Pharmacology, Biochemistry and Behavior 92 (2009) 537–542

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Continuous morphine produces more tolerance than intermittent or acute treatment

Shveta V. Dighe, Priyanka A. Madia, Sunil Sirohi, Byron C. Yoburn *

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, 8000 Utopia Parkway Queens, NY 11439, USA

ARTICLE INFO

Article history:
Received 17 December 2008
Received in revised form 30 January 2009
Accepted 13 February 2009
Available online 25 February 2009

Keywords:
Morphine
Acute treatment
Intermittent treatment
Continuous treatment
Tolerance

ABSTRACT

Dosing protocol and analgesic efficacy have been proposed to be important determinants of the magnitude of opioid tolerance. The present study examined the effect of acute, intermittent and continuous treatment with the low analgesic efficacy agonist morphine on analgesic tolerance. Mice were implanted s.c. with a 25 mg morphine pellet for 1–7 days. Other mice were implanted s.c. with two 25 mg, or one 75 mg morphine pellet for 7 days. The release of morphine from subcutaneous implanted pellets was quantitated using a spectrophotometric assay. In other studies, mice were injected with morphine once (18.5–185 mg/kg/day; ≈10–100 times ED_{50} for morphine analgesia) or once/day for 7 days. Controls were implanted with a placebo pellet or injected with saline. Analysis of drug release from a 25 mg pellet indicated that release was greatest during the first 24 h, declined and then remained relatively constant. The amount of morphine released over 7 days by a 75 mg pellet (23.9 mg) was more than that of a single 25 mg pellet (15.4 mg) but less than two 25 mg pellets (30.8 mg). Following treatment, morphine cumulative dose–response studies were conducted (tailflick). Continuous treatment with morphine using pellet implantation produced a dose-dependent shift in the morphine ED_{50} by 3.3, 5.8 and 8.5 fold for one 25 mg pellet, one 75 mg pellet and two 25 mg pellets, respectively. Acute and intermittent morphine administration produced substantially less analgesic tolerance than continuous release of morphine by implant pellets. The maximum shift in the ED_{50} was 1.6 for acute treatment and 2.7 for 7 day intermittent treatment; despite a larger total daily dose. The present results indicate that continuous treatment with morphine results in greater analgesic tolerance than acute or intermittent morphine treatment even at comparable daily doses. These results are consistent with the suggestion that intermittent dosing has reduced risk of producing opioid tolerance.

1. Introduction

Recent data suggest that receptors can exist in multiple active conformations that can stimulate an array of effectors. Different ligands acting at the same receptor can have distinct efficacy profiles for these effectors and this supports suggestions that efficacy is system and effect dependent (e.g., Urban et al., 2007). This property has been variously termed "functional selectivity", "biased agonism" and "protean agonism" (Galandrin et al., 2007; Jarpe et al., 1998; Kenakin, 1995; Mailman, 2007; Urban et al., 2007). In previous studies, opioid efficacy, as a general drug-related characteristic, has been suggested to play a role in tolerance and opioid receptor regulation (Duttaroy and Yoburn, 1995; Paronis and Holtzman, 1992; Stevens and Yaksh, 1989; Walker and Young, 2001). Since efficacy of an agonist depends on the particular response measured, in recent studies, we have used a quantitative model to specifically estimate opioid analgesic efficacy (Kumar et al., 2008; Pawar et al., 2007; Sirohi et al., 2008; see Mailman, 2007 for discussion).

Studies indicate that analgesic efficacy of opioids is inversely related to the magnitude of opioid tolerance and directly related to the downregulation of μ-opioid receptor density (Duttaroy and Yoburn, 1995; Paronis and Holtzman, 1992; Pawar et al., 2007; Kumar et al., 2008; Sirohi et al., 2008). For example, continuous treatment with lower analgesic efficacy agonists (e.g., morphine, oxycodone or hydromorphone) produces more tolerance compared to higher analgesic efficacy agonists (e.g., DAMGO, etorphine, fentanyl) (Duttaroy and Yoburn, 1995; Kumar et al., 2008; Paronis and Holtzman, 1992; Sirohi et al., 2008; Sosnowski and Yaksh, 1990; Stevens and Yaksh, 1989; Tiano et al., 1998; Walker et al., 1997; Walker and Young, 2001). Higher analgesic efficacy opioid agonists produce internalization and downregulation of μ-opioid receptor in *in vitro* and *in vivo* studies (Arden et al., 1995; Duttaroy and Yoburn, 1995; Keith et al., 1998; Pawar et al., 2007; Sirohi et al., 2008; Stafford et al., 2001; Whistler and von Zastrow, 1998). In contrast, lower analgesic efficacy opioid agonists are generally incapable of producing internalization and downregulation of μ-opioid receptors (Keith et al., 1996, 1998; Kumar et al., 2008; Patel et al., 2002; Pawar et al., 2007; Stafford et al., 2001; Yoburn et al., 2004).

Although analgesic efficacy has a role in tolerance, previous studies (Duttaroy and Yoburn, 1995; Kumar et al., 2008; Sirohi et al., 2008) have

* Corresponding author. Tel.: +1718 990 1623; fax: +1 718 990 6036.
E-mail address: yoburnb@stjohns.edu (B.C. Yoburn).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.02.004

shown that opioid analgesics administered intermittently produce less tolerance compared with continuous treatment approaches despite the fact that the total daily drug dose is comparable. Furthermore, analgesic efficacy is not a critical factor in determining the extent of tolerance produced in intermittent treatment protocols. Both high and low analgesic efficacy agonists administered intermittently produce minimal tolerance compared to continuous treatment protocols. In addition, opioid agonists administered intermittently do not downregulate μ-opioid receptor density *in vivo* (Kumar et al., 2008; Sirohi et al., 2008).

