose determined on day 7 from the log ED_{50} dose determined on day 1. Individual ED_{50} values were then averaged, and converted back to linear values for presentation. Therefore, a relative potency of 1 suggests a lack of tolerance development (i.e., no shift in the morphine dose-effect curve). In contrast, a relative potency greater than 1 suggests that tolerance has developed (i.e., a rightward shift in the morphine dose-effect curve), and a quantitatively greater relative potency is indicative of increased tolerance development.

Interactions between CP-55940 and LY235959 on the attenuation of morphine tolerance were assessed using both graphical and statistical approaches. A one-quarter log shift in the morphine dose-effect curve was chosen as an effect level for analysis, as it is on the linear portion of both the CP-55940 and LY235959 dose-effect curves. Graphically, the distinction between infra-additive, additive, or supra-additive interactions were made with the use of isobolograms. In the current study, isobolograms were constructed by plotting an effective dose of CP-55940 (z_2^*) on the ordinate and an equieffective dose of LY235959 (z_1^*) on the abscissa. A line of additivity from the equation $(z_1/z_1^*) + (z_2/z_2^*) = 1$ represents the loci of dose pairs (z_1,z_2) that are predicted to produce an effect equal to each drug administered alone, if the combination is additive. Dose pairs that fall below the additivity line suggest the effect was reached with lesser quantities of the drugs, suggestive of supra-additivity. In contrast, experimental points representing dose pairs that fall above the line are suggestive of infra-additivity.

Drug interactions were statistically analyzed by comparing the experimentally determined potency for each mixture (z_{mix}) with the predicted additive potency (z_{add}) as described by Tallarida et al. (1997) and Tallarida (2000). The experimentally determined potency was defined as the total drug dose (i.e., dose CP-55940 + dose LY235959) that produced a one-quarter log shift in the morphine dose-effect curve. The predicted additive potency was defined by the equation $z_{add} = fz_1^* + (1 - f)z_2^*$. The value of f ($0 \geq f \geq 1$) is related to the proportion of LY235959 in the mixture (p) and is defined by the equation $f = p(z_2^*)/(z_1^* + p(z_2^*) - p(z_1^*))$. A statistical test of significance was made ($z_{add} - z_{mix}$) as described (Tallarida et al., 1997; Tallarida, 2000). If the difference is significantly different from zero, it was concluded that CP-55940 and LY235959 were interacting in an infra-additive ($z_{add} < z_{mix}$) or supra-additive ($z_{add} > z_{mix}$) manner.

3. Results

3.1. Morphine antinociceptive tolerance

Fig. 1 shows the antinociceptive effects of morphine before (Day 1) and after (Day 7) chronic administration of saline, 32 mg/kg morphine, or 100 mg/kg morphine. Prior to chronic treatment, morphine produced dose-dependent increases in latency to respond on the hot plate, resulting in ED_{50} values (95% CL) of 5.2 (4.0–6.7), 9.6 (7.3–12), and 5.5 (4.5–6.6) for the saline, 32 mg/kg morphine, and 100 mg/kg morphine groups, respectively. After chronic administration of saline, the re-determination of the morphine dose-effect curve resulted in an ED_{50} value of 7.7 (6.1–9.6) and a relative potency (95% CL) of 1.5 (1.0–2.2). Chronic administration of morphine produced dose-dependent rightward shifts in

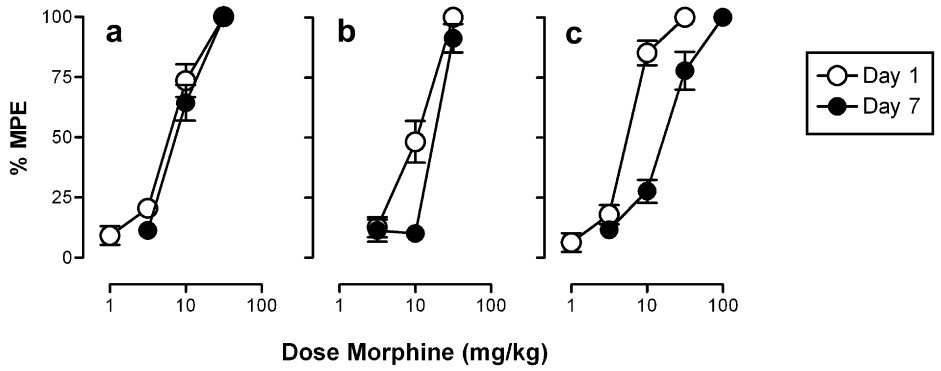

Fig. 1. Morphine antinociception before (open symbols) and after (closed symbols) chronic administration of saline (panel a), 32 mg/kg morphine (panel b), and 100 mg/kg morphine (panel c). Abscissae, cumulative dose of morphine in mg/kg. Ordinate, antinociception as percent maximum possible effect. Each data point represents the mean (±S.E.M.) from 8 mice.

