The Relative Potency of Inverse Opioid Agonists and A Neutral Opioid Antagonist in Precipitated Withdrawal and Antagonism of Analgesia and Toxicity

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ABBREVIATIONS: CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂, cAMP, cyclic adenosine monophosphate.; GPCR, G-protein coupled receptor, [³⁵S]GTPγS, guanosine 5’-O-(3-[³⁵S] thio)triphosphate.
ABSTRACT

Opioid antagonists can be classified as inverse agonists and neutral antagonists. In the opioid dependent state, neutral antagonists are significantly less potent in precipitating withdrawal than inverse agonists. Consequently, neutral opioid antagonists may offer advantages over inverse agonists in the management of opioid overdose. In this study, the relative potency of 3 opioid antagonists to block opioid analgesia and toxicity, and precipitate withdrawal was examined. First, the potency of two opioid inverse agonists (naltrexone, naloxone) and a neutral antagonist (6β-naltrexol) to antagonize fentanyl-induced analgesia and lethality was determined. The order of potency to block analgesia was naltrexone > naloxone > 6β-naltrexol (17, 4, 1); which was similar to that to block lethality (13, 2, 1). Next, the antagonists were compared using withdrawal jumping in fentanyl-dependent mice. The order of potency to precipitate withdrawal jumping was naltrexone > naloxone >>> 6β-naltrexol (1107, 415, 1). The relative potencies to precipitate withdrawal for the inverse agonists compared to the neutral antagonist were dramatically different from that for antagonism of analgesia and lethality. Finally, the effect of 6β-naltrexol pretreatment on naloxone precipitated jumping was determined in morphine and fentanyl-dependent mice. 6β-naltrexol pretreatment decreased naloxone precipitated withdrawal; indicating that 6β-naltrexol is a neutral antagonist. These data demonstrate that inverse agonists and neutral antagonists have generally comparable potencies to block opioid analgesia and lethality, whereas the neutral opioid antagonist is substantially less potent in precipitating opioid withdrawal. These results support suggestions that neutral antagonists may have advantages over inverse agonists in the management of opioid overdose.
INTRODUCTION

Antagonists can display a spectrum of efficacy from zero to negative (Kenakin, 2001; Milligan and Bond, 1997). Antagonists that have negative efficacy can suppress basal signaling (constitutive) activity of receptors and are termed inverse agonists or negative antagonists (Kenakin, 2001; Milligan et al., 1997; Prather, 2004). Antagonists with zero efficacy generally only block agonist-induced effects without altering basal receptor signaling and are termed neutral antagonists; although in the absence of constitutive activity inverse agonists behave as neutral antagonists (Kenakin, 2001; Milligan et al., 1997; Prather, 2004).

Like many GPCR’s, opioid receptors can display basal signaling activity. Constitutive activity has been reported for μ, δ and κ opioid receptors (Burford et al., 2000; Costa and Herz, 1989; Becker et al; 1999) as well as for some opioid receptor mutants (e.g., Huang et al., 2001). In addition, studies have demonstrated that chronic exposure to opioid agonists can increase constitutive signaling activity of μ, δ and κ opioid receptors (Liu and Prather, 2001; Costa and Herz, 1989; Becker et al., 1999). This increase in constitutive activity has been suggested to be associated with the development of tolerance and dependence (Wang et al., 1994; 2001; Sadée et al., 2005; Walker and Sterious, 2005).

In behavioral studies in opioid dependent mice, inverse opioid agonists (e.g., naltrexone and naloxone) precipitate withdrawal jumping, whereas neutral antagonists (e.g., 6β-naltrexol, CTAP) are dramatically less potent (Wang et al., 2001; Sirohi et al., 2007; Raehal et al., 2005; Walker and Sterious, 2005). In biochemical studies, inverse opioid agonists increase cyclic AMP levels and inhibit GTPγS binding in cells previously
exposed to opioid agonists (e.g., Liu and Prather, 2001; Wang et al., 2001; Raehal et al., 2005). In contrast, neutral opioid antagonists do not affect cAMP levels or GTPγS binding (e.g., Wang et al., 2001). The behavioral and biochemical effects of inverse agonists have been attributed to reductions in basal signaling activity induced by prior opioid agonist exposure; whereas neutral antagonists are less effective since they lack the ability to inhibit constitutive receptor signaling (Liu and Prather, 2001; Wang et al., 2001). Taken together, opioid agonist pretreatment appears to reveal the inverse agonist properties of some antagonists, whereas, the effects of neutral opioid antagonists remain mostly independent of prior opioid treatment.

