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A preclinical comparison between different opioids: antinociceptive versus adverse effects

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Abstract

Reduced side-effect liability of opioids may enhance the patients quality of life and decrease the incidence of opioid-insensitive pain. Literature offers few comparative data between different opioids at equianalgesic doses. Therefore morphine, fentanyl, buprenorphine, codeine, hydrocodone and oxycodone were compared for analgesic properties and side-effect profiles in rats. Analgesic efficacy was analysed using a tail withdrawal test for acute thermal nociception, a formalin test for chemically induced inflammatory pain and a von Frey test for mechanical hypersensitivity. For side-effect profiling inhibition of gastrointestinal activity was evaluated in a charcoal and ricinus oil test, arterial PCO_2 was determined for measuring respiratory depression, the discriminative stimulus properties linked to the narcotic cue were assessed using a drug discrimination learning test, and motor coordination was tested through rotarod performance.

ED_{50}'s for the occurrence of side-effects were compared to ED_{50}'s in behavioural pain tests. Fentanyl had a strong analgesic potency and, compared to other opioids, an acceptable side-effect profiling at analgesic ED_{50}'s. Also consistent was the ceiling effect of buprenorphine implying an increased safety margin for side-effects, but a decreased analgesic efficacy. Differences between opioids as observed in this study can have important indications for their use in acute as well as in the onset of chronic treatments.

Keywords: Opioids; Antinociception; Analgesia; Side-effects; Rodent; Fentanyl; Morphine; Buprenorphine; Adverse event

1. Introduction

Opiates can induce a highly selective alteration in the response of humans and animals to strong and otherwise aversive chemical, mechanical or thermal stimuli. This antinociceptive effect is mediated by opioid receptors, as it is characterized by a structure–activity relationship and the possibility to antagonize these effects (Yaksh, 1997). The clinical choice of an opioid compound depends upon the duration and severity of pain, the route of administration, the desired speed of onset and duration of action, and the adverse effect profile (Bowdle, 1998). There is an enormous variation in the spectrum and severity of opioid adverse effects, dependent on the chemical structure, physiochemical properties and kinetic distribution of the opioid compounds (Meert, 1996), and the dose, route and speed of administration (McQuay, 1999). Opioid-induced side-effects occur in different systems such as the gastrointestinal tract where constipation, nausea and vomiting can be observed, the respiratory system which is depressed, and the central nervous system leading among others to abuse potential (Schug et al., 1992). Adverse effects of opioids are multiple, most often opioid receptor-mediated and therefore almost inseparable from their desired analgesic effects (Schug et al., 1992). The importance of side-effect profiling of opioid compounds can be illustrated by the definition of opioid-insensitive pain, which is pain that does not respond progressively to increasing opioid dose. This insensitivity is usually relative, but increasing the opioid dose to an analgesic effect provokes intolerable or under manageable adverse effects (McQuay, 1999). It has been stated that any opioid

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that produces fewer adverse effects than morphine at a dose, which provides the same degree of analgesia, would be an improvement (McQuay, 1999) since it would significantly enhance the quality of life of patients (Schug et al., 1992). Unfortunately, selection of the most suitable opioid for a particular patient is difficult since in literature for most clinically important adverse effects there are no comparative data between different opioid compounds at equianalgesic doses. The key factor for comparison is equianalgesic dosing, differences in occurrence of opioid receptor-mediated side-effects may then be explained by differences in receptor binding, distribution and metabolism (McQuay, 1999).

In the present study different opioids used in clinical settings were compared at equianalgesic doses in rats for their side-effect profiles in different organ systems. Similarities in opioid pharmacology and function between species provide a validating support for the conclusion that animal models reveal mechanisms of processing that are present in the human (Yaksh, 1997). Therefore the data of the present study add important information to the knowledge on some opioid compounds that are frequently used in clinical settings. The opioids that have been evaluated are morphine, fentanyl, buprenorphine, codeine, hydrocodone and oxycodone. To analyse the analgesic efficacy of these drugs, a tail withdrawal test was done for acute thermal nociception, a formalin test for chemically induced inflammatory pain and a von Frey test in an inflammatory model for mechanical hypersensitivity. Several tests were performed for side-effect profiling. To evaluate the inhibition of gastrointestinal activity a charcoal and ricinus oil test were performed, arterial blood was analysed for $PaCO_2$ to evaluate respiratory depression, a drug discrimination learning test was done to determine the discriminative stimulus property linked to abuse potential, and rotarod performance was tested for opioid effect on motor coordination.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan, Eystrup, Germany) weighing 220–240 g were maintained in a climate-controlled environment on a 12 h light/dark cycle at a temperature of 22±1 °C. All experiments were carried out during the light phase. During housing water and food were available ad libitum. Before each experiment animals were starved overnight, tap water remained available ad libitum except during the test period. The animals were habituated to the experimental room for at least 1 h before the start of the experiment. Animals were used only once.

For drug discrimination learning, trained male Wistar rats (Charles River, Sulzfeld, Germany) weighing 240–260 g at the beginning of the experiment, were used. These animals were individually housed in standard rodent cages with water ad libitum but limited access to food.

All tests were performed according to guidelines of the Institutional Ethical Committee for Animal Experiments and guidelines for animal research according to IASP.

2.2. Chemistry

Compounds used were morphine HCl, oxycodone HCl (Belgopia, Louvain-La-Neuve, Belgium), fentanyl citrate, codeine phosphate, hydrocodone-L-tartrate (Janssen Pharmaceutica, Beerse, Belgium), and buprenorphine HCl (UFC Manchester, UK) (Fig. 1). In each treatment group at least 5

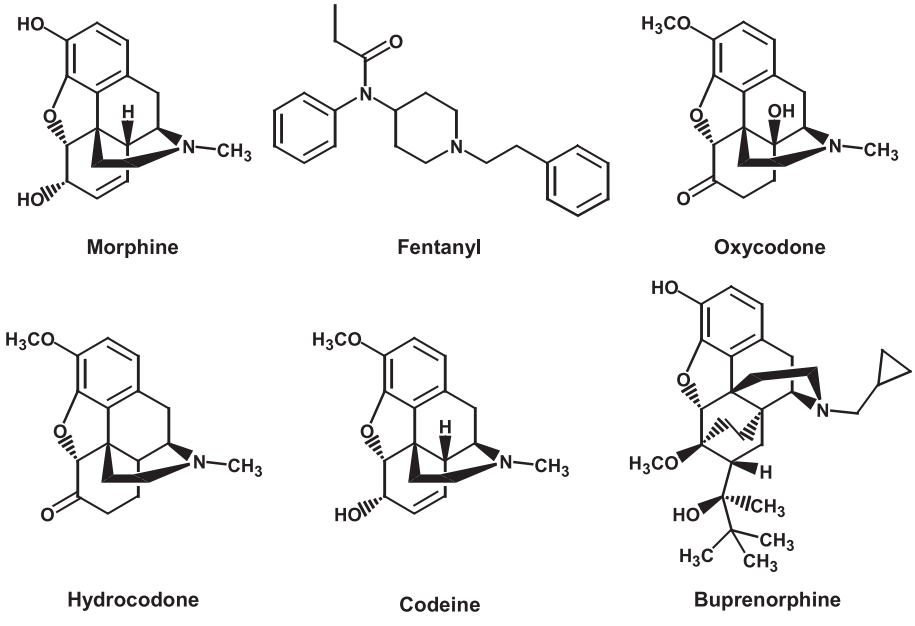

Fig. 1. Chemical structure of different opioid compounds.

animals were tested (additional animals per group are indicated in the figures). Compounds were administered sc, except for the von Frey and rotarod test where compounds were given ip. Dose ranges were selected from the geometrical series 0.00063-0.0025-0.01-.... Opioids were solved in water, except for fentanyl that was solved in 1H2T and for buprenorphine that was solved in water containing 10% cyclodextrine.

2.3. Statistics

Data values are expressed as mean±s.e.m., except where otherwise stated. Using linear regression ED_{50} values and 95% confidence limits were calculated for the different opioids. Differences between groups were evaluated using a Mann–Whitney *U* test (two-tailed). For calculation of ED_{50} values criteria were based on the outcome of control animals. If for a particular compound a ceiling effect was observed—ED_{50}'s were calculated using animals until maximal effect was observed—animals at higher doses were disregarded in the calculations.

To compare the occurrence of a particular side-effect at equianalgesic doses of the different opioids, the ratio was calculated of the ED_{50} for the occurrence of the side-effect, to the ED_{50} for the analgesic activity of that opioid in the tail withdrawal test. If this ratio is equal to 1, the side-effect occurred at the dose at which antinociceptive activity to acute thermal stimuli was obtained. If the ratio is less than 1 the side-effect occurred before antinociception was obtained, and if the ratio is greater than 1 the dose needed to produce a particular side-effect was higher than the antinociceptive dose for that particular opioid.

2.4. Tail withdrawal test

To measure acute thermal nociception a tail withdrawal test was performed (Janssen et al., 1963). The 5 cm distal end of the rat tail was marked with a permanent marker. The withdrawal latency for each rat was measured after emersing the 5 cm distal end of the tail into a recipient filled with water of 55±0.5 °C. A cut-off time of 10 s was used. The tail was gently dried with a soft tissue cloth after each measurement to prevent cooling. The withdrawal latency was measured 5 min before and each 15 min of a 2 h period following sc administration of a compound. The ED_{50}'s ±95% confidence limits for antinociception in the tail withdrawal test were analysed based on the number of animals reaching the criterion for drug effect, which was blockade of the TWR-response or withdrawal latency ≥10 s.

Additionally during this test the cornea and the pinna reflex as well as muscular rigidity were scored every 30 min, in order to evaluate the occurrence of adverse effects of opioid compounds acting on the brain and cranial nerves (Janssen, 1961; Havemann et al., 1980, 1982). This score was 0 for normal reflexes or rigidity to 3 for absence of a reflex or lead-pipe rigid animals. Calculation of ED_{50}'s for μ-opioid related activities was based on score 2 or 3.

