6β-naltrexol preferentially antagonizes opioid effects on gastrointestinal transit compared to antinociception in mice

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A B S T R A C T

Aims: The current studies were designed to compare the in vivo potencies of the opioid antagonists 6β-naltrexol and naltrexone in blocking the effects of the opioid agonist hydrocodone following intravenous (i.v.) or oral (p.o.) administration.

Main methods: Adult male CD-1 mice were used for all experiments. The 55 °C tail-flick assay was used to assess the CNS antinociceptive activity of hydrocodone, and a charcoal meal gastrointestinal transit assay was used to assess the peripheral effects of hydrocodone. Graded antagonist dose–response curves for i.v. and p.o. 6β-naltrexol and naltrexone were generated to determine ID50 antagonist potency estimates against fixed doses of hydrocodone.

Key findings: Both antagonists produced dose-related blockade of hydrocodone-induced antinociception and inhibition of gastrointestinal transit. Naltrexone was between 5- and 13-fold more potent than 6β-naltrexol in blocking a CNS effect of hydrocodone, whereas the drugs were nearly equipotent in blocking inhibition of gastrointestinal transit. Co-administration studies indicated an approximate 10-fold greater potency of 6β-naltrexol for antagonism of hydrocodone-induced inhibition of gastrointestinal transit versus antinociception, whereas naltrexone blocked both effects with near equal potency. 6β-naltrexol produced a longer duration of antagonist blockade and had a slower time to peak effect compared to naltrexone.

Significance: The pharmacology of 6β-naltrexol differentiates it from currently available opioid antagonists. This includes an intermediate selectivity for peripheral versus central opioid receptors, a long duration of action, and neutral antagonist qualities in opioid exposed systems. These properties render it a drug candidate for a co-formulation product with opioid analgesics to reduce peripheral opioid side effects and limit abuse potential.

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Introduction

Opioid analgesics remain the standard of care for management of moderate to severe pain. Hydrocodone, for example, is the most prescribed drug in the U.S. with over 120 million prescriptions filled annually (Manchikanti 2007). The widespread use of opioid analgesics, especially for chronic non-malignant pain, remains controversial (Manchikanti and Singh 2008). Abuse and addiction liability, along with diversion of prescription narcotics, has contributed to opioid phobia and increased regulatory oversight. Furthermore, opioids exhibit a spectrum of adverse side effects that limit therapeutic benefits, decrease patient compliance, and increase patient morbidity (Gutstein and Akil 2006).

One approach for reducing opioid side effects is the administration of an opioid antagonist either as a co-formulation product or as a second stand-alone drug. The general opioid antagonist naloxone has been co-formulated with pentazocine and buprenorphine to reduce intravenous (i.v.) misuse of these drugs (Baum et al. 1987; Simojoki et al. 2008). Low dose oral (p.o.) naloxone has also been evaluated for treatment of opioid-induced bowel dysfunction (McNicol et al. 2008). However, therapeutic benefits can be limited by concomitant reduction of opioid analgesia or precipitation of withdrawal (Culpepper-Morgan et al. 1992; Liu and Wittbrodt 2002; Tofil et al. 2006). Specifically, patients on higher doses of the agonist are more susceptible to aversive CNS effects from typical opioid antagonists (Liu and Wittbrodt 2002).

A second approach has focused on development of peripherally selective opioid antagonists (e.g., methylnaltrexone and alvimopan) (Leslie 2007; Berde and Nurko 2008; Reichle and Conzen 2008). Methylnaltrexone has been approved for the treatment of opioid-induced constipation in advanced-illness patients while alvimopan has received approval for treatment of post-operative ileus associated...
with partial bowel resection surgery. The use of these agents is limited to treatment of peripheral opioid-induced side effects, leaving abuse potential unaffected. Both of these drugs are highly polar, a feature that results in very low p.o. bioavailability. The peripheral antagonist effects, upon p.o. administration, may therefore be limited at least in part to direct action on the GI tract, failing to provide complete protection against other peripheral opioid adverse effects.