the morphine dose-effect curves. When assessed after chronic treatment, the re-determination of the morphine dose-effect curves resulted in ED_{50} values of 17 (13–22) and 15 (12–19) and relative potencies of 1.8 (1.2–2.7) and 2.8 (2.2–3.7) for the 32 mg/kg morphine and 100 mg/kg morphine groups, respectively.

3.2. CP-55940 and LY235959 on morphine antinociceptive tolerance

Fig. 2 shows the antinociceptive effects of morphine before and after chronic administration of 100 mg/kg morphine in combination with CP-55940 (0.32–1.0 mg/kg; panels a–c) or LY235959 (1.0–3.2 mg/kg; panels d–f). Both CP-55940 and LY235959 dose-dependently attenuated the development of morphine antinociceptive tolerance as evidenced by a decrease in the relative potency of morphine assessed on day 1 and day 7 (shown graphically in Fig. 4). A statistical test for parallelism revealed that the CP-55940 dose-effect curve was parallel to the dose-effect curve for LY235959 ($p < 0.05$). The doses of CP-55940 (z_2*; 0.42 mg/kg) and LY235959 (z_1*; 1.1 mg/kg) that produced a one-quarter log shift in the morphine dose-effect curve were used in subsequent analyses (see Data Analysis).

3.3. Fixed-ratio mixtures of CP-55940 and LY235959 on morphine antinociceptive tolerance

Fig. 3 shows the antinociceptive effects of morphine before and after chronic administration of 100 mg/kg morphine in

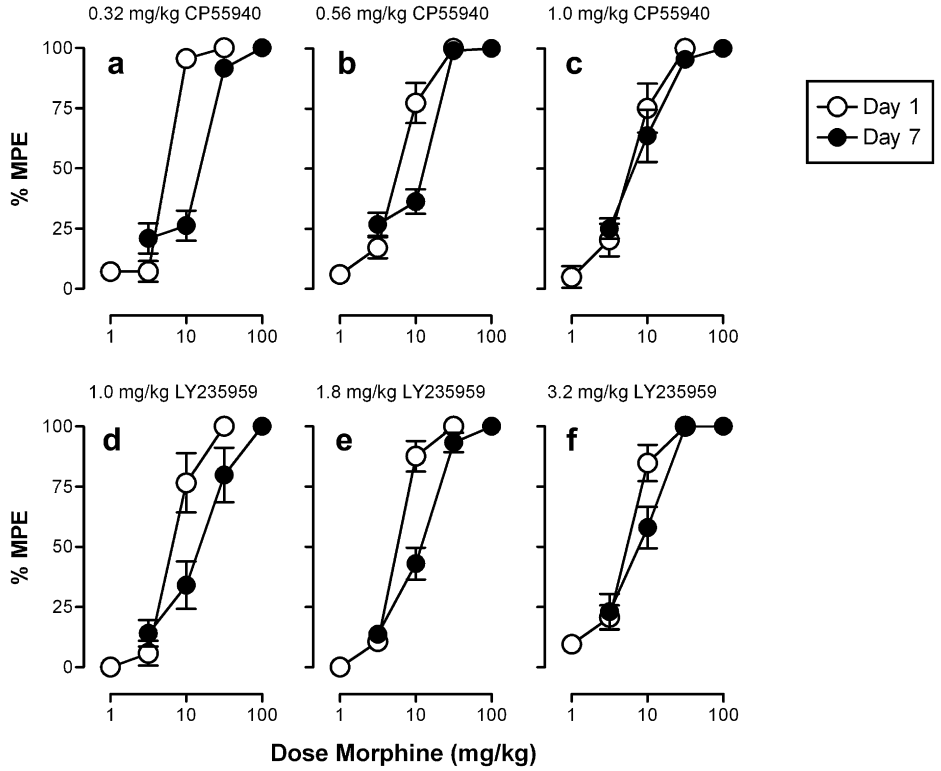

Fig. 2. Morphine antinociception before (open symbols) and after (closed symbols) chronic morphine administration in combination with CP-55940 (panels a–c) or LY235959 (panels d–f). The dose of CP-55940 or LY235959 administered in combination with 100 mg/kg morphine is denoted above each panel. Abscissae, cumulative dose of morphine in mg/kg. Ordinate, antinociception as percent maximum possible effect. Each data point represents the mean (±S.E.M.) from 8 mice.