The utility of opioid antagonists to reverse the action of opioid agonists is well established. Opioid antagonists (e.g., naltrexone and naloxone) have a long clinical history in the management of opioid overdose (Gutstein and Akil, 2001; Clarke et al., 2005; Ling and Wesson, 1990). However, in opioid overdose situations, administration of an opioid antagonist can induce an acute withdrawal syndrome which could be life threatening (e.g., van Dorp et al., 2007). Consequently, precipitated withdrawal by opioid antagonists may be a concern in the management of opioid overdose and treatment of opioid dependence (Ling and Wesson, 1990; van Dorp et al., 2007). In light of these issues, recent data on inverse agonists and neutral antagonists in opioid dependence may be of pragmatic importance. Specifically, neutral antagonists may be preferable to inverse agonists in the clinical management of opioid overdose and dependence.

To explore the utility of employing a neutral opioid antagonist in the management of opioid overdose, the present study compared the activity of inverse agonists (e.g., naltrexone, naloxone) and a neutral antagonist (e.g., 6ß-naltrexol) in blocking analgesia
and lethality induced by fentanyl in the mouse. We also examined the relative potencies of these antagonists to precipitate withdrawal jumping in mice dependent on fentanyl. The data suggest that both the neutral antagonist and the inverse opioid agonists exhibit generally similar potency to block analgesia and lethality. However, the neutral opioid antagonist was dramatically less potent in precipitating withdrawal in fentanyl-dependent mice. These results suggest that neutral antagonists retain efficacy to block or reverse opioid toxicity with reduced potency to precipitate withdrawal and support suggestions (e.g., Wang et al., 2001) that neutral opioid antagonists may be preferred in the management of opioid overdose.
METHODS

Subjects:

Male Swiss Webster mice (25-33 g) obtained from Taconic Farms (Germantown, NY), were used throughout this study. Animals were housed 10 per cage for at least 24 hr after arrival with food and water available ad-libitum. All protocols were approved by the St. John’s University Institutional Animal Care and Use Committee.

General Procedure:

The time to peak effect (15 min), dose-response function and analgesic (tailflick; see below) ED$_{50}$ for fentanyl was determined previously (preliminary studies and Sirohi et al., 2008). The ED$_{50}$ to block fentanyl s.c. induced analgesia (100 μg/kg) was estimated for s.c. naltrexone, naloxone and 6β-naltrexol. The peak effect for all antagonists was previously (Sirohi et al., 2007) determined (naltrexone and naloxone= 40 min, 6β-naltrexol= 70 min). Next, the dose-response function and LD$_{50}$ for fentanyl were determined. Mice were injected with naltrexone, naloxone and 6β-naltrexol 5 min before a lethal s.c. dose of fentanyl (80 mg/kg) and the ED$_{50}$ to block lethality was determined. To examine dependence, mice were infused s.c. for 72 hr with fentanyl (1 mg/kg/day) using osmotic mini pumps. The ED$_{50}$ for naltrexone, naloxone and 6β-naltrexol to precipitate withdrawal jumping was then determined. Finally, to examine the effect of 6β-naltrexol pretreatment on naloxone induced withdrawal jumping, mice were implanted with a mini pump infusing fentanyl (1 mg/kg/day) or a morphine pellet (25 mg) for 72 hr. At the end of fentanyl or morphine treatment, mice were injected with saline or 6β-naltrexol and 70 min (peak effect of 6β-naltrexol) later injected with naloxone and
withdrawal jumping determined. In all studies, pump and pellet implantation were conducted while mice were lightly anesthetized with isoflurane:oxygen (4:96).

**Analgesia Assay:**

Analgesia (antinociception) was determined using the tailflick assay (Model TF6, Emdie Instrument Co., Maidens, VA), in which a beam of light was focused on the dorsal surface of the tail of the mouse, approximately 2 cm from the tip of the tail. The intensity of the light was adjusted so that baseline tailflick latency was typically 1-3 sec. If a mouse did not remove its tail from the heat source by 10 sec, the test was terminated, a latency of 10 sec was recorded and the mouse was defined as analgesic. Analgesia results are presented as both graded (mean tailflick latency) and quantal data. All testing was conducted by an experimenter who was unaware of the treatment of an individual mouse.

