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ORIGINAL INVESTIGATION

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Development of tolerance and sensitization to different opioid agonists in rats

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Abstract *Rationale:* Despite numerous investigations, the mechanisms underlying the development of opioid tolerance are far from clear. However, several in vitro studies implicated a protective role of agonist-induced μ-opioid receptor endocytosis in the development of opioid tolerance. Moreover, we have recently demonstrated that the high-efficacy agonist etonitazene promotes rapid endocytosis of μ-opioid receptors, whereas the agonist morphine and the low-efficacy agonist buprenorphine fail to promote detectable receptor endocytosis in μ-opioid receptor expressing HEK293 cells. *Objectives:* The present study explored the effects of these opioids on the development of tolerance and sensitization in rats in vivo. *Methods:* The opioid effects were quantified using the hot plate, electric tail root stimulation, and the locomotor activity chamber in male Wistar rats. Dose–response curves were generated for each test drug. To induce tolerance, equieffective doses of etonitazene, morphine, and buprenorphine were administered daily for 29 days. *Results:* We found that chronic treatment with the noninternalizing drugs buprenorphine and morphine resulted in a greater development of tolerance than etonitazene. In addition, the sensitization to the locomotor stimulant effect was high after buprenorphine and morphine, but was lacking after chronic etonitazene application. *Conclusion:* The results support a role for the endocytotic potency of agonists in the development of tolerance and addiction during long-term opioid treatment.

Keywords Opioids · Analgesia · Tolerance · Sensitization · Endocytosis · Rats

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Introduction

Opioid administration is associated with tolerance and sensitization. Tolerance is defined as a loss of drug effect whereas behavior sensitization is characterized by progressive augmentation of the enduring motor response (Kalivas and Duffy 1987; Robinson and Berridge 1993; Trujillo et al. 2004; Vanderschuren and Kalivas 2000). Behavioral sensitization is often suggested to be a correlate of drug addiction (Vanderschuren et al. 1999; Kornetzky 2004; Spanagel 1995).

The mechanisms of development of tolerance and sensitization are multifaceted and only partially understood. Important mechanisms involved in opioid tolerance are cellular and molecular adaptation processes like receptor uncoupling from G-protein (desensitization) or receptor internalization leading to a decrease in the binding sites (Hsu and Wong 2000; Freye and Latasch 2003). Another relevant tolerance mechanism is the phospholipase C (PLC)-mediated activation of *N*-methyl-D-aspartate (NMDA) receptor and of nitric oxide synthetase (NOS) entailing antiopioid effects (Hsu and Wong 2000; Freye and Latasch 2003). It has been further demonstrated that NMDA receptor antagonists or NO synthetase inhibitors enhance opioid analgesia and decrease the development of tolerance (Zang and Liu 1999; Jaba et al. 2001). This indicates the close functional connection of opioid receptors, NMDA receptors, and the NO system.

Another important aspect of opioid tolerance is the role of the relative efficacy of μ-agonists, which is measured by the magnitude of G-protein activation (Traynor and Nahorski 1995; Emmerson et al. 1996; Selley et al. 1997). Several studies demonstrated that low-efficacy μ-agonists produce greater tolerance than agonists with higher efficacy (Stevens and Yaksh 1989; Paronis and Holtzman 1992; Duttaroy and Yoburn 1995; Walker et al. 1997; Walker and Young 2001). Moreover, we recently demonstrated that the potency of μ-opioid receptor agonists to induce receptor endocytosis is negatively correlated with the development of tolerance in a cellular model (Koch et al. 2005). In this and previous tolerance

studies, μ-opioid receptor endocytosis has been shown to be an important mechanism for resensitization and recycling of desensitized receptors counteracting the development of opioid tolerance (Koch et al. 1998, 2001, 2004; Law et al. 2000; Schulz et al. 2004). To further elucidate the role of agonist-induced endocytosis and agonist efficacy in the regulation of opioid tolerance, the present study examined in vivo the development of tolerance to the antinociceptive effects and sensitization to the stimulant effects in the same animals during chronic treatment with morphine, buprenorphine, and etonitazene, the drugs which strongly differ in their relative efficacy and endocytotic potency. Morphine is the classic μ-agonist. Buprenorphine is a partial μ-receptor agonist with lower intrinsic activity and with κ-receptor activity (Cowan et al. 1977; Cowan 2003; Walsh and Eissenberg 2003; Pick et al. 1997). Both agonists morphine and buprenorphine fail to induce μ-opioid receptor endocytosis, whereas the higher efficacy agonist etonitazene promotes receptor endocytosis (Koch et al. 2005; Keith et al. 1996). We found that the agonists with low endocytotic potency (morphine and buprenorphine) produced greater tolerance and sensitization than the high endocytotic potency agonist (etonitazene).