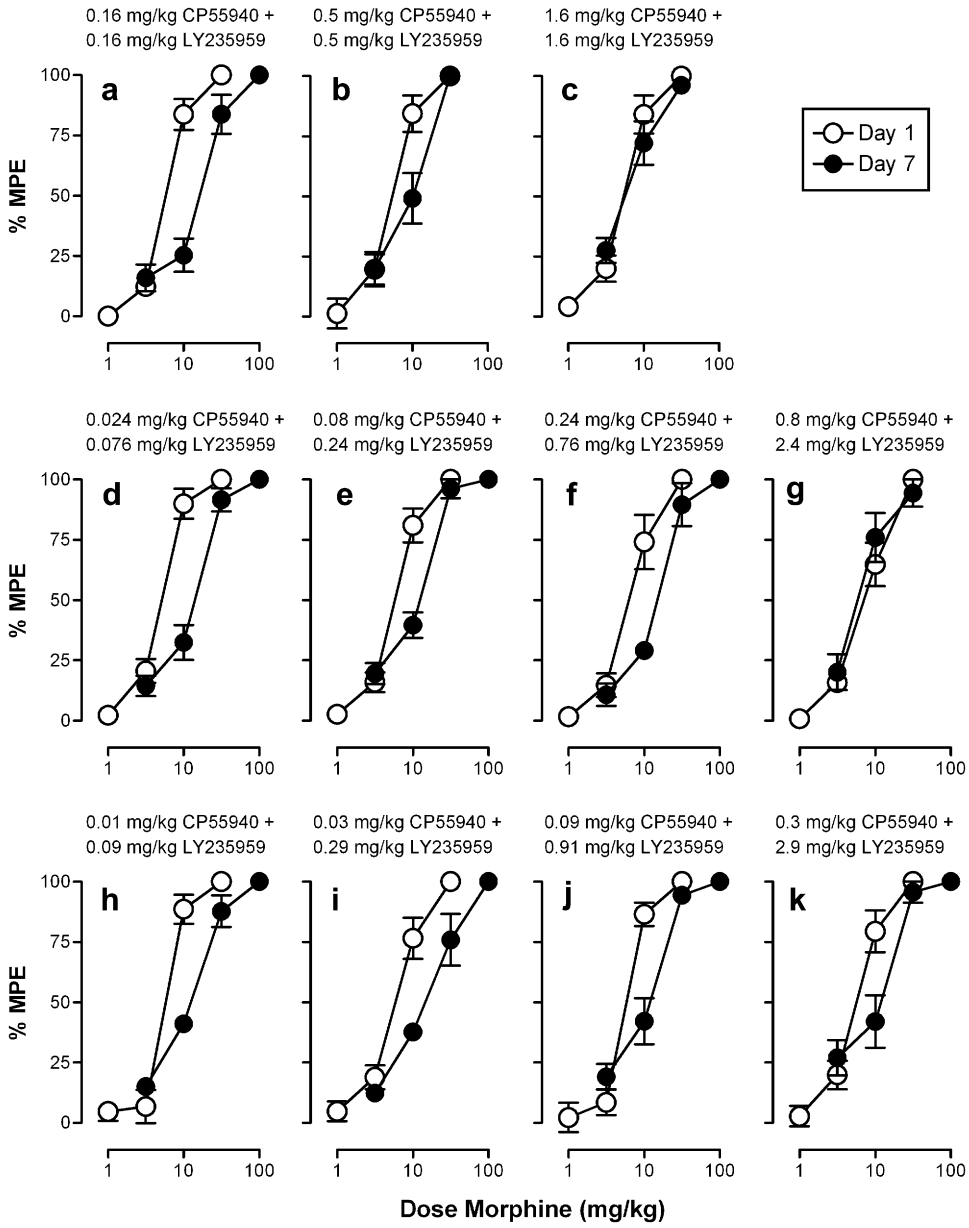

Fig. 3. Morphine antinociception before (open symbols) and after (closed symbols) chronic morphine administration in combination with CP-55940/LY235959 mixtures. Drug mixtures were held at fixed ratios of 1:1 (panels a–c), 1:3.2 (panels d–g), and 1:10 (panels h–k). The doses of CP-55940 and LY235959 administered in combination with 100 mg/kg morphine are denoted above each panel. Abscissae, cumulative dose of morphine in mg/kg. Ordinate, antinociception as percent maximum possible effect. Each data point represents the mean (±S.E.M.) from 7 to 8 mice.