**Antagonism of Analgesia and Lethality:**

Mice (5-20 /dose) were injected s.c. with naltrexone (0.01-0.4 mg/kg), naloxone (0.1 – 1.0 mg/kg) or 6β-naltrexol (0.2- 2.0 mg/kg). Fentanyl (100μg/kg) was injected s.c. 25min following naltrexone and naloxone, and 55min following 6β-naltrexol. Mice were tested for analgesia 15min following fentanyl administration at the time of peak analgesic effect for fentanyl and the time of peak antagonist effect. Controls were injected with saline and then with fentanyl (100μg/kg) and tested for analgesia 15 min later.

For lethality studies, the dose-response function and LD$_{50}$ for fentanyl was determined over a 4hr observation period. Other mice (5-15/dose) were injected s.c. with saline, naltrexone (0.1-10.0 mg/kg), naloxone (1.0-20.0 mg/kg) or 6β-naltrexol (5.0-80.0
mg/kg) 5 min before a lethal s.c. fentanyl dose (80 mg/kg). Mice were observed for lethality for 4 hr following fentanyl administration and time of death recorded.

**Dependence Studies:**

Mice were implanted s.c. with osmotic mini pumps (Alzet Model 2001; DURECT Corporation, CA) delivering fentanyl (1 mg/kg/day) for 72 hr. At the end of treatment, with the pumps in place, mice (5-10/dose) were injected s.c. with saline, naltrexone (0.01-1.0 mg/kg), naloxone (0.01-10.0 mg/kg) or 6β-naltrexol (2.0-200 mg/kg).

Immediately following saline or antagonist injections, mice were placed in a clear container (5L) and observed for withdrawal jumping for 15 min (see below).

Other mice were implanted s.c. with a morphine pellet (25 mg) or a mini pump infusing fentanyl (1mg/kg/day) for 72 hr and then, with pumps and pellets in place, injected s.c. (5-10/dose) with saline or 6β-naltrexol (0.5-2.0 mg/kg) and 70 min (peak effect of 6β-naltrexol) later injected s.c. with naloxone (1.0 mg/kg). Immediately following naloxone injections, mice were observed for withdrawal jumping for 15 min.

Jumping was defined as all 4 paws leaving the bottom of the container. All jumping was observed by an experimenter who was unaware of the treatment of an individual mouse. For the purpose of quantal dose-response analysis, mice that jumped 50 or more times in the 15 min observation period, were defined as positive for withdrawal jumping. The ED$_{50}$ for each antagonist to precipitate withdrawal jumping was estimated. In addition, the mean number of jumps was determined for each condition.

**Drugs:**
Naltrexone HCl, naloxone HCl, 6β-naltrexol HCl, fentanyl citrate and 25 mg morphine pellets were obtained from the Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of NIDA or Spectrum Laboratory Products Inc. (Gardena, CA). Fentanyl and all antagonists were dissolved in 0.9% saline and doses are expressed as the free base.

**Data Analysis:**

Quantal dose response data were analyzed using the BLISS-21 computer program (Department of Statistics, University of Edinburgh). This program uses Probit analysis (Finney, 1973) to calculate ED$_{50}$ values, potency estimates, standard errors and 95% confidence intervals from quantal data. Graded dose response data were analyzed using the 4 parameter logistic equation to calculate the EC$_{50}$, potency estimates, standard errors and 95% confidence limits (Prism ver 5.02). Other data were analyzed by ANOVA and t-tests.
RESULTS

Fentanyl 100 μg/kg produced analgesia in 100% of saline pretreated mice. This fentanyl dose is approximately 5 times the analgesic ED$_{50}$ of fentanyl (see Sirohi et al., 2008). The estimated quantal ED$_{50}$s (95% CL) for naltrexone, naloxone and 6β-naltrexol to block fentanyl induced analgesia (100μg/kg) were 0.08 mg/kg (0.05-0.10), 0.35 mg/kg (0.28-0.44) and 1.38 mg/kg (1.18-1.69), respectively (Fig. 1A). The graded (mean tailflick latency) EC$_{50}$s (95% CL) for naltrexone, naloxone and 6β-naltrexol were 0.08 mg/kg (0.02-0.27), 0.37 mg/kg (0.26-0.51) and 1.37 mg/kg (1.28-1.47), respectively (Fig. 1B). The order of potency for quantal data relative to 6β-naltrexol was naltrexone (17) > naloxone (4) > 6β-naltrexol (1) (see Fig. 4; panel A). Graded relative potency data were similar: naltrexone (17) > naloxone (4) > 6β-naltrexol (1).