2.5. Formalin test

The formalin test is a chemical assay of nociception (Dubuisson and Dennis, 1977). At 30 min after sc injection of the test compound rats were given a single injection of formaldehyde (50μl, 1.75%) in the plantar hypodermis of the skin of the left hind paw. Within seconds the rats started to flinch the paw rapidly, this is the phase I response that is measured during the first 10 min. The following phase II is measured during 50 min and represents a more tonic state. This biphasic flinch behaviour in the rat following the formalin injection was measured in an Automated Nociception Analyser® (UC, San Diego, CA, USA). Therefore the animal was placed in a Plexiglas cylinder mounted above a transmitter/receiver coils assembly. The system detects movement of a small metal band placed on the injected paw. A signal is generated as the band breaks the electromagnetic field of a loop antenna placed underneath the rat. The signal is processed through an algorithm that determines flinch activity using amplitude, zero voltage crossing and duration. The total number of flinches observed during a selected time period was calculated by accumulating flinches of each individual animal over that time period and averaging the treatment groups. To calculate ED_{50}'s, inhibition of the noxious stimulus was considered when the number of flinches was lower than 30 or 300 during phases I and II, respectively.

2.6. Von Frey test in an inflammation model

Inflammation of the left hind paw, a model of prolonged noxious stimulation (Stein et al., 1988), was induced by injection of 50 μl of CFA (complete freunds adjuvant) into the plantar hypodermis. One day after induction of inflammation, animals were tested for the presence of mechanical hypersensitivity by measuring von Frey thresholds 1 h after ip injection of the compound. Gradually increased pressure was applied with a mechanical von Frey probe (1.0 mm of diameter, Senselab® Somedic, Hörby, Sweden) perpendicularly into the mid-plantar surface of the paw. The stimulus was continued until the hind paw was withdrawn or elevated such that the force levelled off. The peak of force in grams was recorded. For each animal 3 measurements of each hind paw were taken with an interval of 60 s, twice before (averaged to the prevalue for each paw) and every 15 min of a 2 h period after opioid administration. To evaluate the presence of mechanical hypersensitivity, the ratio was calculated of the increase in left paw threshold at a certain time point after treatment, to the difference in threshold between left and right paw before treatment (prevalues). This latter difference represents the decreased threshold in the left paw due to inflammation. If

the ratio is 100% then the left paw threshold has increased to normal values.

$$Ratio(\%) = \frac{\text{left paw threshold} - \text{prevalue left paw}}{\text{prevalue right paw} - \text{prevalue left paw}} * 100$$

ED_{50} calculation is based on the number of animals in which the calculated ratio is higher than 70% (partial normalisation) or 90% (complete normalisation), respectively.

2.7. Charcoal test

To measure the effect of opioids on intestinal propulsion 2 ml charcoal solution (10% charcoal in 5% Arabic gum) was administered orally 1 h after sc administration of the test compound. At 30 min after charcoal administration animals were sacrificed by use of CO_2 inhalation. Immediately thereafter the stomach and intestines were removed. The distance travelled by the charcoal meal was recorded as percentage of the total length of the small intestine (measured form the pyloric sphincter to the ileocecal junction). ED_{50}'s ±95% confidence limits for the charcoal test were calculated for inhibition of the gastrointestinal propulsive activity characterized by a ratio lower than 60% (Megens et al., 1989). Also ED_{50}'s for decreasing the propulsive activity even more, <50% are given.

2.8. Ricinus oil test

Opioid-induced constipation was measured using the ricinus oil test (Niemegeers et al., 1972). One hour after sc administration of the test compound 1 ml ricinus oil was administered orally. Thereafter the animals were observed every other hour for the occurrence of diarrhoea (observation 1,2,3,4,5,6,7,8 and, 24 h after the ricinus oil challenge). Number of faeces boli was scored from 0 (no boli) to 3 (many boli) and faeces consistency from 1 (normal) to 3 (fluid). Diarrhoea was considered present when both scores were at least 2. Constipation is seen as an inhibition of ricinus oil-induced diarrhoea that lasts more than 4 h. ED_{50}'s ±95% confidence limits are given for inhibition of the occurrence of diarrhoea at 4 h and at 8 h after treatment, respectively.

2.9. Arterial blood gas analysis

To evaluate respiratory depression, analysis of arterial $PaCO_2$ levels was performed (Verborgh et al., 1997). Under inhalation anaesthesia a polyethylene catheter (PE50) was inserted for 3 mm into the left femoral artery and fixed to the blood vessel. Hereafter the skin was closed and the animals were placed in a Bolman-cage and recovered within 5 min. The experiment was started 2 h after recovery. Just before sc treatment and every 15 min after treatment for a duration of 2 h an arterial blood sample of 0.15 ml was taken using 1 ml heparinized syringes and analysed for $PaCO_2$ using a blood gas analyser (ABL710, Radiometer, Copenhagen). Respiration was considered to be depressed when the $PaCO_2$ level of an animal was increased with 55% compared to pre-treatment value. ED_{50}'s ±95% confidence limits for increase of $PaCO_2$ levels with 55% (on average $PaCO_2$>49 mmHg) and 30% (on average $PaCO_2$>41 mmHg) are given.

2.10. Drug discrimination learning

The drug discrimination assay has been described in detail elsewhere (Meert et al., 1989, 1990). Animals were trained to discriminate between the presence and the absence of the stimulus properties of a specific training compound, which was fentanyl 0.04 mg/kg sc. Animals were habituated to the test box, an operant chamber equipped with two levers and a pellet dispenser, and learned to press a lever for food on a fixed ratio 10 (FR=10) schedule. Thereafter daily discrimination training started. At 30 min before being placed in the test box the rats were now injected sc with either 0.04 mg/kg fentanyl or saline and they obtained food by either pressing the drug lever (DL) or saline lever (SL), respectively. After every 10th press on the correct lever, a food dispenser delivered a 45 mg food pellet. The DL assignment was left in one half and right in the other half of the animals and remained unchanged throughout the study. On each session the FRF value was noted (i.e. the sum of the total number of responses on both levers until 10 responses are made on the appropriate lever). Only animals in which 10 consecutive sessions resulted in an FRF value ≤14 were used for further testing.

To evaluate the discriminative stimulus property linked to narcotics, a generalization experiment was performed in which was tested whether a compound, injected sc at 1 h prior to testing, introduced comparable stimulus properties to the 0.04 mg/kg fentanyl-training drug. The response rate (i.e. the total sum of the responses on both the DL and SL) and the percentage responding on the selected lever (ratio response on selected lever to the response rate) were calculated. For evaluation of opioid discriminative effects, ED_{50}'s were calculated based on the number of animals that showed a complete generalization to the fentanyl (0.04 mg/kg) cue.

2.11. Rotarod

On day 1 animals were trained to run on a rotarod (ENV-575±®, Med Associates, Georgia, US). A first run of 5 min was done at a speed of 8 rounds per min (rpm), 30 min later animals were injected ip with saline, followed by a 5 min run at a speed of 12 rpm and at 60 min a last 5 min run was done at a speed of 16 rpm. On day 2 just before the

experiment animals had to run on the wheel for 5 min at a speed of 16 rpm. During the actual experiment the rotarod turned with a velocity increasing from 4 to 40 rpm. On day 2 immediately after a first session on the rotarod to obtain a prevalue, opioids were administered ip thereafter measurements were done at 30 and 60 min. The time was measured that the animals remained on the turning wheel with a maximum of 5 min. ED_{50}'s were calculated based on the number of animals that fell off the wheel within a period of 150 s.

3. Results

3.1. Antinociceptive activities of opioids in the tail withdrawal test

Saline treated animals exhibited mean latencies of 3.30±0.98 s at the various post-injection time points. After sc administration most opioids resulted in a dose-dependent increase in tail withdrawal latencies (Fig. 2). Buprenorphine had a characteristic dose-response curve with a ceiling in

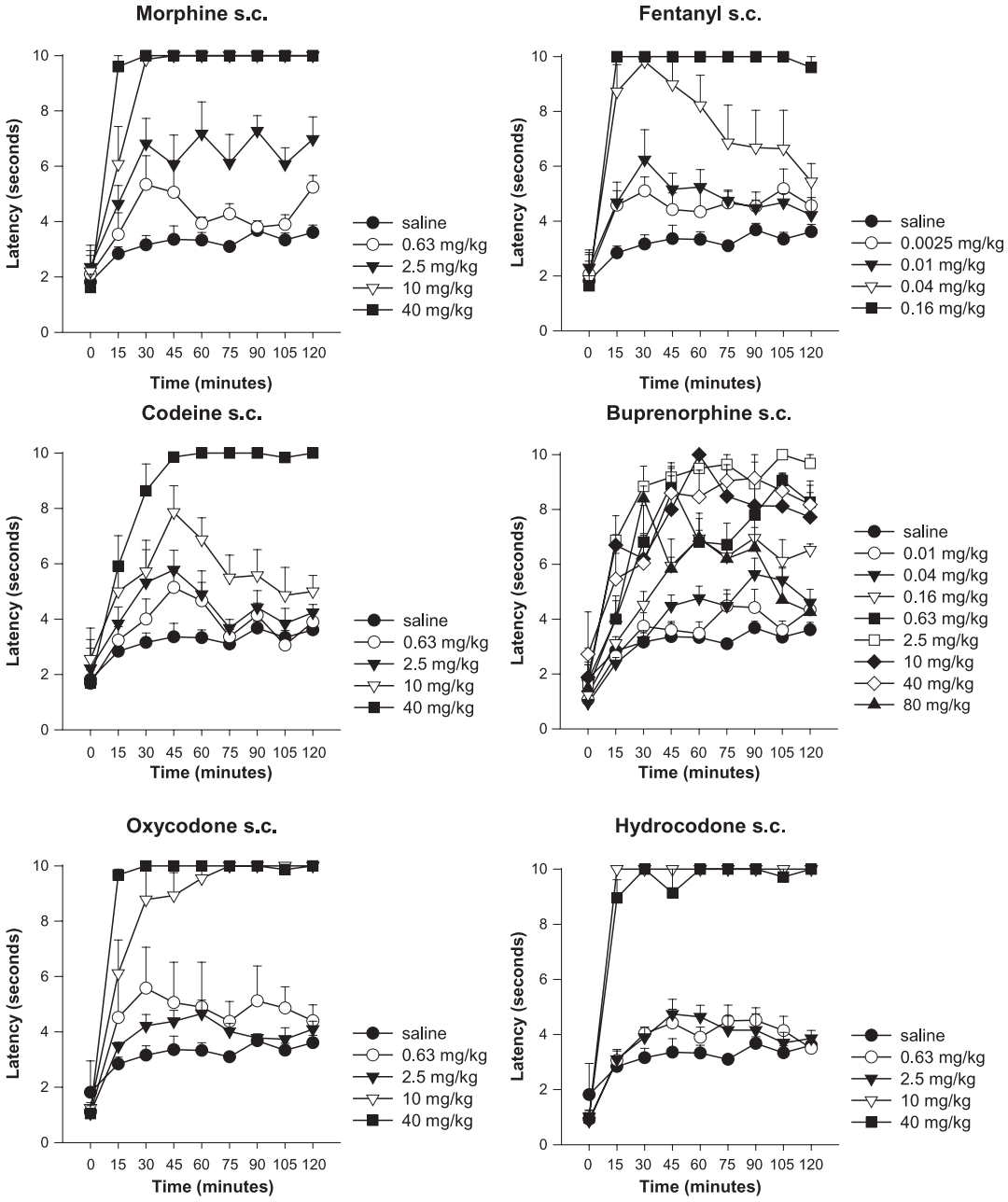

Fig. 2. Effects of sc administration of opioids on response latencies over time in the tail withdrawal test in rats. Given are the mean±s.e.m. response latencies for the different doses tested, n=5 rats per treatment condition and n=11 salines. Both for codeine and fentanyl at the highest dose, 10–40 mg/kg oxycodone and hydrocodone, and for 2.5 and 40 mg/kg morphine, the latency is significantly different from the baseline value at all time points after administration. For 2.5 mg/kg buprenorphine the latency is significantly different from the baseline value at all time points after administration, but less for the 0.63, 10 and 40 mg/kg doses (p<0.001, Mann–Whitney U test two-tailed).