Materials and methods

The experiments were performed according to the requirements of National Act on the Use of Experimental Animals (Germany) and E.U. guidelines.

Animals

For all experiments, male Wistar rats, 8-week-old at the beginning of the experiments, were used. The animals were kept in a humidity- (55–65%) and temperature- (20–22 °C) controlled room maintained on a 12-h light–dark cycle. Commercial rat pellets (Altromin 1326) and water were available ad libitum. The rats were housed in groups of five in Macrolon IV cages.

Apparatus

Hot plate

Analgesia was measured in the hot plate (diameter=19 cm, Ugo Basile, Comerio, Italy). The temperature was maintained at 54 °C. Rats were placed on the plate and the latency to pain reaction (licking or lifting of the hind paws) was measured. The animals were taken off immediately after first signs of reaction or, after 30 s, if the animal did not show a reaction.

Electrical stimulation

A 0.1-mm-thick stainless steel was subcutaneously drawn through the root of the tail under pentobarbital anesthesia (40.0 mg/kg i.p.) 3 days before the start of the experiments. The second electrode was the plate of the restraining tube (diameter=6 cm, length=20/25 cm). The plate of the tube and the subcutaneously implanted electrode were connected with a stimulating current apparatus (TUR RS 12, Dresden, Germany). The current intensity (rectangular pulses, 50 Hz, 50 ms impulse width) was continuously increased until the animal vocalized, the maximum stimulation amperage being 600 mA (impulse peak). After the animals' vocalization or after having reached the impulse peak, the current was immediately switched off.

Locomotor activity box

Locomotor activity was measured using a fully computerized activity meter (47×47×50 cm) (Moti Test, TSE, Bad Homburg, Germany). The illumination level was 30 lux. Horizontal and vertical activities were monitored by infrared cells. Different parameters of activity, such as time of activity (s), traveled distance (m), and number of rearings (*n*) were analyzed.

Drugs

The following opioid receptor agonists were used:

- morphine hydrochloride (Synopharm, Barsbüttel, Deutschland),
- buprenorphine hydrochloride [Reckitt Benchiser Healthcare (UK) Limited, Hull, GB], and
- etonitazene hydrochloride (Novartis Pharma, Basel, Schweiz).

The substances were dissolved in physiological saline and injected subcutaneously in a volume of 1.0 ml/kg body weight.

Dose–response curves

Dose–response curves were determined for the antinociceptive effect of morphine (2.5, 5.0, and 10.0 mg/kg), buprenorphine (0.0325, 0.065, 0.125, 0.25, and 0.5 mg/kg), and etonitazene (2.5, 5.0, and 10.0 μg/kg) using the electrical tail root stimulation. Control animals received saline injections. At the beginning of the session, the basal values were estimated before injection of the substances followed by tests at 30 and 90 min later. From the dose–response curves, the doses of morphine, buprenorphine, and etonitazene for repeated administration were calculated. The intention was to find equieffective doses for the investigation of opioid tolerance and sensitization.

Tolerance development

Tolerance was induced by repeated application of approximately equieffective doses of morphine (10.0 mg/kg), buprenorphine (0.25 mg/kg), and etonitazene (0.010 mg/kg). All tolerance experiments followed the same treatment protocol.

Groups of 15 animals were treated with morphine, buprenorphine, and etonitazene or injected with saline. Animals received daily subcutaneous injections of the substances for 29 days. The animals were tested at weekly intervals (first, eighth, 15th, 22nd, and 29th day of treatment—test day 1 to 5, respectively) according the following schedule:

Time	Enforcement
X−30 min	Measurement of basal intensities for electric tail root stimulation (mA)
X	Injection
X+5 min	Placing in the MOTI test, registration of activity for 15 min
X+25 min	Hot plate test
X+30 min	Electric tail root stimulation

The experimental sessions started with estimation of the basal values for the electrical stimulation before injection. After injection, the locomotor activity of the animals was registered for 15 min. Thereafter, the pain thresholds at the hot plate and for the electrical stimulation were measured. On the other injection days, the animals were returned in their home cage after injection. After 36 days, that means after 1 week without drug applications, the tests were repeated after injection of the test substances once more (test day 6).

Data analysis

The results were statistically analyzed using SPSS with ANOVA (dose–response curves, comparison of experimental day 5 and 6) and repeated measures (development of tolerance and sensitization) and post hoc Bonferroni test.

