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The Effect of Intrinsic Efficacy on Opioid Tolerance

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Background: **The intrinsic efficacy of opioid analgesics has been suggested to play a role in the development of tolerance to these agents. However, the effect of differences in dosing protocol on tolerance to opioid analgesics of high or low efficacy has not been addressed. Therefore, the effect of opioid intrinsic efficacy on tolerance in mice was determined in protocols of continuous and intermittent administration of equieffective doses of opioid agonists.**

Methods: **Initial antinociceptive median effective doses (ED_{50}s) for five opioid agonists that vary in intrinsic efficacy were estimated in untreated mice. Groups of mice received continuous infusions of morphine, fentanyl, or etorphine for 72 h or 7 days from osmotic minipumps implanted subcutaneously. The infusion doses were calculated as multiples of the initial antinociceptive ED_{50}. An inert placebo was implanted subcutaneously in controls. At the end of treatment, the pumps and placebos were removed, and 4–24 h later, mice were tested in dose-response studies (tail flick) using the same drug that had been chronically administered. In another study using intermittent dosing, mice received subcutaneous injections every 24 h for 3 days of saline or morphine, etorphine, fentanyl, oxycodone, or meperidine, or received subcutaneous injections every 24 h for 7 days of saline or morphine, etorphine, or fentanyl. Daily doses were calculated as multiples of the initial antinociceptive ED_{50}. Twenty-four hours after the last injection, mice were tested in dose-response studies.**

Results: **High-intrinsic-efficacy compounds (*e.g.*, etorphine and fentanyl) produced less tolerance than a lower-intrinsic-efficacy drug (morphine) in 72-h and 7-day infusion studies. Tolerance for all compounds after intermittent treatment with equieffective doses was similar, and intrinsic efficacy had no effect on the magnitude of tolerance after intermittent dosing.**

Conclusions: **These results indicate that the intrinsic efficacy of opioid analgesics is inversely related to the degree of tolerance after continuous infusion, but that intrinsic efficacy does not significantly affect tolerance after once-daily intermittent administration of these agents. These findings may be of clinical utility in understanding the development of tolerance to the antinociceptive effects of opioids. (Key words: Analgesia, opioid: continuous infusion; intermittent administration; intrinsic efficacy; tolerance. Analgesics, opioid: etorphine; fentanyl; meperidine; morphine; oxycodone.)**

PREVIOUS studies have suggested that the intrinsic efficacy of an opioid analgesic can determine, in part, the degree of tolerance to that agent. Specifically, animal studies have demonstrated that the tolerance that develops to equieffective doses of opioid analgesics with high intrinsic efficacy is less than the tolerance that develops to lower-intrinsic-efficacy compounds.[1–4]

Intrinsic efficacy is a property of a particular drug-receptor interaction and is directly related to the number of spare receptors (*i.e.*, receptor reserve), such that the larger the receptor reserve, the greater the intrinsic efficacy.[5] In cases in which there is a receptor reserve, a criterion response (*e.g.*, maximal response) can be produced with less than full receptor occupancy. The concept of receptor reserve can be illustrated experimentally by alkylating a fraction of receptors and then demonstrating that the dose-response function is simply shifted to the right without any decrease in the maximal response.[6–9]

With regard to opioid tolerance, it has been suggested that equieffective doses of opioid agonists of high or low intrinsic efficacy should produce differential degrees of tolerance because different fractional receptor occupancy is required.[3] Because it is assumed that tolerance is a consequence of receptor activation, a low-intrinsic-efficacy agonist occupies and produces a "tolerance effect" at more receptors than does a high-efficacy agonist. Therefore, it is predicted that a high-efficacy compound will leave more receptors in the nontolerant state and produce a smaller degree of tolerance than will an equieffective dose of a low-intrinsic-efficacy agonist. In general, this prediction has been supported by studies that show that high-efficacy opioid agonists (*e.g.*, [D-ala^2, N-methyl-phe^4, gly-ol^5] enkeph-

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Table 1. Estimation of Antinociceptive ED_{50}s in Untreated Mice

Drug	ED_{50}
Morphine (mg/kg)	1.25 (0.97–1.75)
Oxycodone (mg/kg)	0.90 (0.78–1.10)
Fentanyl (μg/kg)	13.00 (8.50–15.94)
Etorphine (μg/kg)	0.60 (0.48–0.91)
Meperidine (mg/kg)	5.13 (2.92–8.06)

ED_{50} and 95% confidence limits (in parentheses) for each drug used in tolerance studies. Initial ED_{50}s were estimated using different groups of mice (typically 30–40/group) injected subcutaneously with four or more different doses of each drug and tested for antinociception following administration. Similar ED_{50} estimates were obtained in 1–3 replications for each drug.

alin, sufentanil, and fentanyl) produced less tolerance than intermediate- or low-intrinsic-efficacy agonists (*e.g.*, morphine, meperidine, and [D-ala^2, D-leu^5] enkephalin).[1–4]

That less tolerance may result from equieffective doses of high-intrinsic-efficacy compounds may be an important issue in pain management. However, the majority of studies that have examined this issue in laboratory animals have used continuous administration (*i.e.*, infusion) protocols.[1–4] Because intermittent administration of opioid analgesics is common, it is of interest to determine whether the inverse relation between intrinsic efficacy and tolerance observed in infusion studies holds under noncontinuous administration protocols. Therefore, in the current study we investigated the degree of tolerance after continuous and intermittent administration of equieffective doses of several opioid agonists. In these experiments we show in mice that intrinsic efficacy[6,10–13] interacts with the type of dosing protocol (intermittent *vs.* continuous) in determining the degree of tolerance to the antinociceptive effects of opioids.

Materials and Methods

Subjects

Male Swiss Webster mice (22–35 g) (Taconic Farms, Germantown, NY) were used throughout the study. Mice were housed 10 or 11 per cage with free access to food and water and were used only once. All protocols and procedures were approved by the St. John's University Institutional Animal Care and Use Committee.