Table 1
Given are the ED_{50}'s (±95% confidence limits) in mg/kg of different opioids for blockade of analgesic effect in the tail withdrawal, formalin and von Frey test, respectively

ED_{50}'s in rats (±95% confidence limits) mg/kg for sc opioids (ip in von Frey test)						
	Morphine	Fentanyl	Buprenorphine[a]	Codeine	Oxycodone	Hydrocodone
Tail withdrawal						
Analgesia	2.89 (1.57–5.29)	0.015 (0.008–0.028)	0.42 (0.23–0.76)	8.73 (3.89–19.60)	5.02 (3.27–7.71)	2.89 (1.57–5.29)
Formalin						
Phase I	5.02 (3.27–7.71)	0.061 (0.033–0.111)	≈0.72	≥40.00	1.66 (0.90–3.04)	5.02 (3.27–7.71)
Phase II	3.81 (2.08–6.98)	0.080 (0.052–0.123)	0.03 (0.02–0.06)	20.03 (13.04–30.75)	2.19 (1.19–4.01)	3.81 (2.08–6.98)
Von Frey[b]						
70% normalisation	12.63 (6.80–23.45)	0.144 (0.086–0.24)	≈2.28	20.00 (13.91–28.76)	≈2.76	6.62 (3.61–12.14)
90% normalisation	22.47 (13.34–37.87)	0.213 (0.128–0.355)	≈13.49	20.00 (13.91–28.76)	13.46 (5.98–30.31)	≈26.41
Pinna reflex	3.81 (2.08–6.98)	0.046 (0.025–0.084)	0.14 (0.06–0.31)	20.0 (13.0–30.8)	5.02 (3.27–7.71)	3.81 (2.08–6.98)
Cornea reflex	5.02 (3.27–7.71)	0.035 (0.019–0.064)	0.55 (0.21–1.43)	26.4 (14.4–48.4)	5.02 (3.27–7.71)	3.81 (2.08–6.98)
Rigidity	11.51 (6.28–21.12)	0.035 (0.019–0.064)	6.63 (2.24–19.60)	≥40.00	5.02 (3.27–7.71)	3.81 (2.08–6.98)

For the tail withdrawal test ED_{50}'s for analgesia are based on the number of animals that reach the cut-off time. For the formalin test ED_{50}'s for analgesia are based on the number of animals in which the number of flinches was lower than 30 or 300 during phases I and II, respectively. For the von Frey test ED_{50}'s for analgesia are based on the ratio of the increase in left paw threshold after treatment, to the difference in threshold before treatment between left and right paw. This ratio is higher than 70% (normalisation) or 90% (complete normalisation), respectively. ED_{50}'s for μ-opioid related activities are based on scores of 2 or 3 for pinna or cornea reflex and muscular rigidity.

[a] Ceiling effect observed.

[b] Ip treatment.

analgesic effect at a dose of 2.5 mg/kg, never reaching maximal analgesia, and a decrease in effect at the higher doses of 10–80 mg/kg. The onset of blockade of tail withdrawal occurred fastest for fentanyl, followed by hydrocodone, morphine and oxycodone, while activity of codeine and buprenorphine was highest 60 min after administration. ED_{50}'s ±95% confidence limits for antinociception in the tail withdrawal test are given in Table 1. The ED_{50} criterion was never reached by the control animals (n=11 in current experiment, >1000 animals screened in the essay). The order of potency was fentanyl>buprenorphine>morphine, hydrocodone>oxycodone>codeine.

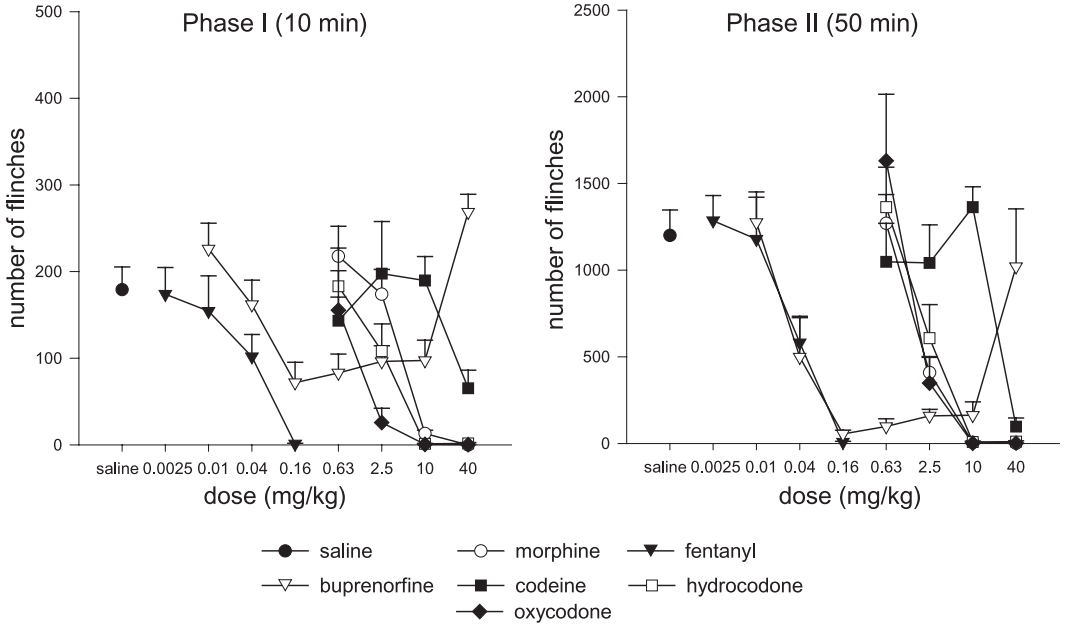

Fig. 3. Dose-response relationships in formalin test after sc injection of opioids. Mean±s.e.m. number of flinches during different phases of the formalin test (Phase I=first 10 min, phase II=next 50 min), n=5 rats per treatment condition and n=8 salines. The maximal opioid effect was observed at the highest dose tested, except for buprenorphine where it was observed at 0.16 mg/kg. This maximal opioid effect was significantly different from saline treated animals in each of the 2 phases of the formalin response (Mann–Whitney U test, 2 sided $p<0.01$, and $p<0.05$ during phase I for buprenorphine and codeine).

3.2. Antinociceptive activities of opioids in the formalin test

After intraplantar formalin injection, saline treated animals exhibited a mean number of flinches of 179±26.3 and 1200±147.5 during phases I and II, respectively. Most opioids, administered sc, resulted in a dose-dependent decrease in number of flinches (Fig. 3). For buprenorphine, after an initial decrease, the number of flinches remained increased at doses of 0.63 mg/kg and higher. Codeine was active only at the highest dose of 40 mg/kg. ED_{50}'s ±95% confidence limits for antinociception during the different phases of the formalin test are given in Table 1. The ED_{50} criterion was never reached by the control animals (n=17 in current experiment, >1000 animals screened). For buprenorphine and codeine no ED_{50}'s were obtained for inhibition of the first phase response because of their low effects. In general fentanyl and buprenorphine were most potent followed by oxycodone, morphine, hydrocodone and, codeine, respectively.