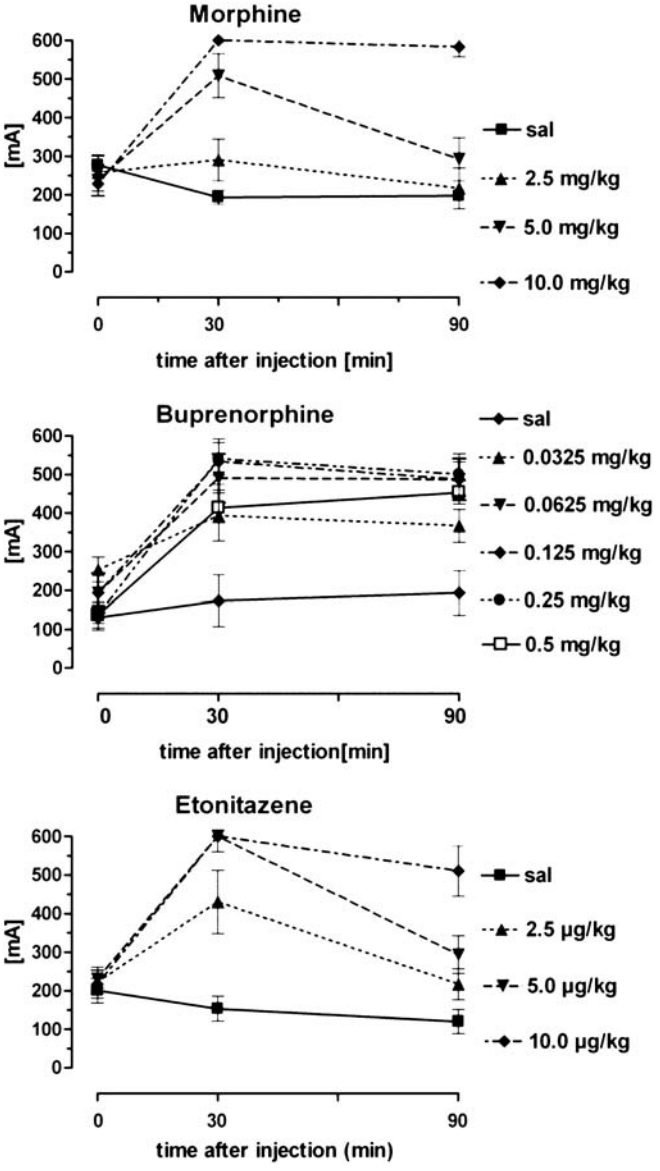

Fig. 1 Dose-response curves electric tail root stimulation. Tests of analgesia before, 30 and 90 minutes after injections. *Ordinate* pain threshold [amperage (mA) mean±SEM]. *Abscissa* time after injection of different doses of the opioids: morphine 2.5 mg/kg (n=6), 5.0 mg/kg (n=8), 10.0 mg/kg (n=8); buprenorphine 0.0325 mg/kg (n=7), 0.0625 mg/kg (n=12), 0.125 mg/kg (n=13), 0.25 mg/kg (n=11), 0.5 mg/kg (n=11); etonitazene 2.5 µg/kg (n=10), 5.0 µg/kg (n=9), 10.0 µg/kg (n=8)

Results

Dose–response curves

Each agonist produced dose-dependent increases of pain thresholds in the electrical stimulation test (Fig. 1).

The 10.0-mg/kg dose of morphine resulted in a higher increase in the intensity of electric stimulation than the 5.0-mg/kg morphine. The dose of 2.5 mg/kg morphine showed post hoc no significant nociceptive effect (ANOVA: $F_{3,25}$=60.81, p=0.001).

Buprenorphine at doses of 0.0325, 0.0625, 0.125, 0.25, and 0.5 mg/kg produced an increase of reaction time (ANOVA: $F_{5,57}$=10,99, p=0.001). However, the higher doses of buprenorphine did not cause a higher increase of current intensity than intermediate doses, demonstrating buprenorphine's ceiling effect in this test method of analgesia.

Etonitazene, a high efficacy agonist, exerted dose-depending effects (ANOVA: $F_{3,30}$=28.58, p=0.001).

On the basis of dose–response curves, doses of 10 mg/kg morphine, 0.25 mg/kg buprenorphine, and 0.010 mg/kg etonitazene were chosen for daily injections to investigate

the development of tolerance. The doses of morphine and etonitazene were maximal in about 80% of the animals. The time curves of the antinociceptive effect were comparable for these opioids. Whereas the experimental doses of 10 mg/kg morphine and 0.010 mg/kg etonitazene showed nearly the same antinociceptive effects 30 and 90 min after injection, the antinociceptive effects of the half doses (5 mg/kg morphine and 0.005 mg/kg etonitazene) diminished in a similar way from 30 to 90 min. In the case of buprenorphine, the dose inducing the highest effect was selected. This dose, but also smaller doses, exerted a nearly constant effect from 30 to 90 min, representing the known long-lasting effectiveness of buprenorphine.

