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Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain

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Abstract

Buprenorphine is a potent opioid analgesic clinically used to treat moderate to severe pain. The present study assessed its analgesic efficacy in a broad range of rodent models of acute and chronic pain. In the phenylquinone writhing, hot plate, and tail flick mouse models of acute pain, full analgesic efficacy was obtained (ED_{50} values: 0.0084–0.16 mg/kg i.v.). Full analgesic efficacy was also obtained in yeast- and formalin-induced inflammatory pain (ED_{50} values: 0.0024–0.025 mg/kg i.v., rats and mice) and in mustard-oil-induced spontaneous pain, referred allodynia, and referred hyperalgesia in mice (ED_{50} values: 0.018–0.025 mg/kg i.v.). Buprenorphine strongly inhibited mechanical and cold allodynia in mononeuropathic rats, as well as mechanical hyperalgesia and cold allodynia in polyneuropathic rats (ED_{50} values: 0.055 and 0.036 mg/kg i.v. and 0.129 and 0.038 mg/kg i.p., respectively). It is concluded that buprenorphine shows a broad analgesic profile and offers the opportunity to treat different pain conditions, including neuropathic pain.

Keywords: Analgesic; Neuropathic pain; Inflammatory pain; Nociception; (Rat, Mouse)

1. Introduction

Buprenorphine is a clinically well-established opioid analgesic which shows complex interactions at the various opioid receptor subtypes. It shows high affinity to μ-, δ-, κ- and ORL1-opioid receptors and slow receptor dissociation (Sadee et al., 1982). In addition, its strong potency and high lipophilicity makes buprenorphine suitable for incorporation in a transdermal formulation, which is used efficiently for the treatment of moderate to severe pain (Evans and Easthope, 2003). In vitro data generated in [^{35}S]-GTPγS and adenylate cyclase assays (Zaki et al., 2000; Huang et al., 2001), as well as in organ bath preparations (Kajiwara et al., 1986; Lattanzi et al., 2001), characterise buprenorphine as a partial agonist at μ-opioid and ORL1 receptors and as an antagonist at κ-opioid and δ-opioid receptors. Although the extent of analgesic efficacy of partial opioid receptor agonists is discussed controversially (Wheeler-Aceto and Cowan, 1991), clinical experience indicates that the compound is a potent and efficient analgesic with a favourable side effect profile (Heel et al., 1979; Walsh et al., 1994; Evans and Easthope, 2003).

Over the years, a large body of data on the analgesic effect of buprenorphine in animals has been published. However, most of these studies were performed in animal models of acute pain (Cowan, 1995, 2003), and it remains to be clarified to what extent the compound is effective in chronic pain models. Thus, with respect to inflammatory, visceral, and neuropathic pain, a broad and thorough preclinical assessment of the analgesic efficacy of buprenorphine appears to be lacking. In addition, variations in test protocols and routes of administration often make a direct comparison in terms of potency and efficacy difficult, and the maximal possible efficacy has not always been assessed. For example, the efficacy of buprenorphine against neuro-

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pathic pain has been tested in photochemically induced central and peripheral mononeuropathic pain without addressing maximal efficacy and effects of supramaximal doses (Kouya et al., 2002).

Preclinical studies have shown that doses of buprenorphine exceeding the maximal effective dose often lead to a decrease in analgesic efficacy (Wheeler-Aceto and Cowan, 1991), as well as in side effects (Cowan, 1992). It has been suggested that the occurrence of an inverted u-shaped (or bell-shaped) dose–response curve, as demonstrated in a mouse model of acute pain, may depend on the intensity of the stimulus used to induce pain (Lutfy et al., 2003), but the generality of this suggestion is still controversial, and the underlying mechanism of this phenomenon remains to be clarified. As it was found that combination with μ-opioid antagonists leads to a rightward shift of the inverted u-shaped curve in models of acute pain (Dum and Herz, 1981), it is possible that this peculiarity of the dose–response curve relates to the μ-opioid mechanism of the compound. Alternatively, noncompetitive autoinhibition, a model based on two receptor populations, one mediating the agonistic properties at low doses and another one mediating the antagonistic properties at high doses, was proposed as a possible molecular mechanism (Cowan et al., 1977; Sadee et al., 1982; Richards and Sadee, 1985). Beside the analgesic effect resulting from activation of μ-opioid receptors, a contribution of ORL-1 receptors has also been suggested based on results obtained with buprenorphine in ORL-1 knock-out mice (Lutfy et al., 2003). However, it should be realised that the inverted u-shaped dose–response curve has been observed only in animal models. Moreover, the apparent loss of efficacy only occurs at high doses of buprenorphine. Therefore, it can be argued that the inverted u-shaped curve observed preclinically is only of limited relevance for the clinical use of buprenorphine as an analgesic.

This study aimed at the assessment of buprenorphine's analgesic efficacy in a broad range of rodent models of acute and chronic pain, including somatic, visceral, inflammatory, and neuropathic pain. Since the experimental outcome in animal models of pain may depend on the test parameters, a broad range of stimulus qualities, such as chemical, thermal, and mechanical stimulation, as well as different stimulus intensities were selected. In some models, the effect of buprenorphine was compared with clinically relevant reference compounds. A preliminary account of the present study was reported previously (Christoph et al., 2003).

2. Material and methods

2.1. Animals

Male NMRI mice (20–35 g) and Sprague–Dawley rats (133–178 g), supplied by commercial breeders (Charles River, Sulzfeld, Germany, Iffa Credo, Brussels, Belgium, Janvier, Genest St. Isle, France), were housed under a 12:12 h light–dark cycle (lights on at 06:00 a.m.) and with room temperature 20–24 °C, relative air humidity 35–70%, 15 air changes per hour, and air movement <0.2 m/s. The animals had free access to standard laboratory food (Ssniff R/M-Haltung, Ssniff Spezialdiäten, Soest, Germany) and tap water. All animals were used only once in all pain models, except for the neuropathic pain models, in which they were tested repeatedly with a wash-out period of at least 1 week between tests. There were at least 5 days between delivery of the animals and the test day or the operation.

Animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain (Zimmermann, 1983) and the German Animal Welfare Law. All study protocols were approved by the local government committee for animal research, which is also an ethics committee.

2.2. Experimental procedures

Animals were assigned randomly to treatment groups. Different doses and vehicle were tested in a randomised fashion. Although the operators performing behavioural tests were not formally 'blinded' with respect to the treatment, they were not aware of the study hypothesis or the nature of differences between drugs.

2.2.1. Writhing test

Writhing was induced by i.p. injection of 0.35 ml of a 0.02% solution of phenylquinone according to the method described by Hendershot and Forsaith (1959). The characteristic writhing response, such as stretching, twisting a hind leg inward, or contraction of abdomen, was observed and counted from 5 to 20 min after phenylquinone administration. During this time, the animals were placed individually in observation boxes. Recording of the writhing reactions (with the help of a press-button counter) started 10 min after i.v. administration.