combination with CP-55940/LY235959 mixtures. Three fixed-ratio combinations were assessed: 1:1 CP-55940/LY235959 (total dose of 0.32–3.2 mg/kg; panels a–c), 1:3.2 CP-55940/LY235959 (total dose of 0.1–3.2 mg/kg; panels d–g), and 1:10 CP-55940/LY235959 (total dose of 0.1–3.2 mg/kg; panels h–k). The proportion of LY235959 in each mixture resulted in f values of 0.28, 0.55, and 0.79, for mixtures of 1:1, 1:3.2, and 1:10 CP-55940/LY235959, respectively. Each mixture of CP-55940 and LY235959 dose-dependently attenuated the development of morphine antinociceptive tolerance, and the relative potency of morphine assessed on day 1 and day 7 is shown graphically in Fig. 4.

Fig. 5 shows the isobolographic analysis of CP-55940/LY235959 drug combinations on the inhibition of morphine tolerance. The mixtures with the largest proportion of CP-55940 relative to LY235959 (i.e., 1:1 CP-55940/LY235959) produced an additive effect, as its isobol fell close to the line of additivity. Statistical comparison of the experimentally determined potency (z_{mix}) and predicted additive potency (z_{add}) confirms this finding (i.e., $z_{add} = z_{mix}$) (Table 1). Graphical analysis of 1:3.2 CP-55940/LY235959 mixtures suggest a trend towards supra-additivity, as its isobol fell below the additivity line, however comparison of z_{add} and z_{mix} values demonstrate that the experimentally determined potency and the predicted additive potency are not statistically different (Table 1). The mixtures with the lowest proportion of CP-55940 relative to LY235959 (i.e., 1:10 CP-55940/LY235959) produced a supra-additive effect, as its isobol fell below the line of additivity, and statistical comparison confirms that the predicted additive potency was greater than the experimentally determined potency ($z_{add} > z_{mix}$) (Table 1).

