Antianalgesia: Stereoselective Action of \textit{dextro-}Morphine over \textit{levo-}Morphine on Glia in the Mouse Spinal Cord

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\textbf{ABSTRACT}

We have previously shown that the naturally occurring \textit{levo}-morphine at a subanalgesic picomolar dose pretreated i.t. induces antianalgesia against \textit{levo}-morphine-produced antinociception. We now report that the synthetic \textit{stereo-enantiomer} \textit{dextro-}morphine, even at an extremely low femtomolar dose, induces antianalgesia against \textit{levo}-morphine-produced antinociception using the tail-flick (TF) test in male CD-1 mice. Intrathecal pretreatment with \textit{dextro-}morphine (33 fmol) time-dependently attenuated the i.t. \textit{levo-}morphine-produced TF inhibition for 4 h and returned to the preinjection control level at 24 h. Intrathecal pretreatment with \textit{dextro-}morphine (0.3–33 fmol), which injected alone did not affect the baseline TF latency, dose-dependently attenuated the TF inhibition produced by i.t.-administered \textit{levo-}morphine (3.0 nmol). The ED\textsubscript{50} value for \textit{dextro-}morphine to induce antianalgesia was estimated to be 1.07 fmol, which is 71,000-fold more potent than the ED\textsubscript{50} value of \textit{levo-}morphine, indicating the high stereoselective action of \textit{dextro-}morphine over \textit{levo-}morphine for the induction of antianalgesia. Like \textit{levo-}morphine, the \textit{dextro-}morphine-induced antianalgesia against \textit{levo-}morphine-produced TF inhibition was dose-dependently blocked by the nonopioid \textit{dextro-}naloxone and its \textit{stereo-enantiomer} \textit{levo-}naloxone, a nonselective \textit{\mu-}opioid receptor antagonist. The antianalgesia induced by \textit{levo-}morphine and \textit{dextro-}morphine is reversed by the pretreatment with the glial inhibitor propentofylline (3.3–65 nmol), indicating that the antianalgesia is mediated by glial stimulation. The findings strongly indicate that the antianalgesia induced by \textit{levo-}morphine and \textit{dextro-}morphine is mediated by the stimulation of a novel nonopioid receptor on glial cells.

Naturally occurring \textit{levo-}morphine, which is isolated from the juice of the opium poppy, \textit{Papaver somniferum}, is stereochemically identified as a levorotatory isoform of morphine. \textit{levo-}Morphine produces potent analgesic and other major pharmacological effects, which are mainly mediated by the stimulation of \textit{\mu-}opioid receptors. The synthetic \textit{dextro-enantiomer} of \textit{levo-}morphine has minimal activity in the \textit{\mu-}opioid receptor binding assay, the electrically stimulated guinea pig ileum assay, and the inhibition of adenylate cyclase activity in the neuroblastoma \texttimes glia hybrid cell homogenates, indicating that it does not interact with \textit{\mu-}opioid receptors (Jacquet et al., 1977). Unlike \textit{levo-}morphine, which produces potent \textit{levo-}naloxone reversible analgesia, \textit{dextro-}morphine microinjected into the periaqueductal gray in rats produces minimal analgesia (Jacquet et al., 1977). In the present study, i.t. pretreatment with \textit{dextro-}morphine, which injected alone does not affect baseline nociceptive latency, attenuates the antinociception produced by i.t.-administered \textit{levo-}morphine. The phenomenon of the attenuation of \textit{levo-}morphine-produced analgesia by \textit{dextro-}morphine has been defined as antianalgesia (Wu et al., 2004b).

Nonselective \textit{\mu-}opioid receptor antagonist \textit{levo-}naloxone and nonopioid receptor antagonist \textit{dextro-}naloxone were used to identify the opioid or nonopioid nature of the \textit{dextro-}morphine-induced antianalgesia. We have previously reported that i.t. pretreatment with \textit{levo-}morphine at a picomolar dose dose-dependently attenuates the antinociception produced by i.t.-administered \textit{levo-}morphine using the thermal tail-flick (TF) test in mice. This antianalgesia is blocked by pretreatment with \textit{dextro-}naloxone, indicating that the antianalgesia induced by \textit{levo-}morphine is not mediated by the stimulation of conventional G protein-coupled \textit{\mu-}opioid receptors (Wu et al., 2004b). On the other hand, the antianalgesia induced by endogenous \textit{\mu-}opioid ligands endomorphin-1 and endomorphin-2 is blocked by \textit{levo-}naloxone but not by \textit{dextro-}naloxone, indicating that the antianalgesia induced by endomorphin-1 and endomorphin-2 is mediated by the desensitization of \textit{\mu-}opioid receptors by endomorphin-1 and endomorphin-2 pretreatment (Wu et al., 2003; Terashvili et al., 2005). Thus, \textit{levo-}morphine has biphasic effects: it produces antinociception or analgesia, which is mediated by the stimulation of