Repeated administrations

Analgesia

Control animals chronically treated with saline showed only minor changes in pain reaction. Comparing the four treatment groups (Fig. 2), a significantly different development of pain reactions with both methods used for measuring analgesia was found (repeated measures with treatment and time, hot plate: $F_{3,51}$=40.796, p=0.001; post hoc Bonferroni: saline vs morphine p=0.001; saline vs buprenorphine p=0.65; saline vs etonitazene p=0.001; morphine vs buprenorphine p=0.013; morphine vs etonitazene p=0.001; buprenorphine vs etonitazene p=0.001; repeated measures, electrical stimulation: $F_{3,51}$=4.01, p=0.013; post hoc Bonferroni: saline vs morphine, buprenorphine, and etonitazene p=0.001, morphine vs buprenorphine p=1.0, morphine vs etonitazene p=0.008, buprenorphine vs etonitazene p=0.001).

Daily treatment with 10.0 mg/kg morphine for 1 week significantly decreased the latency of pain reaction in the hot plate test. Furthermore, after morphine treatment for 2 weeks, the antinociceptive effect of this dose of morphine completely disappeared. Chronic treatment with 0.25 mg/kg buprenorphine per day also induced a decrease of the analgesic effect on hot plate. However, due to a lower intrinsic activity of buprenorphine in this test, no analgesic effect could be detected already after 1-week drug administration (on test day 2). On the other hand, in the electric tail stimulation test, the analgesic effects of both buprenorphine and morphine were comparably diminished during long-term treatment.

As compared to morphine and buprenorphine, daily treatment with 10 μg/kg etonitazene for 4 weeks decreased reaction times on the hot plate and in the electric tail stimulation test to a significantly lesser degree.

One week after termination of drug administration, the antinociceptive effects of the opioids were slightly increased (Fig. 3). Only the effect of buprenorphine was significantly enhanced in the electrical stimulation test, demonstrating a decline of tolerance (T-test-paired, electrical stimulation: T_{13}=−5,472, p=0.001; hot plate: T_{13}=−1,836, p=0.089).

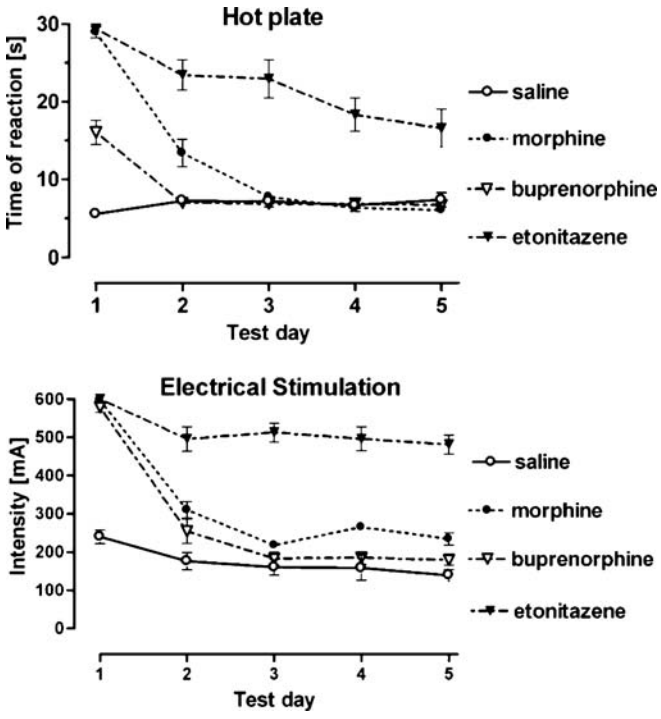

Fig. 2 Development of nociceptive efficacy of morphine 10 mg/kg (n=13), buprenorphine 2.5 mg/kg (n=15), and etonitazene 0.010 mg/kg (n=12) compared to saline control group (n=14) during repeated administration over 29 days; five tests of analgesia in weekly intervals. On the *top*: hot plate [reaction time (s), mean±SEM 25 min after injection]; on the *bottom*: electric tail root stimulation [amperage (mA), mean±SEM 30 min after injection]

A comparison of the basic pain thresholds in the electric stimulation test before drug administration showed a slight decline over time, including the control group. However, this decrease of the basal values after 23 h of abstinence was bigger in the drug-treated groups compared to the control group, which was significant in the morphine and buprenorphine but not in the etonitazene-treated animals (repeated measures: treatment vs time: F_3=3.367, p=0.026; post hoc Bonferroni: saline vs morphine: p=0.016, saline vs buprenorphine: p=0.001, saline vs etonitazene: p=0.071) (Fig. 4).