Our laboratories have been investigating the pharmacology of a series of opioid antagonists structurally related to naltrexone. The most extensively characterized compound, 6β-naltrexol (Fig. 1), has high affinity for the mu opioid receptor and produces dose-related antagonism of opioid agonist effects (Raehal et al. 2005). The compound exhibits a neutral antagonist profile in opioid exposed cells and rodents (Wang et al. 2001, 2004; Raehal et al. 2005), which may offer therapeutic advantages over inverse agonists (e.g., nalorexone and naltrexone) by eliciting reduced withdrawal symptoms (Sadée et al. 2005).

The present studies compared the antagonist properties of 6β-naltrexol and naltrexone. The i.v. and p.o. potencies of the antagonists to inhibit the central and peripheral effects of hydrocodone were determined. The results demonstrated that 6β-naltrexol preferentially blocks peripheral opioid effects, is orally active, and can antagonize CNS effects of hydrocodone at higher doses. These results suggest that 6β-naltrexol can be co-formulated with an opioid agonist to reduce some common adverse effects of opioid analgesics and potentially reduce abuse liability.

Materials and methods

Animals

Adult male CD-1 mice (25–35 g) (Charles River, Wilmington, MA) were used for all experiments. Mice were housed in groups of five in standard Plexiglas containers with food and water available ad libitum. Animals were maintained in a temperature and humidity controlled colony on a 12-h light/dark cycle (lights on at 07:00 AM). All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. All procedures were conducted at the University of New England with Institutional Animal Care and Use Committee approved protocols for all procedures involving animals.

Drug solutions and injections

Naltrexone was obtained through the National Institute on Drug Abuse drug supply program. Mallinckrodt (St. Louis, MO) generously provided hydrocodone bitartrate and 6β-naltrexol. All drugs were dissolved in physiological saline (0.9% NaCl) for both p.o. and i.v. injections. For p.o. administration, an 18-gauge 1.5 inch curved ball tip needle (Popper and Sons, Inc., New Hyde Park, USA) was used to prevent damage to the esophagus and from passing through the glottal opening into the trachea. Conscious mice were manually restrained by firmly gripping a fold of skin on the neck/upper back to immobilize the head. With the neck slightly extended, the feeding needle was passed gently through the mouth and pharynx into the esophagus and the substance was then administered over a 5 second period. For i.v. injections mice were placed into a restraining device and the tail was then immersed in a 38–40 °C water bath for approximately 1-min to warm the tail and dilate the tail veins. A 30-gauge needle was inserted into one of the lateral tail veins, and the solution was slowly injected over 5 s. Once the needle was removed, the injection site was firmly pressed with sterile gauze between the fingers to prevent backflow/bleeding. For multiple i.v. injections, the first injection was made as distal as possible in relation to the hindquarters of the mouse, with subsequent administrations being made progressively more proximal or using the other lateral tail vein. All injections were made using a 1-ml syringe at a volume of 10-ml/kg bodyweight.

Tail-flick assay

Antinociception was assessed using the 55 °C warm-water tail-flick assay described previously (Bilsky et al. 2000; Raehal et al. 2005). The latency to the first sign of a rapid tail-flick was used as the behavioral endpoint. Briefly, each mouse was tested for baseline latency by immersing the distal third of its tail into the water bath and recording the time until a vigorous tail-flick response to the noxious stimulus. Latencies typically averaged between 1.5 and 2.5 s in drug naïve subjects. Any mice that had baseline latencies greater than 5 s were eliminated from further testing. Upon completion of baseline testing mice were injected and retested for tail-flick latencies at various times after injection (typically 10, 20, 30, 45, 60, 90, 120 and 180 min or until the test latency approached the baseline latency, e.g., less than 20% MPE for the group). A maximal score was assigned to mice not responding within 10 s to avoid tissue damage. The percentage of antinociception was calculated as (test latency – baseline latency)/(10 – baseline latency) × 100.