\textbf{ABBREVIATIONS:} TF, tail-flick; ANOVA, analysis of variance; CI, confidence interval.
μ-opioid receptors, and also induces nonopioidergic antianalgesia. However, it is reasonable to believe that levo-morphine at high analgesic doses also induces antianalgesia, but the effect is masked by the analgesic effect of levo-morphine. The finding that a small dose of levo-naloxone or other opioid antagonists enhances levo-morphine-produced analgesia in laboratory animals (Crain and Shen, 1995, 2000, 2001) and humans (Fan et al., 1997; Joshi et al., 1999) supports this view.

By releasing neurotransmitters, including glutamate, ATP, and other extracellular signaling molecules, glia can affect neuronal excitability and synaptic transmission and coordinate activity across networks of neurons (Fields and Stevens-Graham, 2002). There are indications from the literature that opiate effects are not only mediated by opioid systems such as μ, δ, and κ-opioid receptors but also that they are influenced by immune mediators such as cytokines, chemokines, free radicals, and nitric oxide that are released through activation of glial cells. Exposure of the microglia to morphine causes changes in microglial morphology and induces apoptosis, which can be blocked by levo-naloxone (Do-brenis et al., 1995; Magazine et al., 1996; Hu et al., 2002). Chronic levo-morphine treatment induces a marked proliferation and hypertrophy of microglia and astrocytes in the spinal dorsal horn (Raghavendra et al., 2002; Narita et al., 2004). Inhibition of glial activation by the glial modulator propentofylline spares levo-morphine analgesia in neuropathic rats and reverses the development of levo-morphine tolerance and withdrawal hyperalgesia (Raghavendra et al., 2002). The findings indicate that levo-morphine also acts on glia to modulate the analgesic and other pharmacological activities of levo-morphine.

We have previously shown that levo-morphine induces antianalgesia, which is mediated by a nonopioidergic mechanism (Wu et al., 2004b). Present experiments were then undertaken to determine whether the nonopioid levo-morphine enantiomer dextro-morphine would act like levo-morphine and induce antianalgesia. The glial inhibitor propentofylline and dextro-naloxone were used to determine whether the antianalgesic effects induced by dextro-morphine and levo-morphine are mediated by the stimulation of a nonopioid mechanism on glial cells. We now report for the first time that pretreatment with an ultra-low, femtomolar dose of dextro-morphine attenuates the analgesia produced by subsequent injection of levo-morphine, and that the antianalgesia induced by either dextro-morphine or levo-morphine is mediated by the stimulation of a novel nonopioid receptor on glia in the mouse spinal cord.

Materials and Methods

Animals. Male CD-1 mice weighing 25 to 30 g (Charles River Breeding Laboratories, Portage, MI) were used. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. Each animal was used only once. All the experiments were approved by and conformed to the guidelines of the Animal Care Committee of the Medical College of Wisconsin.

Assessment of Analgesia. Analgesic responses were measured with the TF test (D’Amour and Smith, 1941). To measure the latency of the TF response, mice were gently held with the tail put on the apparatus (Model TF6; EMDIE Instrument Co., Maidens, VA). The TF response was elicited by applying radiant heat to the dorsal surface of the tail. The low- and high-intensity heat stimuli were set to provide a predrug TF response time of 8 to 10 s and 3 to 4 s, respectively. The cutoff times for the low- and high-intensity heat stimulation were set at 20 s and 10 s, respectively, to avoid tissue damage. The TF response with a low-intensity heat stimulus was only used in Fig. 1, and high heat intensity was used throughout all the experiments. To calculate the ED50 values of the drug tested, the TF response latencies were then converted to the “percent maximum possible effect,” which was calculated as \[ \left( \frac{T_1 - T_c}{T_0 - T_c} \right) \times 100 \]. \( T_0 \) and \( T_1 \) were the TF latencies before and after i.t. injection of morphine, respectively, and \( T_2 \) was the cutoff time, which was set at 10 s.

