Buprenorphine Blocks ε- and μ-Opioid Receptor-Mediated Antinociception in the Mouse

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ABSTRACT
Antagonistic properties of buprenorphine for ε- and μ-opioid receptors were characterized in β-endorphin- and [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO)-induced antinociception, respectively, with the tail-flick test in male ICR mice. ε-Opioid receptor agonist β-endorphin (0.1–1 μg, μ-opioid receptor agonist DAMGO (0.5–20 ng), or buprenorphine (0.1–20 μg) administered i.c.v. dose dependently produced antinociception. The antinociception induced by 10 μg of buprenorphine given i.c.v. was completely blocked by the pretreatment with β-funaltrexamine (β-FNA) (0.3 μg i.c.v.), indicating that the buprenorphine-induced antinociception is mediated by the stimulation of the μ-opioid receptor. The antinociceptive effects induced by β-endorphin (1 μg i.c.v.) and DAMGO (16 ng i.c.v.) were dose dependently blocked by pretreatment with smaller doses of buprenorphine (0.001–1 μg i.c.v.), but not by a higher dose of buprenorphine (10 μg i.c.v.). β-FNA at a dose (0.3 μg i.c.v.) that strongly attenuated DAMGO-induced antinociception had no effect on the antinociception produced by β-endorphin (1 μg i.c.v.). However, pretreatment with buprenorphine (0.1–10 μg) in mice pretreated with this same dose of β-FNA was effective in blocking β-endorphin-induced antinociception. β-FNA was 226-fold more effective at antagonizing the antinociception induced by DAMGO (16 ng i.c.v.) than by β-endorphin (1 μg i.c.v.). The antinociception induced by δ-opioid receptor agonist [D-Ala²]deltorphin II (10 μg i.c.v.) or κ-opioid receptor agonist trans-3,4-dichloro-N-methyl-N-[2-[1-pyrrolidinyl]cyclohexyl]benzeneacetamide methanesulfonate salt ([(-)]-U50,488H) (75 μg i.c.v.) was not affected by pretreatment with buprenorphine (0.1–1.0 μg i.c.v.). It is concluded that buprenorphine, at small doses, blocks ε-opioid receptor-mediated β-endorphin-induced antinociception and μ-opioid receptor-mediated DAMGO-induced antinociception, and at high doses produces a μ-opioid receptor-mediated antinociception.

Buprenorphine, an oripavine derived from thebaine, has widely been shown to be a partial agonist and antagonist of the μ-opioid receptor, producing both μ-opioid receptor-mediated antinociception and blocking morphine-induced antinociception in laboratory animals (Cowan et al., 1977; Dum and Herz, 1981; Kamei et al., 1995, 1997). Buprenorphine antagonizes the antinociceptive actions of morphine in the mouse and rat with the tail-flick test, but is not effective in the rat tail-pressure test (Cowan et al., 1977), and also suppresses and precipitates abstinence in morphine-dependent dogs, mice, monkeys and rats (Martin et al., 1976; Cowan et al., 1977; Dum and Herz, 1981). Evidence also supports a κ-opioid antagonism associated with buprenorphine. Buprenorphine shows potent κ-opioid antagonist properties in the in vitro [³⁵S]GTPyS binding assay (Romero et al., 1999). Buprenorphine also blocks the κ-opioid agonist U-50,488H-induced inhibition of abdominal stretching induced by intra-peritoneal injection of acetic acid in mice (Leander, 1988). However, less data are known for the involvement of buprenorphine to stimulate κ-opioid receptors or to block δ-opioid receptors, and some findings are contradicting. The antinociception produced by buprenorphine administered intracereally is blocked by the κ-opioid antagonist Win 44,441-3 (Tejwani and Rattan, 2002), whereas buprenorphine given systemically is blocked by pretreatment with selective κ-opioid receptor antagonist nor-binaltorphimine (Kamei et al., 1995). Neilan et al. (1999) demonstrate that buprenorphine acts as a δ-opioid antagonist in the [³⁵S]GTPyS binding assay using C6 glioma cells expressing δ-opioid receptor.
the cloned δ-opioid receptor. On the other hand, no differences were found in the dose-response curves in mice treated with either DPDPE alone, or DPDPE and buprenorphine in the hot water tail-flick technique (Pick et al., 1997).