Antinociception studies for determining time of antagonist peak effect

Time ranging studies were conducted for each antagonist to determine the appropriate parameters for setting up the dose–response measurements and combination experiments with hydrocodone. The duration of naltrexone and 6β-naltrexol effects were assessed by pre-treating mice with antagonists at various times prior to an injection of an approximate A90 dose of hydrocodone (3.2 mg/kg, i.v.). Previous experiments had determined the full time– and dose–response relationships with hydrocodone in the tail-flick assay (data not shown). Based on these studies, mice were tested 10 min after the hydrocodone administration, a time corresponding to agonist peak effect. The doses used for each antagonist (i.v. and p.o. respectively) were 0.56 and 5.6 mg/kg for 6β-naltrexol and 0.10 and 0.56 mg/kg for naltrexone.

Determination of antagonist potencies against hydrocodone antinociception

The p.o. and i.v. potencies of naltrexone and 6β-naltrexol were determined by administering vehicle or various doses of each test compound at appropriate times prior to an A90 dose of hydrocodone (3.2 mg/kg, i.v.). The respective i.v. and p.o. pretreatment times were 90 and 120 min for 6β-naltrexol and 30 and 45 min for naltrexone. Mice were then tested 10 min post hydrocodone injection in the 55 °C tail-flick assay. At least three doses were tested for each antagonist to generate a dose–response curve. The percent antinociception was calculated for each dose and compared to hydrocodone controls.
Antinociception studies for oral co-administration

The tail-flick assay was used to construct full dose– and time–response summaries for each antagonist in combination with hydrocodone. Vehicle or various doses of each test compound were orally co-administered with an A50 dose of hydrocodone (32 mg/kg, p.o.). At least three doses were tested for each antagonist until near maximal inhibition of hydrocodone-induced antinociception was observed. The percentage of inhibition or reversal of the hydrocodone effect was determined for each mouse at the time of peak effect for p.o. hydrocodone (20 min post injection) using methods described below.

GI transit assay

Opioid-induced GI inhibition was measured using a standard protocol (Current Protocols in Pharmacology, number 5.3). Mice were deprived of food for approximately 18 h prior to the start of the experiment. The test compound or vehicle was injected at 1 ml/100 g bodyweight (route and pretreatment time vary) prior to a p.o. delivery of a charcoal suspension (250 µl). Animals were sacrificed 30 min later by light ether anesthesia followed by cervical dislocation. The small intestine (duodenum to cecum) was dissected out and carefully uncoiled. The distance covered by the charcoal was measured and compared to the total length of the small intestine for each animal. The mean percent transit was then calculated with the following formula: (distance covered by the charcoal)/(total length of the small intestine) × 100.

Determination of antagonist potencies against hydrocodone inhibition of GI transit

The ability of each antagonist to block hydrocodone-induced GI inhibition was assessed. Both p.o. and i.v. potencies were determined by administering vehicle or various doses of each test compound prior to an antinociceptive A50 dose of hydrocodone (3.2 mg/kg, i.v.). The pretreatment times corresponded with the time of peak effect found in the antinociception studies. The charcoal meal was then given 10 min following the injection of hydrocodone. The percentage of GI transit was calculated for each mouse and a dose–response curve was constructed for each antagonist. At least three doses were used for each route and the results were compared to saline and hydrocodone controls.

GI transit studies for oral co-administration

Vehicle (saline) or various doses of each test compound were orally co-administered with an antinociceptive A50 dose of hydrocodone (32 mg/kg, p.o.). 6β-naltrexol and hydrocodone were given 60 min prior to charcoal administration whereas the naltrexone combination was given 15 min prior to the charcoal meal. These times were chosen so that the time of peak effect determined in the antinociception experiments for each antagonist would occur during the intestinal transit of the charcoal meal. Hydrocodone was found to produce the same level of GI transit inhibition over a 90 min interval (data not shown). The percentage of GI transit was calculated for each mouse, and a dose–response curve was constructed for each antagonist. At least three doses were used for each antagonist, and the results were compared to saline and hydrocodone controls.