Experimental Protocols. Intrathecal injection was performed according to the procedure of Hylden and Wilcox (1980) using a 25-μl Hamilton syringe with a 30-gauge needle. The injection volume was 5 μl. The following experiments were performed. 1) The effects of dextro-morphine on the TF latency induced by either a low- or high-intensity heat stimulus were determined. Groups of mice were treated with various doses of dextro-morphine, and TF were measured at different times for 2 h at high or low thermal stimulus. 2) The time course and the dose-response relationship of dextro-morphine for the induction of antianalgesia against levo-morphine-produced antinociception were determined. Groups of mice were pretreated i.t. with 33 fmol of dextro-morphine for different times (0–24 h) before i.t. administration of levo-morphine (3.0 nmol). Other mice were used to test different doses (0.3–330 fmol) of dextro-morphine 45 min before i.t. administration of levo-morphine (3.0 nmol). In both cases, TF responses under high heat intensity were measured at different times thereafter. 3) The effects of the pretreatment with nonopioid antagonist dextro-naloxone and nonselective opioid antagonist levo-naloxone on the attenuation of levo-morphine-produced antinociception induced by dextro-morphine-induced antianalgesia were studied. Groups of mice were pretreated i.t. with dextro-naloxone (0.03–280 pmol) or levo-naloxone (0.03–28 pmol) 10 min (Wu et al., 2004b) before i.t. injection of dextro-morphine (33 fmol). Levo-Morphine (3.0 nmol) was injected i.t. 45 min after dextro-morphine injection, and the TF responses under high heat intensity were measured at different times thereafter. 4) The effect of propentofylline, a glial inhibitor (Schubert et al., 2000; Sweitzer et al., 2001), on the attenuation of levo-morphine-produced TF inhibition induced by separate dextro-morphine and levo-morphine pretreatment was determined. Groups of mice were coadministered i.t. propentofylline with dextro-morphine (33 fmol) or levo-morphine (0.3 nmol) 45 min before i.t. injection of levo-morphine (3.0 nmol), and the TF responses under high heat intensity were then measured 15 min thereafter.

![Figure 1](image-url)
Drugs. levo-Morphine sulfate, dextro-morphine base, and dextro-naloxone were obtained from National Institute of Drug Abuse (Baltimore, MD). levo-Naloxone and propentofylline were purchased from Sigma (St. Louis, MO). levo-Morphine, levo-naloxone, dextro-naloxone, and propentofyllin were dissolved in 0.9% saline. The dextro-morphine was dissolved in 10 N hydrochloric acid and then titrated with 1 N sodium hydroxide to a pH of 7.4, which was then diluted to the intended dose in 0.9% saline.

Statistical Analysis. The analgesic responses (TF latencies) were presented as the mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett’s post test or two-way ANOVA followed by Bonferroni’s post tests was used to test the differences between groups. The nonlinear regression model was used to fit the dose-response curve and to calculate the ED50 value and 95% confidence interval (CI). The F test was used to test the difference of logED50 between dextro-morphine- and levo-morphine-induced antinociception. The GraphPad Prism software was used to perform the statistics (version 4.1; GraphPad Software Inc., San Diego, CA).

**Results**

The TF Responses to a High- and Low-Intensity Heat Stimulus after the Intrathecal Injection of Various Doses of dextro-Morphine. The experiment was designed to determine whether dextro-morphine given i.t. caused any change of the TF latency. The intensity of the thermal stimulation was adjusted so that a high and low intensity of heat stimulus induced TF responses in 3 to 4 s and 8 to 10 s, respectively. Groups of male CD-1 mice were injected i.t. with various doses of dextro-morphine (0.3 fmol, 33 fmol, or 3.3 pmol) or saline vehicle, and the TF responses were measured at different times after injection for 2 h using either a high or low intensity of heat stimulus. Intrathecal injection of dextro-morphine at 0.3 fmol, 33 fmol, or 3.3 pmol did not cause any change of the TF latency either induced by a high-intensity heat stimulus or a low-intensity heat stimulus (Fig. 1). Figure 1 also showed that the curves for TF latencies elicited by a high-intensity heat stimulus were more steady and fluctuated less than the TF latencies induced by a low-intensity heat stimulus. The high-intensity heat stimulus was used in the following experiments.