Initial Estimation of Median Effective Doses

Untreated mice were tested for antinociception (tail flick, see below) in dose-response studies, and the median effective doses (ED_{50}s) for morphine, etorphine, fentanyl, meperidine, and oxycodone were calculated (table 1). Four or more different doses of each drug were administered (n = 5–10 mice/dose), and mice were tested for antinociception (see below) 30 min (morphine) or 15 min (all other drugs) after subcutaneous administration. As shown in figure 1, the dose-response functions obtained in untreated mice for each of the five opioids used in these studies appeared parallel to one another. Probit analysis (see Data Analysis, below) confirmed that there was no significant deviation from parallelism for any dose-response function ($P > 0.20$). The dosing protocols for tolerance induction were based on these initial ED_{50}s and were designed to deliver equieffective doses of each drug. Specifically, the dosing schedules were defined as multiples of the initial antinociceptive ED_{50} value. This strategy was based on the principle that the ED_{50} value for all drugs is equieffective, by definition, and that therefore multiples of the ED_{50} also are equivalent. For example, 10 times the ED_{50} for morphine was a dose equivalent to 10 times the ED_{50} for etorphine or fentanyl.

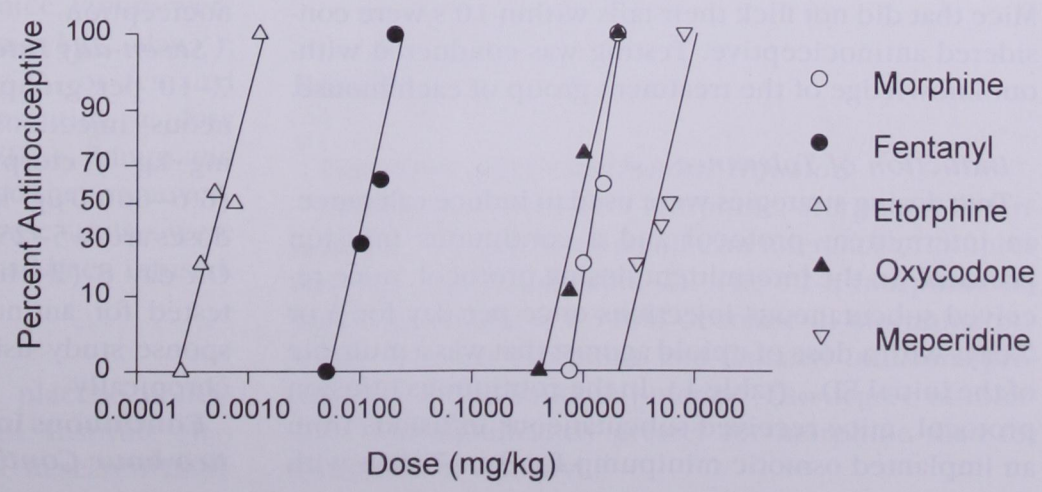

Fig. 1. Representative dose-response functions in untreated mice for five opioid analgesics.

Dose-Response Protocols

Dose-response studies were conducted by means of two procedures: standard and cumulative dosing. In the standard procedure, separate groups of mice (typically 30–40 per group) received subcutaneous injections with three or more different doses of a drug and were tested for antinociception (see below) at the time of peak effect (30 min for morphine and 15 min for all other drugs). Peak effect times were based on time-action profiles determined earlier (data not shown).

In the cumulative dose-response protocol, all mice in a treatment group (typically seven to ten per group) received subcutaneous injections with a starting dose of agonist and were tested for antinociception 30 min (morphine) or 15 min (all other drugs) after administration of opioid agonists. All mice responding to the painful stimulus were given a second dose of the same drug within 5 min of testing and then were retested for antinociception. This cumulative dose-response protocol was continued until all mice failed to respond. The actual doses given for each drug and the strategy for choosing doses in the cumulative dose-response protocol were determined from previous studies.[1,6,14] Data from cumulative dosing are presented such that the percentage of mice that were antinociceptive is plotted against the total (cumulative) dose administered. In most cases, the ED_{50} determined in cumulative dose-response studies was greater than that determined in standard dose-response studies.

Analgesia

Antinociception was determined with a tail-flick assay, as previously described.[15] A beam of light was focused on the dorsal surface of the tail of the mouse and the apparatus adjusted so that baseline tail flicks occurred within 2–4 s. In dose-response studies, a cutoff tail-flick latency (10 s) was used to avoid tissue damage. Mice that did not flick their tails within 10 s were considered antinociceptive. Testing was conducted without knowledge of the treatment group of each mouse.

Induction of Tolerance

Two dosing strategies were used to induce tolerance: an intermittent protocol and a continuous infusion protocol. In the intermittent dosing protocol, mice received subcutaneous injections once per day for 3 or 7 days with a dose of opioid agonist that was a multiple of the initial ED_{50} (table 1). In the continuous infusion protocol, mice received subcutaneous infusions from an implanted osmotic minipump for 3 or 7 days with a dose of opioid agonist that was a multiple of the initial ED_{50}. In all experiments, a parallel control group was included as an internal reference for potency changes for the treated group. To minimize the costs associated with the use of the osmotic infusion pumps, an inert placebo pellet was implanted subcutaneously in control mice. Preliminary studies showed that the implantation of placebo pellets produced no effect on morphine's antinociceptive ED_{50} when compared with the ED_{50} determined in mice with a pump infusing saline for 7 days (ED_{50} 2.54 $mg \cdot kg^{-1}$ [95% confidence limits 1.78–3.42] and ED_{50} 2.70 $mg \cdot kg^{-1}$ [95% confidence limits 1.91–3.63], placebo pellet, saline infusion, respectively).

Intermittent Administration of Opioid Agonists. ***Seventy-two-hour Intermittent Administration.*** Mice (n = 45–50 per group per experiment) received subcutaneous injections once per day for 3 days of saline, morphine, etorphine, fentanyl, meperidine or oxycodone. The dose of each drug for each injection was based on the initial estimate of the ED_{50} such that $\approx$20 times the ED_{50} (table 1) was administered on days 1 and 2 and $\approx$40 times the ED_{50} on day 3. The daily doses for the first 2 days and the 3rd day, respectively, were morphine 25.0 and 50.0 $mg \cdot kg^{-1}$; etorphine 12.0 and 24.0 $\mu g \cdot kg^{-1}$; fentanyl 260 and 520 $\mu g \cdot kg^{-1}$; oxycodone 18.0 and 36.0 $mg \cdot kg^{-1}$; and meperidine 102.6 and 205.2 $mg \cdot kg^{-1}$. On day 4 (72 h after the first injection), mice were tested for analgesia in a dose-response study (standard protocol) using the same drug that was chronically administered. To determine the effect of a larger dose of morphine, mice (n = 30 per group) received subcutaneous injections of saline or a morphine dose $\approx$ 40 times (50 $mg \cdot kg^{-1}$) the ED_{50} on days 1 and 2 and $\approx$80 times (100 $mg \cdot kg^{-1}$) the ED_{50} on day 3. Twenty-four hours after the last injection (72 h after the first injection), mice were tested for antinociception.