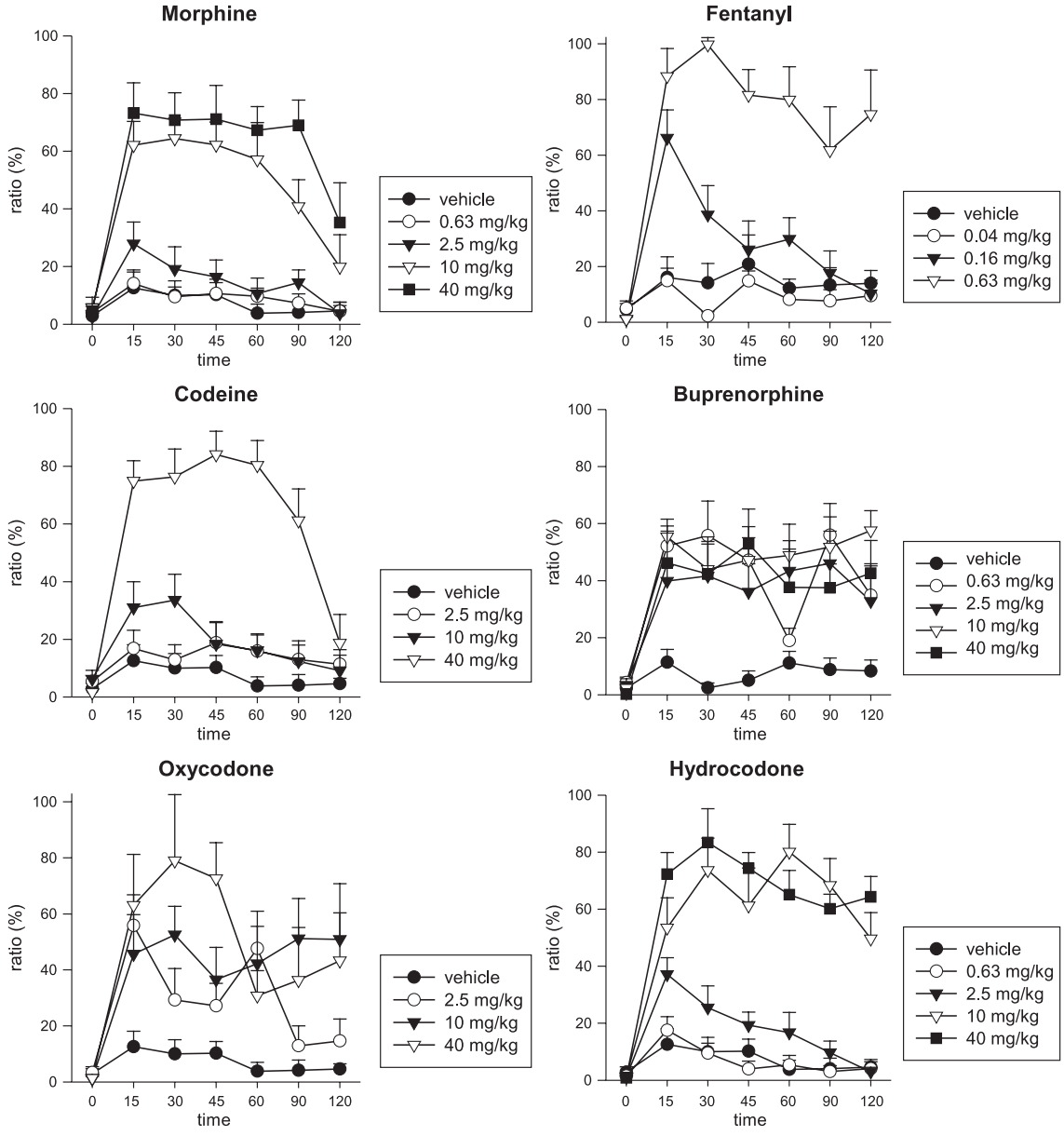

Fig. 4. Effects over time of opioids on von Frey threshold in an inflammation model after ip administration. Presence of mechanical allodynia was measured by comparing the von Frey threshold in the inflamed left paw and the normal right paw. Ratios were calculated of the increase in left paw threshold after treatment to the difference in threshold before treatment between left and right paw (%). Given are the mean±s.e.m. ratios for the different doses tested, n=7 rats/dose, except hydrocodone: 5 rats/dose and morphine: 12 rats/dose. The difference between the ratio at the highest dose tested was significant from vehicle values for hydrocodone during the whole test period (p<0.01), for morphine and codeine until 90 min (p<0.01) and for fentanyl from 15–60 min and 90–120 min (p<0.01 and 0.05, respectively). For buprenorphine significant decreased allodynia was observed 15–30–45 min after treatment (p<0.05, 0.01 and 0.01, respectively) and for oxycodone 15 min (p<0.05) and 30–60 min after treatment (p<0.01) (Mann–Whitney U test, 2 tailed).

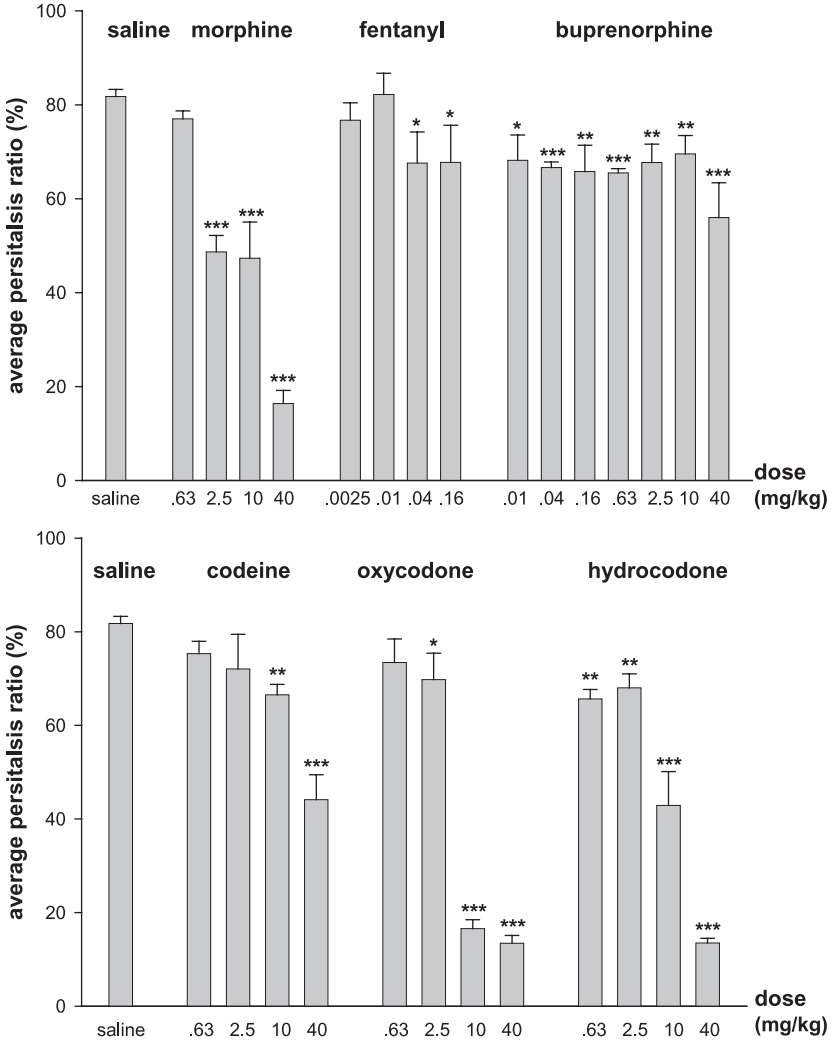

Fig. 5. Effects of sc administration of opioids on charcoal propulsion ratio in rats. Given are the mean±s.e.m. values of the peristalsis ratio of 5 rats per treatment condition (n=20 for controls). Significant differences between the treatment groups and the vehicle controls were calculated using the Mann–Whitney U test (two-tailed; *p<0.05; **p< 0.01; ***p<0.001).

3.3. Reversal of inflammation-induced mechanical hypersensitivity

After ip administration all opioids, except buprenorphine, resulted in a dose-dependent increase in the mechanical von Frey threshold of the inflamed left paw, thus obtaining a higher ratio of the increase in left paw threshold after treatment to the difference in threshold between left and right paw before treatment (Fig. 4). In saline treated animals the mean ratio was 5.9±1.03% at the

Table 2
Given are the ED_{50}'s (±95% confidence limits) in mg/kg of different opioids

ED_{50}'s (±95% confidence limits) in mg/kg				
	Charcoal test		Ricinus oil test	
	<55% propulsion	<60% propulsion	4 h inhibition	8 h inhibition
Morphine	2.89 (1.29–6.48)	1.66 (0.74–3.72)	2.19 (1.19–4.01)	5.02 (2.24–11.27)
Fentanyl	0.18 (0.08–0.41)	0.14 (0.06–0.31)	0.11 (0.06–0.19)	≈0.24
Codeine	20.03 (7.68–52.24)	11.51 (5.13–25.85)	6.62 (2.95–14.86)	8.73 (3.89–19.60)
Buprenorphine[a]	≈20.04	≈6.63	0.02 (0.01–0.03)	0.18 (0.06–0.054)
Oxycodone	5.02 (2.24–11.27)	5.02 (2.24–11.27)	2.19 (0.84–5.71)	6.62 (2.54–17.27)
Hydrocodone	8.73 (4.76–16.01)	3.81 (2.08–6.98)	0.95 (0.43–2.14)	2.19 (0.98–4.91)

ED_{50}'s were calculated based on the number of rats that had less than 55% and 60% charcoal propulsion in the charcoal test, respectively, and that had no diarrhoea after 4 and 8 h in the ricinus oil test, respectively. Opioids were administered sc in 5 animals per treatment group.

[a] No maximal effect observed in the charcoal test and a ceiling effect observed in the ricinus oil test, ED_{50}'s calculated using animals until maximal effect was observed, animals at higher doses were disregarded.

various post-injection time points. The onset of the opioid effects on mechanical hypersensitivity was fast for all opioids after ip administration. A dose-dependent effect on mechanical hypersensitivity was observed, except for buprenorphine that showed a ceiling effect at around 50% of the maximal effect. ED_{50}'s ±95% confidence limits for opioid effect on mechanical hypersensitivity are given in Table 1. The ED_{50} conditions were reached by none of the control animals (n=39). Again fentanyl was most potent followed by oxycodone, hydrocodone, morphine, and codeine, respectively. For buprenorphine only moderate alteration of hypersensitivity was achieved at the different doses tested.

3.4. μ-Opioid related activity

μ-Opioid related activity, i.e. muscular rigidity and absence of cornea and pinna reflex, was mostly observed at the higher doses tested for all opioids, except for buprenorphine for which absence of reflexes was observed at doses between 0.63–10 mg/kg and muscular rigidity at doses of 40–80 mg/kg. ED_{50}'s ±95% confidence limits for