Effects of Different Times and Doses of Pretreatment of dextro-Morphine Given Intrathecally on the TF Latency Produced by Intrathecally Administered levo-Morphine. Groups of mice were pretreated i.t. with dextro-morphine (33 fmol) at various times before i.t. injection of levo-morphine (3.0 nmol), and the TF response was measured 15 min thereafter. Other groups of mice pretreated i.t. with vehicle 0, 0.5, 0.75, 1, 2, 4, 8, and 24 h before i.t. administration of levo-morphine (3.0 nmol), and the TF latency was measured 15 min thereafter. Each column represents the mean, and the vertical bar represents the S.E.M. with 8 to 10 mice in each group. The two-way ANOVA followed by Bonferroni’s post test was used to test the difference between groups. The F interaction, treatment, time = 11.24, 46.65, and 9.08; *, p < 0.01; **, p < 0.001.

![Fig. 2. Effects of different pretreatment times with dextro-morphine given i.t. on the TF inhibition produced by i.t.-administered levo-morphine.](image)

Ex vivo pretreatment of dextro-morphine 45 min before i.t. administration of levo-morphine (3.0 nmol), and the TF response was measured thereafter. Intrathecal injection of levo-morphine 3.0 nmol caused an increase of TF inhibition in mice pretreated with the saline vehicle. The TF inhibition developed in 5 to 10 min, reached a maximum in 15 min, and returned slowly to the control level in 60 min. Intrathecal pretreatment with dextro-morphine at doses from 1.0 to 33 fmol dose-dependently attenuated the TF responses produced by i.t.-administered levo-morphine observed at different times after injection (Fig. 3A). Figure 3B shows that dextro-morphine at doses from 1.0 to 33 nmol dose-dependently attenuated the levo-morphine-produced TF inhibition observed at 15 min after levo-morphine injection. The attenuation reached a maximum at 33 fmol, and a higher dose (330 fmol) of dextro-morphine did not further attenuate the levo-morphine-produced TF inhibition. The ED50 value for dextro-morphine to attenuate levo-morphine-produced antinociception was estimated to be 1.07 fmol (95% CI, 0.61–1.88 fmol). Figure 3B also shows that dextro-morphine at a dose of 33 fmol given alone did not affect the TF latency, which differs from the mice injected with vehicle and levo-morphine at 3 nmol that produced marked TF inhibition.

**Effect of Intrathecal Pretreatments with dextro-Naloxone and levo-Naloxone on the Intrathecal dextro-Morphine-Induced Antinociception against levo-Morphine-Produced TF Responses.** The nonopioid dextro-naloxone and its enantiomer, the nonselective μ-opioid receptor antagonist levo-naloxone, were used to determine whether the antinociception induced by dextro-morphine pretreatment is mediated by the stimulation of a nonopioid or μ-opioid receptor mechanism. We have previously shown that 10 or 30 min, but not 45 or 60 min, of levo-naloxone pretreatment is the most appropriate treatment time for blocking the levo-morphine-induced antinociception against levo-morphine-induced TF inhibition (Wu et al., 2004b). The pretreatment time of 10 min for dextro-naloxone or levo-
naloxone was used in the present study. Intrathecal pretreatment with dextro-naloxone (0.03–280 pmol) 10 min before i.t. dextro-morphine (33 fmol) 45 min before i.t. injection of levo-morphine (3.0 nmol), and TF latencies were measured at different times thereafter. B, TF latency was measured 15 min after levo-morphine (3.0 nmol) administration based on the data from Fig. 3A. Each column represents the mean, and the vertical bar represents the S.E.M. with 8 to 10 mice in each group. The two-way ANOVA followed by Bonferroni’s post test (A) or one-way ANOVA followed by Dunnett’s post test (B) was used to test the difference between groups. The F interaction, treatment, time; F = 5.52, 57.52, and 153 (A); F = 27.68 (B); **, p < 0.01, ***, p < 0.001.