Seven-day Intermittent Administration. Mice (n = 7–10 per group per experiment) received subcutaneous injections of saline, morphine (13.7–27.4 $mg \cdot kg^{-1}$), etorphine (6.2–16.0 $\mu g \cdot kg^{-1}$), or fentanyl (6.6–660.0 $\mu g \cdot kg^{-1}$) every 24 h for 7 days. These daily doses are $\approx$5–25 times the initial estimate of the ED_{50}. On day 8 (24 h after the last injection), mice were tested for antinociception in a cumulative dose-response study using the same drug that was injected chronically.

Continuous Infusion of Opioid Agonists. ***Seventy-two-hour Continuous Infusion.*** In mice (n = 7–10

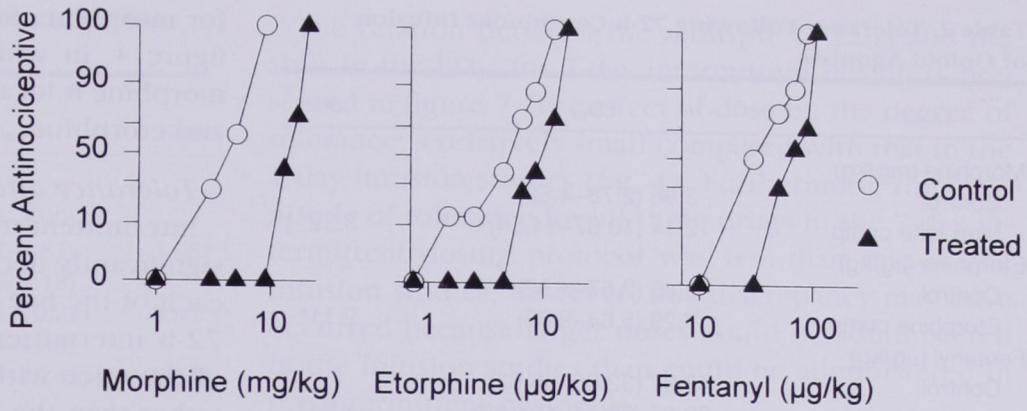

Fig. 2. The effect of intrinsic efficacy on tolerance after 72-h infusion of opioid agonists. Mice received infusions subcutaneously using osmotic minipumps for 72 h with morphine, etorphine, or fentanyl. The daily infusion dose represents ≈27 times the median effective dose (ED_{50}) for each drug as determined previously (table 1). Placebo pellets were implanted subcutaneously in controls. At the end of the infusion the pellets and pumps were removed, and 4 h later all mice were tested in cumulative dose-response studies (tail flick) using the same drug. For ease of comparison, dose is presented on a scale that is equated for distance between units for each drug. Each panel represents a separate representative experiment for each drug (table 2). The cumulative dose range for each drug for the dose response was morphine 1.00–21.00 mg · kg^{-1}, etorphine 0.75–16.50 μg · kg^{-1}, and fentanyl 10.0–180.0 μg · kg^{-1}.

per group per experiment) an osmotic minipump (ALZET 2001, Alza, Palo Alto, CA) that delivered pump contents at a rate of 1.0 μl · h^{-1} was implanted subcutaneously. An inert placebo pellet was implanted subcutaneously in control mice. Pumps were filled with morphine (33.33 mg · kg^{-1} · day^{-1}), etorphine (16 μg · kg^{-1} · day^{-1}), or fentanyl (347.10 μg · kg^{-1} · day^{-1}). The infusion doses were equal to the total dose of drug delivered during the 72-h intermittent dosing protocol (see above). Thus, an equivalent dose was administered continuously rather than intermittently. The daily infusion dose represented ≈27 times the ED_{50} dose for each drug. Pumps and pellets were left in place for 72 h and then removed, and 4 h later, cumulative dose-response studies were conducted using the same drug that had been infused.

Seven-day Continuous Infusion. In mice (n = 7–10 per group per experiment) osmotic minipumps (ALZET 2001 or 2002, Alza) delivering contents at a rate of 1.0 μl · h^{-1} or 0.05 μl · h^{-1}, respectively, were implanted subcutaneously. An inert placebo pellet was implanted subcutaneously in control mice. Pumps were filled with morphine (13.70–41.10 mg · kg^{-1} · day^{-1}), etorphine (15.50–124 μg · kg^{-1} · day^{-1}), or fentanyl (165–1,320 μg · kg^{-1} · day^{-1}), representing a dose range of ≈10–200 times the initial ED_{50}s. Pumps and pellets were left in place for 7 days and then removed, and 24 h later, cumulative dose-response studies were conducted using the same drug that had been infused.

Drugs

Etorphine hydrochloride 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. Fentanyl citrate and oxycodone hydrochloride were obtained from Sigma Chemical (St. Louis, MO). Morphine sulfate and meperidine hydrochloride were supplied by Penick Corporation (Newark, NJ). All drugs were dissolved in 0.9% sodium chloride and administered subcutaneously. Doses are expressed as the base. Pellets and osmotic minipumps were implanted subcutaneously at the nape of the neck. Pumps and pellets were implanted and removed while mice were lightly anesthetized with halothane/oxygen.

Data Analysis

Quantal dose-response data were analyzed by probit analysis[16] with use of a computer program (BLISS 21, Department of Statistics, University of Edinburgh, Edinburgh, Scotland) that estimates ED_{50}s, 95% confidence limits, and relative potencies and determines whether dose-response functions are parallel. Significant differences between potency estimates and ED_{50}s were determined by probit analysis.

Results

Tolerance after Continuous Infusion

Continuous infusion of morphine, etorphine, or fentanyl for 72 h produced significant tolerance to all three agonists (fig. 2 and table 2). The 72-h morphine infusion generated a 3.3-fold decrease in morphine potency, whereas etorphine and fentanyl infusions produced ≈2-fold shifts in potency. The degree of tolerance was significantly greater for morphine than for etorphine or fentanyl.

Table 2. Tolerance Following 72-h Continuous Infusion of Opioid Agonists

Drug	ED_{50}	Shift
Morphine (mg/kg)		
Control	3.66 (2.78–4.69)	
Morphine pump	12.14 (10.07–14.54)	3.32*†
Etorphine (μg/kg)		
Control	3.45 (2.57–4.52)	
Etorphine pump	7.29 (5.64–9.65)	2.11*
Fentanyl (μg/kg)		
Control	43.97 (32.60–56.62)	
Fentanyl pump	80.95 (63.31–101.82)	1.84*

Data presented are the antinociceptive ED_{50} and 95% confidence limits (in parentheses) from two cumulative dose–response studies for each drug. The shift is calculated as the ratio: (ED_{50} for drug group)/(ED_{50} for controls).