Statistical analysis

Dose–response curves were generated for each antagonist to estimate potency to block a central (antinociception) and peripheral (GI transit) effect of hydrocodone. The percentage of inhibition or reversal of the hydrocodone effect in either assay was determined for each mouse. Percent reversal was calculated as ((test value – hydrocodone control)/(saline control – hydrocodone control)) × 100. From this data ID50 values (and 95% confidence intervals) were calculated for each route/antagonist/assay using linear regression (FlashCalc software, Dr. Michael Ossipov, University of Arizona, Tucson, AZ). Other data was analyzed using Prism GraphPad 5.0a to conduct one-way ANOVA followed by post hoc analysis.

Results

The opioid agonist hydrocodone produced dose- and time-dependent antinociception (55 °C tail–flick) and inhibition of gastrointestinal transit following i.v. and p.o. administration (Fig. 2). The calculated antinociceptive A50 values were 1.3 mg/kg and 11 mg/kg after i.v. (10 min) and p.o. (20 min) drug administration, respectively. For the inhibition of GI transit, a one-way ANOVA yielded F values of 21.9 and 16.6 (p<0.001) for i.v. and p.o. administration, respectively. The calculated inhibitory ID50 values in the GI transit assay were 3.3 and 20 mg/kg for the two routes of administration.

For antagonism studies, approximate A90 doses of hydrocodone (3.2 mg/kg i.v. and 32 mg/kg p.o.) were chosen. Based on preliminary dose-ranging studies, a fixed dose of naltrexone (0.10 mg/kg, i.v. or 0.56 mg/kg p.o.) or 6β-naltrexol (0.56 mg/kg, i.v. or 5.6 mg/kg p.o.) was administered at various time points before the hydrocodone administration (Fig. 3). Both antagonists attenuated the antinociceptive effects of i.v. hydrocodone in a time-dependent manner. Naltrexone produced a quicker onset and shorter duration of action than did approximately equipotent doses of 6β-naltrexol (Fig. 3). Naltrexone effects peaked between 30 and 45 min after i.v. administration, whereas 6β-naltrexol effects peaked between 90 and 120 min. In general, the p.o. antagonist administration resulted in a slower onset and longer duration of action compared to the i.v. route for both naltrexone and 6β-naltrexol.

Based on Fig. 3 data, dose–response curves for each antagonist (i.v. and p.o.) were generated using pretreatment times that corresponded to times of peak effect (Fig. 4). The calculated ID50 values for each antagonist and route are presented in Table 1. Upon i.v. administration, naltrexone was five-fold more potent than 6β-naltrexol in reversing the antinociceptive effects of hydrocodone, similar to previous results reported using these antagonists with morphine (Raehal et al. 2005). The p.o. administration of the antagonists also effectively reversed the antinociceptive effects of i.v. (3.2 mg/kg) hydrocodone (Fig. 4, Table 1), with naltrexone exhibiting approximately 4-fold greater potency than 6β-naltrexol. Antagonists were further characterized in their ability to inhibit hydrocodone-induced antinociception following p.o. co-administration with hydrocodone. Dose– and time–response curves were conducted for each antagonist and compared to both vehicle (saline) and hydrocodone controls (Fig. 5). When given as a p.o. co-administration, naltrexone and 6β-naltrexol were 22-fold and 56-fold less potent, respectively, than when given by the i.v. route.

The effects of similar drug combinations (hydrocodone with naltrexone or 6β-naltrexol) were assessed in the gastrointestinal transit assay (Fig. 6 and Table 1). Both antagonists produced dose-related reversal of the hydrocodone effect. Whereas naltrexone was between 5- and 13-fold more potent than 6β-naltrexol in blocking the CNS effects of hydrocodone, 6β-naltrexol was approximately equipotent to naltrexone in blocking the peripheral GI transit effects of hydrocodone (Fig. 6 and Table 1). Fig. 7 summarizes the p.o. co-administration dose–response curves for each antagonist against the GI transit and antinociceptive effects of hydrocodone, highlighting the approximate 10-fold potency difference for 6β-naltrexol for peripheral versus central sites. In contrast, naltrexone was approximately equipotent in blocking both the peripheral and central effect of hydrocodone following p.o. co-administration (Table 1).
Fig. 2. Dose–response curves for hydrocodone i.v. and p.o. in the mouse 55 °C tail-flick assay (panels A and B), and in the mouse GI transit assay (panels C and D). Statistical significance is indicated as differences between saline controls and doses of hydrocodone (Dunnett's post hoc analysis, **p<0.01 and ***p<0.001).