Effect of Intrathecal Administration of the Glial Inhibitor Propentofylline on Intrathecal dextro-Morphine-Induced Antianalgesia against levo-Morphine-Produced TF Inhibition. The glial inhibitor propentofylline was used to determine whether the dextro-morphine-induced antianalgesia is mediated by the stimulation of glial cells. Groups of mice were coadministered i.t. with propentofylline (3.3–65 nmol) and 33 fmol of dextro-morphine or saline vehicle 45 min before i.t. injection of levo-morphine (3.0 nmol) 45 min thereafter. The TF latency was measured 15 min after levo-morphine administration. Each column represents the mean, and the vertical bar represents the S.E.M. with 7 to 10 mice in each group. The one-way ANOVA followed by Dunnett’s post test was used to test the difference between groups. The F = 12.30 (A) and 10.57 (B); **, p < 0.01; ***, p < 0.001.
TABLE 1
The ED<sub>50</sub> values of propentofylline, dextro-naloxone, and levo-naloxone for blocking dextro-morphine-induced and levo-morphine-induced antianalgesia against levo-morphine-produced TF inhibition

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Propentofylline</th>
<th>dextro-Naloxone</th>
<th>levo-Naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>dextro-Morphine</td>
<td>28.7 nmol&lt;sup&gt;a&lt;/sup&gt; (9.28–88.47)</td>
<td>0.1 pmol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 pmol&lt;sup&gt;a&lt;/sup&gt; (0.05–0.21)</td>
</tr>
<tr>
<td>levo-Morphine</td>
<td>16.9 nmol&lt;sup&gt;a&lt;/sup&gt; (0.08–3507)</td>
<td>1.79 pmol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21 pmol&lt;sup&gt;b&lt;/sup&gt; (0.68–4.71)</td>
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<sup>a</sup> ED<sub>50</sub> calculated based on data in Figs. 4 and 5.
<sup>b</sup> Data obtained from Wu et al. (2004b).

µ-opioid receptor-mediated pharmacological effects, the synthetic dextro-morphine does not have any affinity and efficacy to µ-opioid receptor and therefore does not produce analgesia and other levo-morphine-like effects (Jacquet et al., 1977). We have previously shown that subanalgesic doses of levo-morphine induce antianalgesia. The antianalgesia induced by levo-morphine is blocked by a nonopioid dextro-naloxone, indicating the mediation of a nonopioidergic mechanism (Wu et al., 2004b). Like levo-morphine, we found in the present studies that dextro-morphine, at extremely low femtomolar doses, induces antianalgesia to attenuate levo-morphine-produced antinociception. The ED<sub>50</sub> value for dextro-morphine to induce antianalgesia against levo-morphine-produced antinociception was estimated to be 1.07 fmol,
which is approximately 71,000-fold more potent than levomorphine for inducing antianalgesia (Wu et al., 2004b). The extremely high stereoselective action of dextro-morphine over levomorphine to induce antianalgesia strongly supports the view that the antianalgesia induced by dextro-morphine and levomorphine is mediated by the stimulation of a novel receptor and not the traditional G protein-coupled \( \mu \)-opioid receptors.

The effect of dextro-morphine and levomorphine on the baseline nociceptive latency seems to depend on the method of the nociceptive tests used, the route of administration, and different strains of animals. Using the TF response as the nociceptive test, we found in the present study that dextro-morphine, at the same doses used to induce antianalgesia, given i.t. did not produce any change of the baseline TF latency either using a high or low intensity of heat stimulus for the TF response. However, we recently found that dextro-morphine at femtomolar to picomolar doses given i.t. dose-dependently produced thermal hyperalgesia with the thermal paw-withdrawal test and tactile allodynia with the mechanical paw-withdrawal test in mice. We also found that dextro-morphine at an extremely low dose (0.1–10 ng/kg) injected s.c. dose-dependently produced thermal hyperalgesia using the TF test in mice (unpublished observations). Crain and Shen (2001) reported that levo-morphine at a low dose (0.1–1 \( \mu \)g/kg) given s.c. produces a decrease of TF latency (hyperalgesia) for a period more than 3 h in Swiss-Webster male mice but causes an increase of TF latency (analgesia) in 129/SvEvTac mice. The hyperalgesia and allodynia have also been described following various doses of levo-morphine in humans. Sjogren et al. (1994) report that levo-morphine-induced hyperalgesia disappears after discontinuing or substituting levo-morphine with other opioid agonists, indicating that levo-morphine itself, but not other \( \mu \)-opioid agonists, plays a role in hyperalgesia and allodynia.