The objective of the present experiment was to study the effect of acute, intermittent and continuous treatment with morphine on analgesic tolerance. Morphine is a prototypical μ-opioid agonist used in the clinical management of moderate to severe pain and is generally classified as a lower analgesic efficacy opioid (Adams et al., 1990; Pawar et al., 2007; Morgan and Picker, 1998). In previous studies, using osmotic pumps it was not possible to infuse higher doses of morphine due to the lower solubility of this drug. To overcome this limitation, subcutaneous implant pellets can be used for continuous treatment with higher doses of morphine (e.g., Stafford et al., 2001; Yoburn et al., 1985). However, the actual dose administered using the s.c. morphine pellet implantation procedure is difficult to determine. Therefore, we developed a spectrophotometric assay to determine the residual morphine in the pellets following implantation. This allowed calculation of the dose and provided an opportunity to correlate dose with the magnitude of tolerance. The results of the present study indicate that continuous treatment with morphine produces greater analgesic tolerance than acute or intermittent treatment with comparable doses. These results are consistent with previous data that intermittent dosing produces less opioid tolerance and suggest that clinically employed continuous administration formulations of opioid analgesics may foster the development of opioid tolerance.

2. Materials and methods

2.1. Drugs

Morphine sulphate was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA). Morphine pellets (containing 25 or 75 mg morphine base) and inert placebo pellets were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse. Morphine sulphate was dissolved in 0.9% saline and doses are expressed as the free base. All implant pellets were wrapped in nylon mesh before subcutaneous implantation.

2.2. Subjects

Male Swiss Webster mice (26–40 g) obtained from Taconic Farms (Germantown, NY, USA) were used throughout this study. Animals were housed 10 per cage for at least 24 h before use with food and water available *ad-libitum*. All animal protocols were approved by the St. John's University Institutional Animal Care and Use Committee.

2.3. General procedure

Initially, the analgesic (tailflick) ED_{50} of morphine was estimated using standard (5–7/dose) and cumulative dosing (8–10/group) protocols (see below). Next, mice (8/group) were implanted s.c. with a 25 mg morphine pellet for 1, 2, 3, 4, 5, 6 or 7 days. Other groups of mice (8/group) were implanted s.c. with two 25 mg pellets or one 75 mg morphine pellet for 7 days. Control mice were implanted with placebo pellets. Pellets were removed at the end of treatment and 16 h later morphine cumulative dose–response studies were conducted. Pellets were implanted and removed while mice were lightly anesthetized using isoflurane:oxygen (4:96) (Webster Veterinary Supply Inc, Sterling, MA). The excised pellets were analyzed using a spectrophotometric method (see below). In other studies, mice (8–10/group) were injected with morphine (18.5–185 mg/kg/day; ≈10–100 times the ED_{50} for morphine analgesia determined using the standard dosing protocol). Mice were injected once (acute treatment) or once/day for 7 days (intermittent treatment). Control mice were injected with saline. Twenty four h following the last injection, morphine cumulative dose–response studies were conducted.

2.4. Analgesia assay

Antinociception (analgesia) was estimated using the tailflick assay (Model TF6, Emdie Instrument Co., Maidens, VA). In this assay, a beam of light was focused on the dorsal surface of the tail of the mouse (approximately 2 cm from the tip of tail). The intensity of light was adjusted so that the baseline tailflick latency was typically 1–3 s. The maximum tailflick latency was set to 10 s. Following drug administration, a mouse was defined as analgesic if it failed to remove its tail from the heat source by 10 s. Testing was by an experimenter who was unaware of the specific treatment protocol of each individual mouse.

2.5. Dose–response protocol

Dose–response studies were conducted using standard and cumulative dosing protocols. In standard dosing studies, mice ($N = 5$–7/dose) were injected s.c. with a single dose of morphine (0.5–4.0 mg/kg) and tested for analgesia 30 min later. For the standard dosing protocol, mice were injected once and tested once following drug administration. For cumulative dosing studies, mice ($N = 8$–10/group) were injected s.c. with a starting dose of morphine (0.5 mg/kg) and tested 30 min following injection for analgesia. If the mouse was analgesic it was not tested further. Otherwise mice were immediately injected again with another dose of morphine and tested again (the sequence of doses was 0.5, 1, 1.5, 1.5, 2, 3, 4, 4, 4, 4, 8, 8, 8, 8 mg/kg yielding cumulative dose of 0.5, 1.5, 3, 4.5, 6.5, 9.5, 13.5, 17.5, 21.5, 25.5, 29.5, 37.5, 45.5, 53.5, 61.5 mg/kg). The cumulative dosing protocol was based on previous studies (Duttaroy et al., 1997). Cumulative dosing with morphine was continued until all mice were determined as analgesic.

