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The Analgesic Efficacy of Fentanyl: Relationship to Tolerance and μ -Opioid Receptor Regulation

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Abstract

This study determined if fentanyl analgesic efficacy predicts the magnitude of tolerance and μ -opioid receptor regulation. To estimate efficacy, mice were injected i.p. with saline or clocinnamox (CCAM), an irreversible μ -opioid receptor antagonist, (0.32 – 25.6 mg/kg) and 24 hr later fentanyl cumulative dose response studies were conducted. CCAM dose dependently shifted the fentanyl dose-response function to the right. The apparent efficacy (τ) of fentanyl, based on the operational model of agonism, was estimated as 58, indicating that fentanyl is a high analgesic efficacy agonist. Next, mice were infused with fentanyl (1, 2 or 4 mg/kg/day) for 7 days. Controls were implanted with placebo pellets. At the end of 7 days, morphine cumulative dose response studies or μ -opioid receptor saturation binding studies were conducted. Fentanyl infusions dose-dependently decreased morphine potency with the highest fentanyl dose reducing morphine potency by $\approx$ 6 fold. Chronic infusion with fentanyl (4mg/kg/day) significantly reduced μ -opioid receptor density by 28% without altering affinity, whereas lower infusion doses had no effect. Taken together, the present results strengthen the proposal that opioid analgesic efficacy predicts μ -opioid receptor regulation and the magnitude of tolerance.

Keywords

Fentanyl; opioid agonist; analgesia; efficacy; tolerance; μ -opioid receptor; receptor downregulation

INTRODUCTION

Efficacy has been defined as the ability of a drug to impart a physiological response to a biological system at a given receptor occupancy (Kenakin, 2002). Recent formulations of this concept have suggested that there are multiple efficacies for ligands at the same receptor; a property which has been variously termed 'functional selectivity', 'biased agonism' and 'protean agonism' (e.g., Kenakin, 2007; Galandrin and Bouvier, 2006; Mailman, 2007). Consistent with this concept, opioid analgesics have been shown to vary in terms of efficacy for a variety of effects. For example, opioid agonists have differential effects on μ -opioid

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receptor regulation (e.g., internalization, downregulation), regulation of trafficking proteins (e.g., β -arrestin, Dynamin) and the magnitude of tolerance (Bohn et al., 2004; Duttaroy and Yoburn, 1995; Keith et al., 1998; Whistler and von Zastrow, 1998; Pawar et al., 2007).

Previously, opioid agonist efficacy has been considered as a characteristic of a drug irrespective of the outcome measure. Many experiments using this unitary measure of efficacy have suggested that chronic treatment (e.g., continuous s.c. infusion) with agonists proposed as having higher efficacy (e.g., etorphine, DAMGO) can induce internalization and downregulation of μ opioid receptors in vitro and in vivo (Keith et al., 1998; Duttaroy and Yoburn, 1995; Pawar et al., 2007; Stafford et al., 2001; Whistler and von Zastrow, 1998). Conversely, agonists with lower efficacy (e.g., morphine, oxycodone) are less likely to produce either internalization or downregulation (Keith et al., 1998; Pawar et al., 2007; however; see Haberstock-Debic et al., 2005; Rodríguez-Muñoz et al., 2007). Furthermore, the ability of opioid analgesics to internalize and downregulate μ opioid receptors appears to be inversely related to the magnitude of tolerance. Higher efficacy opioid agonists produce substantially less tolerance in vivo than lower efficacy opioid agonists at equieffective (e.g., equi-multiples of the ED₅₀) doses (Duttaroy and Yoburn, 1995; Paronis and Holtzman, 1992; Pawar et al., 2007; Walker and Young, 2001).