2.2.2. Hot plate test

The hot plate test was adapted from Eddy and Leimbach (1953). The device consisted of an electrically heated surface and an open Plexiglas tube (17 cm high×22 cm diameter) to confine the animals to the heated surface. The temperature was kept at either 48.0±0.5 or 58.0±0.5 °C (48 and 58 °C hot plate test, respectively) to analyse supraspinally and spinally evoked nocifensive reactions. Mice were placed on the hot plate, and the time until either licking of the hind paw or jumping occurred was recorded by a stopwatch. Habituation was used to minimize learning effects. At the 48 °C hot plate, animals were tested two times, at the 58 °C hot plate, animals were tested once before baselines were taken. The animals were tested prior to drug administration and 30 and 60 min after i.v. administration. The predrug latencies in the 48 °C hot plate were between 17

and 45 s and in the 58 °C hot plate test were between 2 and 6 s. The maximum possible antinociceptive effect (MPE) was defined as the lack of a nociceptive response during the exposure to the heat stimulus, and the percentage of MPE was calculated according to the formula: $[(T1-T0)/(T2-T0)]\times 100$, where $T0$ and $T1$ were the latencies obtained before and after drug injection, and $T2$ was the cut-off time (120 s 48 °C, 15 s 58 °C).

2.2.3. Tail flick test

The tail flick test was carried out in mice using a modification of the method described by D'Amour and Smith (1941). The tail flick latency defined by the time (in seconds) to withdraw the tail from a radiant heat source (bulb, 8 V/50 W) was measured using a semiautomated device (tail flick analgesiemeter Typ 50/08/1.bc, Labtec, Dr. Hess, Germany). The heat source was adjusted to produce a baseline tail flick latency of 3–5 s prior to any of the experiments and was left at a constant setting thereafter. A cut-off time of 12 s was set to avoid tissue damage of the tail. The increase in tail flick latency was defined as antinociception and calculated as % MPE. The maximum possible antinociceptive effect was reached when the animals did not show a tail flick reaction within the cut-off time of 12 s. % MPE was calculated according to the formula: $[(T1-T0)/(T2-T0)]\times 100$, where $T0$ and $T1$ were the latencies obtained before and after drug application, and $T2$ was the cut-off time. Animals were tested before and 20, 40, and 60 min after i.v. administration of the test compound. A single habituation test was used prior to baseline test to minimize learning effects.

2.2.4. Randall Selitto test

The yeast model of inflammatory pain was performed according to Randall and Selitto (1957). For induction of inflammation, 0.1 ml of 20% yeast was injected s.c. into the plantar surface of the right hind paw of the rat. The mechanical nociceptive threshold was measured using an algesiometer (Ugo Basile, Italy). The device generated a mechanical force with a linear increase over time. The force was applied to the dorsal surface of the inflamed rat hind paw via a cone-shaped stylus with a rounded tip (2 mm^2). The nociceptive threshold was defined as the force (in g) at which the rat vocalised (cut-off force 450 g). Compounds or vehicle was given 4 h after yeast injection. The mechanical nociceptive threshold was measured before and at 15, 30, 45, and 60 min after drug or vehicle administration. The drug effects were expressed as % MPE calculated as follows: [(nociceptive threshold drug−nociceptive threshold control)/(cut-off−nociceptive threshold control)×100].

2.2.5. Formalin test

The formalin test was used as a model of chemically induced persistent pain. The test was carried out in an open glass cylinder (16 cm in diameter) with a mirror placed behind to allow an unobstructed view of the animals. Each animal was pretreated with 20 μl of 1% formalin in 0.9% NaCl (mouse) or 50 μl of 5% formalin in 0.9% NaCl (rat) injected s.c. into the dorsal surface of the right hind paw. After placing the animal back into the chamber, the behaviour was observed for 30 min starting directly after formalin injection and the amount of time spent licking and biting the injected paw was counted (score 3) (Dubuisson and Dennis, 1977). The first 15 min after formalin injection were considered as phase I; whereas reactions between 15 and 30 min after formalin administration were considered as phase II. In the rat formalin test, the observation period started 21 min after the injection (21st to 27th min). Characteristic pain behaviour during this 6-min period was scored (0—normal behaviour, 1—paw lifting, 2—flinching, 3—licking and biting of the injected paw). Buprenorphine or vehicle was administered i.v. 5 min before the formalin injection.

2.2.6. Mustard-oil-induced visceral pain

Acute colitis was induced by mustard oil (Laird et al., 2001), and animals were monitored in Plexiglas boxes (14.5×14.5 cm, height: 10 cm) on a grid. Rectal administration of mustard oil (50 μl, 3.5%) in mice induced spontaneous visceral pain behaviour (scored as follows: 1—licking of abdominal wall, 2—stretching, squashing, mounting, backward-movement, or contraction of the flank muscles; number of reactions 2–12 min after mustard oil) and referred hypersensitivity. Prior to rectal administration (baseline) and 20 min thereafter, the frequency of withdrawal reactions to the application of von Frey filaments to the abdomen was examined. Five filaments with stimulus intensities of 1, 4, 8, 16, and 32 mN were applied 10 times each in ascending order, and the number and intensity of the responses were recorded. The filament was applied for 1–2 s, with an interstimulus interval of 5–10 s. Consecutive stimulations were performed at different locations of the abdomen. Pretest values were subtracted from values taken 20 min after mustard oil for each individual animal. Beside the response frequency against the different von Frey stimuli (calculated for each animal, with 100% being animals responding to each of the 10 stimulations with the respective filament), referred allodynia (counting of withdrawal reactions against 10 stimulations on the abdomen with a 1 mN von Frey filament; 20 min after mustard oil) and referred hyperalgesia (counting and scoring of withdrawal reactions against 10 stimulations on the abdomen with a 16 mN von Frey filament; 20 min after mustard oil) were measured. Scoring of withdrawal reactions for hyperalgesia was as follows: 1—lifting of abdomen, licking, and movement, 2—extrusion or flinching of hind paws, slight jumping, and strong licking, 3—strong jumping and vocalisation. As control animals without colitis were unresponsive towards the von Frey filament of 1 mN, this stimulus intensity was considered to be adequate for testing of allodynia. One group of control animals received rectal

administration of mustard oil vehicle, another group received drug vehicle i.v. before mustard oil. Drug or drug vehicle was given i.v. 5 min before rectal administration.

2.2.7. Chronic constriction injury

The chronic constriction injury model of neuropathic pain was adapted from Bennett and Xie (1988). Under pentobarbital anesthesia (Narcoren®, 60 mg/kg i.p., Merial, Hallbergmoos, Germany), the right common sciatic nerve was exposed by blunt dissection at the level of midthigh, and four loose ligatures (softcat®chrom USP 4/0, metric 2; Braun Melsungen, Germany) were placed around the nerve, taking care not to interrupt the epineural circulation.