2.6. Spectrophotometric analysis

Analysis of morphine was performed using a spectrophotometric method. The wavelength used for absorbance was 284 nm based on U. S. Pharmacopeia XXIII National Formulary XVIII (1995) and preliminary analysis in our lab. Initially, a known amount of morphine sulphate was dissolved in 99.9% methanol. Serial dilutions were made and the absorbance (Spectronic 601 spectrophotometer, Milton Roy USA, Ivyland, PA) was linear in the concentration range of 10–500 μg ml^{-1}. To generate a standard curve for morphine pellets, individual unimplanted morphine pellets (25 or 75 mg morphine base) wrapped in nylon mesh (as were implanted pellets) were crushed and transferred to a volumetric flask containing 40 ml of 99.9% methanol. The resultant solution was sonicated (Branson Ultrasonic Corporation, Danbury, CT) for 20 min and brought to a final concentration of 500 μg ml^{-1}. The solution was filtered through nylon membrane filters (0.45 μm pore size, 47 mm diameter; Pall Life Sciences, Ann Arbor, MI) using vacuum filtration and serial dilutions were made. Calibration curves for morphine pellets were linear in the range of 10–500 μg ml^{-1}. We compared these curves to those from the known concentrations of morphine sulphate in methanol (see above). This comparison indicated that the mean (±SD) recovery of morphine from 25 and 75 mg morphine pellets was 93.9 ± 0.6% and 96.8. ± 0.9% respectively. All results from unknowns were corrected for recovery using these data.

Pellets removed from mice were stored at −20 °C until analysis. On the day of analysis, the pellets were thawed, suspended in 99.9% methanol and analyzed as described above. The concentration of unknown samples was determined using linear regression from standard curves included in each assay.

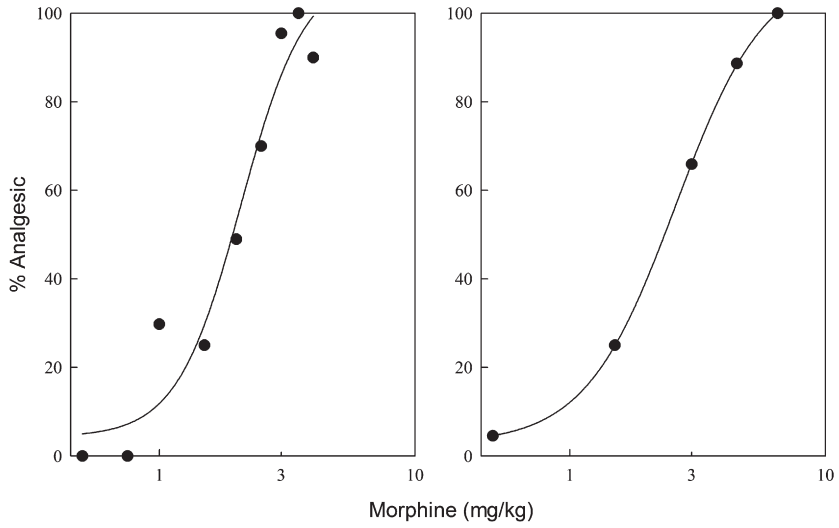

Fig. 1. Dose–response functions for the analgesic effect of morphine using standard (left panel) and cumulative (right panel) dosing protocols. For standard dosing studies, mice were injected once s.c. with 0.5–4.0 mg/kg (5–7/dose) and tested for analgesia 30 min later. For cumulative dosing studies, mice ($N=8$) were injected s.c. with cumulative doses of morphine and tested for analgesia 30 min following each injection as described in Materials and methods. The mean (±SEM) ED_{50} for morphine based on 7 independent experiments using the standard dosing protocol was 1.9 ± 0.2 mg/kg. The mean (±SEM) ED_{50} for morphine cumulative dosing studies based on 5 independent experiments was 2.2 ± 0.2 mg/kg. In the figure, the combined results from all studies for each dosing protocol are plotted and the data were fit with the 4 parameter logistic equation (Sigmaplot version 10; Systat Software, Inc., San Jose, CA).

2.7. Data analysis

Quantal dose–response studies were analyzed using the program BLISS-21 (Department of Statistics, University of Edinburgh) which uses Probit Analysis (Finney, 1973) to calculate ED_{50} values, standard errors, potency ratios and 95% confidence intervals. The shift in ED_{50} was defined as: (ED_{50} treated group/ED_{50} control group). ED_{50} shift data were analyzed by ANOVA and appropriate post-hoc tests. In order to statistically compare data, nonlinear regression was used to fit either the logistic equation or the exponential growth curve using Sigmaplot (version 10; Systat Software, Inc., San Jose, CA) or Prism (version 5.01, Graph Pad Software, San Diego, CA).

3. Results

The analgesic ED_{50} for morphine was determined using standard and cumulative dosing protocols. The mean (±SEM) ED_{50} for morphine based on 7 independent experiments using the standard dosing protocol (Fig. 1 left panel) was 1.9 (±0.2) mg/kg. The mean (±SEM) ED_{50} for morphine determined in cumulative dosing studies (Fig. 1 right panel) based on 5 independent experiments was 2.2 (±0.2) mg/kg.