Buprenorphine is also identified to exhibit ε-opioid receptor binding activity using the nonselective ligand (–)-[3H]ethylketocyclazocine, in the presence of selective μ-, δ-, and κ-opioid receptor agonists (Nock et al., 1990, 1993; Nock, 1995). Unlike β-endorphin, whose affinity to the ε-opioid receptor is decreased by high concentration of NaCl, the affinities of buprenorphine to the ε-opioid receptor are increased by high concentration of NaCl, suggesting that buprenorphine might be an antagonist for the ε-opioid receptor (Nock et al., 1990). Buprenorphine blocks the increase of [35S]GTPγS binding induced by ε-agonist β-endorphin in the pons/medulla membrane obtained from μ-opioid receptor knockout mice (Mizoguchi et al., 2002). This finding indicates that buprenorphine acts as an ε-opioid receptor antagonist to block the β-endorphin-induced G protein activation in the pons/medulla membrane of mice that genetically lack μ-opioid receptors.

The present study was designed to further characterize the antagonistic properties of buprenorphine for ε- and μ-opioid receptor-mediated antinociception induced by ε-opioid receptor agonist β-endorphin and μ-opioid receptor agonist [d-Ala2,N-Me-Phe4,Gly5-ol]-enkephalin (DAMGO), in mice. We report here for the first time that buprenorphine blocks the tail-flick response. Doses of buprenorphine (up to 1 g dose dependently attenuated the tail-flick response. The inhibition induced by 10 and 20 μg of buprenorphine reached a peak in 10 or 20 min after injection and the tail-flick latency returned to the preinjection level within 60 min after injection (Fig. 1B). Buprenorphine given i.c.v. at doses of 3, 10, and 20 μg, but not 1 μg, also dose dependently inhibited the tail-flick response. The inhibition induced by 10 and 20 μg of buprenorphine reached a peak in 10 or 20 min after injection and the tail-flick latency returned to preinjection level within 60 min after injection (Fig. 1C). Buprenorphine at a low dose (1 μg) did not significantly cause any inhibition of the tail-flick response. Doses of buprenorphine (up to 1 μg) were then used to study the effects of buprenorphine in blocking the tail-flick inhibition induced by β-endorphin or DAMGO. In the following experiments, the β-endorphin- and DAMGO-induced tail-flick inhibitions were investigated at 20 min after i.c.v. injection of β-endorphin or DAMGO.

Effect of i.c.v. Pretreatment with Buprenorphine on the Tail-Flick Inhibition Induced by i.c.v. Administered β-Endorphin. Groups of mice were pretreated i.c.v. with buprenorphine (0.001–10 μg) or vehicle (0.1% DMSO/saline, 4 μl) 10 min before β-endorphin (1 μg i.c.v.) administration, and the tail-flick response was measured 20 min after β-endorphin administration. Pretreatment with buprenorphine at doses of 0.01 to 1 μg dose dependently attenuated the β-endorphin-produced tail-flick inhibition. Buprenorphine at 0.1 μg markedly reduced the β-endorphin-induced tail-flick inhibition to 12.7% MPE from 90.5% MPE in the vehicle pretreatment. However, i.c.v. pretreatment with a high dose (10 μg) of buprenorphine did not attenuate the tail-flick inhibition induced by β-endorphin (Fig. 2A).

In another experiment, the effect of pretreatment with 0.1 μg of buprenorphine on the tail-flick inhibition induced by various doses of β-endorphin (0.1–4 μg) was studied. β-Endorphin at doses between 0.1 and 4 μg given i.c.v. dose dependently inhibited the tail-flick response in mice pretreated with vehicle for 10 min. The i.c.v. pretreatment with 0.1 μg of buprenorphine for 10 min significantly attenuated the tail-flick latency induced by 0.1 to 4 μg of β-endorphin (Fig. 2B).
the tail-flick inhibition induced by \( \beta \)-endorphin and the dose-response curve for \( \beta \)-endorphin-induced tail-flick inhibition was significantly shifted to the right 3.5-fold compared with that of vehicle-pretreated mice (Fig. 3A; Table 1).