After operation, animals were allowed to recover for 1 week. Cold allodynia was stable for several weeks and was tested on a metal plate cooled by a water bath to a constant temperature of 4 °C. Animals were observed for periods of 2 min before and 30, 60, and 90 min after i.v. or 15, 30, 45 and 60 min after p.o. administration of the test compound, and the number of brisk withdrawal reactions was counted. % MPE of each time point was calculated according to the formula: $[(T0-T1)/T0]\times 100$, where $T0$ and $T1$ were numbers of paw withdrawal reactions before and after drug administration, respectively. Testing was done 1 to 3 weeks after operation.

2.2.8. Spinal nerve ligation

The spinal nerve ligation model of neuropathic pain was adapted from Kim and Chung (1992). Under pentobarbital anesthesia (Narcoren®, 60 mg/kg i.p., Merial), the left L5 and L6 spinal nerves were exposed by removing a small piece of the paravertebral muscle and a part of the left spinous process of the L5 lumbar vertebra. The L5 and L6 spinal nerves were then carefully isolated and tightly ligated with silk (NC-silk black, USP 5/0, metric 1, Braun Melsungen). After checking homeostasis, the muscle and the adjacent fascia were closed with sutures, and the skin was closed with metal clips.

After operation, animals were allowed to recover for 1 week. For the assessment of mechanical allodynia, which was stable for at least 5 weeks, the rats were placed on a metal mesh covered with a plastic dome and were allowed to habituate until the exploratory behaviour diminished. Threshold for mechanical allodynia was measured with an electronic von Frey anesthesiometer (Somedic, Malmö, Sweden). Animals were tested before and 0.5, 1, and 3 h after i.v. administration of the test compound. Withdrawal thresholds of the injured paws were assessed and expressed as % MPE comparing predrug threshold of ligated animals (i.e., 0% MPE) and control threshold of sham animals (i.e., 100% MPE). Buprenorphine was tested in weeks 1 to 5 after operation.

2.2.9. Streptozotozin model

The streptozotozin model of neuropathic pain was adapted from Courteix et al. (1993). Rats were injected with streptozotozin (75 mg/kg i.p.; Sigma, Bad Dreieich, Germany) dissolved in citrate solution (pH 4.6). Control animals received citrate solution. Diabetes was confirmed 1 week after injection by measurement of tail vein blood glucose levels with Haemoglukotest 20-800R glucose and a reflectance colorimeter (Boehringer Mannheim, Mannheim, Germany). Animals with a blood glucose level of >17 mM were considered to be diabetic.

Tests took place during weeks 3 and 4 after the induction of diabetes. At that time, the abnormal pain behaviour was at a stable maximum (Courteix et al., 1993). Mechanical hyperalgesia was assessed using an algesiometer (Ugo Basile) by measuring withdrawal thresholds to an increasing pressure on the dorsal surface of the right paw via a cone-shaped pusher with a rounded tip (2 mm^2). The cut-off was set at 250 g, and the behavioural read out was paw withdrawal, vocalisation, or overt struggling. The reaction latencies were measured before and 15, 30, 45, and 60 min after i.p. administration of compound. The value before administration of substance was used as 0% MPE, and 100% MPE represented full antihyperalgesic efficacy (i.e., cut-off).

2.2.10. Vincristine model

The vincristine model of neuropathic pain was adapted from Authier et al. (1999). Rats were injected on five alternate days (days 4, 6, 8, 10, and 12) with saline or vincristine (200 μg/kg i.v.; Sigma), using an injection volume of 1 ml/kg. Thus, the cumulative vincristine dose was 1 mg/kg.

Tests took place 3 days after the last injection and continued over the next 3 weeks. For the assessment of cold allodynia, which was stable for at least 3 weeks, the rats were placed on a metal mesh covered with a plastic dome and were allowed to habituate until the exploratory behaviour diminished. Cold allodynia was measured as the number of foot withdrawal responses after application of stimuli to the plantar surface of the paw. A drop of acetone (10 μl) was gently applied to the heel of the animal with a Hamilton syringe connected to a thin polyethylene tube. A brisk foot withdrawal response (shaking, tapping, or licking) after the spread of acetone over the plantar surface of the paw was considered a sign of cold allodynia. Acetone was applied five times (once every 5 min) on the left paw, and the number of reactions (shaking, tapping, or licking) was counted during 30 s. The score was expressed as the accumulated numbers of reactions over the five trials and determined before and 60 and 180 min after i.p. administration of compound or vehicle. Each individual test was expressed as % MPE compared to the mean of the time-matched weekly vehicle control.

2.2.11. Hole board test

Explorative activity was measured in a plastic box with a size of 45×45 cm and 40-cm-high walls (MotiSystem, TSE, Bad Homburg, Germany). Sixteen holes with a diameter of

2.5 cm were distributed evenly over the floor. The board was elevated so that the mouse, when poking its nose into the hole, could not see the bottom. Each hole was provided with an infrared-emitter and an opposed receiver, both 1.5 cm below the upper panel. A head dip was scored if both eyes disappeared into the hole. The interruptions were measured automatically by a computer connected to the exploration box. Five minutes after administration of the test compound, the mouse was placed individually into the centre of the hole board, and the exploration activity was measured as the number of head dips within 5 min. Mean values (±S.E.M.) were calculated for each and expressed as percent change versus control.

2.2.12. Open field test

Explorative activity of male Sprague–Dawley rats was measured in an open field with a computerized ActiMot system (TSE) placed in a plastic box (45×45 cm, height: 40 cm) with transparent walls. Detection of animal location was performed with infrared sensor pairs arranged in strips. The exploration activity in the open field was determined as distance covered of horizontal movements and explorations. Fifteen minutes after i.v. and 30 min after i.p. administration of the test compound, naive rats were placed individually in the centre of the test box. Thereafter, behaviour in the open field was analysed for 5 min. Mean values (±S.E.M.) were calculated for each and expressed as percent change versus control.

2.3. Test compounds

Buprenorphine hydrochloride (Lohmann, Andernach, Germany), morphine hydrochloride (Merck, Darmstadt, Germany), and gabapentin (Grünenthal, Aachen, Germany) were dissolved in 0.9% NaCl (Fresenius, Bad Homburg, Germany). Ibuprofen (Sigma) was dissolved in 1% carboxymethyl cellulose (Clariant, Wiesbaden, Germany) and 10% Tween 80 (ICI, Cleveland, UK). Compounds and vehicle were administered i.v. as a bolus, p.o., or i.p. in an application volume of 10 ml/kg in mice and 5 ml/kg in rats.