* Significant potency shift *versus* control ($P < 0.05$).

† Significantly different from the potency shift for all others ($P < 0.05$).

In the 7-day infusion study, morphine, etorphine, and fentanyl produced significant tolerance after all or most dosing schedules (fig. 3 and table 3). However, doses of morphine much smaller than those of fentanyl or etorphine were required to produce equivalent tolerance. The relation between the multiple of ED_{50} (*i.e.*, chronic dose) and the shift in the ED_{50} (*i.e.*, tolerance) for morphine, fentanyl, and etorphine is presented in figure 4, in which it can be seen that the curve for morphine is located to the left of the curves for fentanyl and etorphine.

Tolerance after Intermittent Dosing

Intermittent treatment with opioid agonists for 72 h significantly decreased the antinociceptive potency of each of the five opioids (fig. 5 and table 4A). (In this 72-h intermittent administration experiment, testing of the mice with the standard dose-response protocol rather than the cumulative protocol accounts for the lower ED_{50} estimates; see Materials and Methods.) The dosing schedule of ≈20 times the ED_{50} on days 1 and 2 and ≈40 times the ED_{50} on day 3 produced similar tolerance for the five agonists (potency shift range 1.52–1.95). There were no significant differences among these potency shifts. When a larger intermittent dosing schedule for morphine was used (fig. 5, top middle, and table 4B), greater tolerance was observed (3.40-fold). Dosing at higher multiples of the ED_{50} was not attempted for the other drugs because of toxicity. Mortality using the above dosing schedule was 0% for oxycodone, <1% for both morphine dosing protocols, 11% for meperidine, 6% for etorphine, and 16% for fentanyl.

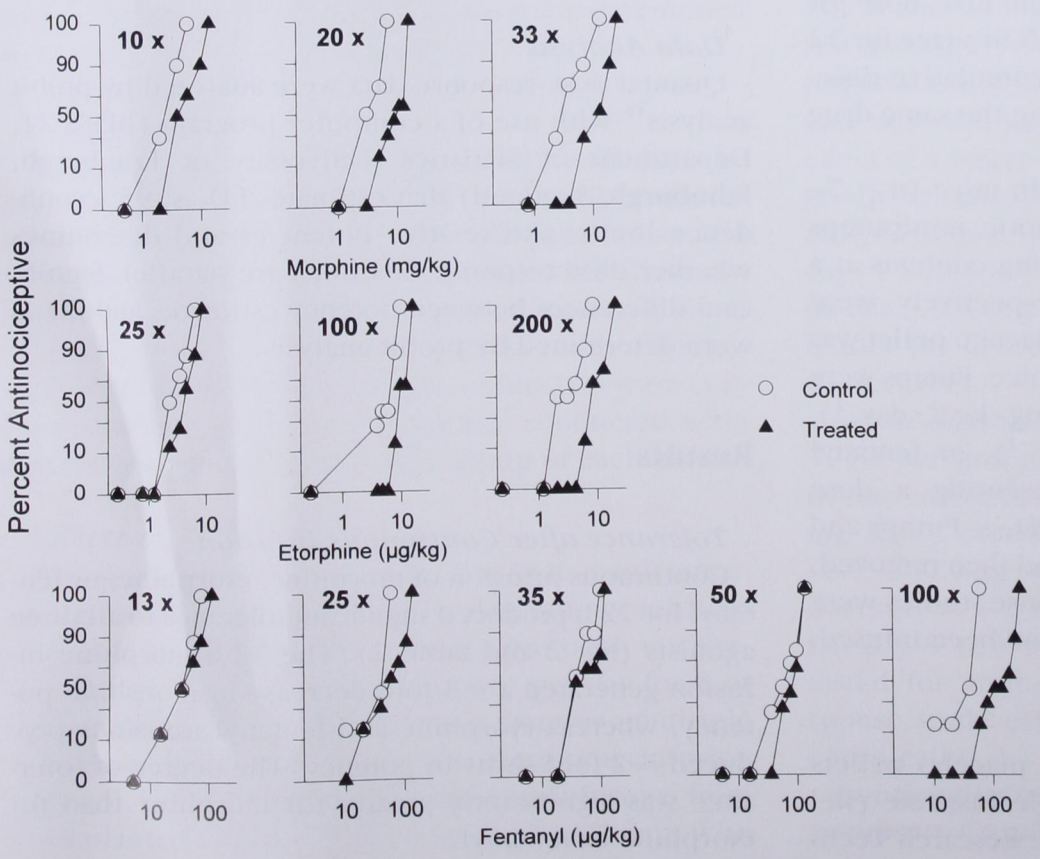

Fig. 3. The effect of intrinsic efficacy on tolerance after 7-day infusion of opioid agonists. Mice received subcutaneous infusions from osmotic minipumps for 7 days with morphine, etorphine, or fentanyl. The daily infusion dose represents a multiple (top left of each panel) of the median effective dose (ED_{50}) dose for morphine, etorphine and fentanyl, as determined previously (table 1). Placebo pellets were implanted in controls. At the end of the infusion the pellets and pumps were removed, and 24 h later all mice were tested in cumulative dose-response studies (tail flick) using the same drug. For ease of comparison, dose is presented on a scale that is equated for distance between units for each drug. Each panel represents a separate experiment (table 3). The cumulative dose range for each drug for the dose-response was morphine 0.5–19.50 $mg \cdot kg^{-1}$, etorphine 0.24–20.80 $\mu g \cdot kg^{-1}$, and fentanyl 5.0–290.0 $\mu g \cdot kg^{-1}$.