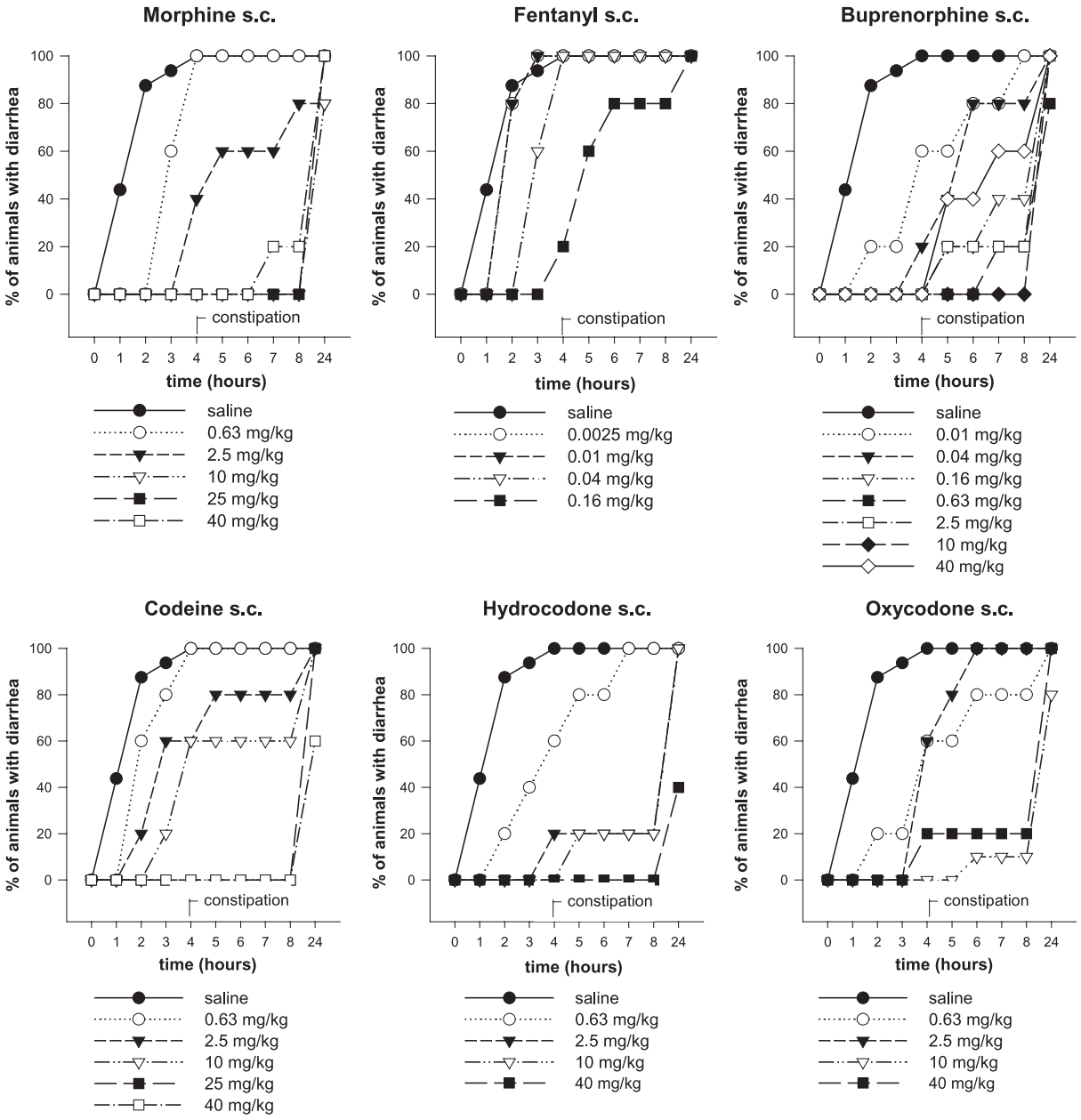

Fig. 6. Effects of sc administration of opioids on occurrence of diarrhoea in the ricinus oil test in rats. Given over time is the percentage of animals with ricinus oil-induced diarrhoea for 5 animals per treatment group (n=17 for controls). Constipation is seen as an inhibition of ricinus oil-induced diarrhoea that lasts more than 4 h.

the occurrence of μ-opioid related activities are given in Table 1.

3.5. Effects of opioids on gastrointestinal activity

After sc administration all opioids resulted in an inhibition of the gastrointestinal propulsive activity. This was observed in the charcoal test as a decrease in propulsion ratio (Fig. 5). In the control animals the average propulsion ratio was 81.7±1.47%. The opioid-induced decrease was rather low for fentanyl and buprenorphine, as compared to the other opioids for which the decrease was clearly dose-dependent. Morphine had the most potent effect, followed by oxycodone, hydrocodone and codeine, respectively. In Table 2, ED_{50}'s ±95% confidence limits are given for inhibition of the gastrointestinal propulsion. ED_{50}'s conditions were reached by none of the control animals (n=20 in current experiment, >1000 animals screened).

In the ricinus oil test opioids induced a decrease in number of animals in which ricinus oil-induced diarrhoea was observed at specific time points (Fig. 6). At 4 h after oil administration diarrhoea had occurred in all control animals

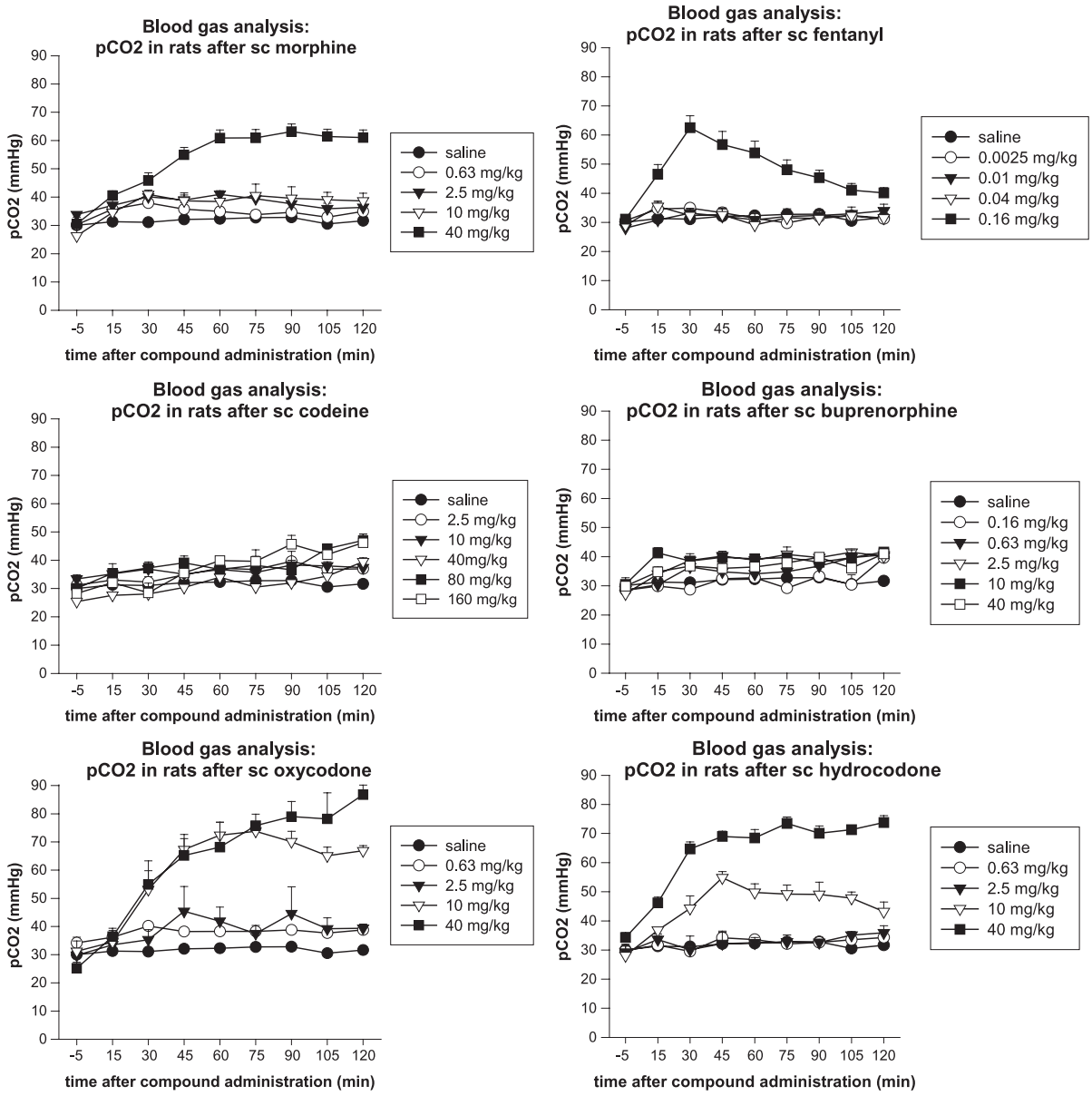

Fig. 7. Effects of sc administration of opioids on arterial $PaCO_2$ levels in rats. Given are the mean±s.e.m. levels of $PaCO_2$ over time. The number of animals tested was 5 per dose (16/dose for salines and 15 for highest dose of morphine and fentanyl). Both for morphine and fentanyl at the highest dose, and for 10 and 40 mg/kg hydrocodone, the $PaCO_2$ is significantly different from the baseline value at all time points after administration. For 10 and 40 mg/kg oxycodone and 10 mg/kg buprenorphine the $PaCO_2$ is significantly different from the baseline value at all time points later than 15 min after administration. For 180 mg/kg codeine the $PaCO_2$ is significantly different from the baseline value at all time points later than 45 min after administration (p<0.001, Mann–Whitney U test two-tailed).

Table 3
Given are the ED_{50}'s (±95% confidence limits) in mg/kg of different opioids

ED_{50}'s (±95% confidence limits) in mg/kg				
	Respiratory		Drug	Rotarod[a]
	30% increased $PaCO_2$	55% increased $PaCO_2$[b]	discrimination 100% fentanyl cue	<150 s rotarod performance
Morphine	2.89 (1.11–7.53)	8.73 (3.35–22.78)	2.33 (1.72–3.16)	11.51 (6.28–21.12)
Fentanyl	0.035 (0.016–0.078)	0.061 (0.033–0.111)	0.021 (0.016–0.029)	0.167 (0.098–0.286)
Codeine	6.60 (2.93–14.83)	80.0 (35.59–179.85)	6.16 (3.81–9.95)	26.41 (17.20–40.55)
Buprenorphine	≈0.18[c]	6.65 (2.02–21.92)[c]	0.028 (0.016–0.048)	34.83 (13.35–90.86)[c]
Oxycodone	0.95 (0.43–2.14)	3.81 (2.08–6.98)	0.51 (0.30–0.88)	5.04 (3.29–7.74)
Hydrocodone	≈0.95	2.89 (1.29–6.48)	1.54 (1.14–2.09)	5.01 (3.26–7.69)

To evaluate respiratory depression ED_{50}'s were calculated based on the number of rats that had an increased arterial $PaCO_2$ level of at least 30% and 55% above prevalue. For evaluation of opioid abuse potential, ED_{50}'s were calculated based on the number of animals that showed a complete generalization to the fentanyl (0.04 mg/kg) cue. Opioids were administered sc in 5 animals per treatment group. To evaluate motor impairment rotarod performance of rats after ip administration of opioids was measured. ED_{50}'s were calculated based on the number of animals that remained on the rotarod for less than 150 s in a 5 min period.

[a] Ip treatment.

[b] Respiratory depression: $PaCO_2$ level 55% increase compared to pre-treatment value.