2.4. Statistical analysis

Data were analysed by means of analysis of variance (ANOVA) with or without repeated-measures ANOVA, depending on the experimental design. Significance of treatment effect, time effect, or treatment×time interaction was analysed by means of Wilks' Lambda statistics. In case of a significant treatment effect, pair-wise comparison was performed at the time of maximal effect by Fisher's least

Table 1
Overview of the analgesic effect of buprenorphine and reference compounds in rodent models of acute and chronic pain

Model	Species, route of administration	ED_{50} (mg/kg)	Mean maximal efficacy in % MPE (dose in mg/kg)	Bell-shaped dose–response curve
Acute pain				
Hot plate 48 °C	Mouse, i.v.	0.037	85% (0.1)	Yes
Hot plate 58 °C	Mouse, i.v.	0.28	83% (1.0)	Yes
Tail flick	Mouse, i.v.	0.16	96% (2.15)	Yes
Tail flick, morphine	Mouse, i.v.	1.44	100% (6.81)	N.D.
Inflammatory pain				
Randall Selitto	Rat, i.v.	0.019	100% (0.1)	N.D.
Randall Selitto, ibuprofen	Rat, i.p.	N.D.	35% (146)	N.D.
Formalin test phase I	Mouse, i.v.	0.006	93% (0.1)	No
Formalin test phase II	Mouse, i.v.	0.025	89% (0.464)	No
Formalin test phase II	Rat, i.v.	0.0024	100% (0.1)	No
Visceral pain				
Phenylquinone writhing	Mouse, i.v.	0.0084	100% (0.0464)	No
Mustard oil SP	Mouse, i.v.	0.019	100% (1.0)	No
Mustard oil 1 mN rAD	Mouse, i.v.	0.027	99% (1.0)	Yes
Mustard oil 4 mN	Mouse, i.v.	0.101	97% (1.0)	Yes
Mustard oil 8 mN	Mouse, i.v.	0.121	97% (1.0)	Yes
Mustard oil 16 mN	Mouse, i.v.	0.170	97% (1.0)	Yes
Mustard oil 32 mN	Mouse, i.v.	0.212	96% (1.0)	Yes
Mustard oil rHA	Mouse, i.v.	0.018	100% (1.0)	Yes
Neuropathic pain				
Spinal nerve ligation	Rat, i.v.	0.055	98% (0.464)	Yes
Chronic constriction injury	Rat, i.v.	0.036	74% (0.1)	Yes
Chronic constriction injury, gabapentin	Rat, p.o.	178	58% (316)	N.D.
Streptozotozin polyneuropathy	Rat, i.p.	0.129	99% (0.464)	No
Vincristine polyneuropathy	Rat, i.p.	0.038	82% (0.1)	No

N.D., not determined; SP, spontaneous pain behaviour; rAD, referred allodynia; rHA, referred hyperalgesia.

Table 2
Outcome of ANOVAs on the analgesic effect of buprenorphine and reference compounds in rodent models of acute and chronic pain

Model	Treatment	Time[a]	Treatment×Time[a]
Acute pain			
Hot plate 48 °C	$F(6,63)=14.96$, $P<0.0001$	$F(2,62)=52.47$, $P<0.0001$	$F(12,124)=4.37$, $P<0.0001$
Hot plate 58 °C	$F(8,81)=28.26$, $P<0.0001$	$F(1,81)=85.36$, $P<0.0001$	$F(8,81)=5.75$, $P<0.0001$
Tail flick	$F(11,108)=10.08$, $P<0.0001$	$F(2,107)=5.22$, $P=0.0069$	$F(22,214)=0.76$, $P=0.77$
Tail flick, Morphine	$F(7,72)=21.85$, $P<0.0001$	$F(2,71)=22.43$, $P<0.0001$	$F(14,142)=0.95$, $P=0.051$
Inflammatory pain			
Randall Selitto	$F(4,45)=122.05$, $P<0.0001$	$F(3,43)=22.86$, $P<0.0001$	$F(12,114)=2.84$, $P=0.0019$
Randall Sellito, Ibuprofen	$F(3,36)=18.34$, $P<0.0001$	$F(3,34)=7.10$, $P=0.0008$	$F(9,83)=1.08$, $P=0.39$
Formalin test mouse phase I	$F(12,117)=30.54$, $P<0.0001$	N.A.	N.A.
Formalin test mouse phase II	$F(12,117)=15.44$, $P<0.0001$	N.A.	N.A.
Formalin test rat phase II	$F(10,99)=20.09$, $P<0.0001$	N.A.	N.A.
Visceral pain			
Phenylquinone writhing	$F(10,99)=21.17$, $P<0.0001$	N.A.	N.A.
Mustard oil SP	$F(8,61)=6.20$, $P<0.0001$	N.A.	N.A.
Mustard oil 1–32 mN	$F(9,60)=60.43$, $P<0.0001$	$F(4,57)=4.14$, $P=0.0051$	$F(36,215)=3.69$, $P<0.0001$
Mustard oil rHA	$F(8,61)=6.21$, $P<0.0001$	N.A.	N.A.
Neuropathic pain			
Spinal nerve ligation	$F(9,90)=37.28$, $P<0.0001$	$F(2,89)=9.06$, $P=0.0003$	$F(18,178)=1.73$, $P=0.038$
Chronic constriction injury	$F(5,54)=10.08$, $P<0.0001$	$F(2,53)=0.75$, $P=0.78$	$F(10,106)=1.53$, $P=0.14$
Chronic constriction injury, gabapentin	$F(4,45)=17.21$, $P<0.0001$	$F(3,43)=7.18$, $P=0.0005$	$F(12,114)=4.02$, $P<0.0001$
Streptozotozin polyneuropathy	$F(7,55)=56.34$, $P<0.0001$	$F(2,54)=15.87$, $P<0.0001$	$F(14,108)=3.38$, $P=0.0002$
Vincristine polyneuropathy	$F(8,76)=12.41$, $P<0.0001$	$F(1,76)=7.21$, $P=0.0089$	$F(8,76)=0.56$, $P=0.81$

N.A., not applicable; SP, spontaneous pain behaviour; rAD, referred allodynia; rHA, referred hyperalgesia.
[a] Corresponds to force (mN) in the mustard oil colitis model.

significant difference test. Results were considered statistically significant if $P<0.05$. ED_{50} values and 95% confidence intervals (95% CI) were determined at the time of the peak effect for each drug by semilogarithmic regression analysis or according to Litchfield and Wilcoxon (1949). ED_{50} values with nonoverlapping 95% CI were considered to be significantly different. Group numbers were $n=10$ (hot plate, tail flick, formalin, writhing, Randall Selitto, spinal nerve ligation, chronic constriction injury, open field, and hole board model), $n=7$ (mustard-oil-induced colitis model), $n=5$–10 (streptozotozin model), and $n=6$–10 (vincristine model).