In a previous report (Pawar et al., 2007), we have used the operational model of agonism (Black and Leff, 1983) to estimate the analgesic efficacy of several opioid analgesics. This model, which is based on the use of an irreversible antagonist to deplete receptors, calculates the parameter τ . τ is used to compare analgesic efficacy; with larger values representing agonists with greater efficacy. Morphine and oxycodone were found to have relatively low τ values, whereas etorphine was identified as a higher efficacy opioid analgesic. This more quantitative estimate of analgesic efficacy confirmed previous results suggesting a relationship between opioid efficacy, μ -opioid receptor regulation and tolerance (e.g., Duttaroy and Yoburn, 1995; Paronis and Holtzman, 1992; Pawar et al., 2007).

In the present study, we have extended this approach to the clinically used opioid analgesic fentanyl. The analgesic efficacy of fentanyl was quantitatively estimated (i.e., τ) and this was used to predict tolerance and μ -opioid receptor regulation. In addition, in a previous paper (Duttaroy and Yoburn, 1995), we reported that opioid efficacy was not a major factor in predicting the magnitude of tolerance when opioid analgesics were administered intermittently. Therefore, in this study we also examined tolerance and μ -opioid receptor regulation following intermittent treatment with fentanyl.

MATERIALS AND METHODS

Subjects

Male Swiss Webster mice (23–35 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

Initially, the analgesic ED₅₀ (tailflick; see below) was estimated using standard and cumulative dosing protocols (see below), at the time of peak effect of fentanyl. Next, mice were injected i.p. with saline (8–10/group) or CCAM (0.32 – 25.6 mg/kg) (5–10/group) and 24 hr later fentanyl cumulative dose response studies were conducted. In subsequent studies, tolerance to morphine following fentanyl treatment was determined. Morphine was used as the test drug, as we have done previously, so that the magnitude of potency shifts induced by fentanyl could

be directly compared among opioid drugs (see Pawar et al., 2007). For chronic treatment studies, mice (8–10/group) were implanted s.c. with osmotic mini pumps (Alzet Model 2001; DURECT Corporation, CA) infusing fentanyl (1, 2 or 4 mg/kg/day) for 7 days. Controls were implanted with placebo pellets. At the end of 7 days, pumps and pellets were removed and 16 hr later morphine cumulative dose response studies were conducted or, 16 – 24 hr later mice were sacrificed and spinal cords were collected for radioligand binding studies. For acute (single injection) and intermittent (one injection/day for 7 days) studies, mice (8–10/group) were injected with fentanyl (100 or 200 µg/kg) and 24 hr later, morphine cumulative dose response studies were conducted or mice were sacrificed and spinal cords were collected for radioligand binding studies. Pump and pellet implantation and removal were conducted while mice were lightly anesthetized with isoflurane: oxygen (4:96).

Analgesia Assay

Antinociception was assayed 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 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 analgesic. All testing was conducted by an experimenter who was unaware of the treatment of an individual mouse.

Dose Response Protocol

Dose-response studies were conducted according to standard and cumulative dosing protocols (see Duttaroy et al., 1997). Briefly, in the standard dosing protocol, mice (5–6/dose) were injected with fentanyl (10 – 40 µg/kg) and tested once for analgesia at the time of peak effect of fentanyl (15 min; as determined previously).

In the cumulative dosing protocol (available on request), mice (8/group) were injected s.c. with a starting dose of morphine or fentanyl and tested for analgesia 30 and 15 min later, respectively. If the mouse displayed a tailflick latency of 10 sec, it was defined as analgesic and not tested further. Otherwise, the mouse was immediately injected s.c. with a second dose (increment dose) of morphine or fentanyl and retested. This procedure was continued until all mice were analgesic. The cumulative dosing protocol is useful to reduce the number of mice required and the cost of supplies (e.g. osmotic pumps).