The dose response function for fentanyl-induced lethality is presented in Fig 2A. The LD$_{50}$ (95% CL) was 28.5mg/kg (14.5-74.1). In all subsequent lethality antagonism studies, 80mg/kg fentanyl was used and the ED$_{50}$ for each antagonist to block fentanyl induced lethality was determined. Mice were observed for 4hr to determine the time-course of toxicity. In each experiment, 100% lethality was observed in control groups in which saline was injected 5 min before fentanyl. The estimated ED$_{50}$s (95% CL) for naltrexone, naloxone and 6β-naltrexol to block fentanyl-induced lethality were 1.18 mg/kg (0.69-1.95), 7.19 mg/kg (4.60-11.22) and 15.34 mg/kg (9.31-24.98), respectively (Fig. 2B). The order of potency relative to 6β-naltrexol was naltrexone (13) > naloxone (2) > 6β-naltrexol (1) (see Fig. 4; panel B). The mean (± S.E.M.) time to death in controls (46.7min ±3.6) was not significantly different (F$_{3,97}$ =1.00, p>0.05) from that for 6β-naltrexol (45.0min±2.2), naltrexone (62.2min± 13.3), or naloxone (52.8min ±8.1) pre-
treated mice. These data suggest that the potency differences among the 3 antagonists to antagonize opioid overdose was not due to delayed effects of fentanyl.

To determine the ED$_{50}$ of each antagonist to precipitate withdrawal jumping, mice were infused with fentanyl (1mg/kg/day) for 72 hr and then injected with saline or an antagonist and observed (see methods). Naltrexone, naloxone and 6β-naltrexol all produced dose-dependent withdrawal jumping in mice dependent on fentanyl (Fig. 3). Naloxone at doses higher than 1.0 mg/kg produced overt signs of ataxia and shaking that were distinctly different behaviors from the doses that elicit jumping and therefore doses higher than 1.0 mg/kg for naloxone were excluded from the analysis. Similar results were obtained when mice implanted with a 25 mg morphine pellet for 72 hr were injected with higher doses of naloxone (data not shown) indicating that this was not specifically related to fentanyl dependence. In controls, no withdrawal jumping was observed when saline was injected in mice dependent on fentanyl (data not shown). The quantal ED$_{50s}$ (95 % CL) for naltrexone, naloxone and 6β-naltrexol to precipitate withdrawal jumping were 0.09 mg/kg (0.03-0.26), 0.24 mg/kg (0.12-0.47) and 99.65 mg/kg (28.74-383.40), respectively (Fig. 3A). The graded (mean number of jumps) EC$_{50s}$ (95 % CL) for naltrexone, naloxone and 6β-naltrexol to precipitate withdrawal jumping were 0.08 mg/kg (0.02-0.40), 0.25 mg/kg (0.12-0.51) and 119.1 mg/kg (6.83-2078), respectively (Fig. 3B). The order of potency for quantal data relative to 6β-naltrexol was naltrexone (1107) > naloxone (415) > 6β-naltrexol (1) (see Fig. 4; panel C). Graded relative potency data were similar: naltrexone (1489) > naloxone (476) > 6β-naltrexol (1).

Since 6β-naltrexol is believed to function as a neutral antagonist, we examined the effect of 6β-naltrexol pretreatment on naloxone precipitated withdrawal jumping.
Mice were implanted with a 25 mg morphine pellet for 72 hr and injected first with saline or 6β-naltrexol (0.5-2.0 mg/kg) and 70 min later (peak effect of 6β-naltrexol) injected with naloxone 1.0 mg/kg (see methods). In the saline pretreatment group, withdrawal jumping was observed in 93% of mice. On the other hand, 6β-naltrexol pretreatment before naloxone administration dose dependently decreased naloxone precipitated withdrawal jumping (Fig. 5A,B). Other mice were treated with fentanyl (1mg/kg/day) for 72 hr and then injected with saline or 6β-naltrexol (2.0mg/kg), followed by naloxone (1.0mg/kg). 6β-naltrexol completely blocked naloxone precipitated withdrawal in fentanyl-dependent mice (Fig 5C,D).
DISCUSSION