Table 3. Tolerance Following 7-day Continuous Infusion of Opioid Agonists

Drug	Daily Dose (multiple of ED_{50})	ED_{50}	Shift
Morphine (mg/kg)			
Control		2.38 (1.72–3.09)	
Morphine pump	10X	4.65 (3.61–5.81)	1.95*
Control		2.37 (1.64–3.18)	
Morphine pump	20X	8.09 (6.46–10.22)	3.41*
Control		3.38 (2.48–4.48)	
Morphine pump	33X	11.96 (9.27–15.45)	3.54*
Etorphine (μg/kg)			
Control		2.75 (2.11–3.41)	
Etorphine pump	25X	4.30 (3.48–5.24)	1.56*
Control		5.53 (4.73–6.42)	
Etorphine pump	100X	10.20 (8.82–11.89)	1.84*
Control		3.04 (2.38–3.82)	
Etorphine pump	200X	9.08 (7.23–11.61)	2.99*
Fentanyl (μg/kg)			
Control		30.82 (21.20–42.27)	
Fentanyl pump	13X	35.61 (24.79–47.90)	1.16
Control		31.42 (21.54–45.96)	
Fentanyl pump	25X	51.28 (37.19–68.88)	1.63*
Control		31.38 (18.87–48.52)	
Fentanyl pump	35X	67.46 (41.83–108.94)	2.15*
Control		50.65 (36.46–66.77)	
Fentanyl pump	50X	83.02 (61.81–115.35)	1.64*
Control		32.66 (23.13–45.43)	
Fentanyl pump	100X	131.19 (103.11–169.79)	4.02*

Data presented are the antinociceptive ED_{50} and 95% confidence limits (in parentheses) from 1–2 cumulative dose–response studies for each condition. The shift is calculated as the ratio: (ED_{50} for drug group)/(ED_{50} for controls).

* Significant potency shift ($P < 0.05$).

In the 7-day intermittent administration study (fig. 6 and table 5), equieffective dosing produced approximately similar tolerance for all drugs, suggesting that there was little relation between intrinsic efficacy and the degree of tolerance. For example, 10 times the ED_{50} treatment for morphine and etorphine produced no significant tolerance, but 20 times the ED_{50} dose produced equivalent and significant tolerance. (However, 10 times the ED_{50} treatment for fentanyl resulted in significant tolerance.) Doses of fentanyl and etorphine 25 times the ED_{50} also produced equivalent and significant tolerance. Larger doses of fentanyl and etorphine could not be used for acute daily administration because of the toxicity of larger doses. Specifically, mortality was 0% for all doses of morphine, 20% at 25 times the ED_{50} for etorphine, and 26% for 25 times the ED_{50} for fentanyl; mortality was 0% for the lower dosing schedules for both fentanyl and etorphine.

The relation between the multiple of ED_{50} and the shift in the ED_{50} for 7-day intermittent dosing is presented in figure 7. The effect of dose on the degree of tolerance is relatively small compared with that in the 7-day infusion studies (fig. 4). Furthermore, the magnitude of tolerance for all three drugs in the 7-day intermittent dosing protocol was less than that in the infusion studies; however, this discrepancy may have occurred because larger doses could be administered in the infusion studies than could be administered in the intermittent protocols.

Discussion

The contribution of the intrinsic efficacy of opioid agonists to the development of antinociceptive tolerance in the mouse was examined in the current study in continuous infusion and intermittent dosing protocols. Intrinsic efficacy was inversely related to tolerance after continuous infusion of morphine, etorphine, or fentanyl. These results confirm in mice the findings of previous investigators in rats, in which infusions of high-intrinsic-efficacy opioids (*e.g.*, fentanyl and sufentanil) were associated with less tolerance than were infusions of lower-intrinsic-efficacy opioids (*e.g.*, morphine).[1–4]

In contrast to continuous infusion, intermittent administration of equieffective doses of opioid agonists produced approximately the same degree of tolerance

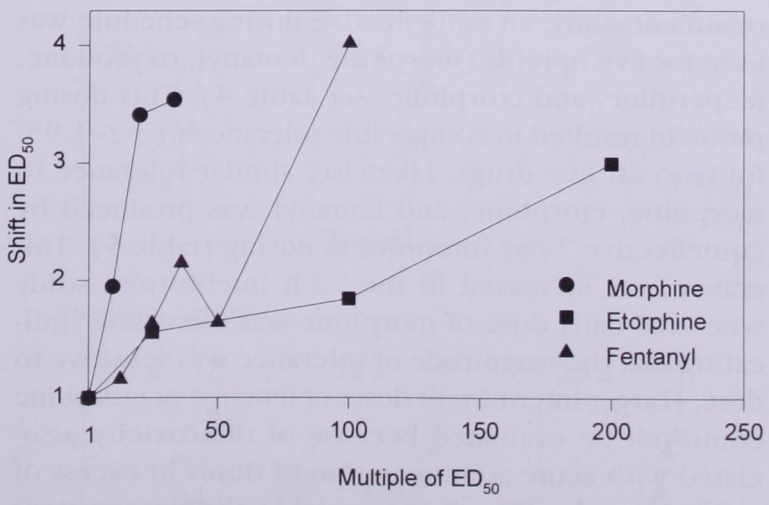

Fig. 4. The effect of intrinsic efficacy on the shift in the median effective dose (ED_{50}) after 7-day infusions of morphine, etorphine, or fentanyl. Mice were treated as described in the legend to figure 3. The shift in the ED_{50} is plotted against the multiple of the initial ED_{50}s. The shift in the ED_{50} is the ratio (ED_{50} for drug group)/(ED_{50} for controls) from table 3.

Table 4. Tolerance Following Intermittent Opioid Agonist Treatment for 72 h

Drug	ED_{50}	Shift
(A)		
Morphine (mg/kg)		
Control	1.28 (0.94–1.70)	
Morphine	2.50 (1.93–3.69)	1.95*
Oxycodone (mg/kg)		
Control	0.77 (0.60–0.98)	
Oxycodone	1.17 (0.94–1.49)	1.52*
Fentanyl (μg/kg)		
Control	14.12 (10.96–17.90)	
Fentanyl	24.32 (18.56–33.70)	1.72*
Etorphine (μg/kg)		
Control	0.92 (0.72–1.17)	
Etorphine	1.51 (0.96–1.93)	1.64*
Meperidine (mg/kg)		
Control	6.76 (5.30–8.59)	
Meperidine	12.07 (9.37–16.39)	1.79*
(B)		
Morphine (mg/kg)		
Control	1.62 (1.05–2.38)	
Morphine	5.50 (3.72–8.84)	3.40*,†

Data presented are the antinociceptive ED_{50} and 95% confidence limits (in parentheses) from 1–4 studies using the standard dose–response protocol for each drug. The shift is calculated as the ratio: (ED_{50} for drug group)/(ED_{50} for controls).

(A) Mice were injected with ≈20 times the ED_{50} on day 1 and 2; ≈40 times the ED_{50} on day 3. (B) Mice received injections with ≈40 times the ED_{50} on day 1 and 2; ≈80 times the ED_{50} on day 3.

* Significant potency shift ($P < 0.05$).