Radioligand Binding Studies

Mice (10/dose) were infused with fentanyl (1, 2 or 4 mg/kg/day) for 7 days. For all treatment groups, controls were implanted with placebo pellets for 7 days. At the end of treatment, the pumps and pellets were removed and 16 or 24hr later mice were sacrificed. Other mice (10/dose) were injected s.c. with fentanyl 200 µg/kg once a day for 7 days. Controls were injected s.c. with saline. Twenty four hr after the last injection, mice were sacrificed, spinal cords were rapidly removed. Spinal cord was used to examine changes in µ-opioid receptors since numerous studies have shown that opioids produce analgesic effects at the spinal level, and this tissue is readily removed and relatively homogenous compared to brain (e.g., Shah et al., 1994; Patel et al., 2003). Spinal cords were pooled in tubes containing 15ml ice cold 50mM TRIS buffer (pH 7.4). Samples were homogenized (Brinkmann Polytron Homogenizer, Westbury, NY) at 20,000 rpm on ice for 40 sec. Homogenates were centrifuged at 15,000 rpm ($\approx 26,000 \times g$) for 15 min (3–9°C). The supernatant was discarded and pellets were stored (–80°C) until analysis. On the day of assay, the pellet was thawed on ice, re-suspended in 15ml TRIS buffer and centrifuged at 15,000 rpm for 15 min (3–9°C) and the supernatant discarded. The pellet was re-suspended in 35ml TRIS buffer and incubated for 30 min in a shaking water bath (25°C). Samples were centrifuged again for 15min, the supernatant discarded and pellets were

suspended in 18 ml of ice cold 50 mM potassium phosphate buffer (pH 7.2). An aliquot of homogenate was assayed in triplicate in saturation binding assays using [^{3} H] DAMGO (range: 0.02–10nM) (μ -opioid receptor ligand; PerkinElmer Life Sciences, Boston, MA). Non-specific binding was determined in triplicate in the presence of levorphanol (1000nM). Tubes were incubated for 90min at 25°C. Incubation was terminated by the addition of ice cold phosphate buffer and filtering the samples over GF/B filters (Brandel, Gaithersburg, MD). Filters were washed three times with phosphate buffer and then placed in vials containing scintillation cocktail and counted. Counts per minute (cpm) were converted into disintegration per minute (dpm) using the external standard method. Protein was assayed by the Bradford method (Bradford, 1976) using reagent from Bio-Rad (Richmond, CA). Binding studies were conducted 1–8 times for each treatment.

Drugs

Fentanyl hydrochloride was obtained from the Sigma Chemical Company (St. Louis, MO). Morphine sulphate and inert placebo pellets were obtained from the Research Triangle Institute (Research Triangle Park, NC). Cloccinnamox mesylate was obtained from Tocris Bioscience (Ellisville, MO). Inert placebo pellets were wrapped in nylon mesh before implantation. Fentanyl and morphine were dissolved in 0.9 % saline and doses are expressed as the free base. Cloccinnamox (CCAM) was dissolved in dH₂O with $\approx$ 4 % DMSO (dimethyl sulfoxide) to enhance solubility and CCAM doses are expressed as the salt.

Data Analysis

Dose-response studies were analyzed as quantal (percent analgesic) or graded (tailflick latency) data. The estimation of agonist ED₅₀ necessitates the use of quantal data. Quantal dose response data were analyzed with the program BLISS-21 (Department of Statistics, University of Edinburgh). This program uses Probit Analysis (Finney, 1973) to calculate ED₅₀ values, standard errors, potency ratios and 95% confidence intervals. Potency changes are based on the ratio of the ED₅₀ value in the treated groups relative to control. This change is referred to as the shift in the ED₅₀. The calculation of agonist efficacy following receptor depletion using the operational model requires graded data (see details below). Dose-response data were fit using nonlinear regression (4 parameter logistic) using Sigmaplot (version 10; Systat Software, Inc., San Jose, CA).

The operational model of agonism (Black and Leff, 1983; Black et al., 1985; Leff et al., 1990), allows quantitative comparison of various agonists in terms of efficacy. In this model, apparent efficacy is represented by τ , a dimensionless proportionality factor which is generally considered to be analogous to traditional estimates of efficacy (Motulsky and Christopoulos, 2004). In this model, as in other models that estimate efficacy (Furchgott, 1966; Furchgott and Bursztyn, 1967), the experimental protocol requires partial receptor inactivation or depletion. Cloccinnamox (CCAM), an irreversible μ opioid receptor antagonist is a reasonable choice for estimating τ since it has been shown to dose dependently decrease the μ opioid receptor density (Burke et al., 1994; Chan et al., 1995).