Effect of i.c.v. Pretreatment with Buprenorphine on the Tail-Flick Inhibition Induced by i.c.v. Administered DAMGO. Groups of mice were pretreated i.c.v. with buprenorphine (0.001–10 \( \mu \)g) or vehicle (0.1% DMSO/saline; 4 \( \mu \)l) 10 min before the i.c.v. injection of \( \beta \)-endorphin (1 \( \mu \)g) or DAMGO (16 ng), and the tail-flick responses were measured 20 min after the injection. The data represent the mean and S.E.M. The statistical significance of differences between the groups was assessed with one-way ANOVA followed by Bonferroni’s test. * \( p < 0.05 \) versus vehicle-treated mice. The \( F \) values of one-way ANOVA for \( \beta \)-endorphin- and DAMGO-induced antinociception are \( F(5,49) = 17.40 \) and \( F(5,54) = 5.013 \), respectively.

Fig. 2. Effects of buprenorphine pretreatment on the antinociception induced by \( \beta \)-endorphin (A) and DAMGO (B) in mice. Groups of mice were pretreated i.c.v. with vehicle (4 \( \mu \)l) or various doses of buprenorphine (1 ng–10 \( \mu \)g) 10 min before the i.c.v. injection of \( \beta \)-endorphin (1 \( \mu \)g) or DAMGO (16 ng), and the tail-flick responses were measured 20 min after the injection. The data represent the mean and S.E.M. The statistical significance of differences between the groups was assessed with two-way ANOVA followed by Bonferroni’s test. * \( p < 0.05 \) versus vehicle-treated mice. A, the \( F \) values of two-way ANOVA for \( \beta \)-endorphin (0.3, 0.6, and 1 \( \mu \)g) treatment in comparison with vehicle treatment are \( F(1,90) = 13.00 \), \( F(1,140) = 16.76 \), and \( F(1,85) = 101.8 \), respectively. B, the \( F \) values of two-way ANOVA for DAMGO (5, 10, and 16 ng) treatment in comparison with vehicle treatment are \( F(1,90) = 5.679 \), \( F(1,90) = 11.58 \), and \( F(1,90) = 23.05 \), respectively. C, the \( F \) values of two-way ANOVA for buprenorphine (3 and 10 \( \mu \)g) treatment in comparison with vehicle-treatment are \( F(1,100) = 12.00 \) and \( F(1,89) = 31.10 \), respectively.
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**Fig. 3.** Dose-response curves of the \( \beta \)-endorphin (A) - and DAMGO (B) induced antinociception in mice pretreated with saline vehicle or buprenorphine. Groups of mice were pretreated i.c.v. with vehicle (4 \( \mu l \)) or buprenorphine (0.1 \( \mu g \)) 10 min before the i.c.v. injection of \( \beta \)-endorphin (0.1–4 \( \mu g \)) or DAMGO (5–40 ng), and the tail-flick response was measured 20 min after the treatment. The data represent the mean and S.E.M. The ID\(_{50}\) value was calculated by computer-assisted curve-fit program (Prism). A, the ID\(_{50}\) value for \( \beta \)-endorphin to produce antinociception in mice pretreated with vehicle and buprenorphine was 0.58 and 2.03 \( \mu g \), respectively. The statistical significance of the differences between the groups was assessed with \( F \) test. The \( F \) value and \( p \) value are 13.40 and 0.017, respectively. B, the ID\(_{50}\) value for DAMGO to produce antinociception in mice pretreated with vehicle and buprenorphine was 12.17 and 23.95 ng, respectively. The statistical significance of differences between the groups was assessed with \( F \) test. The \( F \) value and \( p \) value are 13.46 and 0.003, respectively.