Locomotor activity

The activity of the animals registered in the activity meter was drastically changed after repeated administration and differed significantly between the treatment groups (Fig. 5). The first injection of the opioids exerted depressive effects on all parameters of activity in the investigated time interval from 5 to 20 min after treatment compared to saline injection. However, all parameters of activity, like total times of activity, traveled distance, and number of rearings, continuously increased until a maximum in morphine- and buprenorphine-treated rats. In contrast, the locomotor activity of etonitazene-treated animals increase only scarcely and never reached the level of control animals (repeated measures: time of activity: treatment and

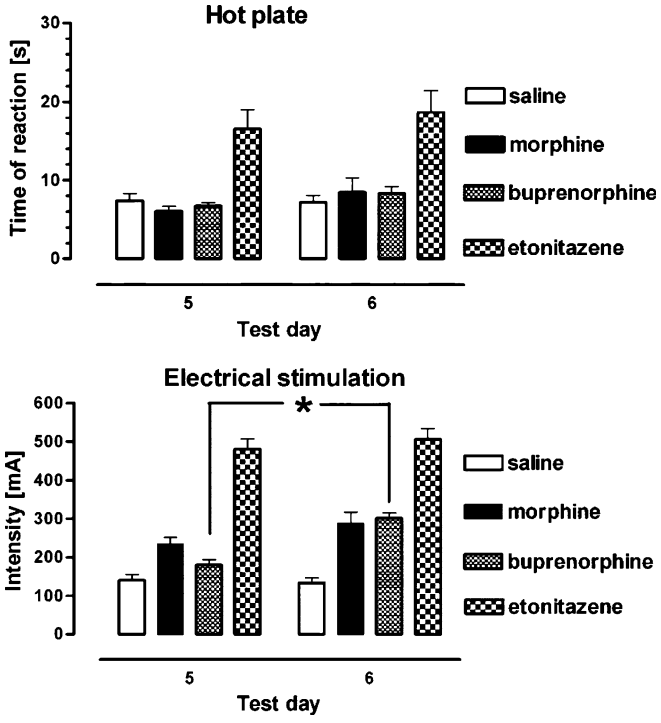

Fig. 3 Comparison of the nociceptive effects of 10 mg/kg morphine, 2.5 mg/kg buprenorphine, and 0.01 mg/kg etonitazene on the last day of repeated administration and after 1 week of abstinence (test days 5 and 6). On the *top*: hot plate [reaction time (s), mean±SEM 25 min after injection]; on the *bottom*: electrical simulation [amperage (mA), mean±SEM 30 min after injection]

time $F_{3,50}$=25.686, p=0.001; treatment $F_{3,50}$=62.22, p=0.001; post hoc Bonferroni saline vs morphine, buprenorphine, and etonitazene p=0.001, morphine vs buprenorphine p=0.063, morphine vs etonitazene p=0.001, buprenorphine vs etonitazene p=0.001; traveled distance: treatment and time $F_{3,50}$=40.69, p=0.001, treatment $F_{3,50}$=61.62, post hoc Bonferroni saline vs morphine and buprenorphine p=0.001, saline vs etonitazene p=0,189, morphine vs buprenorphine p=0.011, morphine vs etonitazene p=0.001; number of rearings: treatment and time $F_{3,50}$=17.65, p=0.001, treatment $F_{3,50}$=21.48, p=0.001, post hoc Bonferroni saline vs morphine p=0.025, saline vs buprenorphine p=0.003, saline vs etonitazene p=0.04, morphine vs buprenorphine p=1.0, morphine vs etonitazene p=0.001, buprenorphine vs etonitazene p=0.001). The locomotor activity of saline-treated rats was nearly unchanged during the time of treatment, indicating lacking effects of habituation under control conditions.

After 1 week of withdrawal, the activity of the treatment groups became different. Whereas the time of activity and traveled distance were not significantly changed in the control and morphine and etonitazene groups, we found a nearly complete normalization in the buprenorphine group (Fig. 6).