Calculation methods for the operational model of agonism described by Zernig et al., (1996) were employed to estimate the efficacy of fentanyl. τ is equal to the ratio of the total concentration of receptors (R_0) and the concentration of receptor needed to be occupied by an agonist to provoke half maximal response (K_E). The irreversible μ -opioid receptor antagonist CCAM was used to deplete the fraction of receptors available for an agonist to interact which can be denoted as q (Furchgott, 1996). The q value can be obtained by dividing the τ value of a CCAM treated group by the τ value of control. According to Black et al., 1985, non-rectangular hyperbolic curves relate agonist dose $[A]$ to the effect E :

$$E = \frac{E_m \times \tau^n \times [A]^n}{(K_A + [A])^n + \tau^n \times [A]^n}$$

Where E is the graded effect (mean tailflick latency), E_m is the maximum attainable response (analgesia) (10 sec). $[A]$ is the dose of agonist, K_A is the apparent dissociation constant, and n is the slope factor of the transducer function. A semi-logarithmic form of the above equation (Zernig et al., 1995) is used that incorporates a new constant c , which is the baseline response (base line tailflick latency in the present case), and replaces τ with $(q \times \tau_{\text{Control}})$:

$$E = E_m / (((10^{(\log(K_A) - \log[A])}) + 1) / (q * \tau_{\text{control}}))^n + 1) + c$$

Using a non linear fitting program developed by Zernig et al., (1995), and a general mathematical software package (Mathematica, Wolfram Research, Champaign, USA, Wolfram 1991), all fentanyl dose response curves obtained from controls and CCAM treated groups were simultaneously fit to the above equation.

Binding data from saturation studies were analyzed by Prism ver 5.01 (Graph Pad Software, San Diego, CA) using nonlinear regression. All binding data were best fit by a one-site model.

RESULTS

Both standard and cumulative dosing protocols were used to determine the ED_{50} for fentanyl (Fig. 1). The ED_{50} for fentanyl in two independent studies using the standard dose protocol was 18.1 ± 0.07 and 22.0 ± 0.06 $\mu\text{g/kg}$ ($\pm$ SE from Probit Analysis). The mean ($\pm$ SEM) ED_{50} for fentanyl in 8 independent studies for controls using the cumulative dosing protocol was 40.8 ± 5.2 $\mu\text{g/kg}$ (Fig. 1). Next, the efficacy of fentanyl was determined using CCAM (Fig. 2). Mice were injected with CCAM and 24 hr later fentanyl cumulative dose response studies were conducted. CCAM dose dependently shifted the fentanyl dose-response function to the right. The apparent efficacy (τ) of fentanyl based on the operational model of agonism was estimated as 58 (54–62, 95 % CL).

To examine the effect of fentanyl on μ -opioid receptor density in spinal cord, mice were infused with fentanyl (1, 2 or 4 mg/kg/day) for 7 days or injected once/day for 7 days (200 $\mu\text{g/kg/day}$). Chronic infusion with fentanyl (4mg/kg/day) significantly reduced μ opioid receptor density by 28% without altering affinity, whereas 1 or 2mg/kg/day fentanyl infusions had no significant effect (Fig. 3). When mice were injected daily for 7 days with fentanyl (200 $\mu\text{g/kg}$) there were no significant changes in B_{MAX} or K_D (data not shown). Daily injections of fentanyl at doses higher than 200 $\mu\text{g/kg}$ could not be used because of lethality (≈ 10 –20% lethality at 500 $\mu\text{g/kg}$).

The effect of 7 day fentanyl treatment on morphine potency was examined (Fig. 4). Fentanyl infusions (1, 2 or 4 mg/kg) dose-dependently decreased morphine potency with the highest fentanyl dose reducing morphine potency by ≈ 6 fold. However, neither acute nor 7 days of intermittent fentanyl (100 or 200 $\mu\text{g/kg}$) injections had any significant effect on morphine potency.