To determine the release of morphine from 25 mg and 75 mg s.c. implanted pellets, a spectrophotometric assay was developed (see methods). Standard curves were linear over the range of 10–500 μg ml^{-1}

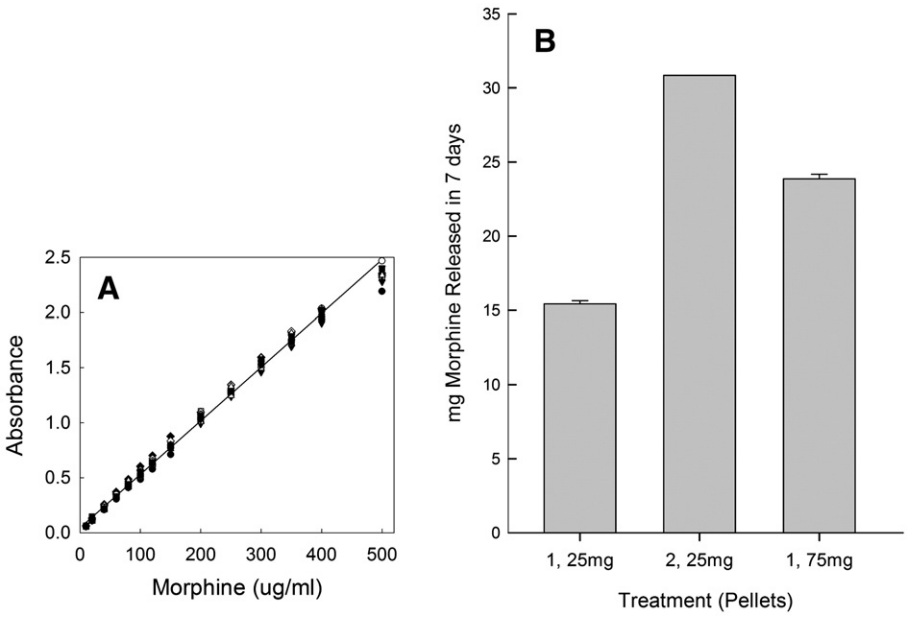

Fig. 2. Amount of morphine released following s.c. implantation of morphine pellets for 7 days. A: Standard curves used to estimate morphine remaining in 25 mg and 75 mg morphine pellets following s.c. implantation of pellet. The r^2s for all 16 curves were >0.98. B: The mean (±SE) amount of morphine released over 7 days from s.c. implanted pellets. The value for two 25 mg pellets was calculated based on the release data for a single 25 mg morphine pellet.

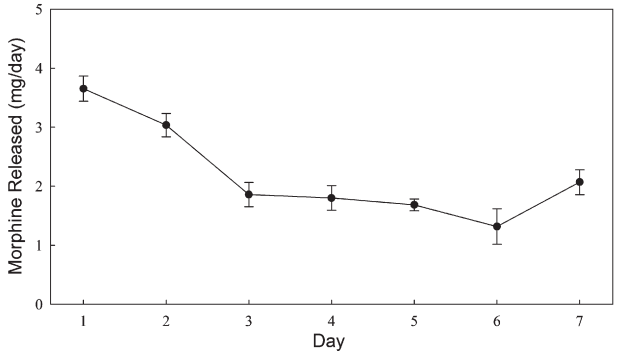

Fig. 3. Daily release (mg/day) of morphine from a single 25 mg s.c. implanted pellet. Mice (8/group) were implanted with a morphine pellet and 1, 2, 3, 4, 5, 6 or 7 days later the pellet was removed and analyzed using a spectrophotometric method (see Materials and methods). The data are the mean (±SEM) mg of morphine released each day.

(Fig. 2A). Over the 7 day implant period (Fig. 2B), the average (±SEM) amount of morphine released from a 25 mg and a 75 mg morphine pellet was 15.4 (±0.2) and 23.9 (±0.3) or an average daily release of 2.2 and 3.4 mg/day, respectively. The amount of morphine released from two 25 mg morphine pellet over 7 days implantation (Fig. 2B) was estimated from the analysis of release of morphine from a single 25 mg pellet.

Mice were implanted with a single 25 mg pellet for 1–7 days and morphine release was determined daily. The amount of morphine released from a 25 mg morphine pellet (Fig. 3) was greatest during the first 24 h (3.7 mg±0.2). Daily morphine release declined and then remained relatively constant (≈2 mg/day). At the end of 1–7 days of 25 mg morphine pellet implantation, the pellets were removed and 16 h later morphine cumulative dose–response studies were conducted. Tolerance following 1–7 days treatment with a 25 mg morphine pellet increased for 3 days and then reached a steady state (Fig. 4). Treatment with one 25 mg pellet, one 75 mg pellet or two 25 mg morphine pellets for 7 days produced a dose-dependent shift in the morphine ED_{50} (±SE's) by 3.3 (±0.1), 5.8 (±1.4) and 8.5 (±0.5) (Fig. 5).

Tolerance following acute and intermittent morphine injections was also determined (Fig. 6). Mice were injected with morphine

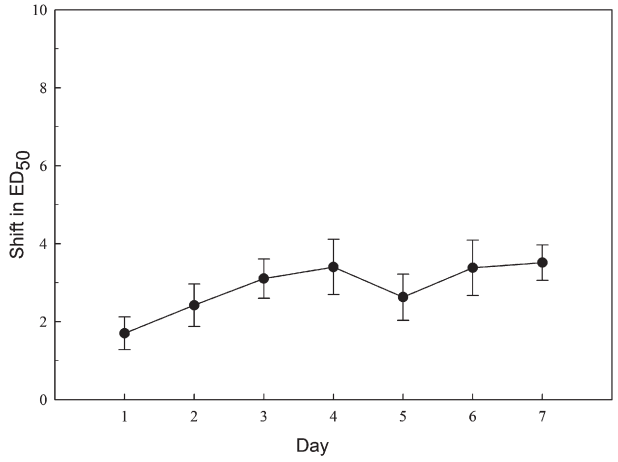

Fig. 4. Tolerance (shift in ED_{50}) following chronic treatment with a 25 mg morphine pellet for 1–7 days. Mice (8/group) were implanted s.c. with one 25 mg morphine pellet, pellets were removed 1, 2, 3, 4, 5, 6 or 7 days later and 16 h following pellet removal morphine cumulative dose–response studies were conducted (tailflick). Controls were implanted with placebo pellets. The results plotted are the calculated shift in ED_{50} (±SE) as determined by Probit Analysis. The data are based on a single experiment for each time point, except for Days 3 and 7 which are the combined results from 2 and 3 experiments, respectively.