[c] Ceiling effect observed, ED_{50}'s calculated using animals until maximal effect was observed, animals at higher doses were disregarded.

(n=17 in current experiment, >1000 animals screened). For fentanyl inhibition of diarrhoea occurred only at the highest dose tested (0.16 mg/kg). For the other opioids an antidiarrhoeal effect was observed at all doses tested, except at the lowest dose (0.63 mg/kg) of morphine and codeine. For buprenorphine inhibition of diarrhoea occurred until a later time point at lower doses of 0.16–10 mg/kg than at the highest dose tested (40 mg/kg), indicating a biphasic antidiarrhoeal effect. In Table 2, ED_{50}'s ±95% confidence limits are given for inhibition of the occurrence of diarrhoea at 4 h and at 8 h after treatment, respectively. In this test buprenorphine was most potent followed by fentanyl, hydrocodone, morphine, oxycodone, and codeine, respectively.

3.6. Effects of opioids on respiration

In saline treated animals mean arterial $PaCO_2$ levels were 31.9±0.30 mmHg at the various post-injection time points. After sc administration the highest dose of morphine (40 mg/kg), fentanyl (0.16 mg/kg), hydrocodone (40 mg/kg) and oxycodone (10–40 mg/kg) resulted in a considerable increase in $PaCO_2$ levels >60 mmHg (Fig. 7). This increase in $PaCO_2$ levels occurred faster after fentanyl administration but was of a shorter duration. After 10 mg/kg buprenorphine a slight increase in $PaCO_2$ levels was observed, to values just below 40 mmHg, that became significant as compared to baseline values in the period 30–120 min after administration. For 160 mg/kg codeine the $PaCO_2$ slightly increased to mean values of 39 mmHg and higher, which is significant compared to baseline at all time points later than 45 min. ED_{50}'s ±95% confidence limits are given in Table 3. ED_{50} conditions were reached only at 1 time point by 2 of the control animals (n=15), yet their absolute $PaCO_2$ values remained lower than 40 mmHg. To increase $PaCO_2$ levels with at least 30%, oxycodone was most potent followed

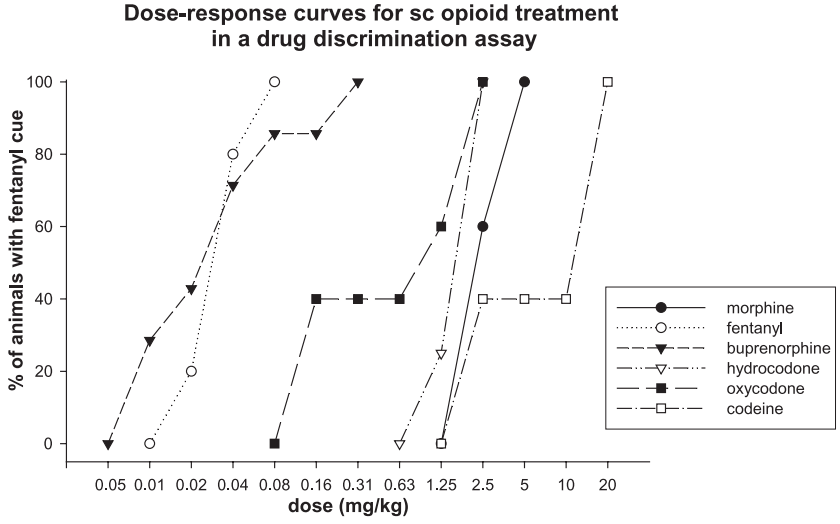

Fig. 8. Effects of sc administration of opioids on drug discrimination in rats. The number of animals tested was 5 per dose. For all opioids after subcutaneous administration a dose was obtained at which all animals showed a complete (100%) generalization to fentanyl 0.04 mg/kg cue.

by buprenorphine, hydrocodone, codeine, morphine, and fentanyl, respectively.

3.7. Drug discrimination of the different opioids

In the drug discrimination assay all opioids tested induced comparable subjective effects as the training drug, thus showing a complete generalization to the 0.04 mg/kg fentanyl cue (Fig. 8). Fentanyl was most potent for this effect followed by buprenorphine, oxycodone, hydrocodone, morphine and codeine, respectively. Based on the dose response curves, ED_{50}'s for generalization were calculated (Table 3). Vehicle treatment did not result in generalization to the fentanyl cue (n=30). The average FRF values were 10.6±1.63 in the control animals and 10.7±1.46 for the animals treated with the different doses of the opioids.

3.8. Rotarod

Rotarod performance was decreased after ip administration of the different opioids, with buprenorphine having the lowest effect (Fig. 9). ED_{50}'s ±95% confidence limits for rotarod performance after ip opioid administration are given in Table 3. None of the control animals reached the ED_{50}

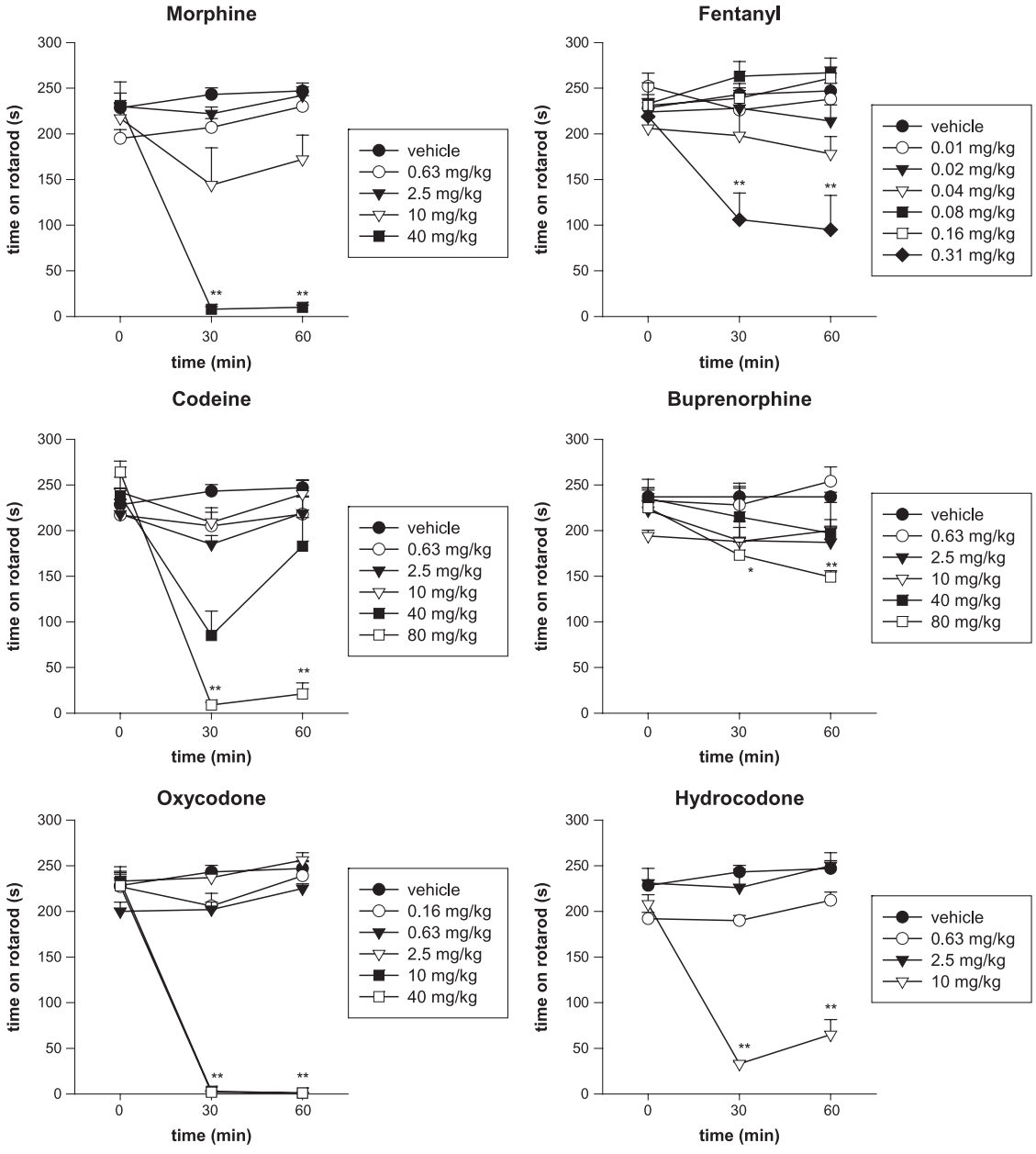

Fig. 9. Effects of ip administration of opioids on motor impairment measured by means of rotarod performance in rats. Given are the mean±s.e.m. levels of time that the animals remain on the wheel. The number of animals tested was 5 per dose (20/dose for vehicles and 5 vehicles for buprenorphine). Except for buprenorphine, mean rotarod performance was lower than 150 s at the highest dose tested. If values at the highest effective dose are compared to vehicle values all opioid effects are significantly different from vehicle (Mann–Whitney U test, 2 sided, $^{**}p<0.01$, $^{*}p<0.05$).

Table 4
To compare the occurrence of a particular side-effect at equianalgesic doses of the different opioids, the ratio was calculated between the ED_{50} for the occurrence of the side-effect after administration of an opioid to the ED_{50} for the analgesic activity of that opioid in the tail withdrawal test

Ratio ED_{50} side-effect/ED_{50} analgesia in TWR test							
Antinociception		Morphine	Fentanyl	Buprenorphine[a]	Codeine	Oxycodone	Hydrocodone
Tail withdrawal	Analgesia	1	1	1	1	1	1
Formalin	Phase I	1.74	4.00	1.74	≥5	**0.33**	1.74
	Phase II	1.32	5.28	**0.08**	2.29	**0.44**	1.32
Von Frey	ratio >90%	7.79	14.07	32.46	2.29	2.68	9.15
	ratio >70%	4.37	9.49	5.48	2.29	**0.55**	2.29
μ-Receptor mediated							
	Pinna	1.32	3.03	**0.33**	2.29	1	1.32
	Cornea	1.74	2.29	1.32	3.02	1	1.32
	Rigidity	3.99	2.30	15.96	5.26	1	1.32
Gastrointestinal							
Charcoal	ratio <55%	1	11.98	48.23	2.29	1	3.02
	ratio <60%	**0.58**	9.09	15.96	1.32	1	1.32
Ricinus	4 h diarrhoea	**0.76**	6.96	**0.04**	**0.76**	**0.44**	**0.33**
	8 h diarrhoea	1.74	16.00	**0.44**	1	1.32	**0.76**
Respiratory							
$PaCO_2$	>30%	1	2.30	**0.44**	**0.76**	**0.19**	**0.33**
Increase	>55%	2.29	4.00	16.00	9.16	**0.76**	1
Other							
DDL	100%	**0.81**	1.41	**0.07**	**0.71**	**0.10**	**0.53**
Rotarod	<150 s	3.99	11.02	83.82	3.02	1	1.74

Ratio ≤1: the side-effect will occur before or together with the analgesic effect, ratio >1: the dose needed to produce a particular side-effect is higher than the analgesic dose for that particular opioid in the tail withdrawal test. Opioids were administered sc except for the von Frey test and rotarod test where administration route was ip.
Bold numbers are those lower than 1, indicating occurence of analgesic effect before occurence of side-effect.
[a] For buprenorphine frequently a ceiling in effect was observed (see previous tables), in that case ED_{50}'s were calculated using animals until maximal effect was observed, animals at higher, less effective, doses were disregarded.

criterion (n=25 in current experiment, >1000 animals screened). Fentanyl had the lowest ED_{50} followed by hydrocodone, oxycodone, morphine, codeine and buprenorphine, respectively.