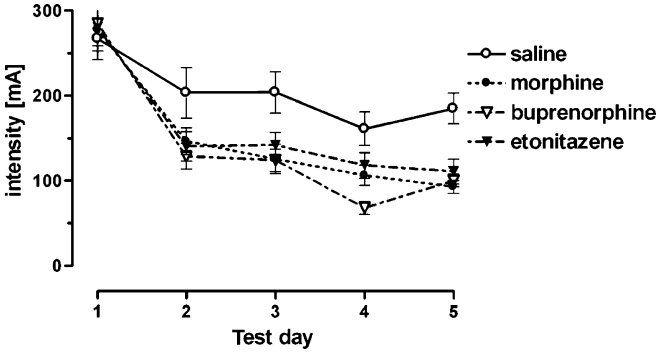

Fig. 4 Basic pain thresholds for electric tail stimulation [amperage (mA)] before injection of the substances during the schedule of repeated administration of 10 mg/kg morphine (n=13), 2.5 mg/kg buprenorphine (n=15), 0.01 mg/kg etonitazene (n=12), and saline (n=14) for 29 days experiments, estimations in weekly intervals (*left*), and after one-weekly withdrawal (*right*); noninjection measurements. *Ordinate*: amperage (mA) mean±SEM

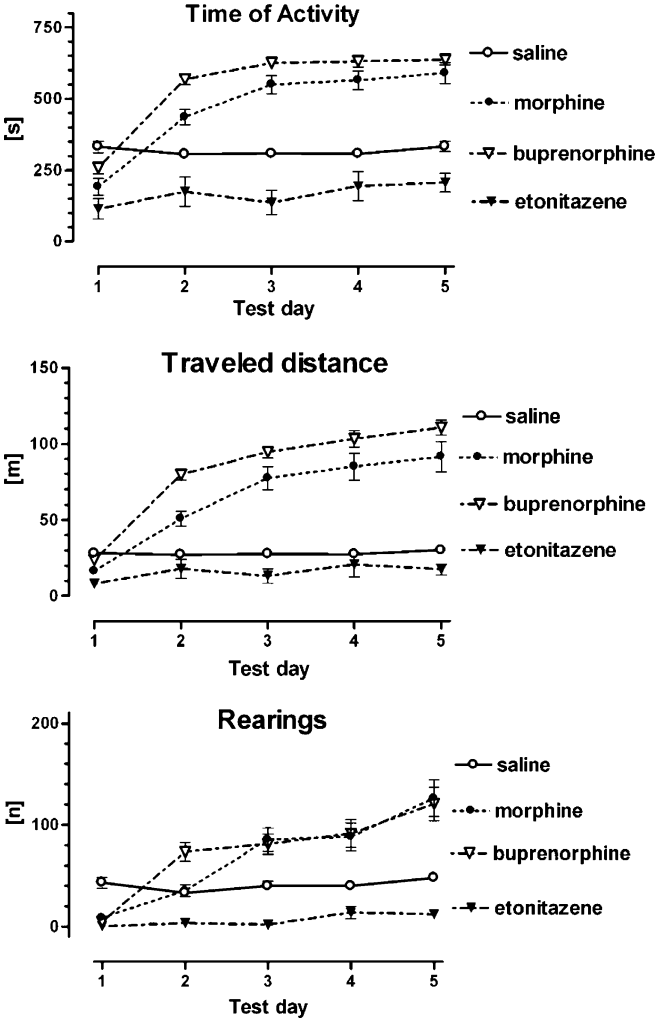

Fig. 5 Development of locomotor activity during repeated application of morphine (10 mg/kg, n=13), buprenorphine (2.5 mg/kg, n=15), etonitazene (0.010 mg/kg, n=12), or saline (n=14) over 29 days; experiments in weekly intervals. On the *top*: time of activity (s)/15 min; in the *middle*: horizontal activity as traveled distance (m)/15 min, mean±SEM; on the *bottom*: vertical activity as rearings (n)/15 min, mean±SEM

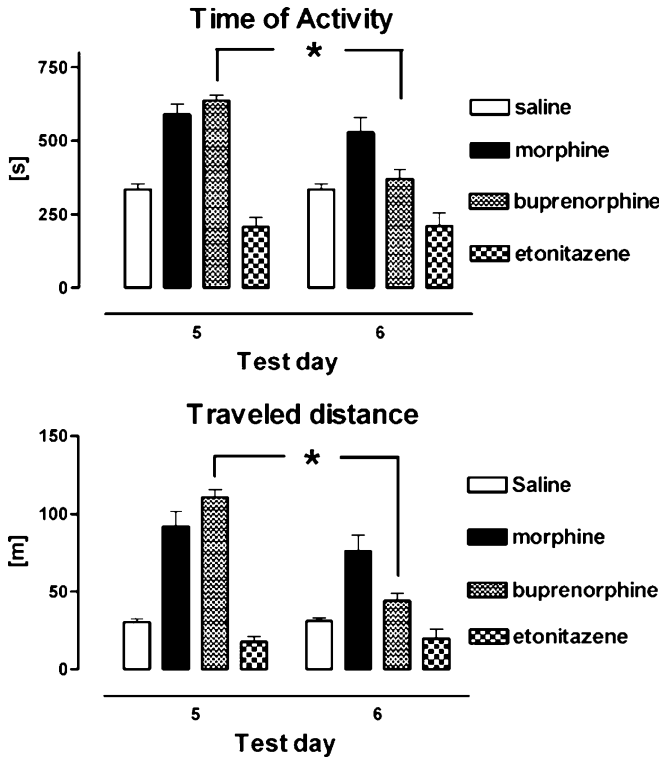

Fig. 6 Comparison of the locomotor activity of the different treated groups on the last day of repeated administration and after 1 week of abstinence (test day 5 and 6). On the *top*: time of activity (s)/15 min, mean±SEM; On the *bottom*: traveled distance (m)/15 min, mean±SEM