3. Results

3.1. Animal models of acute pain

Buprenorphine showed dose-dependent analgesic efficacy in several mouse models of acute pain (data summarised in Table 1; outcome of repeated-measure ANOVA summarised in Table 2). Different heat stimulus intensities were used to investigate a possible influence on antinociceptive potency and efficacy. Increase in heat intensity led to decreased potency in terms of ED_{50} values and maximal effective dose (Figs. 1 and 2A). ED_{50} values (95% CI) were 0.037 (0.032–0.043), 0.28 (0.26–0.33), and 0.16 (0.11–0.24) mg/kg i.v. for the hot plate 48 °C (latency time 120 s), hot plate 58 °C (latency time 15 s), and tail flick (latency time 12 s), respectively, and the maximal effective dose was 0.1, 1.0, and 2.15 mg/kg i.v., respectively. Although potency clearly depended on stimulus intensity, no change in maximal efficacy was seen. Thus, buprenorphine reached full efficacy in all models with a mean % MPE of 85%, 83%, and 96% for the hot plate 48 °C, hot plate 58 °C, and tail flick test, respectively. Latency times of vehicle groups (mean±S.E.M.) were 28.2±2.6, 34.1±3.1, and 32.8±3.1 s for baseline and 30 and 60 min at the 48 °C hot plate, 3.7±0.4,

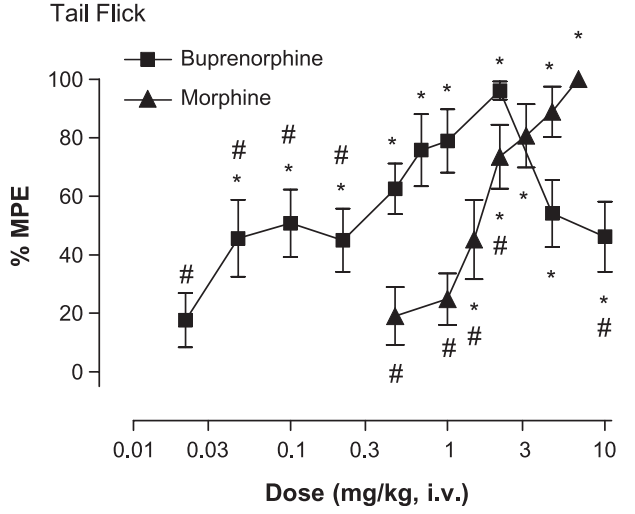

Fig. 1. Effect of buprenorphine and morphine in the mouse tail flick model of acute thermal pain. Data are expressed as mean (±1 S.E.M.) percentage maximal possible effect (% MPE). *$P<0.05$ versus vehicle control; #$P<0.05$ versus the maximal effective dose.

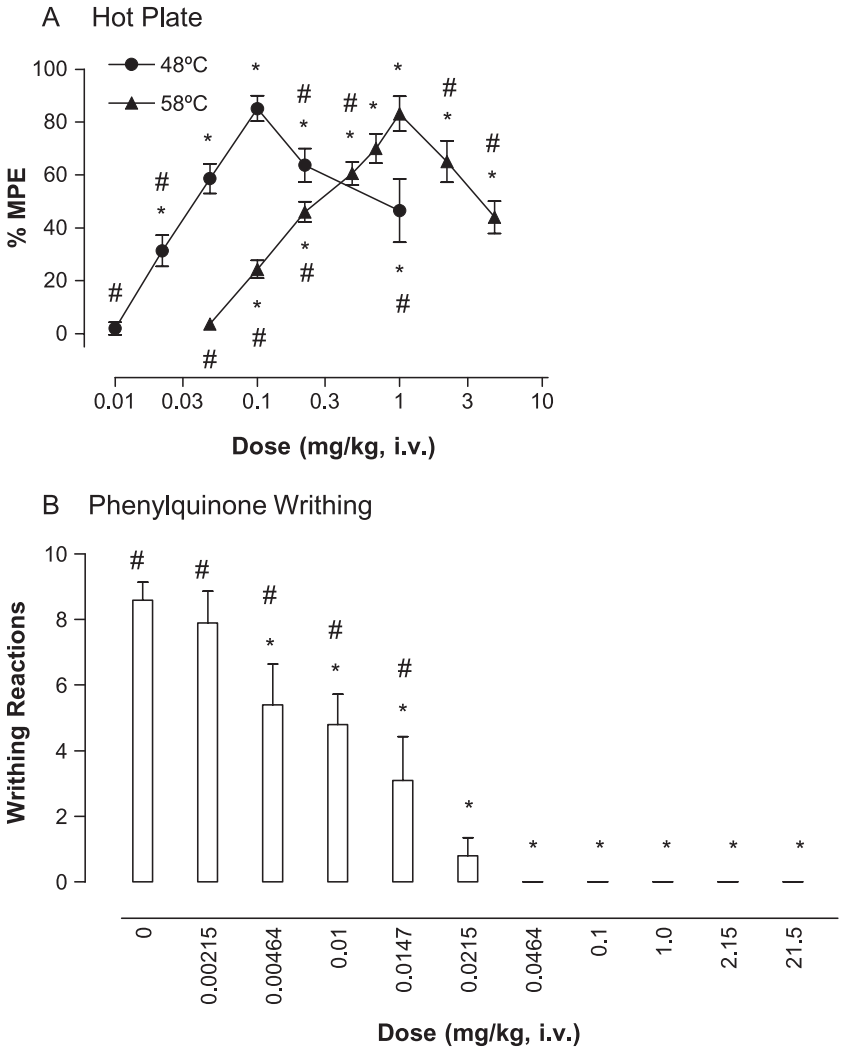

Fig. 2. Effect of buprenorphine in the mouse (A) hot plate 48 and 58 °C model of acute thermal pain and (B) phenylquinone writhing model of acute visceral pain. Data are expressed as (A) mean (±1 S.E.M.) % MPE or (B) mean (+ 1 S.E.M.) number of writhing reactions. *P<0.05 versus vehicle control; #P<0.05 versus the maximal effective dose.

2.8±0.3, and 2.6±0.4 s for baseline and 30 and 60 min at the 58 °C hot plate, and 3.3±0.1, 3.7±0.2, 3.6±0.2, and 3.4±0.3 s for baseline and 20, 40, and 60 min in the tail flick, respectively. In the phenylquinone-induced writhing test, buprenorphine showed highly potent and efficient analgesia, with an ED_{50} value (95% CI) of 0.0084 (0.0060–0.0112) mg/kg i.v., and 100% MPE was obtained at 0.0464 mg/kg i.v. (Fig. 2B). Supramaximal doses showed a decrease in efficacy in the hot plate tests and the tail flick test, whereas no reduction in efficacy was seen in the writhing test. The reference compound morphine showed full efficacy in the tail flick test with an ED_{50} value (95% CI) of 1.44 (1.10–1.79) mg/kg i.v., reaching 100% MPE at 6.81 mg/kg (Fig. 1).

3.2. Animal models of inflammatory pain

In the Randall Selitto test, buprenorphine showed a dose-dependent antinociceptive effect with full efficacy (100% MPE at 0.1 mg/kg i.v.) and an ED_{50} value (95% CI) of 0.019 (0.016–0.021) mg/kg i.v. (Fig. 3A). The reference compound ibuprofen showed a relatively weak antinociceptive effect (35% MPE) when tested up to 146 mg/kg i.p. (Fig. 3A). Because ibuprofen induced a dose-dependent sedative effect, which possibly confounded measurement of analgesia, higher doses were not tested.