† Significantly different potency shift *versus* all others ($P < 0.05$).

in all of the agents tested. In the 72-h intermittent treatment study, an equieffective dosing schedule was used for five opioids (morphine, fentanyl, oxycodone, meperidine, and etorphine; see table 4). This dosing protocol resulted in comparable tolerance (1.52–1.95-fold) to all five drugs. Likewise, similar tolerance to morphine, etorphine, and fentanyl was produced by equieffective 7-day intermittent dosing (table 5). Tolerance was increased in the 72-h intermittent study when the daily dose of morphine was increased, indicating that the magnitude of tolerance was sensitive to dose. (Larger intermittent doses of fentanyl or etorphine could not be evaluated because of the toxicity associated with acute administration of doses in excess of ≈25 times the ED_{50}.) It is notable that intermittent administration of morphine in rats is associated with a reduced level of naloxone-precipitated withdrawal compared with that associated with continuous administration of an equivalent amount of drug.[17] Thus, both tolerance and dependence appear to be related to the degree of intermittency of drug administration.

Intrinsic efficacy is a property of a drug-receptor interaction and is proportional to the number of receptors required to produce a given effect.[5] Measurement of intrinsic efficacy is a laborious process, and the intrinsic efficacy *in vivo* of the vast majority of opioid analgesics has not been determined. However, in studies with irreversible μ-receptor antagonists, it has been shown that the intrinsic efficacy of morphine is lower than that of fentanyl.[6,12] Behavioral data from a drug discrimination study suggest that the intrinsic efficacy of meperidine is lower than that of morphine.[13] Although data on the intrinsic efficacy *in vivo* of etorphine are not currently available, parallel binding and analgesia studies imply that etorphine has greater intrinsic efficacy than does morphine.[10] Finally, there is no information currently concerning the intrinsic efficacy of oxycodone. Although the intrinsic efficacies of all opioid analgesics are not known, the finding that all five opioid agonists produced similar tolerance after 72-h intermittent administration suggests that intrinsic efficacy is not an important determinant of tolerance in this dosing protocol. For morphine, etorphine, and fentanyl, which have been shown to differ in intrinsic efficacy, there was a clear difference in the magnitude of tolerance after continuous infusion. Of interest, it has been reported that the degree of analgesic tolerance in rats was similar after acute (8-h) infusions of opioids that vary in intrinsic efficacy (morphine, sufentanil, and alfentanil).[18,19] Thus, the effect of intrinsic efficacy on tolerance after continuous administration appears by 72 h but is not present after an 8-h infusion.

In the 7-day administration studies, the range of dosing schedules for intermittent and continuous administration permitted comparison of the relation between the multiple of ED_{50} (*i.e.,* dose) and the degree of tolerance for continuous and intermittent dosing. As might be predicted, the larger the dose, the greater the degree of tolerance. This relation was most striking for the infusion studies (fig. 4), although there was a weaker relation between dosing and tolerance in the intermittent protocol (fig. 7).

The effect of dosing protocol on the capacity of a drug to produce tolerance can be demonstrated by examining the shift in the ED_{50} for continuous and intermittent administration. In general, tolerance to morphine (low efficacy) was greater after infusion than after intermittent administration. Specifically (tables 2 and 4), acute intermittent administration of morphine for

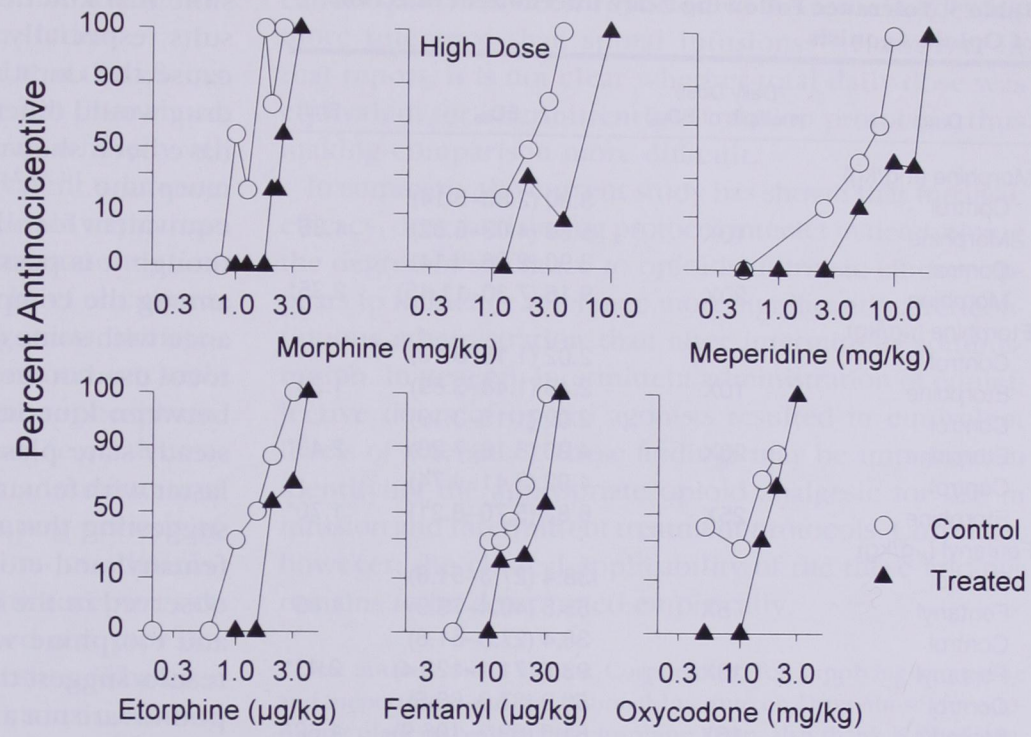

Fig. 5. The effect of intrinsic efficacy on tolerance after daily intermittent opioid agonist administration for 3 days. Mice received subcutaneous injections every 24 h for 3 consecutive days with saline or with ≈20 times (days 1 and 2) and ≈40 times (day 3) the median effective dose (ED_{50}) for morphine, fentanyl, etorphine, oxycodone, or meperidine. Other mice received injections of ≈40 times (days 1 and 2) and ≈80 times (day 3) the ED_{50} for morphine (High Dose). ED_{50}s were based on initial estimates (table 1). Twenty-four hours after the last injection all mice were tested in dose-response studies (tail flick) using the same drug. For ease of comparison, dose is presented on a scale that is equated for distance between units for each drug. Each panel represents a separate experiment for each drug (table 4). The dose range for each drug for the dose response was morphine 0.5–4.50 $mg \cdot kg^{-1}$, etorphine 0.125–4.0 $\mu g \cdot kg^{-1}$, fentanyl 4.0–50.0 $\mu g \cdot kg^{-1}$, oxycodone 0.125–4.0 $mg \cdot kg^{-1}$, and meperidine 0.5–20.0 $mg \cdot kg^{-1}$.