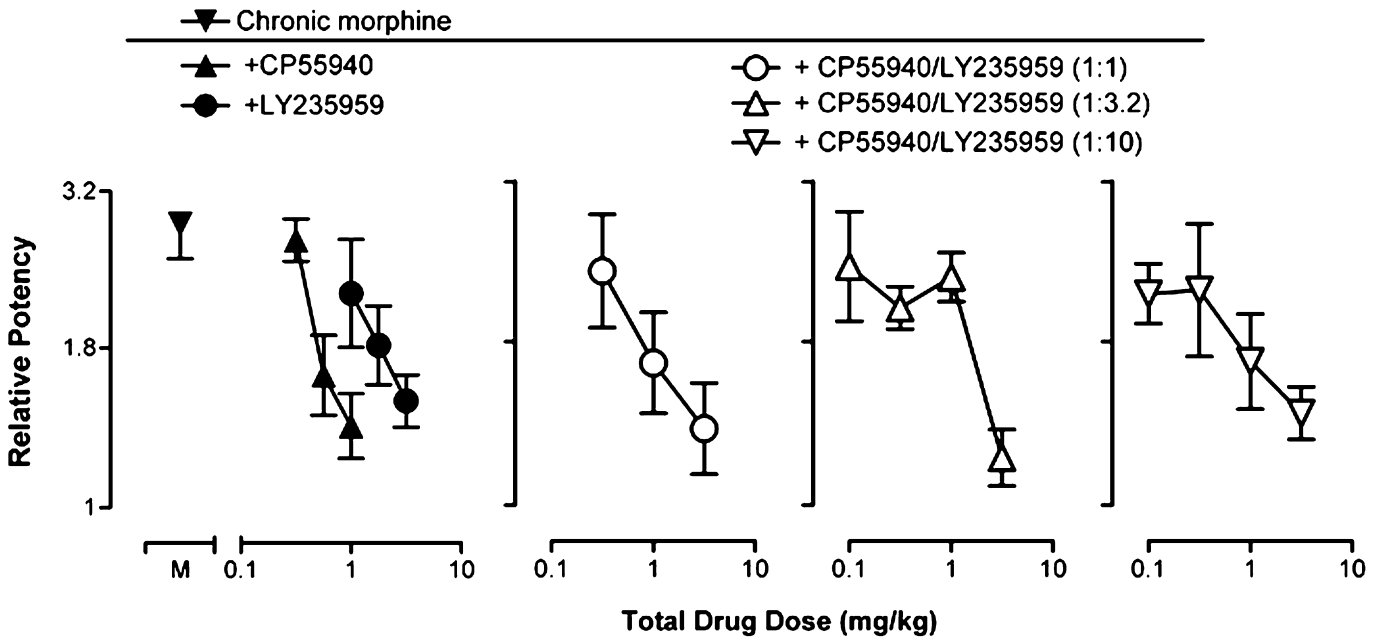

Fig. 4. Attenuation of morphine antinociceptive tolerance by CP-55940, LY235959, or CP-55940/LY235959 mixtures. Abscissae, total drug dose (mg/kg) of CP-55940, LY235959, or CP-55940 and LY235959 administered in combination with 100 mg/kg morphine during the chronic regimen. Ordinate, relative potency of morphine as assessed before and after chronic drug administration. Data point above "M" represents the relative potency after chronic administration of 100 mg/kg morphine alone. Each data point represents the mean (±S.E.M.) from 7 to 8 mice.

4. Discussion

The purpose of the present study was to assess the effects of the CB_1 receptor agonist CP-55940, the NMDA receptor antagonist LY235959, and CP-55940/LY235959 mixtures on the attenuation of morphine antinociceptive tolerance. The main findings from these experiments were that 1) CP-55940 and LY235959 both attenuated morphine tolerance and 2) fixed-ratio CP-55940/LY235959 mixtures also attenuated morphine tolerance, and did so in an additive or supra-additive manner, depending on their relative proportions. Taken together, these data implicate CB_1 and NMDA receptors in morphine tolerance, and further suggest that these receptor subtypes functionally interact during this process. These data also provide additional evidence that the interactive effects of drug mixtures depend on their relative proportions.

In the current study, the CB_1 receptor agonist CP-55940 attenuated the development of tolerance to the antinociceptive effects of morphine. Previously, the role of the cannabinoid system in the development of morphine tolerance has been assessed with the naturally occurring cannabinoid agonist Δ^9-tetrahydrocannabinol