DISCUSSION

Opioid agonist efficacy has been proposed to be an important factor in μ -opioid receptor regulation and in determining the magnitude of opioid tolerance. Previous studies have shown that higher efficacy opioid agonists downregulate μ -opioid receptors in vivo and, when infused at equi-effective doses, produce less tolerance compared to lower efficacy agonists (e.g., Duttaroy and Yoburn, 1995; Pawar et al., 2007). To most directly address the potential role of opioid agonist efficacy in tolerance and receptor regulation, it is important to accurately quantitate efficacy. In the current study, we used a modification of the operational model of

agonism (Zernig et al., 1995; 1996) to estimate the efficacy (τ) of fentanyl to produce analgesia in the tailflick assay in the mouse. We have previously used this approach to estimate analgesic efficacy for morphine, etorphine and oxycodone (Pawar et al., 2007). In that study, the analgesic efficacy of these agonists was predictive of the magnitude of tolerance and opioid agonist induced downregulation of spinal μ -opioid receptors.

In the present study, the efficacy of fentanyl ($\tau = 58$) in the mouse tailflick assay was found to be relatively high compared to oxycodone and morphine (Pawar et al., 2007). This outcome agrees with a study that examined the analgesic efficacy of fentanyl relative to morphine using the operational model (Zernig et al., 1995). Similarly, data from other studies that did not quantitatively estimate efficacy, suggest that fentanyl is a high analgesic efficacy agonist compared to morphine (e.g., Adams et al., 1990). On the other hand, fentanyl's relative efficacy in the GTP γ S binding assay is generally similar to that of morphine, although this can vary depending on the cells or tissue examined (e.g., Clark et al., 2006; Peckham and Traynor, 2006; Selley et al., 1997, 1998). However, in a drug discrimination procedure in the pigeon, fentanyl was reported to have a relatively low τ value, although etorphine retained its high efficacy in that assay (Barrett et al., 2003). Taken together, these data indicate that relative efficacy of opioid agonists can vary depending on the outcome measure, which is consistent with the concept of functional selectivity (Mailman, 2007). Thus, it is important to estimate efficacy for each effect, rather than to assume that efficacy determined in one assay, or in one tissue source, accurately reflects efficacy for other effects.

Since the τ value for fentanyl indicated it is a high efficacy agonist in a model of analgesia in the mouse, we predicted that it would be effective in downregulating μ -opioid receptors. Consistent with previous studies demonstrating fentanyl induced μ opioid receptor internalization and downregulation (Keith et al., 1998; Koch et al., 2005; Yoburn et al., 1993), fentanyl infusion was found to downregulate spinal μ -opioid receptors. We have previously shown that etorphine which has a comparable τ value (52) to fentanyl, also effectively downregulates spinal μ -opioid receptors (Pawar et al., 2007; Patel et al., 2002). In addition, we predicted that fentanyl would produce less tolerance than low analgesic efficacy opioids when infused at equi-effective doses (i.e., equi-multiples of the analgesic ED₅₀). To evaluate this prediction, Fig. 5 plots the shift in the ED₅₀ for fentanyl, as well as oxycodone and etorphine ($\tau=20, 52$, respectively; data from Pawar et al., 2007). As expected of a higher efficacy opioid agonist, fentanyl behaved similarly to etorphine and produced less tolerance than oxycodone, a lower efficacy agonist. Therefore, the current results with fentanyl are consistent with the inverse relationship between τ and tolerance; and the direct relationship between τ and μ -opioid receptor downregulation in vivo. Taken together, these results, and the results of previous studies (e.g., Keith et al., 1998; Pawar et al. 2007;) are consistent with the concept of functional selectivity (Mailman, 2007) since opioid agonists can produce antinociception and receptor regulation (e.g., etorphine, fentanyl), while others, acting at the same receptor, produce only antinociception (e.g., morphine, oxycodone).