Nociceptive behaviour in the mouse formalin test was inhibited dose-dependently with ED_{50} values (95% CI) of 0.0060 (0.0033–0.010) and 0.025 (0.013–0.049) mg/kg i.v. for the acute phase I and the more persistent phase II, respectively (Fig. 3B). Full efficacy of 93% and 89% MPE was obtained at 0.1 and 0.464 mg/kg i.v. for phase I and II, respectively. In rats, only the second phase of the formalin test was measured, and it was found that the compound dose-dependently inhibited pain behaviour with an ED_{50} value (95% CI) of 0.0024 (0.0014–0.0036) mg/kg i.v., and 100% MPE was obtained at 0.1 mg/kg i.v. At supramaximal doses, a

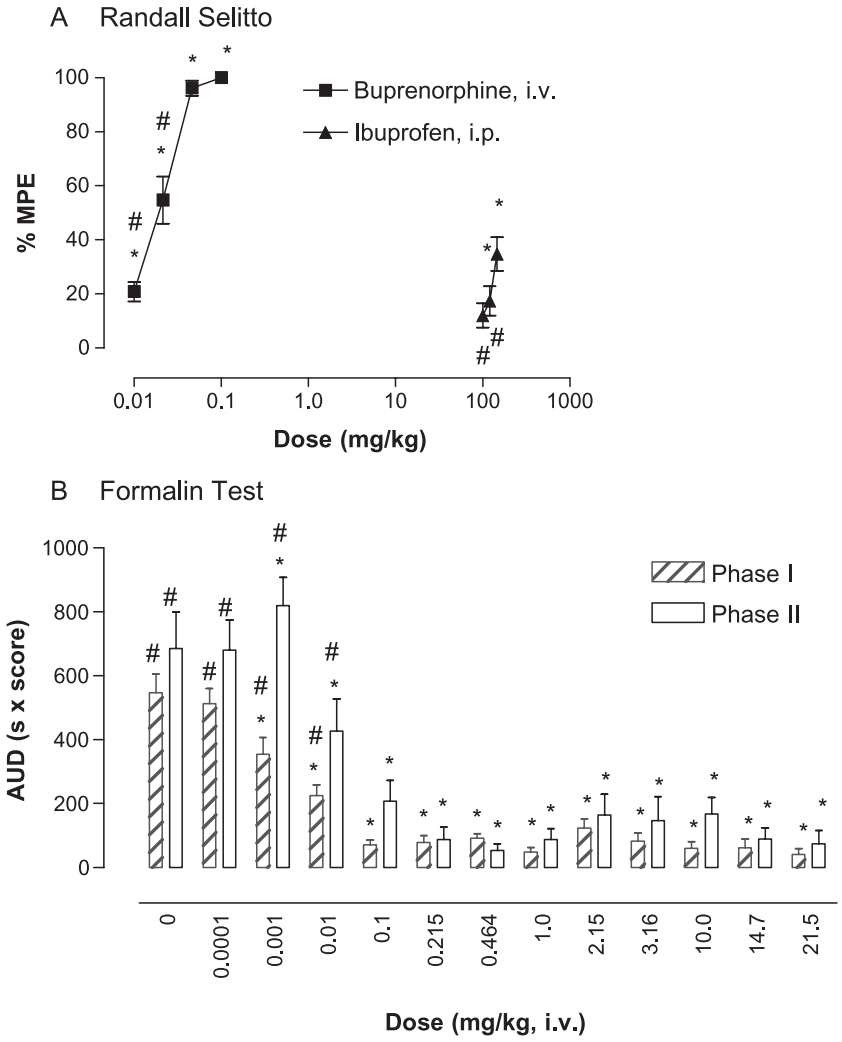

Fig. 3. Effect of (A) buprenorphine (i.v.) and ibuprofen (i.p.) in the Randall Selitto model of acute inflammatory pain in rats and (B) buprenorphine in the early and late phase of the formalin test in mice. Data are expressed as (A) mean (±1 S.E.M.) % MPE and (B) mean (+ 1 S.E.M.) area under the data (AUD, s×score). *P<0.05 versus vehicle control; #P<0.05 versus the maximal effective dose.

nonsignificant trend for a reduction in analgesic efficacy was observed.

3.3. Animal models of visceral pain

In the mustard-oil-induced colitis model in mice, buprenorphine showed a dose-dependent inhibition, with comparably high potencies, of both the acute visceral pain behaviour and the referred mechanical stimulation (Fig. 4). An increase in the intensity of the mechanical stimulus, resulted in decreased potencies, while full efficacy of 100% MPE was maintained at all intensity levels (maximal effective dose of 1.0 mg/kg i.v.). Spontaneous pain behaviour was inhibited with an ED_{50} value (95% CI) of 0.019 (0.009–0.032) mg/kg i.v. (Fig. 4A). Referred pain was inhibited with ED_{50} values (95% CI) of 0.027 (0.009–0.080), 0.010 (0.048–0.210), 0.121 (0.058–0.255), 0.170 (0.081–0.355), and 0.212 (0.107–0.419) mg/kg i.v. for 1, 4, 8, 16, and 32 mN von Frey filaments, respectively. As the dose–response curves were similar for all intensity levels, only one representative data set is shown (i.e., 1 mN von Frey filament; Fig. 4B). Referred hyperalgesia, as measured by scoring the withdrawal reactions towards the 16 mN von Frey filament was inhibited with full efficacy and an ED_{50} value (95% CI) of 0.018 (0.012–0.026) mg/kg i.v. A tendency for reversal of the effect at doses beyond 1 mg/kg i.v. was seen for spontaneous pain behaviour (Fig. 4A), whereas almost complete reversal of the effect was seen in the mechanically evoked responses (Fig. 4B,C).

3.4. Animal models of neuropathic pain

In the spinal nerve ligation model, buprenorphine showed potent and dose-dependent inhibition of mechanical allodynia with an ED_{50} value (95% CI) of 0.055 (0.040–0.075) mg/kg i.v., and a maximal effective dose of 98% MPE was obtained at 0.464 mg/kg (Fig. 5A). Baseline withdrawal thresholds (mean±S.E.M.) were 21.1±0.45 g