Discussion

In the present study, great differences were observed in the development of tolerance to the antinociceptive effects and in the sensitization to the locomotor activity after chronic treatment with etonitazene, morphine, and buprenorphine.

The antinociceptive effects disappeared completely during chronic treatment with 2.5 mg/kg buprenorphine and 10.0 mg/kg morphine in two different methods for testing antinociceptive effects. On the other hand, the higher efficacy agonist etonitazene produced less tolerance than the lower efficacy agonists morphine and buprenorphine.

A similar differential tolerance to the antinociceptive effects of μ-opioids was recently reported by Walker and Young (2001), who found that etonitazene treatment produced less tolerance than does treatment with morphine and buprenorphine.

It is well established that the μ-opioid receptor agonist etonitazene has a very high receptor affinity and intrinsic activity (Moolten et al. 1993; Emmerson et al. 1996; Walker et al. 1998). Thus, it is reasonable to speculate that these pharmacological characteristics of etonitazene might be primarily responsible for the observed negligible development of tolerance compared to morphine and buprenorphine (Walker et al. 1993).

In terms of this agonist efficacy hypothesis, the partial μ-opioid agonist buprenorphine, which has a lower efficacy and intrinsic activity than full μ-agonists (Walsh and Eissenberg 2003), should lead to a clear lower tolerance than the full agonist morphine. However, in the present study, we did not observe a significant difference in the opioid tolerance development between both agonists. This indicates that the efficacy of opioids cannot be the only critical parameter influencing the development of opioid tolerance. Therefore, other explanations for the observed effects have to be taken into consideration. Presumably, the receptor specificity of the agonists might affect the tolerance development. Etonitazene has a high μ-opioid receptor specificity (Moolten et al. 1993), whereas morphine interacts to a certain extent also with κ- and δ-receptors which are all contributing to analgesia, respiratory depression, and miosis. Moreover, cellular adaptation mechanisms appear to be responsible for the development of tolerance to opioids. For instance, in vitro experiments on cells have demonstrated that agonist-induced internalization of the μ-opioid receptor has a protective role against the development of opioid tolerance. However, contradictory theories exist about the mechanism how receptor endocytosis reduces the development of opioid tolerance. The RAVE theory of Whistler's group suggests that agonist-induced endocytosis results in a fast uncoupling of the receptors from G-proteins reducing counterregulatory mechanisms (e.g., cAMP upregulation) that are responsible for the development of tolerance (Whistler et al. 1999; He et al. 2002). However, we and others have demonstrated in cell cultures that agonist-induced receptor

Table 1 Potency for opioid agonist stimulation of adenylate cyclase (AC) inhibition, induction of internalization, and receptor desensitization in MOR1-expressing HEK293 cells (from Koch et al. 2005)

Agonist	Endo-cytosis (%)	AC inhibition (%)	RAVE	EC_{50}			Desensitization (%)
				AC inhibition (nM)	Endocytosis nM	Ratio	
Morphine	5±2	64±5	9.8	25±4	>1 mM	>40,000	65±4
Buprenorphine	7±2	31±7	3.36	36±3	>1 mM	>27,777	69±5
Etonitazene	47±4	72±3	1.18	1.9±0.6	0.7±0.35 nM	0.37	10±4

For determination of receptor endocytosis, signaling, and desensitization, HEK293 cells expressing MOR1 were exposed for 1 h with receptor saturating opioid doses (Koch et al. 2005). RAVE was defined as relative activity vs endocytosis. Similar to Whistler et al. (1999), the peptide DAMGO was defined as having activity and endocytosis of 1 and thus a RAVE of 1. Based on the DAMGO values, morphine has a relative activity of 0.98 and a relative endocytosis of 0.1, resulting in a RAVE of 9.8. For EC_{50} calculation, μ-opioid receptor-expressing HEK293 cells were incubated with increasing amounts of agonists (10^{-13}–10^{-4} M), and adenylate cyclase inhibition and internalization data were analyzed (sigmoidal dose–response curve fit analysis; Graph Pad Software, San Diego, CA, USA)