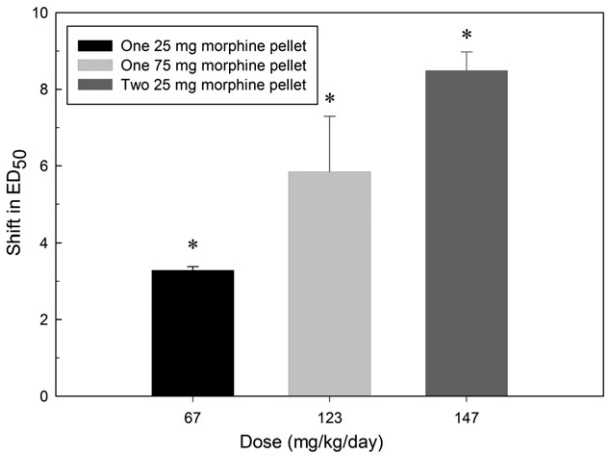

Fig. 5. Tolerance (shift in ED_{50}) following chronic treatment with s.c. implanted morphine pellets for 7 days. Mice (8/group) were implanted s.c with one or two 25 mg, or one 75 mg morphine pellet (67–147 mg/kg/day; ≈36–80 times the ED_{50} for morphine analgesia) for 7 days. Data are based on the total drug released over a 7 day period and the mean animal body weight. Controls were implanted with placebo pellets. Pellets were removed after 7 days and 16 h later, morphine cumulative dose–response studies were conducted (tailflick). Data are expressed as shift in ED_{50} (±SE) as determined by Probit Analysis based on combined results of 3 experiments for one 25 mg and two 25 mg morphine pellets and 2 experiments for one 75 mg morphine pellet. The mg/kg dose for two 25 mg morphine pellets is extrapolated from the analysis of the release of morphine for a single 25 mg pellet (see Fig. 3).

(18.5–185 mg/kg/day; ≈10–100 times the standard ED_{50} for morphine analgesia) once or once/day for 7 days. Injection doses higher than 100 times the ED_{50} for morphine could not be used because of lethality. There was significantly greater tolerance following intermittent treatment compared to acute treatment ($F_{1,9}=9.6$, $p<0.05$), but no significant dose effect or dose X treatment effect (F's<1.9, $p>0.05$). The maximum mean (±SEM) shift in the ED_{50} for acute treatment was 1.6 (±0.4). The maximum shift in the ED_{50} (±SE's) for 7 day intermittent treatment was 2.7 (±0.5). Overall, acute and intermittent treatment with morphine produced minimal tolerance compared to continuous treatment (Fig. 5), despite the fact that daily dosing was comparable or greater.

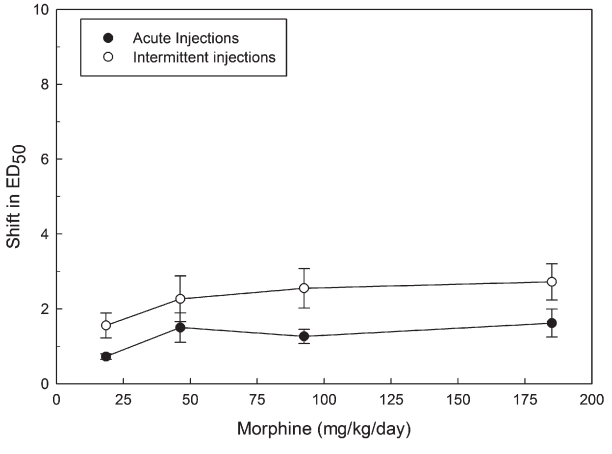

Fig. 6. Tolerance (shift in ED_{50}) following acute (single injection) or intermittent (7 daily injections) morphine treatment. Mice (8–10/group) were injected with morphine (18.5–185 mg/kg/day; ≈10–100 times the ED_{50} for morphine analgesia determined using the standard dosing protocol) once or once/day for 7 days. Controls were injected with saline. Twenty four h following the last injection, morphine cumulative dose–response studies were conducted. The data presented for acute injections are the mean (±SEM) shift in the ED_{50} based on 3–4 independent experiments for each dose. The data presented for intermittent treatment are based on a single experiment for each dose and the shift in the ED_{50} (±SE) was determined by Probit Analysis.

4. Discussion

It has been hypothesized that opioid agonist analgesic efficacy is directly correlated with μ-opioid receptor downregulation and inversely related to the magnitude of tolerance (Duttaroy and Yoburn, 1995; Kumar et al., 2008; Paronis and Holtzman, 1992; Pawar et al., 2007; Sirohi et al., 2008; Stevens and Yaksh, 1989; Walker and Young, 2001). Previous studies in our lab (e.g. Duttaroy and Yoburn, 1995; Kumar et al., 2008) suggested that analgesic efficacy plays a less significant role in predicting the magnitude of tolerance following intermittent opioid administration compared to continuous administration approaches. In the present study, we have further examined this possibility and compared acute, intermittent and continuous dosing protocols using morphine. The present results indicate that acute and intermittent treatment with morphine produces minimal tolerance compared to continuous treatment (Figs. 5 and 6). Fig. 7 compares the present results with data from two previous studies. It is clear that when morphine is administered continuously, there is substantially more analgesic tolerance at roughly equivalent daily doses. This is especially pronounced at doses over 100 mg/kg/day where the function relating tolerance following continuous morphine treatment diverges from that for acute and intermittent treatment. These results are not confined to morphine and agree with previous studies on dosing protocol using hydromorphone and fentanyl (Kumar et al., 2008; Sirohi et al., 2008).