The ED₅₀ for fentanyl in the mouse tailflick assay was estimated using both standard and cumulative dosing protocols. The standard dosing protocol typically provides an accurate estimate of the ED₅₀. On the other hand, the ED₅₀ determined by cumulative dosing is dependent on the dosing schedule (starting dose and increment doses) and can vary substantially if different doses are used (Duttaroy et al., 1997). Therefore, we used the standard ED₅₀ value in this and previous studies (Duttaroy and Yoburn, 1995; Pawar et al., 2007) as the metric for determining equi-effective treatments as the multiple of the ED₅₀ (see Fig. 5). This allows relatively straight-forward comparison of dosing among various opioids. In addition, tolerance following fentanyl treatment was examined using morphine, which is primarily a μ -opioid receptor agonist that lacks activity in μ -opioid receptor knockout mice (Kieffer, 1999).

By using morphine to assess tolerance, the effects of treatment with various opioid agonists can be directly compared.

Earlier work from our lab indicated that tolerance is most robust following continuous treatment. Intermittent administration of several opioid agonists produces moderate tolerance that appears to be unrelated to efficacy and is not dramatically affected by agonist dose (Duttaroy and Yoburn, 1995). In the present study, we intended to examine this issue using acute and intermittent fentanyl dosing. However, when doses of 25 times the fentanyl ED₅₀ were used, this was lethal for $\approx$ 10–20% of mice. Data were available for 5 and 10 times the fentanyl ED₅₀ but this produced no tolerance and minimal changes in μ -opioid receptors, as we have reported previously (Duttaroy and Yoburn, 1995). We are currently examining the role of intermittency in tolerance using other opioid agonists. Nevertheless, taken together, fentanyl infusions dose-dependently produced substantial tolerance while acute or intermittent fentanyl injections produced minimal tolerance in all dosing groups, irrespective of duration of treatment.

Various hypotheses have been proposed to explain why higher efficacy opioid agonists produce less tolerance than lower efficacy agonists at equi-effective dosing. One hypothesis suggests that the lower efficacy agonists occupy more receptors compared to higher efficacy agonists to produce a desired effect. Thus, more receptors are desensitized by lower efficacy agonists (Waldhoer et al., 2004). Others have suggested that lower efficacy agonists don't regulate trafficking proteins, such as dynamins and GRK and hence do not induce receptor internalization (Patel et al., 2002; Stafford et al., 2001; Martini and Whistler, 2007). Internalization has been suggested to be a process by which a desensitized receptor undergoes endocytosis and subsequent dephosphorylation and resensitization (Ferguson et al., 1996; 1998). Failure of an agonist to promote receptor internalization may increase tolerance, as the desensitized receptor remains at the plasma membrane, and is not resensitized. Other explanations have focused on more robust development of compensatory signaling by lower efficacy agonists (Whistler et al., 1999). Recent studies raise the possibility that high efficacy opioids, including fentanyl, may engage different signaling pathways and that this may be important in inducing tolerance (e.g., Zheng et al., 2008). In any case, it is clear that a reduction in receptor density is not required for, but may contribute to, opioid tolerance (Stafford et al., 2001; Pawar et al., 2007). Thus, both "receptor-density independent mechanisms" (desensitization) and "receptor-density dependent mechanisms" (downregulation) can contribute tolerance (see Stafford et al., 2001). The mechanism by which acute and intermittent treatment may produce reduced tolerance is not entirely clear. However, this may be related to periodic versus continuous opioid receptor occupancy. Relatively short periods of activation of receptors and downstream events might engage the mechanisms for opioid tolerance in a less than maximal manner.

In conclusion, the current study demonstrates that fentanyl is a higher efficacy opioid analgesic. Fentanyl infusion produced μ opioid receptor downregulation and tolerance similar to that of other high efficacy opioid agonists. These results support suggestions that opioid analgesic efficacy is predictive of μ -opioid receptor regulation and tolerance.

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Fig 1.