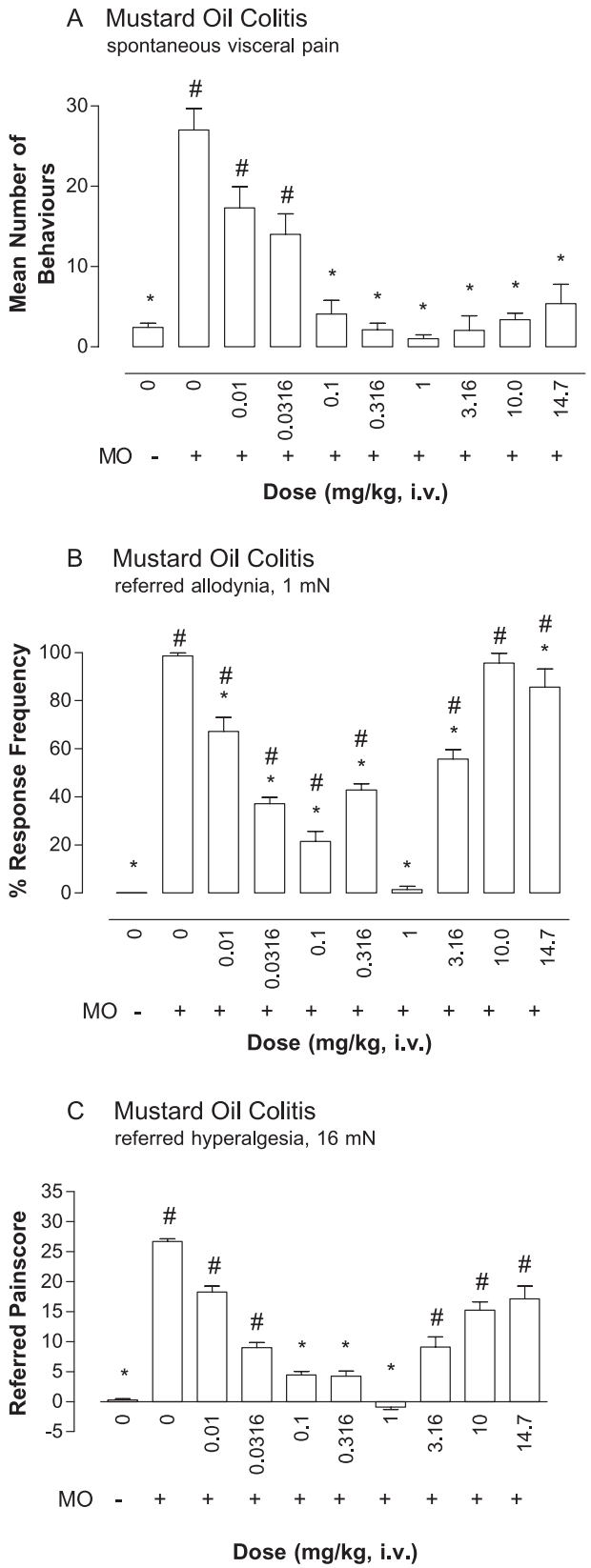

Fig. 4. Effect of buprenorphine in the mouse mustard-oil (MO)-induced visceral (A) spontaneous pain behaviour, (B) referred allodynia (1 mN), and (C) referred hyperalgesia (16 mN). Data are expressed as mean (+ 1 S.E.M.) (A) number of pain behaviours, (B) % response frequency, and (C) referred pain score. *P<0.05 versus vehicle control; #P<0.05 versus the maximal effective dose.

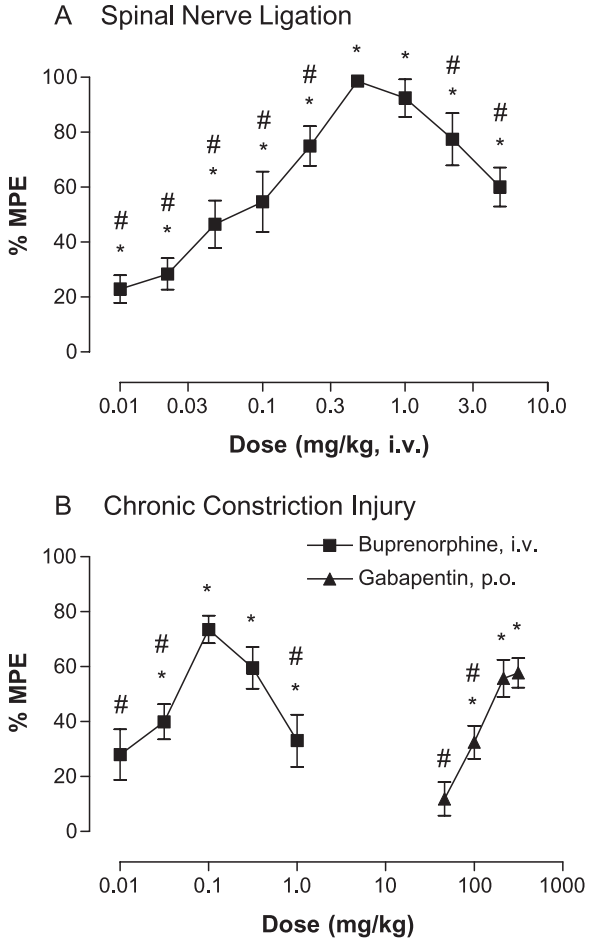

Fig. 5. Effect of (A) buprenorphine (i.v.) on mechanical allodynia in the rat spinal nerve ligation model of neuropathic pain and of (B) buprenorphine (i.v.) and gabapentin (p.o.) on cold allodynia in the rat chronic constriction injury model of neuropathic pain. Data are expressed as mean (±1 S.E.M.) % MPE. *P<0.05 versus vehicle control; #P<0.05 versus the maximal effective dose.

for ligated animals and 73.2±3.94 g for sham animals. In the chronic constriction injury model, cold allodynia was inhibited dose-dependently with an ED_{50} value (95% CI) of 0.036 (0.023–0.062) mg/kg i.v. and a maximal effective dose of 74% MPE at 0.1 mg/kg (Fig. 5B). A group of nine sham animals followed for 6 weeks after sham operation showed mean±S.E.M number of paw withdrawals of 0.1±0.11 and 0±0 in weeks 1 and 5 and weeks 2, 3, 4, and 6, respectively. In contrast, the ligated animals showed baselines of (mean±S.E.M.) 26.9±0.53 (n=60) and 24.1±0.58 (n=50) paw withdrawal reactions for buprenorphine and gabapentin, respectively. In both models, doses beyond the maximal effective dose were clearly less efficient.

The reference compound gabapentin showed a less potent and efficient antiallodynic effect in the chronic constriction injury model, with an ED_{50} value (95% CI) of 178 (134–298) mg/kg p.o. and a maximal effective dose of 58% MPE at 316 mg/kg (Fig. 5B). Because gabapentin induced dose-dependent behavioural agitation on the cold

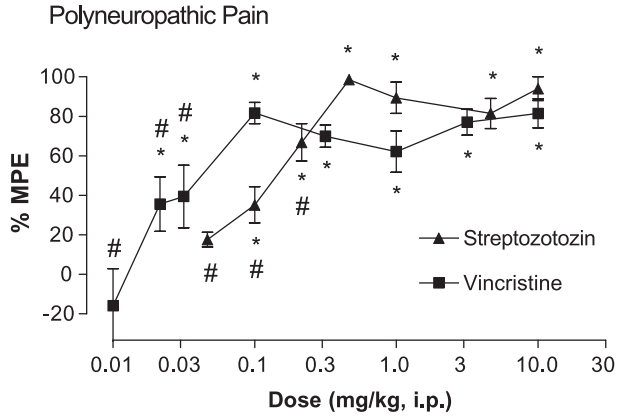

Fig. 6. Effect of buprenorphine (i.p.) on mechanical hyperalgesia in the rat streptozotozin model of polyneuropathic pain and on cold allodynia in the rat vincristine model of polyneuropathic pain. Data are expressed as mean (±1 S.E.M.) % MPE; *P<0.05 versus vehicle control for streptozotozin and between groups after analysis of pretests between dose groups and vehicle control groups for vincristine; #P<0.05 versus the maximal effective dose.

plate starting at 316 mg/kg, which possibly confounded measurement of analgesia, doses higher than 316 mg/kg were not tested.