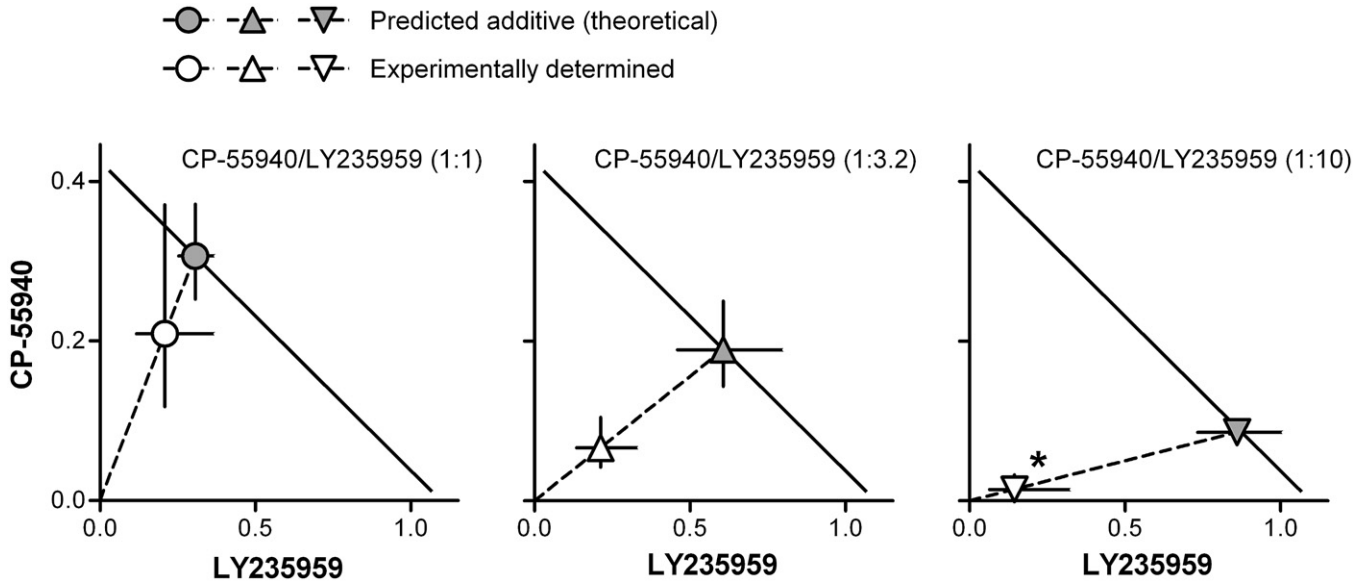

Fig. 5. Isobolograms for CP-55940/LY235959 mixtures. Open symbols, experimentally determined potency to attenuate morphine tolerance; closed symbols, predicted additive potency to attenuate morphine tolerance. Abscissae, potency of LY235959 in mg/kg. Ordinate, potency of CP-55940 in mg/kg. The asterisk represents a statistically significant difference from additivity.

Table 1
Predicted additive potency (z_{add}) and experimentally determined potency (z_{mix}) of CP-55940/LY235959 mixtures administered in combination with 100 mg/kg morphine during the chronic regimen on the attenuation of morphine tolerance.

Drug combination	z_{add} (±S.E.M.)	z_{mix} (±S.E.M.)	γ[a]
CP-55940/LY325959 (1:1)	0.61 (0.50–0.74)	0.42 (0.24–0.74)	0.68
CP-55940/LY325959 (1:3.2)	0.80 (0.60–1.1)	0.28 (0.18–0.44)	0.35
CP-55940/LY325959 (1:10)	0.95 (0.81–1.1)	0.16 (0.07–0.36)[b]	0.17

[a] Interaction index (i.e., $z_{mix} \div z_{add}$).
[b] An experimentally determined potency significantly different from the predicted additive potency ($P < 0.05$).

(Δ^9-THC) (e.g., Cichewicz and Welch, 2003). These initial studies were conducted from observations that CB_1 and opioid receptors are co-localized in brain regions important for the expression of morphine dependence (e.g., Navarro et al., 1998; see also Inoue et al., 2003; Adam et al., 2008), and from data suggesting that cannabinoid agonists decrease naloxone-precipitated withdrawal in rodents (Hine et al., 1975; Bhargava, 1976; Vela et al., 1995). The results from the current study provide further evidence that CB_1 receptors are involved in morphine-induced changes after chronic drug administration, and suggest that morphine antinociceptive tolerance can be attenuated by a CB_1 receptor agonist other than Δ^9-THC.

Morphine tolerance was also attenuated by the NMDA receptor antagonist LY23939. Relative to the cannabinoid system, there has been an abundant pre-clinical literature implicating a role for NMDA receptors in the development of tolerance and dependence following chronic morphine administration. To date, multiple demonstrations of the ability of NMDA receptor antagonists to interfere with the development of morphine tolerance provide evidence that NMDA receptors are important mediators of the behavioral plasticity that is associated with chronic morphine administration (for review, see Trujillo, 2000). Various antagonists with differential affinity for binding sites on the NMDA receptor complex, including competitive, noncompetitive and glycine sites, attenuate morphine tolerance across a range of experimental conditions (e.g., Allen and Dykstra, 2000; Kozela et al., 2003; Adam et al., 2006; Mendez and Trujillo, 2008). Together with the results presented here, these data provide converging evidence for a role of NMDA receptors in the development of morphine tolerance.

To our knowledge, the interactive effects of a CB_1 receptor agonist and an NMDA receptor antagonist on the attenuation of morphine antinociceptive tolerance have not been assessed. Previous research has identified a role for CB_1 cannabinoid receptors as pre-synaptic mediators of excitatory glutamate receptor systems, where they reduce activity at NMDA glutamate receptors (Auclair et al., 2000; Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2002; Brown et al., 2004; Godino et al., 2007). Therefore, additive effects of such mixtures may be based on serial enhancement of the LY235959-induced attenuation of glutamate signaling by CP-55940. In the present study fixed-ratio mixtures of 1:1 and 1:3.2 CP-55940/LY235959 produced an additive attenuation of morphine tolerance. While the present study does not directly assess the mechanism of action underlying CP-55940/LY235959 interactions, the supra-additive effect of the 1:10 CP-55940/LY235959 mixtures suggests that CB_1 agonism and NMDA antagonism may also work through parallel mechanisms to attenuate morphine tolerance. For example, results from Smith et al. (2007) suggest that administration of Δ^9-THC blocks the development of tolerance by preventing chronic agonist-induced changes in μ-opioid receptor-mediated G-protein activation, an effect that may augment the effects of NMDA antagonism on neuroplasticity that leads to an attenuation of morphine tolerance.