3.9. Comparison of occurrence of side-effects relative to the antinociceptive doses of different opioids

The ratios of the ED_{50} for the occurrence of the side-effect, to the ED_{50} for the analgesic activity of that opioid in the tail withdrawal test are given in Table 4 for all opioids. As can be observed different side-effects in this study were most easily obtained at antinociceptive doses for oxycodone, followed by buprenorphine, hydrocodone, morphine and codeine, respectively. For fentanyl all ratios were higher than 1. At doses twice the antinociceptive ED_{50} for the tail withdrawal test most side-effects in this study could be obtained for oxycodone, followed by hydrocodone, morphine, buprenorphine and codeine, respectively. For fentanyl only the opioid cue has an ED_{50} less than twice the antinociceptive ED_{50} as determined in the tail withdrawal test. In Fig. 10 a visual representation of all the ED_{50}'s for both the nociceptive tests and side-effect models are given for the different opioids.

4. Discussion

The aim of the study was to compare different opioids for analgesic efficacy and more specifically for side-effect profiling at equianalgesic doses. It was determined whether for a certain opioid compound the occurrence of side-effects is inherent to its analgesic activity, and more important if side-effects occurred when the different compounds were given at a dose that produced a comparable analgesic effect. To establish the analgesic efficacy of the opioid compounds, different behavioural pain tests were performed in which clear differences were observed between compounds concerning potency and maximal analgesic effect. The nature of the pain test also influenced the results, due to factors such as different stimulus modalities and intensities, variations between tests in time courses of measurements or intervals between opioid administration and measurement. Between nociceptive tests morphine, fentanyl, hydrocodone and codeine had their highest potency in the tail withdrawal test that measures opioid activity on acute pain, followed by the formalin test which mimics tonic pain and von Frey test in an inflammation model, respectively. Buprenorphine and oxycodone were more potent for analgesia in the formalin test followed by the tail withdrawal and von Frey test,

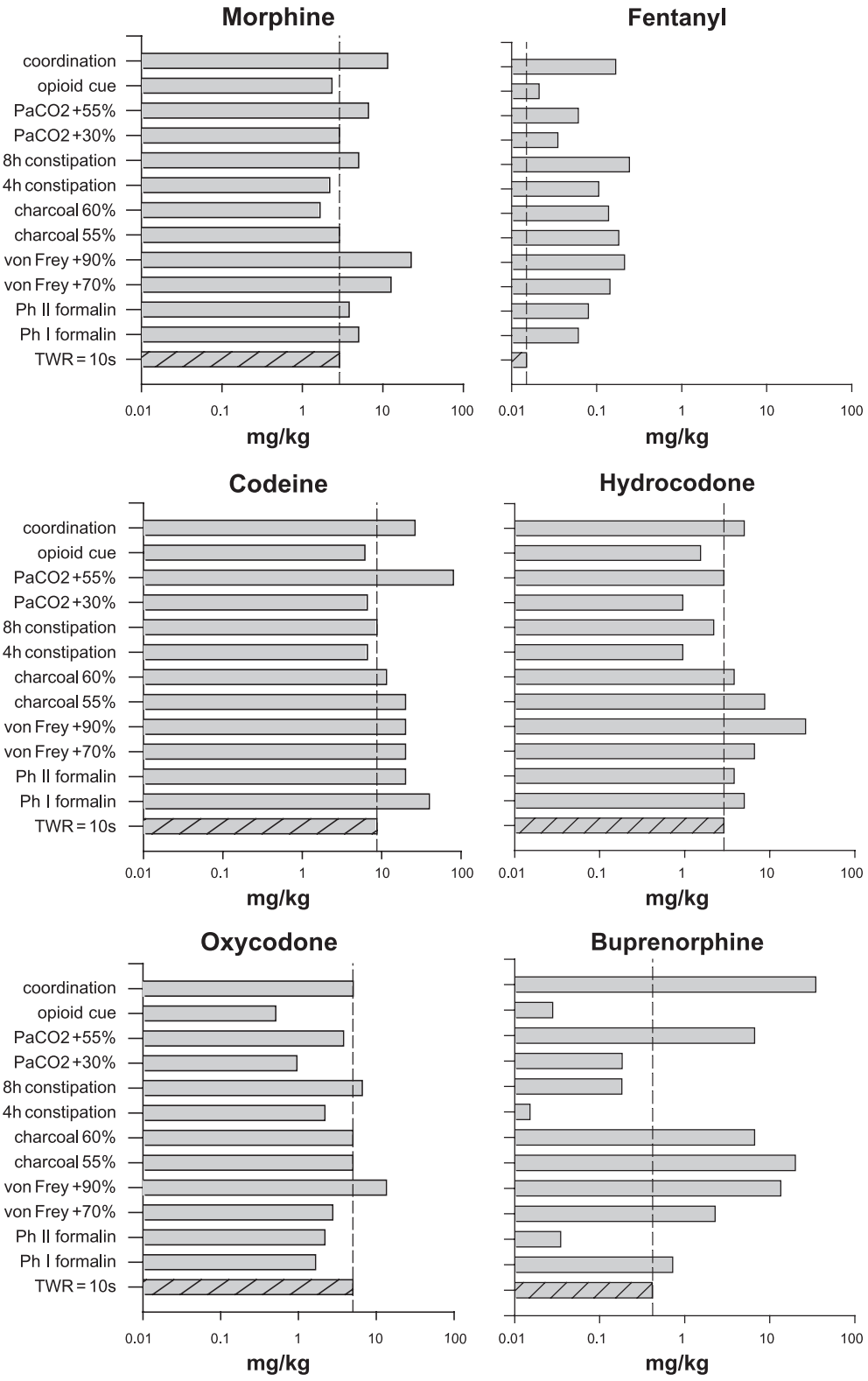

Fig. 10. ED_{50}'s (mg/kg) of different opioids for different kinds of analgesia and side-effects. Opioids are administered sc, except ip administration for inflammation-induced mechanical hypersensitivity and evaluation of coordination through rotarod performance. (TWR=tail withdrawal reflex).

respectively. In general and across the analgesic tests, fentanyl was most potent followed by buprenorphine, oxycodone, morphine, hydrocodone, and finally codeine. Orders of potency found in the present study are in agreement with potencies as derived from dosing tables for treatment of pain in humans (Jacox et al., 1994; Cherny

and Portenoy, 1994; Reisine and Pasternak, 1996). In humans the most potent after intramuscular administration is fentanyl>buprenorphine>morphine>oxycodone>codeine, and after oral administration oxycodone>hydrocodone>morphine>codeine. Equianalgesic doses were based on first single dose administrations and produce an analgesic effect that lasts for about 4 h, except for fentanyl where the duration is about 1 h (Reisine and Pasternak, 1996). It is mentioned that the less potent opioids codeine, oxycodone and hydrocodone are indicated especially for moderate pain (Reisine and Pasternak, 1996; Joranson et al., 2000), while according to Cherny and Portenoy (1994) codeine and oxycodone are usually combined with a non-opioid.

A consistent finding in this study was that buprenorphine showed a ceiling effect, both in behavioural testing and most adverse effect tests. These bell-shaped or flattened dose response curves that reflect a limited or decreased efficacy of the drug at the upper end of the curve are characteristic for partial agonists (Dum and Herz, 1981; Walsh et al., 1995; Bowdle, 1998). Buprenorphine is a mixed agonist–antagonist, acting as an opioid antagonist under some conditions because of its high affinity for μ-receptors but low intrinsic activity. Such biphasic effect includes limitations for treatment when using buprenorphine (Walsh et al., 1995), since opiate agonists with low efficacy may be unable to achieve a maximum effect after interaction with their specific receptors (Yaksh, 1997; Zuurmond et al., 2002).

After obtaining ED_{50}'s for antinociception, the same opioid compounds were compared for occurrence of various side-effects, with emphasis on the side-effect profile at equianalgesic doses. Although side-effects were observed after administration of all the opioids tested, the intensity of the side-effects as well as relative doses at which the effects occurred differed.