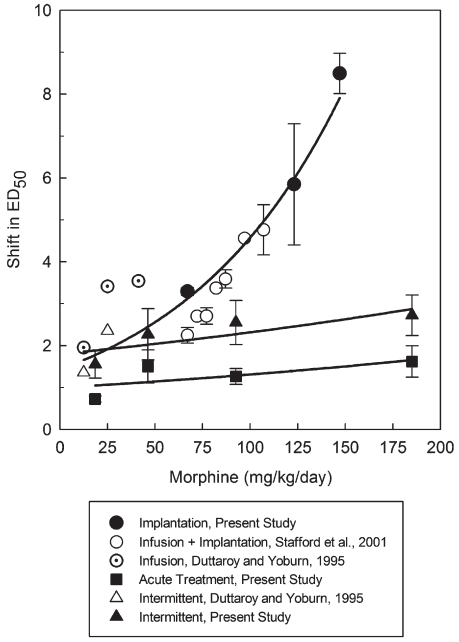

Fig. 7. Comparison of morphine tolerance following acute (single injection), intermittent (7 daily injections) and continuous morphine treatment. The data from the present experiment (● ■ ▲) were compared with previous studies from our lab (Duttaroy and Yoburn, 1995 ⊙ △; Stafford et al., 2001 ○). In continuous (● ○ ⊙) morphine administration studies, (pellet implantation, pellet implantation plus minipump infusion, or minipump infusions), mice were treated for 7 days, then pumps and pellets were removed and 4–24 h later morphine tolerance was determined using morphine cumulative dose–response studies (present study, Stafford et al., 2001; Duttaroy and Yoburn, 1995). For intermittent (▲ △) studies, (present study, Duttaroy and Yoburn, 1995) mice were injected s.c. once per day with morphine and 24 h later tested for tolerance using morphine cumulative dose–response studies. Finally, data for acute (■) morphine treatment (single s.c. injection) from the present study are presented. The shift in ED_{50} was calculated as: ED_{50} treated group/ED_{50} control group. The combined results from all studies for different treatment protocols are plotted and the data were fit with the two parameter exponential growth curve: $y = ae^{kx}$ where, y is the shift in ED_{50}; a is the y-intercept; x is the daily dose of morphine (mg/kg/day) and k is the rate constant. Curve fitting indicated that the a values did not differ ($p>0.05$) among the three conditions, whereas the k value for continuous treatment (0.015 ± 0.001) was significantly different ($p<0.05$) from that for intermittent (0.006 ± 0.001) and acute treatment (0.003 ± 0.001). Where available, mean data are plotted. Otherwise, single observations are presented. In some cases, SE's were not available from previous studies.

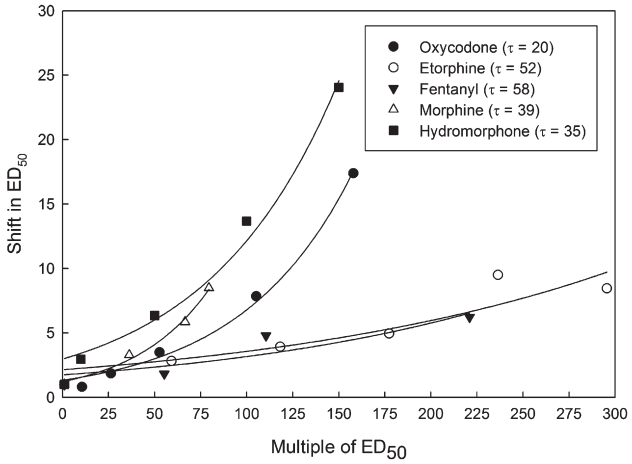

Fig. 8. The effect of analgesic efficacy on analgesic tolerance following continuous administration of opioid agonists. The results from the present study on morphine tolerance are compared with those of oxycodone, etorphine (Pawar et al., 2007), fentanyl (Sirohi et al., 2008) and hydromorphone (Kumar et al., 2008). The shift in the ED_{50} following treatment was plotted against the agonist dose expressed as a multiple of the analgesic ED_{50} that was infused per day. The analgesic ED_{50} was determined in untreated mice using standard dosing studies (see methods). By using the multiple of the analgesic ED_{50}, different potency drugs can be equated in terms of effect. Thus, for example, infusion of a dose that is 100 times the analgesic ED_{50} for each drug is defined as an equi-analgesic dose. In order to compare the data statistically, each data set was fit using nonlinear regression with the two parameter exponential growth curve (see Fig. 7); where, y is the shift in ED_{50}; a is the y-intercept; x is the multiple of the analgesic ED_{50} and k is the rate constant. The a values ($\pm$SEM) for oxycodone, etorphine, fentanyl, morphine and hydromorphone were not significantly different ($p>0.05$). The k values ($\pm$SEM) for oxycodone, morphine and hydromorphone (0.016 ± 0.001, 0.0243 ± 0.002 and 0.014 ± 0.001) were significantly greater ($p<0.05$) than the k values for etorphine and fentanyl (0.005 ± 0.001, 0.006 ± 0.002). The τ values represent the analgesic efficacy estimates determined previously using the operational model of agonism (Black and Leff, 1983) for each opioid agonist (Kumar et al., 2008; Pawar et al., 2007; Sirohi et al., 2008).