Polyneuropathic pain was assessed in rats with streptozotozin-induced diabetes and in rats with vincristine-induced polyneuropathic pain, using mechanical hyperalgesia and cold allodynia, respectively. Buprenorphine showed dose-dependent inhibition of mechanical hyperalgesia and cold allodynia, with ED_{50} values (95% CI) of 0.13 (0.10–0.16) and 0.038 (0.02–0.07) mg/kg i.p., respectively (Fig. 6).

3.5. Behavioural side effects of buprenorphine

In general, buprenorphine did not induce obvious behavioural side effects when tested in the diverse pain models. Only in the rat formalin and vincristine model higher doses (i.e., 4.64 and 10 mg/kg i.v.) resulted in stereotypic behaviours which might have interfered with the outcome measures.

When tested in a dose range of 2.15–21.5 mg/kg i.v., buprenorphine did not affect explorative behaviour in the hole board test in mice [$F(4,45)=1.94$, $P=0.12$; data not shown]. When tested in rats in a dose range from 2.15–14.7 mg/kg i.v. and 4.64–21.5 mg/kg i.p., buprenorphine did not affect horizontal movements in the open field test [$F(4,45)=1.67$, $P=0.17$ and $F(3,36)=1.35$, $P=0.27$, respectively; data not shown].

4. Discussion

The present study investigated the analgesic efficacy of the opioid analgesic buprenorphine in a broad panel of rodent models of acute and chronic pain. The compound showed full analgesic efficacy against acute thermal and visceral pain, as well as against persistent/chronic inflammatory and neuropathic pain. Buprenorphine was more potent, and in some models also more efficient, than the clinically established reference compounds morphine, ibuprofen, and gabapentin.

Buprenorphine was found to induce potent analgesia and full efficacy in mouse models of acute somatic (i.e., the hot plate, tail flick, and formalin model) and visceral pain (i.e., the phenylquinone writhing and mustard-oil-induced colitis model). Therefore, it can be concluded that the analgesic efficacy of buprenorphine is not limited by the nature and the intensity of the painful stimulus. It was previously reported that intensity of thermal and chemical painful stimuli influenced the level of efficacy and the shape of the dose–response curve of buprenorphine in rats (Cowan, 1995) and mice (Lutfy et al., 2003). In the present study, increasing stimulus intensities in heat- or pressure-induced acute nociception in mice only affected the potency of the compound, whereas maximal efficacy was maintained at virtually 100% MPE. Furthermore, the inverted u-shape of the dose–response curve was maintained across the different levels of stimulus intensity (Table 1). As reported previously (Cowan, 1995), the dose–response curve appeared to be inverted u-shape in acute pain models based on thermal painful stimuli. On the other hand, as an inverted u-shaped curve was not found in the three models using chemical stimuli (i.e., the mouse phenylquinone writhing, the early phase of the formalin test, and the spontaneous visceral pain behaviour in mustard-oil-induced colitis), it can be hypothesised that the shape of the dose–response curve depends on the nature of the painful stimulus rather than on its intensity.

It has been reported that the opioid system shows adaptive changes under chronic pain conditions. For example, μ-opioid receptor up-regulation was demonstrated in dorsal root ganglia of polyarthritic rats (Ballet et al., 2003), whereas δ-opioid receptor up-regulation was seen in the spinal cord of monoarthritic rats (Cahill et al., 2003). Likewise, ORL-1 receptor expression was reported to be up-regulated in the spinal cord of monoarthritic rats (Jia et al., 1998). In neuropathic pain models, spinal μ-opioid receptor expression appears to be reduced (Chen et al., 2002; Stone et al., 2004), whereas an increased number of μ-opioid receptors was found at the site of nerve lesion (Truong et al., 2003). The possible impact of such complex changes in opioid receptor expression for the efficacy of opiates against chronic pain is not well understood. Therefore, it seemed of particular interest to test whether buprenorphine exhibits the same analgesic potency and efficacy in models of chronic pain as compared to models of acute pain. It was found in the present study that buprenorphine showed potent analgesic effects in models of chronic pain. These models included models of inflammatory, as well as neuropathic pain. Despite the previously demonstrated decrease in opioid receptor expression in neuropathic pain models (Chen et al., 2002), buprenorphine showed strong efficacy and potency in models of mononeuropathic pain (i.e., the

spinal nerve ligation and chronic constriction injury model) and polyneuropathic pain (i.e., the streptozotozin and vincristine model). It can be speculated that the decreased receptor expression in the spinal cord is a sign of nerve degeneration, whereas the increased receptor expression observed at the site of injury (Truong et al., 2003) may be the result of counterregulation. Consequently, it is possible that the maintained analgesic efficacy against neuropathic pain is the result of activation not only of supraspinal but also of peripheral opioid receptors. Interestingly, it was found that, in both models of mononeuropathic pain, the dose–response curve was inverted u-shape, whereas no reduction in efficacy at higher dose was found in models of polyneuropathic pain. This suggests that, in contrast to acute nociception, the shape of the dose–response curve is rather affected by the extent or degree of neuropathic pain than by the nature of the painful stimulus. The efficacy of opioids against neuropathic pain has been discussed controversially, but, recently, several studies have indicated that opioids are effective against diverse symptoms of neuropathic pain in various rat models (Ollat and Cesaro, 1995; Suzuki et al., 1999; Tsai et al., 2000), as well as in patients (Attal, 2001; Sindrup et al., 1999; Watson, 2000; Watson et al., 2003). The present study clearly demonstrates that buprenorphine is highly effective both in mono- and polyneuropathic pain models. Thus, pronounced inhibition of the clinically relevant symptoms, such as cold and mechanical allodynia, as well as mechanical hyperalgesia was obtained. It was previously reported that buprenorphine, but not morphine, is able to reduce central sensitisation after repetitive C-fibre stimulation in rats (Kouya and Xu, 2004), a phenomenon which is considered to be closely related to the development and maintenance of neuropathic pain (Woolf, 2004). Moreover, in contrast to other opioids, such as morphine, which activates pertussis toxin-sensitive pathways, buprenorphine was found to activate pertussis-toxin-insensitive pathways of pain transmission (Wheeler-Aceto and Cowan, 1991). Although further experiments are needed to substantiate the suggestion that buprenorphine may be superior to other opiates against neuropathic pain, these molecular findings may offer a possible mechanism.

In conclusion, it was found that buprenorphine has a broad analgesic profile in various rodent models of acute and chronic pain. Strong potency and full efficacy, irrespective of the intensity and the type of pain stimulus, support the clinical use of buprenorphine to treat pain conditions of different intensity and origin. Further studies to test its efficacy against diverse neuropathic pain conditions seem warranted.

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