The activity of opioid antagonists has been shown to range from neutral efficacy to negative efficacy. Studies strongly suggest that the commonly used opioid antagonists naloxone and naltrexone display negative efficacy and are therefore classified as inverse agonists (Costa and Herz, 1989; Marczak et al., 2007; Wang et al., 2001). While all opioid antagonists are capable of reversing or blocking the effects of opioid agonists, only inverse agonists inhibit signaling of constitutively active opioid receptors (e.g., Sadée et al., 2005; Sirohi et al., 2007; Wang et al., 2004). However, in the absence of constitutive receptor activity, inverse agonists are generally indistinguishable from neutral antagonists.

Previous studies have demonstrated that opioid agonist pretreatment can increase the constitutive activity of opioid receptors and it has been suggested that this may play an important role in opioid tolerance and dependence (e.g., Sadée et al., 2005; Wang et al., 2001). In the opioid dependent state, inverse opioid agonists are substantially more potent in precipitating withdrawal and activating putative biochemical pathways that mediate dependence compared to neutral opioid antagonists (Wang et al., 2001; Sadée et al., 2005; Sirohi et al., 2007). Based on these findings, it seems plausible that neutral opioid antagonists may offer advantages over inverse agonists in the clinical management of opioid overdose and dependence (Wang et al., 2004; Raehal et al., 2005). To date there has been no comparison between inverse agonists and neutral antagonists in terms of reversal of opioid toxicity. In in vivo studies the most widely studied neutral opioid antagonist is 6β-naltrexol. Therefore, the present study compared the potency of two opioid inverse agonists (i.e., naltrexone and naloxone) and a neutral antagonist (i.e., 6β-
naltrexol) to block fentanyl-induced analgesia and lethality. The relative potency of these antagonists to precipitate withdrawal jumping in mice dependent on fentanyl was also determined.

Initially, the potencies of each antagonist to block fentanyl-induced analgesia (Fig. 1, 4A) and lethality (Fig. 2B, 4B) were determined. The order of potency to block fentanyl induced analgesia, relative to 6β-naltrexol, was naltrexone > naloxone > 6β-naltrexol. The rank order of potencies to block analgesia was similar to that to block lethality. Next, the potency to precipitate withdrawal jumping in fentanyl dependent mice was estimated for each antagonist. Naltrexone and naloxone were >1000 and >400 times, respectively, more potent than 6β-naltrexol in precipitating withdrawal (Fig. 3; 4C).

Overall, there was good agreement for the relative potencies of the antagonists for quantal and graded analyses of the data. These results are consistent with previous studies (Wang et al., 2001; Sirohi et al., 2007) in which 6β-naltrexol was found to be substantially less potent in precipitating withdrawal jumping than antagonizing morphine analgesia. In the present study, we extend this observation to fentanyl and to the reversal of opioid toxicity. The neutral opioid antagonist was dramatically less potent in precipitating withdrawal jumping in fentanyl-dependent mice compared to antagonism of fentanyl-induced analgesia and toxicity. Taken together, these results suggest that a neutral opioid antagonist such as 6β-naltrexol might be preferable to the commonly employed inverse agonists (i.e., naloxone, naltrexone) in the clinical management of opioid overdose.