Taken together, at equivalent daily doses of opioids, intermittent dosing is associated with much less analgesic tolerance than continuous administration approaches.

Subcutaneous implantation of morphine pellets is a standard approach for producing opioid tolerance and dependence in rodents. In the present study, the release of morphine in the mouse was determined using a spectrophotometric assay allowing estimation of the actual dose administered. These data were not available previously. In earlier studies, the solubility of morphine was insufficient to allow evaluation of tolerance at higher doses using osmotic pumps for continuous drug administration (e.g. Pawar et al., 2007). An alternative technique to produce high dose treatment used by Stafford et al., 2001, combined pumps and an implanted morphine pellet. However, lack of information about the release of morphine from the pellets made it impossible to determine the dose administered. The spectrophotometric method developed in the present study resolves this issue. We used this information to estimate morphine dose in the present study and dosing from previous studies from our lab (see Fig. 7).

The 75 mg pellet released more morphine than a single 25 mg pellet, but less than two 25 mg pellets over 7 days (Fig. 2). Determination of the daily release characteristics of morphine from one 25 mg pellet for 7 days indicated that the amount of morphine released was greatest during the first 24 h, declined and then remained relatively constant (Fig. 3). Tolerance following treatment with one 25 mg morphine pellet for 1–7 days was also determined. These data show that the magnitude of tolerance increased for 3 days and then was constant (Fig. 4); suggesting that despite continued exposure to morphine the mechanisms that mediate analgesic tolerance had reached equilibrium.

Analgesic efficacy can be quantitatively estimated with the Operational Model of Agonism using the parameter τ (Black and Leff, 1983). Previous reports indicate that morphine has low analgesic efficacy (e.g.

Pawar et al., 2007; Pitts et al., 1998; Walker et al., 1998, Zernig et al., 1995). The magnitude of tolerance produced by continuous morphine treatment in the present study was comparable to other lower analgesic efficacy agonists such as hydromorphone ($\tau = 35$) and oxycodone ($\tau = 20$) (Kumar et al., 2008; Pawar et al., 2007). Higher analgesic efficacy opioids, such as etorphine ($\tau = 52$) and fentanyl ($\tau = 58$) produce significantly less tolerance (Pawar et al., 2007; Sirohi et al., 2008) when infused. In order to compare opioid drugs with different potencies and efficacies, the shift in the ED_{50} (i.e., tolerance) can be plotted against the daily infusion dose expressed as a multiple of the analgesic ED_{50} determined in untreated mice. This approach normalizes drug treatment so that opioids can be compared using equi-analgesic doses. When these data are analyzed in this manner (Fig. 8), the inverse relationship between analgesic efficacy and tolerance is apparent. In Fig. 8, five opioid analgesics (including morphine from the present study) with different τ values are compared. Lower analgesic efficacy opioids produce more tolerance than higher efficacy agonists at equi-analgesic doses (multiple of analgesic ED_{50}). Taken together, these data support the suggestion that opioid agonist efficacy (τ) plays an important role in the magnitude of opioid tolerance produced by continuous administration (Adams et al., 1990; Duttaroy and Yoburn, 1995; Kumar et al., 2008; Pawar et al., 2007; Sirohi et al., 2008; Stevens and Yaksh, 1989).

The molecular mechanisms by which acute and intermittent opioid agonist treatment produce less tolerance than continuous treatment remain unclear. Possible explanations include phasic versus continuous receptor stimulation. Brief exposure to an opioid agonist will result in periodic activation of the receptor. This may allow recovery of down-stream events involved in tolerance, which would not occur in continuous treatment approaches that produce sustained receptor occupancy. Nevertheless, these results with morphine, taken together with results from other opioid analgesics (Kumar et al., 2008; Sirohi et al., 2008) suggest that sustained release preparations of opioids for pain may foster more tolerance compared to intermittent treatment approaches.

In summary, the present findings suggest that morphine is a lower analgesic efficacy opioid agonist and that the effects of intermittent dosing on the magnitude of tolerance are different from that of continuous treatment protocols. The analgesic efficacy of opioid agonists strongly impacts on the magnitude of tolerance following continuous treatment. On the other hand, analgesic efficacy seems to be less of a determinant of the magnitude of tolerance following intermittent administration.

Acknowledgements

The authors are grateful to Dr. M.T. Turnock for continuous encouragement during this study. Supported in part by a grant from the National Institute on Drug Abuse (DA 19959, to BCY).