Nonstereoselective Action of dextro-Naloxone and levon-Naloxone in Blocking the Antianalgesia Induced by dextro-Morphine against levo-Morphine-Produced Antinociception. dextro-Naloxone, an enantiomer of the nonselective \( \mu \)-opioid receptor antagonist levo-naloxone, has been shown not to have any affinity to \( \mu \)-opioid receptors or to block \( \mu \)-opioid-mediated analgesia (Iijima et al., 1978). We found in the present study that pretreatment with dextro-naloxone dose-dependently blocked dextro-morphine-induced antianalgesia. The finding provides additional evidence that dextro-morphine-induced antianalgesia is not mediated by the stimulation of \( \mu \)-opioid receptors. We have reported that the levo-morphine-induced antianalgesia is blocked by dextro-naloxone (Wu et al., 2004b). This neural mechanism of antianalgesia induced by dextro-morphine and levo-morphine is completely different from that of antianalgesia induced by selective \( \mu \)-opioid ligand endomorphin-2. The antianalgesia induced by endomorphin-2 is selectively blocked by levo-naloxone but not by dextro-naloxone, indicating that the endomorphin-2-induced antianalgesia is mediated by an opioidergic mechanism (Wu et al., 2004b). However, we also found that the nonselective \( \mu \)-opioid antagonist levo-naloxone was also effective with equal potency to dextro-naloxone in blocking dextro-morphine-induced antianalgesia. Thus, in addition to blocking the \( \mu \)-opioid receptors, levo-naloxone also shares with dextro-naloxone the ability to nonselectively block the novel receptor stimulated by dextro-morphine. The finding with levo-naloxone clearly indicates that levo-naloxone is nonselective as an \( \mu \)-opioid receptor blocker and should not be used as a reliable marker for identifying the opioidergic mechanism.

Thus, dextro-morphine and levo-morphine are considered to be agonists to stimulate stereoselectively this novel non-opioid receptor for inducing antianalgesia, and levo-naloxone and dextro-naloxone are antagonists to block nonstereoselectively this receptor and reverse the antianalgesia induced by dextro-morphine and levo-morphine. Thus, levo-morphine has biphasic effects; at high doses, it produces analgesia, which is mediated by \( \mu \)-opioid receptors, and at low doses, it induces nonopioidergic antianalgesia. However, it is reasonable to believe that levo-morphine at analgesic doses also induces antianalgesia, but the effect is masked by the analgesic effect of levo-morphine. This view is supported by the findings that a small dose of levo-naloxone or other opiate antagonists enhances the levo-morphine-produced analgesia in mice (Crain and Shen, 1995; Shen and Crain, 1997) and in humans (Gan et al., 1997; Joshi et al., 1999).

The fundamental principles of enantiometric selectivity were delineated long before investigators contemplated to isolation of receptors (Taylor and Insel, 1990). Easson and Stedman (1933) suggest that if selectivity of enantiomeric pairs could be seen in a biologic system, then a three-point attachment must occur between the enantiomer and a disymmetric surface. We found that dextro-morphine exhibits extremely high stereoselective action over levo-morphine in a more than four orders of magnitude for stimulating this receptor to induce antianalgesia, whereas dextro-naloxone and levo-naloxone block nonstereoselectively the dextro-morphine-induced and levo-morphine-induced antianalgesia. This finding suggests that at least three-point attachment on the receptor for the agonists and only a single or two binding sites on the receptor for the antagonists.

Antianalgesia Induced by dextro-Morphine and levo-Morphine Is Mediated by the Glial Stimulation. Propentofylline, a methylxanthine derivative, exhibits neuroprotective effects through multiple mechanisms, which include an inhibition of glutamate release (Miyashita et al., 1992), an increase in nerve growth factor secretion (Shinoda et al., 1990), and an attenuation of glial activation (Schubert et al., 2000). The specific mechanism by which propentofylline exhibits such diverse effects is not understood because of its multiple mechanisms of actions, which include nonspecific inhibition of phosphodiesterase enzyme (Meskini et al., 1994; Schubert et al., 1997) and its ability to inhibit adenosine reuptake (Parkinson et al., 1993). Both molecular mechanisms exert neuroprotective effects. The glial inhibitory property of propentofylline (Raghavendra and DeLeo, 2004) was then used to determine whether dextro-morphine-induced and levo-morphine-induced antianalgesia is mediated by glial stimulation.