internalization results in a fast receptor reactivation and recycling counteracting the development of receptor desensitization and opioid tolerance thus enhancing compensatory cAMP upregulation (Koch et al. 1998, 2001, 2004, 2005; Law et al. 2000; Borgland 2001; Cox 2005) (Table 1). Thus, opioids that do not cause receptor internalization, like morphine and buprenorphine, induce robust tolerance development (Koch et al. 2001, 2004, 2005; Borgland et al. 2003). These in vitro findings are consistent with the present in vivo data showing that etonitazene induced less opioid tolerance than morphine and buprenorphine during long-term treatment in rats. Therefore, our results support the hypothesis that differences in the endocytotic potency of agonists as demonstrated in a cellular model might also be relevant for modulating opioid tolerance in vivo. Although our in vitro and in vivo tolerance data are in good agreement, further in vivo investigations demonstrating differences in the agonist-induced internalization of μ-receptors are necessary to establish this hypothesis. In the electric tail root stimulation test, basal pain threshold values measured before drug injection were decreased during chronic treatment, showing a hyperalgesia for all treatment groups. This decrease of pain threshold in the saline-treated animals is most likely the consequence of a learning effect after repeated measurements. However, a more pronounced development of hyperalgesia was observed after treatment with the three opioids. Buprenorphine induced the greatest hyperalgesia, followed by morphine. This hyperalgesia during chronic opioid administration measured 23 h after the last opioid injection might be interpreted as a sign of abstinence.

In the present experiments, buprenorphine and morphine induced a comparable tolerance. It is interesting to note that 1 week after abstinence, only the buprenorphine-treated rats showed a significant decline of tolerance. The mechanistic basis for the observed differences in the recovery from opioid tolerance between buprenorphine, morphine, and etonitazene is presently unknown. Probably, a κ-antagonistic effect of buprenorphine (Pick et al. 1997) is involved in this effect.

The development of tolerance to the nociceptive effects was compared with the sensitization to the locomotor stimulation by the same opioids in the same animals. The locomotor activity measured in the activity box 5 to 20 min after opioid application represented tolerance development to the depressive effect together with sensitization of locomotor stimulation. The depressive effects of morphine and buprenorphine quickly disappeared during the repeated treatment, whereas etonitazene-treated rats showed a depressed activity all the time. The rats treated with morphine and buprenorphine showed a marked sensitization already in the second activity test (eighth injection). It is interesting to note that an escalation of locomotor activity during chronic opioid treatment could only be observed in our study, when also a development of tolerance was evident. Etonitazene, the opioid with the least development of tolerance to the analgesic effect, did not show sensitization to the locomotor effect. However, it cannot be excluded that the lacking tolerance to the depressive effect in etonitazene-treated animals masked signs of sensitization. Furthermore, in the present experimental protocol, it was not possible to investigate the locomotor activity at later intervals after drug administration. However, different time curves at later periods cannot be ruled out.

The phenomenon of behavioral sensitization is thought to be a correlate of certain aspects of drug addiction (Segal and Schuckit 1983; Robinson and Berridge 1993; Trujillo et al. 2004; Vanderschuren and Kalivas 2000). A relationship between drug-seeking behavior and drug sensitization was postulated from animal studies (Vanderschuren et al. 1999; Kornetzky 2004), and behavioral sensitization may have important implications for the understanding of addictive processes (Spanagel 1995). The high degree of locomotor sensitization seen after chronic buprenorphine contrasts with previous findings suggesting a lower dependence potential for buprenorphine compared with other potent μ-opioid receptor agonists (Mello et al. 1993; Tzschentke 2002). On the other hand, the fast reversal of locomotor sensitization after 1 week withdrawal which was not seen for morphine indicates that buprenorphine might have lower dependence liability than morphine.

The present study does not confirm the hypothesis that the magnitude of tolerance is exclusively related to intrinsic efficacy. A strong development of tolerance was found not only with buprenorphine, a low-efficacy opioid, but also with morphine, a higher efficacy opioid. The highest efficacy opioid etonitazene induced the lowest tolerance in our treatment schedule with only one injection per day. Differences in the duration of receptor occupancy seem not likely because etonitazene and morphine showed similar time courses of drug action. However, the results support an important role of the endocytotic potency of the agonists not only for the development of tolerance to the nociceptive effects but also for the sensitization to locomotor stimulation.

Regarding the slight or lacking development of tolerance and sensitization to etonitazene, some advantages can be assumed for such a substance in clinical use, to diminish the risks of tolerance and addiction. However, investigations using more opioid agonists with different abilities to induce endocytosis are necessary to support this hypothesis.

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