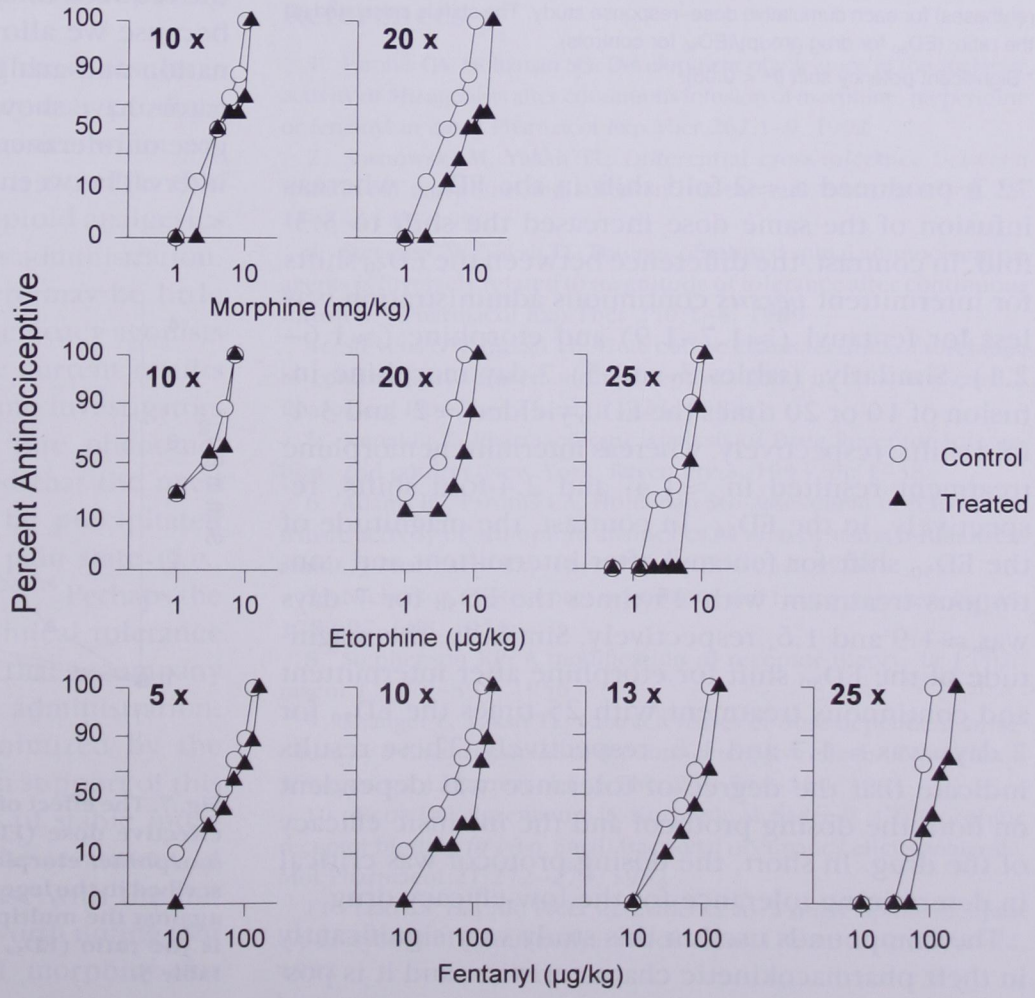

Fig. 6. The effect of intrinsic efficacy on tolerance after 7-day intermittent opioid agonist administration. Mice received subcutaneous injections every 24 h for 7 days with morphine, etorphine or fentanyl. The daily injection dose represents a multiple (top left of each panel) of the median effective dose (ED_{50}) for morphine, etorphine, and fentanyl, as determined previously (table 1). Controls received injections of saline. Twenty-four hours after the last injection all mice were tested in cumulative dose-response studies (tail flick) using the same drug. For ease of comparison, dose is presented on a scale that is equated for distance between units for each drug. Each panel represents a separate experiment for each drug (table 5). The cumulative dose range for each drug for the dose response was morphine 1–18 $mg \cdot kg^{-1}$, etorphine 0.5–11.25 $\mu g \cdot kg^{-1}$, and fentanyl 10–240 $\mu g \cdot kg^{-1}$.

Table 5. Tolerance Following 7-day Intermittent Injection of Opioid Agonists

Drug	Daily Dose (multiple of ED_{50})	ED_{50}	Shift
Morphine (mg/kg)			
Control		3.90 (2.88–5.14)	
Morphine	10X	5.30 (4.03–6.82)	1.36
Control		3.90 (2.88–5.14)	
Morphine	20X	9.16 (7.30–11.45)	2.35*
Etorphine (μg/kg)			
Control		2.02 (1.15–3.19)	
Etorphine	10X	2.49 (1.48–3.89)	1.23
Control		2.02 (1.15–3.19)	
Etorphine	20X	4.90 (3.19–7.26)	2.43*
Control		4.02 (3.41–4.74)	
Etorphine	25X	6.82 (5.70–8.21)	1.70*
Fentanyl (μg/kg)			
Control		38.4 (27.3–51.8)	
Fentanyl	5X	55.5 (40.4–73.9)	1.45
Control		38.4 (27.3–51.8)	
Fentanyl	10X	93.5 (71.1–122.4)	2.43*
Control		50.2 (37.0–66.5)	
Fentanyl	13X	81.5 (62.1–106.9)	1.62*
Control		66.2 (53.5–81.3)	
Fentanyl	25X	122.8 (104.3–143.8)	1.85*

Data presented are the antinociceptive ED_{50} and 95% confidence limits (in parentheses) for each cumulative dose–response study. The shift is calculated as the ratio: (ED_{50} for drug group)/(ED_{50} for controls).

* Significant potency shift ($P < 0.05$).