The current data suggest that 6β-naltrexol might be useful clinically in treating opioid overdose, while limiting the risk of precipitating withdrawal. If neutral antagonists
have a role in reversing opioid overdose clinically, it is important that activity be demonstrated in primates. In light of this suggestion, it is notable that Ko et al. (2006) and Li et al. (2008) propose that 6β-naltrexol does not act as a neutral antagonist in the rhesus monkey. Li et al. (2008) found that naltrexone, 6β-naltrexol and 6α-naltrexol did not differ in pA₂ values in dependent and nondependent monkeys. Ko et al. (2006) report that pretreatment with 6β-naltrexol did not shift the dose response function for naltrexone-induced increases in respiration which is a model of morphine dependence. While these results suggest caution in concluding that 6β-naltrexol, and neutral opioid antagonists in general, might be useful in man, there are substantial procedural differences between our studies and the primate studies. An important difference is that morphine dependence was produced using intermittent injections (Ko et al., 2006; Li et al., 2008) and monkeys were tested for withdrawal 18hr (Ko et al., 2006) following the final injection; unlike the present results in which mice were tested in the presence of morphine or fentanyl. It is worth noting that the protocol used in the present study is analogous to what might occur in clinical opioid overdose situations. In addition, Ko et al. (2006) used a cumulative dose approach that might reduce the effect of the 6β-naltrexol compound. Specifically, it might be anticipated that the effects of 6β-naltrexol would be waning as the experiment using cumulative dosing continues. These studies may be best conducted using a single pretreatment 6β-naltrexol dose, followed by a single naltrexone dose. Nevertheless, it is important that the potential translational value of 6β-naltrexol and other neutral antagonists be thoroughly studied. Finally, Divin et al. (2008) report that 6β-naltrexol did not block withdrawal precipitated by the putative inverse agonist naltrexone in the mouse. The reason for this outcome is not clear, but
there are several studies, including the present report, that have demonstrated that inverse agonist effects, including precipitated withdrawal, are inhibited by neutral antagonists such as 6β-naltrexol (e.g., Marczak et al., 2007; Raehal et al., 2005; Walker and Sterious, 2005; Wang et al., 2007). Furthermore, in the present study 6β-naltrexol was effective in inhibiting withdrawal in both morphine and fentanyl-dependent mice which suggests that this effect is likely to extend to dependence on other opioid agonists.

In a recent study, 6β-naltrexol was reported to be equipotent to naltrexone and naloxone to up-regulate μ-opioid receptor density and to produce functional supersensitivity (Sirohi et al., 2007). Thus, these data indicate that naloxone, naltrexone and 6β-naltrexol share similar efficacy to block opioid analgesia and lethality, and to induce opioid receptor upregulation. These results strongly indicate that these effects depend only upon occupancy of the receptor and do not require changes in receptor signaling. On the other hand, the fact that 6β-naltrexol is substantially less potent than naloxone and naltrexone in precipitating withdrawal jumping, suggests that this outcome is dependent to some degree on changes (reductions) in opioid receptor activity by inverse agonists. These data support the idea of functional (protean) selectivity of ligands and extend this idea to antagonist effects (e.g., Urban et al., 2007). Thus, a given antagonist acting at a receptor can produce a profile of effects that differs from that of another antagonist acting at the same receptor in terms of the relative potencies to produce these effects. That 6β-naltrexol functions as a lower efficacy antagonist is supported by blockade of inverse agonist mediated effects. 6β-naltrexol pretreatment dose-dependently blocked naloxone precipitated withdrawal jumping in mice dependent on morphine and fentanyl (Fig 5). These data, taken together with previously reported
results (Sirohi et al., 2007; Raehal et al., 2005; Wang et al., 2001), substantiates suggestions that 6β-naltrexol is a neutral antagonist.

Taken together, inverse agonists and the neutral antagonist have relatively similar potencies to block opioid analgesia and lethality. Conversely, inverse agonists are substantially more potent in precipitating opioid withdrawal compared to the neutral antagonist. These results suggest that neutral antagonists may be preferred over inverse agonists in the management of opioid overdose. Furthermore, the reduced potency of neutral antagonists to precipitate withdrawal in the opioid dependent state may allow the initiation of antagonist treatment for addiction without the opioid free interval that is typically required in the case of inverse opioid agonists. Overall, the present results support suggestions (e.g., Wang et al., 2001) that neutral opioid antagonists may have advantages in the clinical management of opioid overdose, addiction and dependence.

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REFERENCES


FOOTNOTES:

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LEGENDS FOR FIGURES:

Figure 1. Dose-response functions for antagonism of fentanyl induced analgesia by naltrexone, naloxone and 6β-naltrexol. Mice (5-20 /dose/drug) were injected s.c. with naltrexone (0.01-0.4 mg/kg), naloxone (0.1 – 1.0 mg/kg) or 6β-naltrexol (0.2- 2.0 mg/kg). Fentanyl (100μg/kg) was injected s.c. 25min following naltrexone and naloxone, and 55min following 6β-naltrexol. Mice were tested for analgesia 15 min following fentanyl administration at the time of peak effect for fentanyl and each antagonist. Each mouse was tested only once. A tailflick latency of less than 10 sec was recorded as blockade of fentanyl analgesia. (A)The percent of mice with blockade of fentanyl analgesia and (B) the mean (-S.E.M) tail flick latency are plotted versus dose for each antagonist. For ease of comparison with the quantal data, mean tail flick latency data are plotted on an inverted ordinate.