Opioid effects on the delay in gastrointestinal transit time were evaluated in the charcoal and the ricinus oil test. The problem of nausea and/or vomiting was not included in this study, as these cannot be directly measured in the rat. An increased gastrointestinal transit time resulting in constipation is a side-effect of all opioids and is opioid receptor-mediated with both central and peripheral mechanisms (Ferrante, 1996; McQuay, 1999; Bowdle, 1998). Since tolerance to this effect does not develop, constipation can become a major problem during chronic opioid administration (Schug et al., 1992; McQuay, 1999, Portenoy, 1996). Opioids reduce gastrointestinal motility by increasing sphincter tone and reducing propulsive peristaltic contractions (Ferrante, 1996). This antipropulsive effect occurs in the stomach and small intestine and is centrally mediated, and it can be measured with the charcoal test (Megens et al., 1989). The delay in passage of intestinal contents allows for greater absorption of water in the large intestine, increased viscosity, and desiccation of intestinal contents (Ferrante, 1996). An additional antidiarrhoeal effect, measured using the ricinus oil test, results from direct opioid influence on fluid transport in the large intestine. So there is a dissociation between antipropulsive and antidiarrhoeal properties of the opioids (Megens et al., 1989). This explains why in the present study sometimes clear differences were observed between the ED_{50}'s obtained in the charcoal as compared to the ricinus oil test. For instance both fentanyl and buprenorphine caused rather small effects on charcoal propulsion, whereas in the ricinus oil test buprenorphine caused long lasting antidiarrhoeal effects, while fentanyl did not. In assessing the constipation-inducing potential of an opioid the key factor is the gut selectivity of the opioid (Megens et al., 1989). An opioid with a high constipation/analgesia dosing ratio can therefore be predicted not only to be preferred by patients but also to be titrated at higher dosages with the potential of better pain control (Haazen et al., 1999). The chance on simultaneous analgesic effects on tail withdrawal and antipropulsive effects was high for morphine and oxycodone. Antidiarrhoeal effects tended to occur before analgesia for all opioids, except for fentanyl, and were long lasting for buprenorphine and hydrocodone, respectively, and to a lesser extent for codeine. Clinical trials have consistently shown less constipation with transdermal fentanyl treatment as compared to patients receiving oral morphine (Donner et al., 1996; Ahmedzai and Brooks, 1997; Grond et al., 1997; Haazen et al., 1999) or oxycodone controlled release (Kloke et al., 2000; Ackerman et al., 2003; Staats et al., 2003). Due to its high lipophilicity fentanyl rapidly penetrates into the brain, reducing the intestinal effect since less opioid remains available in the gastrointestinal tract to block local opioid receptors (Twycross, 1997; Megens et al., 1998; Haazen et al., 1999). The reduction of gastrointestinal side-effects significantly enhances the quality of life of the patients. In the case of morphine the brain penetration required for the analgesic effect is obtained at doses that are much higher than those required for the intestinal effects, therefore excessive stimulation of peripheral opioid receptors and appearance of corresponding side-effects will occur at analgesic dose (Megens et al., 1998).

Potentially the most serious acute opioid adverse effect is respiratory depression (Takeda et al., 2001; Inturrisi, 2002, Bowdle, 1998), mediated via μ-receptors although δ-receptors might also play a role (Morin-Surun et al., 1984; Matthes et al., 1998; Su et al., 1998). All μ-agonists and the partial agonist buprenorphine produce a dose-dependent reduction in the responsiveness of brainstem respiratory centres to increases in PCO_2 and depress the pontine and medullary centres involved in regulating the rhythmicity of breathing, resulting in prolonged apnoea between breaths, delayed exhalation and periodic breathing (Ferrante, 1996). In clinical settings opioids reduce respiratory rate, and can result in a slight diminution in tidal volume and finally in an increased alveolar partial PCO_2 (Schug et al., 1992). In the present experiment arterial PCO_2 measurement was used for assessment of adequacy of ventilation (Jordan, 1982) demonstrating clear differences in effects of the various opioid compounds on respiration. The effects on respiration

were less pronounced for buprenorphine and codeine, although for most opioids PCO_2 levels increased at doses lower than the analgesic ED_{50}. The increase for fentanyl was fast but of shorter duration as compared to the other opioids. Comparable observations were made by van den Hoogen et al. (1988) using whole body plethysmography in rats breathing both air and 8% CO_2 in air. Clinically respiratory depression is an important issue after acute opioid treatment and initially in chronic treatments, while later on during chronic treatment tolerance will develop. Meanwhile it is important to realize that when treating a patient with opioids individual titration to the optimal dose is extremely effective and safe (Schug et al., 1992; McQuay, 1999) as opioid-induced respiratory depression is multifactorial, varies inter- and intra-individually in a wide range (Schug et al., 1992). Respiratory depression is more likely to occur after excessive doses or doses given in absence of pain (McQuay, 1999), since in chronic pain states the strongest antagonist against respiratory depression is pain itself (Schug et al., 1992). Agonist–antagonist agents, such as buprenorphine in the present study, are considered to have the advantage of increased safety in the case of accidental over dosage since they show a ceiling effect for respiratory depression, which still can be significant and severe (Zuurmond et al., 2002). However, this higher safety is paralleled by limited analgesic efficacy as respiratory depression due to opioids is mainly a uniform dose-related effect of an opioid on the μ-receptor (Schug et al., 1992). Moreover, because of the tight receptor binding of buprenorphine (Hambrook and Rance, 1976), naloxone-mediated reversal of the effects of buprenorphine might be incomplete and slow even at high doses, while administration of opioid antagonists reverses the respiratory depression induced by full opioid agonists both promptly and completely (Heel et al., 1979; Reisine and Pasternak, 1996; Bowdle, 1998).

To have an idea of the impact of opioid treatment on motor function, effects of opioids on forced locomotor activity were evaluated. Buprenorphine and fentanyl had the lowest effect on locomotion, followed by morphine, codeine, hydrocodone and oxycodone, respectively. For all opioids, effects on locomotion occurred at a dose higher than the analgesic ED_{50}, except for oxycodone where effects occurred at the same dose. Motor function was also evaluated in the tail withdrawal test by controlling pinna and cornea reflexes and muscular rigidity. Blockade of the pinna and cornea reflexes are characteristic effects of opiates on the tenth and fifth cranial nerves (Janssen, 1961; Havemann et al., 1980, 1982). Rigidity of skeletal muscles, although attenuated by supraspinal δ_1- and κ_1-receptors, is primarily mediated by central μ-receptors (Vankova et al., 1996) located in brainstem midline nuclei and the basal ganglia (Janssen, 1961; Havemann et al., 1980, 1982; Weinger et al., 1995). All opioids induced increased muscular rigidity and decreased reflexes, except for codeine. Again fentanyl was most potent but ED_{50}'s exceeded analgesic ED_{50}'s two to four times, while for oxycodone, hydrocodone and to a lesser extent buprenorphine and morphine, ED_{50}'s for decreased reflexes were rather close to analgesic activity. The use of opioids for the treatment of acute pain is often associated with varying degrees of cognitive impairment (Sabatowski et al., 2003) while during chronic treatment tolerance will develop (Portenoy, 1994). This is illustrated by the fact that in opioid dependent/tolerant patients, for instance receiving long-term morphine or transdermal fentanyl treatment with stable doses, opioids do not impair driving related skills (Vainio et al., 1995; Fishbain et al., 2003; Sabatowski et al., 2003) and are not associated with significant impairments in psychomotor and cognitive performance. Driving skills are important for patients since they form an indispensable feature of modern living and contribute considerably to an independent lifestyle (Sabatowski et al., 2003). On the other hand, pain also may impair cognitive and motor function (Lorenz et al., 1997) and opioids might therefore reduce pain-related impairment of psychomotor and cognitive performance (Sabatowski et al., 2003).

One part of abuse potential of opioids, the discriminative stimulus properties, was measured in rats by assessing whether the animals that were not in any kind of pain state were able to recognise the narcotic cue of 0.04 mg/kg fentanyl. A rat can be trained to make a specific response after the administration of narcotic drugs and to execute a different response following solvent injection. This narcotic cue is defined as a subjectively experienced stimulus arising from the drug central narcotic action and is independent from its peripheral effects. It relates intimately to and can serve as a preclinical model for opiate-like subjective effects in man and is not subject to tolerance development. Therefore this discriminative stimulus provides an original means by which to investigate subjectively experienced drug effects (Colpaert et al., 1975; Colpaert and Niemegeers, 1975; Colpaert, 1978). Abuse of prescribed opioids has harmful ramifications for the legitimate and appropriate users of opioids including stigmatisation, opiophobia and under-treatment of pain (Zacny et al., 2003). Addiction in humans is a psychic and sometimes also physical state characterized by behavioural and other responses that always include a compulsion to search and take the drug in order to experience its psychic effects and sometimes to avoid the discomfort of its absence. Thus addiction implies compulsive behaviour and psychological dependence and is conceptually and phenomenologically distinct from tolerance and physical dependence. Development of addiction is related to μ-receptor activity (Ferrante, 1996; Inturrisi, 2002) and can thus be related to analgesia. Only for fentanyl the ED_{50} for abuse potential was higher than that in the tail withdrawal test. For the other opioids this narcotic cue ED_{50} lay close to the analgesic dose, thus indicating that all these opioids had a similar abuse potential in terms of ability to discriminate. Oxycodone and buprenorphine had an ED_{50} for the narcotic cue that was lower than that for

analgesia. As such, the central narcotic discriminative stimuli and thus the sense for abuse potential will be present before the opioid analgesic dose will be reached. This indicates that both opioids can have a higher abuse potential than the other ones. In a study comparing prescription and abuse of opioid compounds prescribed in the US, the emergency department mentions for morphine and fentanyl are several orders of magnitude lower than for hydrocodone and oxycodone, with the highest illicit use/licit use ratio for oxycodone (Zacny et al., 2003). Still, in the use of opioids in the medical setting, addiction seems to be a far smaller problem than generally assumed (Schug et al., 1992; Portenoy, 1996). Data from the drug abuse warning network (DAWN) database indicate that the trend of increasing medical use of opioid analgesics to treat pain does not appear to contribute to increases in the health consequences of opioid analgesic abuse (Joranson et al., 2000). Recent administration modalities that cause a slow onset and prolonged effect with a slowly tapering offset, such as oral administration of controlled/sustained release formulations and possibly transdermal administrations of fentanyl or buprenorphine, may be more easily controlled for preventing the risk of compliance problems and pseudo-addictive and addictive behaviour (Breivik, 2003). This is illustrated by a study of Dellemijn et al. (1998) finding psychological dependence in none of the patients on chronic transdermal fentanyl treatment.

In conclusion, ED_{50}'s for the occurrence of different adverse effects due to opioid administration were compared to ED_{50}'s for these opioids in different behavioural pain tests including acute thermally induced pain, acute and tonic chemically induced pain and allodynia due to inflammation. Striking were the strong potency of fentanyl in the different tests and the fact that its side-effect profiling compared to the other opioids was acceptable when compared to analgesic effectiveness. Also consistent was the ceiling effect of buprenorphine in most tests except for abuse potential. This ceiling effect implies an increased safety margin for different adverse effects, on the other hand also indicating a decreased analgesic efficacy at higher doses, or in more serious pain states in which higher doses are required. The question was raised during the study how the presence of a chronic pain state or the development of tolerance to some side-effects, as frequently seen in human patients receiving opioid therapy, can influence the outcome of the opioids profiles and activities. Nevertheless, the differences observed here among the opioids after an acute administration can have important indications for the use of these drugs in acute as well as in the beginning phase of a chronic treatment.

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