Fig. 3. Time ranging studies for naltrexone and 6β-naltrexol against hydrocodone-induced antinociception. Antagonists were administered i.v. (left panel) or p.o. (right panel) at various times prior to an A90 dose of hydrocodone (3.2 mg/kg, i.v., t=0 min). Antinociception was determined at time of hydrocodone peak effect (t=10 min).
The pharmacological profile of 6β-naltrexol may make it an ideal candidate for combining with an opioid agonist for the treatment of chronic pain. First, as the primary metabolite of naltrexone, there is a significant database on the clinical pharmacology and safety of the compound. Second, the physiochemical properties of 6β-naltrexol suggest CNS bioavailability in humans that would be between that of naltrexone (highly lipophilic) and methylnaltrexone (highly polar). This property may allow for preferential blockade of peripheral versus central opioid receptors. Third, the long half-life of 6β-naltrexol in humans (~14 h) may lead to higher plasma (and CNS) levels of the drug with excessive dosing, thereby blunting the reinforcing effects of a co-formulated product. Fourth, the metabolism of 6β-naltrexol is primarily through glucuronidation and renal secretion, which is expected to avoid the large interindividual variability seen with naltrexone metabolism in humans (Rollason et al. 2008). Finally, because of its neutral antagonist profile, dosing with 6β-naltrexol

**Discussion**

The pharmacological profile of 6β-naltrexol may make it an ideal candidate for combining with an opioid agonist for the treatment of chronic pain. First, as the primary metabolite of naltrexone, there is a significant database on the clinical pharmacology and safety of the compound. Second, the physiochemical properties of 6β-naltrexol suggest CNS bioavailability in humans that would be between that of naltrexone (highly lipophilic) and methylnaltrexone (highly polar). This property may allow for preferential blockade of peripheral versus central opioid receptors. Third, the long half-life of 6β-naltrexol in humans (~14 h) may lead to higher plasma (and CNS) levels of the drug with excessive dosing, thereby blunting the reinforcing effects of a co-formulated product. Fourth, the metabolism of 6β-naltrexol is primarily through glucuronidation and renal secretion, which is expected to avoid the large interindividual variability seen with naltrexone metabolism in humans (Rollason et al. 2008). Finally, because of its neutral antagonist profile, dosing with 6β-naltrexol

**Table 1**

Summary of calculated antagonist ID$_{50}$ values for naltrexone and 6β-naltrexol following various combinations of drug administration.

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>GI transit ID$_{50}$ (95% CI)</th>
<th>Antinociception ID$_{50}$ (95% CI)</th>
<th>Fold-shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>6β-naltrexol (i.v.)/hydrocodone (i.v.)</td>
<td>0.044 (0.028–0.071)</td>
<td>0.22 (0.19–0.25)</td>
<td>5.0</td>
</tr>
<tr>
<td>6β-naltrexol (p.o.)/hydrocodone (i.v.)</td>
<td>0.41 (0.31–0.54)</td>
<td>2.4 (2.1–2.6)</td>
<td>5.7</td>
</tr>
<tr>
<td>6β-naltrexol (p.o.)/hydrocodone (p.o.)</td>
<td>1.3 (0.99–1.7)</td>
<td>12 (10–15)</td>
<td>9.5</td>
</tr>
<tr>
<td>Naltrexone (i.v.)/hydrocodone (i.v.)</td>
<td>0.046 (0.028–0.076)</td>
<td>0.041 (0.032–0.053)</td>
<td>0.90</td>
</tr>
<tr>
<td>Naltrexone (p.o.)/hydrocodone (i.v.)</td>
<td>0.19 (0.14–0.28)</td>
<td>0.53 (0.47–0.61)</td>
<td>2.8</td>
</tr>
<tr>
<td>Naltrexone (p.o.)/hydrocodone (p.o.)</td>
<td>1.4 (1.1–1.8)</td>
<td>0.91 (0.63–1.3)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The fold-shift in peripheral vs. central potency was calculated as (antinociception ID$_{50}$/GI transit ID$_{50}$). Fold-shifts greater than one represent drugs that have greater potency in blocking a peripheral versus central effect of hydrocodone.
Fig. 5. Oral co-administration of hydrocodone (32 mg/kg, p.o.) with increasing doses of naltrexone (left panel) or 6β-naltrexol (right panel) in the 55 °C tail-flick. Tail-flick latencies and percent antinociception were determined at various times after the drug administration.