Figure 2. Dose-response functions for fentanyl induced lethality and blockade of fentanyl induced lethality by naltrexone, naloxone and 6β-naltrexol. (A) Mice (5/dose) were injected with fentanyl (1-80 mg/kg) and observed for 4 hr. The percent lethality is plotted versus fentanyl dose. The LD$_{50}$ (95% CL) was 28.5mg/kg (14.5-74.1). (B) Mice (5-15/dose/drug) were injected s.c. with saline, naltrexone (0.1-10.0 mg/kg), naloxone (1.0-20.0 mg/kg) or 6β-naltrexol (5.0-80.0 mg/kg) 5 min before a lethal dose of fentanyl s.c. (80mg/kg) and mice were observed for 4 hr. The percentage of mice that survived is plotted versus antagonist dose.
Figure 3. Dose-response functions of naltrexone, naloxone and 6β-naltrexol to precipitate withdrawal jumping. Mice were implanted s.c. with osmotic mini pumps delivering fentanyl (1 mg/kg/day) for 72 hr. At the end of treatment, mice (5-10/dose/drug) were injected s.c. with naltrexone (0.01-1.0 mg/kg), naloxone (0.01-1.0 mg/kg) or 6β-naltrexol (2.0-200 mg/kg), and observed for withdrawal jumping for 15min (see methods). (A) Percent of responders (50 or more jumps) and (B) mean number of jumps (± S.E.M) are plotted versus antagonist dose.

Figure 4. Relative potencies of naltrexone, naloxone and 6β-naltrexol for antagonism of analgesia, lethality and to precipitate withdrawal jumping. The data in this figure are based on quantal dose-response results presented in Figs 1, 2 and 3; and potencies were calculated using Probit analyses. (A) Mice were treated as described in Fig 1 and tested for antagonism of fentanyl induced analgesia. Quantal relative potencies (±SE) of naltrexone, naloxone and 6β-naltrexol to block fentanyl (100μg/kg) induced analgesia are plotted. (B) Mice were treated as described in Fig 2 and tested for antagonism of fentanyl induced lethality. Relative potencies (±SE) of naltrexone, naloxone and 6β-naltrexol to block fentanyl (80 mg/kg) induced lethality are plotted. (C) Mice were treated as described in Fig 3 and tested for precipitated withdrawal following fentanyl treatment. Quantal relative potencies (±SE) of naltrexone, naloxone and 6β-naltrexol to induce withdrawal jumping in mice dependent on fentanyl (1 mg/kg/day) are plotted.
Figure 5. Effect of 6β-naltrexol on naloxone precipitated withdrawal jumping in morphine and fentanyl-dependent mice. (A, B) Mice (5-10/dose) were implanted s.c. with a morphine pellet (25 mg) for 72 hr. At the end of treatment, mice were injected s.c. with saline (control) or 6β-naltrexol (0.5-2.0 mg/kg) and 70 min later (peak antagonist effect of 6β-naltrexol) injected s.c. with naloxone (1.0 mg/kg). The doses of 6β-naltrexol used in this study do not produce a withdrawal response (see Fig 3 and Sirohi et al., 2007). Control (C) mice were injected with saline followed by naloxone. Mice were observed for withdrawal jumping for 15min following naloxone treatment. A. Percent of responders for withdrawal jumping (50 or more jumps) and B. mean number of jumps (± S.E.M) are plotted versus antagonist dose. (C, D) Mice (5/group) were implanted with s.c. osmotic mini pumps that infused fentanyl (1.0 mg/kg/day) for 72 hr. At the end of treatment, mice were injected with saline or 6β-naltrexol (2.0 mg/kg) and 70 min later injected with naloxone (1.0 mg/kg). Mice were observed for withdrawal jumping for 15min following naloxone treatment. C. Percent of responders for withdrawal jumping (50 or more jumps) and D. mean number of jumps (± S.E.M) are presented for each group.
Figure 1
Figure 2

(A) Percent lethality of fentanyl (mg/kg) vs. fentanyl concentration.

(B) Percent survival vs. dose (mg/kg) for different opioids:
- Naltrexone
- Naloxone
- 6β-Naltrexol
Figure 3
Figure 4
Figure 5