Table 1

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>ID(_{50}) for ( \beta )-Endorphin</th>
<th>ID(_{50}) for DAMGO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (4 ( \mu l ) i.c.v.)</td>
<td>( 0.58 \pm 0.03 )</td>
<td>( 12.17 \pm 0.95 )</td>
</tr>
<tr>
<td>Buprenorphine (0.1 ( \mu g ) i.c.v.)</td>
<td>( 2.03 \pm 0.32 )</td>
<td>( 23.95 \pm 1.50 )</td>
</tr>
<tr>
<td>Potency ratio</td>
<td>3.50</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Dependently inhibited the tail-flick response in mice pretreated with vehicle. Pretreatment with 0.1 \( \mu g \) of buprenorphine attenuated the tail-flick inhibition by DAMGO, and the dose-response curve for DAMGO-induced tail-flick inhibition was significantly shifted to the right 1.96-fold (Fig. 3B; Table 1).

**Time Course of the i.c.v. Pretreatment with Buprenorphine on the Tail-Flick Inhibition Induced by i.c.v. Administered \( \beta \)-Endorphin.** Groups of mice were pretreated with 0.1 or 10 \( \mu g \) of buprenorphine given i.c.v. at various times before i.c.v. injection of \( \beta \)-endorphin (1 \( \mu g \)) (Fig. 4, A and B), respectively, and the tail-flick inhibition was measured 20 min after injection. Another group of mice was pretreated i.c.v. with vehicle and challenged with the same dose of \( \beta \)-endorphin to serve as controls. The i.c.v. administration of \( \beta \)-endorphin (1 \( \mu g \)) produced consistent 83 to 91\% MPE of the maximum tail-flick inhibition in mice pretreated i.c.v. with vehicle. Pretreatment with 0.1 \( \mu g \) of buprenorphine for 10 min, but not 30 min or 1 h, attenuated the \( \beta \)-endorphin-induced tail-flick inhibition (Fig. 4A). On the other hand, pretreatment with 10 \( \mu g \) of buprenorphine for 3 h only, but not 10 min, 1 h, 2 h, or 4 h, attenuated the \( \beta \)-endorphin-induced tail-flick inhibition (Fig. 4B).

Effects of i.c.v. Pretreatment with \( \beta \)-FNA on the Tail-Flick Inhibition Induced by i.c.v. Administered \( \beta \)-Endorphin, DAMGO, or Buprenorphine. We have previously demonstrated that i.c.v. pretreatment with \( \beta \)-FNA at a dose of 2.5 \( \mu g \) for 24 h completely blocks the tail-flick inhibition induced by i.c.v.-administered morphine, but not \( \beta \)-endorphin (Suh and Tseng, 1988). The present experiment was extended to determine the relative potency of \( \beta \)-FNA, administered i.c.v., for blocking tail-flick inhibition induced by i.c.v.-administered \( \beta \)-endorphin and DAMGO. Groups of mice were pretreated i.c.v. with various doses of \( \beta \)-FNA 24 h before i.c.v. injection of \( \beta \)-endorphin (1 \( \mu g \)) or DAMGO (16 ng), and the tail-flick response was measured 20 min after injection. \( \beta \)-FNA at 0.001 to 0.1 \( \mu g \) and 0.3 to 10 \( \mu g \) dose dependently blocked the tail-flick inhibition induced by DAMGO and \( \beta \)-endorphin, respectively. \( \beta \)-FNA at 0.1 \( \mu g \), which markedly blocked the tail-flick inhibition induced by DAMGO, did not affect the tail-flick inhibition induced by \( \beta \)-endorphin. \( \beta \)-FNA even at a dose up to 0.3 \( \mu g \) did not have any effect on \( \beta \)-endorphin-induced tail-flick inhibition. The ID\(_{50}\) value of \( \beta \)-FNA for attenuating the tail-flick inhibition induced by DAMGO and \( \beta \)-endorphin was 0.023 and 5.2 \( \mu g \), respectively; \( \beta \)-FNA was 226-fold more effective in blocking the antinociception induced by DAMGO than \( \beta \)-endorphin (Fig. 5). \( \beta \)-FNA (0.3 \( \mu g \)) was then chosen as the dose to use in the following experiment to eliminate the \( \mu \)-opioid component of \( \beta \)-endorphin antinociception.