It has been documented that levo-morphine stimulates glial cells to induce the release of cytokines, chemokines, and free radicals. Exposure of microglia to levo-morphine caused a marked change in cellular morphology, including assumption of a rounded shape and retraction of cytoplasmic process. These morphological changes can be blocked by the opioid antagonist levo-naloxone. In contrast, several opioid peptides do not produce effects (Dobrenis et al., 1995; Magazine et al., 1996). Levo-Morphine causes the
release of proinflammatory cytokines such as tumor necrosis factor (Chao et al., 1994). Chronic administration of levo-morphine also activates spinal microglia and astrocytes and up-regulates proinflammatory cytokines. Chronic systemic treatment with levo-morphine given in rats causes a significant increase in complement receptor type 3a-subunit (OX-42) and glial fibrillary acidic protein immunoreactivity and increases the mRNA for interleukin-1β, interleukin-6, and tumor necrosis factor-α in the lumbar spinal cord of rats (Raghavendra et al., 2002). The increase of spinal glial fibrillary acidic protein, as well as antiinflammatory tolerance (antianalgesia) to i.t. levo-morphine induced by i.t. subanalgiesis levo-morphine treatment, is blocked by cotreatments with fluorocitrate, a specific and reversible inhibitor of glial cells (Song and Zhao, 2001). Inhibition of glia activation by chronic treatment with the glial inhibitor propentofylline spares levo-morphine analgesia in neuropathic rats and reverses the development of levo-morphine-induced antiinflammatory tolerance and withdrawal-induced hyperalgesia. Attenuation of pain behaviors by propentofylline is associated with the attenuation of glial activation and the subsequent proinflammatory immune activation in the lumbar spinal cord (Sweitzer et al., 2001; Raghavendra et al., 2002). In the present studies, we found that glial inhibition with propentofylline (Schubert et al., 1997; Sweitzer et al., 2001) inhibited dextro-morphine-induced and levo-morphine-induced antianalgesia with similar potency. The results of the studies provide the evidence that antianalgesia induced by dextro-morphine and levo-morphine is mediated by the stimulation of glial cells. The stimulation of glial cells by dextro-morphine or levo-morphine may subsequently cause the release of cytokines, chemokines, and other free radicals for the induction of antianalgesia.

Damage to the peripheral nerves or nerve roots produces intense microglial and astrocyte activation in the central nervous system (Gehrmann et al., 1991; Colburn et al., 1999; Hashizume et al., 2000). The glial activation leads to the development of hyperalgesia and allodynia and the ineffectiveness or attenuation of morphine-produced analgesia (antianalgesia) (Arner and Meyerson, 1988; Watkins et al., 2001; Rowbotham et al., 2003; Raghavendra and DeLeo, 2004). Similarly, activation of glia by spinal injection of lipopolysaccharide induces hyperalgesia and antianalgesia, which is blocked by glial inhibitor (Wu et al., 2004a; Johnston and Westbrook, 2005). These observations are consistent with the present finding that activation of glia by dextro-morphine causes the induction of antianalgesia against levo-morphine-produced analgesia.

It is concluded that dextro-morphine at femtomolar doses or levo-morphine at picomolar doses given spinal induces antianalgesia against spinal levo-morphine-produced analgesia. The antianalgesia induced by dextro-morphine or levo-morphine is mediated by the stimulation of a novel and nonopioid receptor on glial cells. Blockade of the receptor by dextro-naloxone or inhibition of the glia by propentofylline reverses the attenuation of levo-morphine-produced analgesia induced by dextro-morphine or levo-morphine.

References


Gehrmann J, Monoco S, and Kreutzberg GW (1991) Spinal cord microglia cells and cytokines, chemokines, and other free radicals for the inducement or attenuation of morphine-produced analgesia against spinal dextro-morphine analgesia in neuropathic rats and reversion of the development of levo-morphine-induced antiinflammatory tolerance and withdrawal-induced hyperalgesia. Attenuation of pain behaviors by propentofylline is associated with the attenuation of glial activation and the subsequent proinflammatory immune activation in the lumbar spinal cord (Sweitzer et al., 2001; Raghavendra et al., 2002). In the present studies, we found that glial inhibition with propentofylline (Schubert et al., 1997; Sweitzer et al., 2001) inhibited dextro-morphine-induced and levo-morphine-induced antianalgesia with similar potency. The results of the studies provide the evidence that antianalgesia induced by dextro-morphine and levo-morphine is mediated by the stimulation of glial cells. The stimulation of glial cells by dextro-morphine or levo-morphine may subsequently cause the releases of cytokines, chemokines, and other free radicals for the induction of antianalgesia.

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