References

Adams JU, Paronis CA, Holtzman SG. Assessment of relative intrinsic activity of mu-opioid analgesics in vivo by using beta-funaltrexamine. J Pharmacol Exp Ther 1990;255:1027–32.
Arden JR, Segredo V, Wang Z, Lameh J, Sadée W. Phosphorylation and agonist-specific intracellular trafficking of an epitope-tagged mu-opioid receptor expressed in HEK 293 cells. J Neurochem 1995;65:1636–45.
Black JW, Leff P. Operational models of pharmacological agonism. Proc R Soc Lond B Biol Sci 1983;220:141–62.
Duttaroy A, Yoburn BC. The effect of intrinsic efficacy on opioid tolerance. Anesthesiology 1995;82:1226–36.
Duttaroy A, Kirtman R, Farrell F, Phillips M, Philippe J, Monderson T, et al. The effect of cumulative dosing on the analgesic potency of morphine in mice. Pharmacol Biochem Behav 1997;58:67–71.
Finney DJ. Probit analysis. 3rd ed. London: Cambridge University Press; 1973.
Galandrin S, Oligny-Longpré G, Bouvier M. The evasive nature of drug efficacy: implications for drug discovery. Trends Pharmacol Sci 2007;28:423–30.
Jarpe MB, Knall C, Mitchell FM, Buhl AM, Duzic E, Johnson GL. Substance P acts as a biased agonist toward neuropeptide and chemokine receptors. J Biol. Chem 1998;273:3097–104.
Keith DE, Murray SR, Zaki PA, Chu PC, Lissin DV, Kang L, et al. Morphine activates opioid receptors without causing their rapid internalization. J Biol Chem 1996;271:19021–4.
Keith DE, Anton B, Murray SR, Zaki PA, Chu PC, Lissin DV, et al. Mu-opioid receptor internalization: opiate drugs have differential effects on a conserved endocytic mechanism in vitro and in the mammalian brain. Mol Pharmacol 1998;53:377–84.
Kenakin T. Agonist-receptor efficacy. II. Agonist trafficking of receptor signals. Trends Pharmacol. Sci 1995;16:232–8.
Kumar P, Sunkaraneni S, Sirohi S, Dighe SV, Walker EA, Yoburn BC. Hydromorphone efficacy and treatment protocol impact on tolerance and mu-opioid receptor regulation. Eur J Pharmacol 2008;597:39–45.
Mailman RB. GPCR functional selectivity has therapeutic impact. Trends Pharmacol Sci 2007;28:390–6.
Morgan D, Picker MJ. The mu opioid irreversible antagonist beta-funaltrexamine differentiates the discriminative stimulus effects of opioids with high and low efficacy at the mu opioid receptor. Psychopharmacology 1998;140:20–8.
Paronis CA, Holtzman SG. Development of tolerance to the analgesic activity of mu agonists after continuous infusion of morphine, meperidine or fentanyl in rats. J Pharmacol Exp Ther 1992;262:1–9.
Pawar M, Kumar P, Sunkaraneni S, Sirohi S, Walker EA, Yoburn BC. Opioid agonist efficacy predicts the magnitude of tolerance and the regulation of mu-opioid receptors and dynamin-2. Eur J Pharmacol 2007;563:92-101.
Patel MB, Patel CN, Rajashekara V, Yoburn BC. Opioid agonists differentially regulate mu-opioid receptors and trafficking proteins in vivo. Mol Pharmacol 2002;62:1464–70.
Pitts RC, Allen RM, Walker EA, Dykstra LA. Clocinnamox antagonism of the antinociceptive effects of mu opioids in squirrel monkeys. J Pharmacol Exp Ther. 1998;285:1197–206.
Sirohi S, Dighe SV, Walker EA, Yoburn BC. The analgesic efficacy of fentanyl: relationship to tolerance and mu-opioid receptor regulation. Pharmacol Biochem Behav 2008;91:115–20.
Stafford K, Gomes AB, Shen J, Yoburn BC. Mu-opioid receptor downregulation contributes to opioid tolerance in vivo. Pharmacol Biochem Behav 2001;69:233–7.
Sosnowski M, Yaksh TL. Differential cross-tolerance between intrathecal morphine and sufentanil in the rat. Anesthesiology 1990;73:1141–7.
Stevens CW, Yaksh TL. Potency of infused spinal antinociceptive agents is inversely related to magnitude of tolerance after continuous infusion. J. Pharmacol Exp. Ther 1989;250:1–8.
Tiano MJ, Walker EA, Dykstra LA. Cross-tolerance to etorphine differentiates μ-opioid agonists in a rat tail withdrawal assay. Analgesia 1998;3:251–7.
Walker EA, Richardson TM, Young AM. Tolerance and cross-tolerance to morphine-like stimulus effects of mu opioids in rats. Psychopharmacology 1997;133:17–28.
Walker EA, Zernig G, Young AM. In vivo apparent affinity and efficacy estimates for mu opiates in a rat tail-withdrawal assay. Psychopharmacology 1998;136:15–23.
Walker EA, Young AM. Differential tolerance to antinociceptive effects of mu opioids during repeated treatment with etonitazene, morphine, or buprenorphine in rats. Psychopharmacology 2001;154:131–42.
Whistler JL, von Zastrow M. Morphine-activated opioid receptors elude desensitization by beta-arrestin. Proc Natl Acad Sci 1998;95:9914–9.
Yoburn BC, Chen J, Huang T, Inturrisi CE. Pharmacokinetics and pharmacodynamics of subcutaneous morphine pellets in the rat. J Pharmacol Exp Ther 1985;235:282–6.
Yoburn BC, Purohit V, Patel K, Zhang Q. Opioid agonist and antagonist treatment differentially regulates immunoreactive mu-opioid receptors and dynamin-2 in vivo. Eur J Pharmacol 2004;498:87–96.
Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, et al. Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 2007;320:1-13.
U.S. Pharmacopeia 23 National Formulary 18. The Official Compendia of Standards; authority of the United States Pharmacopeial Convention meeting at Washington D.C. The United States Pharmacopeial Convention, Inc; 1995. p. 1038–9.
Zernig G, Issaevitch T, Broadbear JH, Burke TF, Lewis JW, Brine GA, et al. Receptor reserve and affinity of mu opioid agonists in mouse antinociception: correlation with receptor binding. Life Sci. 1995;7:2113–25.