Dose response functions for fentanyl using the standard (left) and cumulative (right) dose response protocols. For standard dosing, mice (5–6/dose) were injected s.c. with fentanyl as described in the methods. For cumulative dosing, mice (N=8) were injected cumulatively s.c. with fentanyl as described in the methods. The ED_{50} 's for standard dosing for fentanyl from 2 independent studies were 18.1 and 22.0 $\mu\text{g/kg}$. The mean ($\pm\text{sem}$) ED_{50} for cumulative dosing for fentanyl was estimated as $40.8 \pm 5.2 \mu\text{g/kg}$ based on 8 independent studies. Data presented are the results of a single representative experiment for each protocol. Dose-response data were fit using nonlinear regression (4 parameter logistic) using Sigmaplot (version 10; Systat Software, Inc., San Jose, CA).

Fig 2.

The effect of clocinnamox (CCAM) pretreatment on fentanyl analgesia. Mice were injected with saline (N=8–10) or 0.32 – 25.6 mg/kg CCAM (N=5–10/CCAM dose) i.p., and 24 hr later, fentanyl cumulative dose response studies (see methods) were conducted. Each CCAM dose was evaluated with a corresponding saline treated group (control). The results for each CCAM dose were determined 1–3 times. The data presented are the combined mean tail flick latencies from all experiments. Dose-response data were fit using nonlinear regression (4 parameter logistic) using Sigmaplot (version 10; Systat Software, Inc., San Jose, CA).

Fig 3.

The effect of chronic fentanyl treatment on μ -opioid receptor density in mouse spinal cord. Mice (10/group) were infused s.c. with fentanyl (1, 2 or 4 mg/kg/day) for 7 days. Controls were implanted with placebo pellets. At the end of treatment, pumps and pellets were removed and 16–24 hr later mice were sacrificed. Spinal cords were removed and assayed in [^{3} H] DAMGO saturation binding studies (see materials and methods). The histogram (left panel) presents the mean B_{MAX} ($\pm$ SEM) as percent change from control from 8 independent experiments. A representative scatchard plot for control and fentanyl (1 or 4 mg/kg/day) is shown right to the histogram. * significantly different from control ($p < 0.05$).

Fig 4.

The effect of fentanyl treatment on morphine analgesic potency. **A.** The effect of 7 days fentanyl infusion on morphine potency. Mice (8–10/group) were infused with fentanyl (1, 2 or 4 mg/kg/day) for 7 days. Controls were implanted with placebo pellets. At the end of treatment, pumps and pellets were removed and 16 hr later morphine cumulative dose response studies were conducted. The histograms show the shift relative to control in morphine's ED₅₀ (+ upper 95% CL) for each fentanyl dose from one experiment for each dose. **B.** Effect of acute (single dose) or 7 days intermittent fentanyl treatment on morphine potency. Mice (8–10/group) were injected s.c. once (acute) or once a day for seven days (intermittent) with fentanyl (100 and 200 µg/kg). Controls were injected s.c. with saline. Twenty four hr after the last injection,

morphine cumulative dose response studies were conducted. The histogram shows the shift in morphine's ED_{50} (+ sem) for each dose based on 3 experiments for each fentanyl dose.

Fig 5.

The effect of continuous infusions with 3 opioid analgesics on morphine's analgesic potency. The results from the present experiments on fentanyl are compared with those of oxycodone and etorphine from previous studies from our lab (Pawar et al., 2007). The shift in ED₅₀ was calculated as ED₅₀ treated/ED₅₀ control (C). To compare data statistically each data set was fit using nonlinear regression (Prism ver 5.01, Graph Pad Software, San Diego, CA) with the equation:

$$y = ae^{kx}$$

where y is the shift in the ED₅₀; a is the y-intercept; and k is the rate constant. a values ($\pm$ sem) for oxycodone, etorphine and fentanyl were 1.32 ± 0.17 , 2.14 ± 0.71 and 1.74 ± 0.72 respectively were not significantly different ($p > 0.05$). The k value ($\pm$ sem) for oxycodone, etorphine and fentanyl were 0.016 ± 0.001 , 0.005 ± 0.001 and 0.006 ± 0.002 . The k for oxycodone was significantly ($p < 0.05$) greater than for fentanyl and etorphine.