Fig. 6. Reversal of hydrocodone-induced inhibition of GI transit. Increasing doses of naltrexone (left panel) or 6β-naltrexol (right panel) were orally co-administered with an antinociceptive A90 dose of hydrocodone (32 mg/kg) at t = −15 min and t = −60 min. At t = 0 min a charcoal meal was gavaged and 30 min later mice were sacrificed and the small intestine dissected out. Charcoal transit was measured from the duodenum to the cecum (defined as 100%). Mean % GI Transit was calculated and compared to vehicle (saline) or hydrocodone (HC) treated mice. Statistical significance is indicated as differences between saline controls (asterisks) and hydrocodone controls (pound signs) with Dunnett’s post hoc analysis (** or ## p<0.01 and ### or #### p<0.001).

Fig. 7. Comparison of antagonist potencies of naltrexone (left panel) and 6β-naltrexol (right panel) in p.o. co-administration studies with hydrocodone (32 mg/kg) antinociception and GI transit inhibition.
should be less problematic than with inverse agonists such as naloxone and naltrexone in patients who are receiving opioid analgesics (Sadée et al. 2005).

The current study compared the in vivo antagonist activity of β3-naltrexol and the prototypic opioid antagonist naltrexone. Hydrocodone was used as the opioid agonist because (a) it is currently the most prescribed drug in the United States, (b) it produces a number of peripherally mediated opioid side effects, (c) there are significant concerns regarding its misuse and abuse, and (d) it may be an ideal opioid analgesic to co-formulate with an opioid antagonist. As predicted from previous studies with morphine, β3-naltrexol was able to produce dose- and time-related antagonism of hydrocodone. The results extend the previous work by demonstrating that β3-naltrexol is active by both the i.v. and p.o. routes of administration. Furthermore, the drug can block both peripheral (inhibition of GI transit) and central (antinociception) effects of hydrocodone. β3-naltrexol was approximately equipotent with naltrexone in blocking the inhibition of GI transit. In contrast, β3-naltrexol was between 4.4- and 13-fold less potent in blocking the antinociceptive effects of hydrocodone compared to naltrexone.

The CNS/PNS potency profile of β3-naltrexol differs from the CNS-penetrating antagonists naloxone and naltrexone, as well as from the peripherally selective antagonists methylnaltrexone and alvimopan. We surmise that these differences might have been caused by the greater polarity of β3-naltrexol compared to naltrexone. We cannot exclude the possibility that transport mechanisms also could play a role, such as interaction with MDR1A, an extrusion pump highly concentrated at the blood brain barrier, and for which opioids tend to have a degree of affinity (Mercer et al. 2007, 2008; Cunningham et al. 2008). Having modest to moderate selectivity for peripheral opioid receptors may allow for a product co-formulated with an opioid that when taken as directed reduces many of the peripheral side effects of the opioid (opioid-induced bowel dysfunction, effects on immune function, etc.). If the co-formulated product is intentionally misused (unsanctioned dose escalation or i.v. injection), enough of the antagonist may accumulate and/or penetrate the CNS to attenuate or block the agonist effects of the analgesic. Blockade of CNS opioid receptors may reduce the euphoric effects of the opioid and/or precipitate withdrawal.