The present study also demonstrates that the interactive effects of CP-55940 and LY235959 are not only a property of the drugs under study, but also depend on their relative concentrations. This is in agreement with previous research assessing interactions between drugs with activity at other receptor systems (Stevenson et al., 2003, 2005; Fischer and Dykstra, 2006; Fischer et al., 2008; Ward et al., 2008). Assessment of drug interactions across various concentrations can also provide some insight into the receptor mechanisms that may mediate any observed deviation from additivity. When drugs are administered concurrently, two drugs in a mixture can be defined by the fraction (f) of the mixture's constitutes (see methods). As a result, drug mixtures that are tested in proportions that lead to f values less than 0.5 are predicted to have their effects mediated predominately by the drug whose potency is denoted z_2^* (e.g., as the value of f approaches 0, the equation $z_{add} = f z_1^* + (1 - f) z_2^*$ reduces to $z_{add} = z_2^*$). In contrast, drug mixtures that are tested in proportions leading to an f value greater than 0.5 are predicted to have their effects mediated predominately by the drug whose potency is denoted z_1^*.

In the present study, CP-55940/LY235959 mixtures were tested across a range of f values (0.28, 0.55, and 0.80 for fixed-ratio mixtures of 1:1, 1:3.2, and 1:10 CP-55940/LY235959, respectively). It is interesting to note that deviation from additivity was directly correlated with increases in the f value across the CP-55940/LY235959 mixtures examined. Specifically, drug mixtures that were tested at lower f values (i.e., 1:1 and 1:3.2 CP-55940/LY235959) produced additive effects, whereas the drug mixture with the larger f value (1:10 CP-55940/LY235959) produced a supra-additive effect. In addition, quantitatively greater differences in z_{add} and z_{mix} values, as measured by the interaction index of each mixture (Tallarida, 2002), were observed as the f value corresponding to each mixture was increased (Table 1). These observations are consistent with the hypothesis that supra-additive effects of CP-55940/LY235959 mixtures result from CB_1 receptor agonist activity potentiating an effect that is primarily mediated by an NMDA receptor antagonist.

Although the present study demonstrates a supra-additive interaction between CP-55940 and LY235959, it is important to note that this effect may or may not extend to other behavioral endpoints. Indeed, previous research has suggested that the interactive effects of two drugs may vary as a function of the experimental endpoint under study (e.g., Stevenson et al., 2003, 2005; Fischer and Dykstra, 2006; Fischer et al., 2008). CB_1 and NMDA receptor systems in particular have both been implicated in learning and memory (Newcomer and Krystal, 2001; Wise et al., 2008; Robinson et al., 2008), drug reinforcement (Allen et al., 2005, 2007; Soria et al., 2005), nociception (Pertwee, 2001; De Vry et al., 2004; Pelissier et al., 2008), and psychotic disorders such as schizophrenia (Ujike and Morita, 2004; Nabeshima et al., 2006), among others. Therefore, it may be of clinical interest to assess CP-55940/LY235959 interactions on endpoints related to these disorders. Interestingly, co-administration of LY235959 and the noncompetitive NMDA antagonist dextromethorphan also produced a synergistic enhancement of CB_1 agonism-evoked hypothermia in rats (Rawls et al., 2002).

In summary, the present results demonstrate a novel interaction between the cannabinoid CB_1 and NMDA receptor systems on the attenuation of morphine antinociceptive tolerance. The supra-additive behavioral effects after co-administration of CP-55940 and LY235959 suggests an important interaction between CB_1 and NMDA receptor-mediated signals. It is likely that agonism at CB_1 receptors results in the potentiation of an NMDA receptor-mediated effect, although additional studies are necessary to identify mechanisms underlying the supra-additive cannabinoid/NMDA receptor interactions. In addition, further investigation is warranted to

determine whether the observed supra-additive effect applies to other behavioral endpoints.

Acknowledgments

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