72 h produced a ≈2-fold shift in the ED_{50}, whereas infusion of the same dose increased the shift to 3.3-fold. In contrast, the difference between the ED_{50} shifts for intermittent *versus* continuous administration was less for fentanyl (≈1.7–1.9) and etorphine (≈1.6–2.1). Similarly, (tables 3 and 5) 7-day morphine infusion of 10 or 20 times the ED_{50} yielded ≈2- and 3.4-fold shifts, respectively, whereas intermittent morphine treatment resulted in ≈1.4- and 2.4-fold shifts, respectively, in the ED_{50}. In contrast, the magnitude of the ED_{50} shift for fentanyl after intermittent and continuous treatment with 25 times the ED_{50} for 7 days was ≈1.9 and 1.6, respectively. Similarly, the magnitude of the ED_{50} shift for etorphine after intermittent and continuous treatment with 25 times the ED_{50} for 7 days was ≈1.7 and 1.6, respectively. These results indicate that the degree of tolerance was dependent on both the dosing protocol and the intrinsic efficacy of the drug. In short, the dosing protocol was critical in determining tolerance for the low-efficacy drug.

The compounds used in this study vary significantly in their pharmacokinetic characteristics, and it is possible that kinetic differences may have affected the results, especially after intermittent administration because the duration of exposure of receptors to each drug would differ. For example, etorphine and fentanyl have been shown to have much shorter half-lives than morphine.[10,20,21] In general, however, tolerance was equivalent for all agonists after intermittent dosing. Although it is possible that pharmacokinetic differences among the compounds may affect the degree of tolerance with some dosing protocols, the intermittent protocol used in the current study did not reveal a relation between kinetics and tolerance. In infusion studies, steady-state plasma concentrations would be reached faster with fentanyl and etorphine than with morphine, suggesting that more tolerance might be induced for fentanyl and etorphine. In fact, just the opposite was observed in the infusion studies: tolerance to fentanyl and etorphine was less than that to morphine. These results suggest that kinetic differences among the compounds are not a significant variable in determining the degree of tolerance. In infusion studies the washout of fentanyl and etorphine would be more rapid than that of morphine. This difference may have contributed to the reduced tolerance seen for etorphine and fentanyl, because we allowed 4–24 h between infusion termination and analgesic testing. However, other investigators have shown that intrinsic efficacy affects the degree of tolerance after infusions even when there is no interval between dosing termination and testing.[4] Thus,

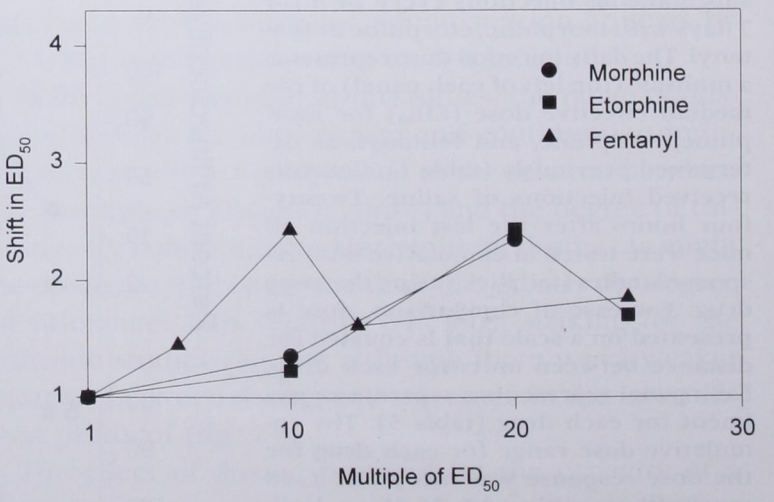

Fig. 7. The effect of intrinsic efficacy on the shift in the median effective dose (ED_{50}) after 7-day intermittent injections of morphine, etorphine or fentanyl. Mice were treated as described in the legend to figure 6. The shift in the ED_{50} is plotted against the multiple of the initial ED_{50}s. The shift in the ED_{50} is the ratio (ED_{50} for drug group)/(ED_{50} for controls) from table 5.

our studies extend previous results on the role of intrinsic efficacy in tolerance in infusion studies.

Overall, the current findings indicate that the effects of intermittent administration are different from those of continuous infusion. The mechanism whereby all agonists tested produced roughly equivalent tolerance after intermittent administration is not readily apparent but may be related to phasic *versus* continuous stimulation of receptors. Perhaps relatively brief elicitation of an equivalent effect by several agonists initiates comparable tolerance mediated by common intracellular substrates (*e.g.*, cyclic adenosine monophosphate, ion flux). Receptor-based contributions (*e.g.*, decoupling) to tolerance after intermittent administration may be minimal because of the alternating periods of receptor occupancy. In contrast, continuous administration may induce changes close to the receptor site (*e.g.*, decoupling) due to uninterrupted receptor occupancy, as well as at other intracellular sites. Whatever the mechanism, infusions of the high-intrinsic-efficacy agonists etorphine and fentanyl produced less tolerance, whereas an equieffective infusion of morphine produced greater tolerance. These findings clearly support the role of intrinsic efficacy in tolerance during continuous administration.

The potential clinical importance of these results is that analgesics of high intrinsic efficacy may produce less tolerance than low-efficacy analgesics when administered as infusions. Therefore, it is possible that there is a clinical advantage to using opioid analgesics of high intrinsic efficacy for continuous administration. However, our results suggest that there may be little benefit in the use of intermittent high-efficacy agonists to reduce tolerance. Furthermore, the current results may be relevant to observations by some investigators that clinical tolerance is a relatively rare phenomenon.[22] Some clinicians have suggested that the need for increased opioid analgesics may be precipitated more by a change in the underlying pain state (*i.e.*, disease progression) than by tolerance.[23–25] Perhaps the modest incidence or magnitude of clinical tolerance is related to the phasic plasma profiles that accompany intermittent oral and acute parenteral administration. Thus, clinical tolerance may be minimized by the phasic nature of intermittent dosing. In support of this suggestion are clinical case examples of stable intermittent opioid (morphine) use for the treatment of pain, followed by increasing opioid use with the initiation of continuous infusions.[24] It is worth noting that intermittent spinal administration of morphine for cancer pain has been reported to be associated with more tolerance than spinal infusions.[26] However, in that report, it is not clear whether total daily dose was equivalent for intermittent and infusion protocols, thus making comparison more difficult.

In summary, the current study has shown that intrinsic efficacy, dose, and dosing protocol interact in determining the degree of tolerance to opioids. Intrinsic efficacy appears to influence tolerance more significantly after continuous administration than after intermittent administration. In general, intermittent administration of equieffective doses of opioid agonists resulted in equivalent levels of tolerance. These findings may be important in identifying the appropriate opioid analgesic for use in infusion and intermittent treatment protocols. Currently, however, the clinical applicability of the these findings remains to be determined empirically.

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