Several additional properties of β3-naltrexol make it an attractive drug candidate for co-formulation. The metabolism of β3-naltrexol in humans is expected to be simpler and less variable than the metabolism of naltrexone (Porter et al. 2000). The longer duration of action of β3-naltrexol versus naloxone or naltrexone also makes it better suited for a co-formulated product with extended release opioid agonists (e.g., Oxycotton or Opana ER) or in cases where unsanctioned dose-escalations may occur. Finally, our group and others have provided evidence that β3-naltrexol has neutral antagonistic properties in the opioid exposed/dependent state (Sadée et al. 2005). This profile, in contrast to inverse agonist effects of naltrexone and naloxone, is expected to reduce the aversive effects and withdrawal observed with conventional antagonists in the opioid exposed/dependent patient (e.g., chronic pain patients receiving high dose opioid analgesics, opioid addicts, etc.).

Other pharmacokinetic properties of β3-naltrexol are also worth mentioning. First, the current studies used the CD-1 strain of mice for all of the in vivo work. Because mice fail to metabolize naltrexone to β3-naltrexol (Misra 2001), a main pathway in primates and humans, the pharmacological profiles of the two compounds can be studied without the potential confound of naltrexone conversion to β3-naltrexol in vivo. Second, beyond the preferential blockade of peripheral opioid receptors, β3-naltrexol also displayed a rather long duration of action, compared to naltrexone. This finding is consistent with the observation in humans that naltrexone is rapidly metabolized to β3-naltrexol and other metabolites, the latter possibly accounting for the long duration of action of naltrexone therapy (Crabtree 1984). Surprisingly, the time of peak action in the CNS of mice was significantly delayed with β3-naltrexol (nearly 2 h), indicating slow access to central receptors at low doses, or slow equilibration with the receptor. The latter is consistent with a ‘receptor micro-compartment model’ we had proposed for the in vivo interaction of potent antagonists with their target CNS receptors (Perry et al. 1980). While the mechanism of this pharmacodynamic delay remains to be clarified, the timing of measuring the pharmacological action of these opioid agonists and antagonists is critical to revealing the true in vivo potencies.

While the present study was performed with hydrocodone, this concept can apply to most other commonly used opioid analgesics. In a previous study we showed that β3-naltrexol is equally potent in mice against morphine compared to hydrocodone (Raehal et al. 2005). Lastly, while showing peripheral selectivity, the difference in potencies between oral and i.v. administration suggests an approximately 10% p.o. bioavailability, similar to naltrexone, and consistent with 14% bioavailability as determined in rats (Yancey-Wrona, personal communication). This is in contrast to the extremely low p.o. bioavailability of the peripherally selective antagonists alvimopan and methylnaltrexone (<1%), which are thought to act mainly by directly targeting intestinal opioid receptors without going through the systemic circulation if taken orally (Boyd and Yuan 2005; Foss and Schmidt 2005). Our results suggest that β3-naltrexol acts at least in part by first reaching the systemic circulation, and then GI opioid receptors. Because of its p.o. bioavailability, β3-naltrexol can be considered for treatment of other peripheral effects that require systemic absorption (e.g., immune dysregulation, pruritus, and bone loss). Alternatively, if given at high enough doses, it can also be considered as a replacement for naltrexone treatment of drug addictions, with the goal to reduce the variability in metabolism and aversive effects incurred with naltrexone.

Conclusion

The preclinical pharmacology of β3-naltrexol differentiates this opioid antagonist from naloxone and naltrexone, as well as from the peripherally selective antagonists methylnaltrexone and alvimopan. β3-naltrexol displays intermediate selectivity for peripheral opioid receptors following systemic administration. It may be possible to co-formulate β3-naltrexol with opioid analgesics to reduce many of the peripherally mediated side effects associated with opioid therapy. If unsanctioned escalations in dosing occurs, or if the product is injected rather than taken orally, the CNS levels of β3-naltrexol may be sufficient to blunt or block the reinforcing effects of the agonist. A similar strategy has been used with buprenorphine-naloxone coformulations (Mendelson and Jones 2003). Additional preclinical and early-stage clinical studies are needed to better understand the pharmacology of β3-naltrexol. Having this information will help in guiding the development of β3-naltrexol as a co-formulated product or as a stand-alone opioid antagonist for the treatment of opioid-mediated side effects and/or addiction.

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References