Groups of mice were pretreated i.c.v. with \( \beta \)-FNA (0.3 \( \mu g \)) for 24 h and various doses (0.001–10 \( \mu g \)) of buprenorphine for 10 min, and the tail-flick response for i.c.v. administration of 1 \( \mu g \) of \( \beta \)-endorphin was measured 20 min thereafter. Pretreatment with \( \beta \)-FNA (0.3 \( \mu g \)) did not have any effect on the tail-flick inhibition induced by 1 \( \mu g \) of \( \beta \)-endorphin in mice pretreated with saline i.c.v. for 10 min. However, the tail-flick inhibition induced by \( \beta \)-endorphin was markedly blocked by 0.1 to 10 \( \mu g \) of buprenorphine in mice pretreated with \( \beta \)-FNA (Fig. 6).

In another experiment, two groups of mice were pretreated i.c.v. with \( \beta \)-FNA (0.3 \( \mu g \)) or saline (4 \( \mu l \)) 24 h before the i.c.v.
injection of 10 μg of buprenorphine, and the tail-flick response was measured 20 min after injection. The tail-flick inhibition induced by 10 μg of buprenorphine was completely blocked by the pretreatment with β-FNA (buprenorphine produced 53.4 ± 13.5% MPE in saline-pretreated mice versus 10.2 ± 3.1% MPE in β-FNA pretreated mice).

**Effect of i.c.v. Pretreatment with Buprenorphine on the Tail-Flick Inhibition Induced by i.c.v. Administered [d-Ala²]Deltorphin II and U50,488H.** Groups of mice were pretreated i.c.v. with buprenorphine (0.1 or 1 μg) or vehicle (0.1% DMSO/saline; 4 μl) 10 min before i.c.v. injection of [d-Ala²]deltorphin II (10 μg) or U50,488H (75 μg), and the tail-flick response was measured 20 min after the injection. The tail-flick inhibition induced by [d-Ala²]deltorphin II or U50,488H was not affected by pretreatment with buprenorphine (Fig. 7).

**Discussion**

The antinociception induced by buprenorphine is mediated by the stimulation of μ-opioid receptors. Buprenorphine seems to be a μ-opioid receptor agonist and produces analgesia that is qualitatively similar to that of morphine. We found in the present study that buprenorphine at high doses (3–20 μg) given i.c.v. dose dependently produced antinociception in a manner similar to that produced by μ-opioid agonist DAMGO. The antinociception induced by buprenorphine was completely blocked by the pretreatment with μ-opioid receptor antagonist β-FNA. Our present finding is consistent with the reports of others (Kamei et al., 1995, 1997) that the antinociception induced by buprenorphine is mediated by the stimulation of μ-opioid receptors. Buprenorphine, currently used clinically as an analgesic (Cowan and Lewis, 1995), is reported to show weaker partial agonistic property for the μ-opioid receptor in cell membranes expressed with cloned μ-opioid receptors than morphine or fentanyl (Selley et al., 1997).

**Buprenorphine at Small Doses Blocks Both ε- and μ-Opioid Receptor-Mediated β-Endorphin- and DAMGO-Induced Antinociception, Respectively.** It has been documented that the antinociception induced by β-endorphin given supraspinally is mediated by the stimulation of the ε-opioid receptors (for reviews, see Tseng, 1995, 2002). This view is supported by the finding that the antinociception induced by β-endorphin given i.c.v. is not blocked by the pretreatment with the μ-opioid receptor antagonists D-Phe-Cys-Tyr-Om-Thr-Pen-Thr-NH₂ or β-FNA, the δ-opioid receptor antagonist naltrindole, or the κ-opioid receptor antagonist nor-binaltorphimine, but is blocked by the ε-opioid receptor antagonist β-endorphin(1–27) (Suh et al., 1988; Suh and Tseng, 1990; Tseng and Collins, 1991; Tseng, 2002).

β-Endorphin(1–27) was the only compound previously used as an ε-opioid receptor antagonist to characterize the
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Fig. 6. Effect of buprenorphine on the $\beta$-endorphin-induced antinociception in mice pretreated with $\beta$-FNA. Groups of mice pretreated i.c.v. with $\beta$-FNA (0.3 $\mu$g) for 24 h were injected i.c.v. with vehicle (4 $\mu$g) or buprenorphine (1 ng-10 $\mu$g) 10 min before the i.c.v. treatment with $\beta$-endorphin (1 $\mu$g). The tail-flick inhibition induced by $\beta$-endorphin was measured 20 min after the treatment. The data represent mean and S.E.M. The statistical significance of differences between the groups was assessed with one-way ANOVA followed by Bonferroni’s test. * $p < 0.05$ versus $\beta$-FNA + vehicle-pretreated mice. The F value of one-way ANOVA is $F(5,50) = 5.394$.

Fig. 7. Effect of buprenorphine pretreatment on the antinociception induced by $[\text{D-Ala}^2]\text{deltorphin II}$ and U50,488H. Groups of mice were pretreated i.c.v. with vehicle (4 $\mu$g) or buprenorphine (0.1 or 1 $\mu$g) 10 min before the i.c.v. injection of $[\text{D-Ala}^2]\text{deltorphin II}$ (10 $\mu$g) and U50,488H (75 $\mu$g), and the tail-flick response was measured 10 min after the treatments. The data represent the mean and S.E.M. The statistical significance of differences between the groups was assessed with one-way ANOVA followed by the Bonferroni test.

$\varepsilon$-receptor properties. We report here for the first time that buprenorphine blocks the $\varepsilon$-mediated antinociception induced by i.c.v.-administered $\beta$-endorphin. We found that antinociception induced by $\beta$-endorphin given supraspinally was not affected by the pretreatment with $\mu$-opioid receptor antagonist $\beta$-FNA (0.3 $\mu$g), but was effectively blocked by the pretreatment with buprenorphine in mice pretreated with the same dose of $\beta$-FNA. These findings indicate that buprenorphine blocks the antinociception by blocking the $\varepsilon$-opioid receptors stimulated by $\beta$-endorphin.

We found that the i.c.v. pretreatment with buprenorphine at doses 0.01, 0.1, and 1 $\mu$g, which given alone did not produce any tail-flick inhibition, effectively and dose dependently attenuated the antinociception induced by i.c.v.-administered $\beta$-endorphin. In addition, buprenorphine at 0.1 $\mu$g, but not 0.01 or 1 $\mu$g, also attenuated the antinociception induced by $\mu$-opioid receptor agonist DAMGO. Thus, buprenorphine at small doses blocks both $\varepsilon$- and $\mu$-opioid receptors.

**Buprenorphine at Doses that Block $\varepsilon$- and $\mu$-Opioid Receptors Does Not Block $\kappa$- and $\delta$-Opioid Receptors.** Pretreatment with these same doses (0.1 and 1 $\mu$g) of buprenorphine given i.c.v., which blocked antinociception induced by i.c.v.-administered $\beta$-endorphin or DAMGO, did not affect the antinociception induced by i.c.v. injected $\delta$-opioid receptor agonist $[\text{D-Ala}^2]\text{deltorphin II}$ or $\kappa$-opioid receptor agonist U50,488H. Our results are consistent with the work done by Pick et al. (1997) showing buprenorphine is unable to block the antinociceptive effects of $\kappa$-opioid agonist U50,488H or $\delta$-opioid agonist DPDPE. Although other groups have shown $\kappa$-opioid (Leander, 1988; Pick et al., 1997; Romero et al., 1999) and $\delta$-opioid (Neilan et al., 1999) antagonistic effects with buprenorphine, we simply conclude that buprenorphine given i.c.v. at doses 0.1 to 1 $\mu$g does not show any significant $\kappa$- or $\delta$-opioid antagonistic components with the respective agonists we administered. Inconsistency in the literature is possibly due to different subtypes of opioid receptors involved in buprenorphine action, different routes of administration and the receptor density at administration sites, and different efficacy requirements for different experiments.

In conclusion, buprenorphine at high doses produced antinociception, which is mediated by stimulation of $\mu$-opioid receptors. At low doses, buprenorphine blocked the antinociception induced by $\epsilon$-opioid receptor agonist $\beta$-endorphin and $\mu$-opioid receptor agonist DAMGO.

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