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Pharmacology, Biochemistry and Behavior 74 (2002) 73–86

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Dextromethorphan and ketamine potentiate the antinociceptive effects of μ- but not δ- or κ-opioid agonists in a mouse model of acute pain

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Received 29 October 2001; received in revised form 30 April 2002; accepted 17 July 2002

Abstract

Animal and clinical studies have reported potentiation of opioid antinociception by NMDA receptor antagonists such as ketamine and dextromethorphan. The aim of this study was to compare these clinically available NMDA antagonists in combination with classical morphine, μ-selective fentanyl-like opioids, the δ-opioid agonist SNC80 and the κ-opioid agonist U50,488H. Using a mouse hot-plate test, dose–response relationships were first determined for all compounds individually and then for opioids co-administered with fixed doses of ketamine or dextromethorphan. All compounds were administered intraperitoneally ED_{50} values were calculated from the proportion of animals failing to exhibit any response within a fixed cut-off criterion of 30 s. To varying degrees, all compounds produced increases in response latencies over time. Dextromethorphan produced lower ED_{50} values for morphine, fentanyl and sufentanil but exerted no effect on the potency of SNC80 or U50,488H. Similarly, ketamine potentiated the antinociceptive potency of morphine, fentanyl and sufentanil but not SNC80 or U50,488H. In summary, these results support the use of μ-opioid agonists in combination with NMDA antagonists, but suggest that there may be no advantage in combining dextromethorphan or ketamine with δ- or κ-opioids in the management of acute pain.

Keywords: Morphine; Fentanyl; Sufentanil; SNC80; U50,488H; Ketamine; Dextromethorphan; Hot-plate; Nociception; Mice

1. Introduction

N-Methyl-D-aspartate (NMDA) receptor antagonists exhibit antinociceptive properties in a wide range of tests and can potentiate the antinociceptive properties of spinal morphine in the rat (Chapman and Dickenson, 1992; Yamamoto and Yaksh, 1992; Advokat and Rhein, 1995; Grass et al., 1996). NMDA antagonists are a chemically diverse class of compounds. The noncompetitive NMDA antagonist dizocilpine (MK-801), the competitive agent LY235959 and the glycine-site antagonist (+)-HA-966 have been compared and were all found to potentiate the antinociceptive effects of morphine (Allen and Dykstra, 2001). Studies in an acute rat model revealed that dextromethorphan was more effective than ketamine in potentiating morphine-induced antinociception (Plesan et al., 1998).

In addition, NMDA antagonists can attenuate tolerance to morphine (Trujillo and Akil, 1991; Allen and Dykstra, 2000), the δ agonists DPDPE and deltorphin II (Bhargava and Zhao, 1996a; Zhao and Bhargava, 1996) and the κ agonist U50,488H (Bhargava and Thorat, 1994). These studies highlight the close relationship between nociception, the NMDA system and opioid control.

In clinical practise, only ketamine and dextromethorphan are used, which are both noncompetitive NMDA antagonists (Lipton, 1993). The widespread use of ketamine is restricted due to psychotomimetic side-effects (White et al., 1982). Ketamine combined with morphine for postoperative analgesia has generated inconsistent results (Parkhouse and Marriott, 1977; Bristow and Orlikowski, 1989; Edwards et al., 1993; Javery et al., 1996; Adriaenssens et al., 1999). Trials with dextromethorphan monotherapy in pain patients have also demonstrated mixed results (McQuay et al., 1994; Price et al., 1994; Ilkjaer et al., 1997; Kinnman et al., 1997). In addition, dextromethorphan showed no activity in experimental ischemic pain (Plesan et al., 2000). However, dextromethorphan is generally effective in reducing postoperative pain and opioid consumption after a variety of surgical procedures (Helmy and Bali, 2001).

Antinociceptive properties of the peptidic δ-opioid receptor agonist DPDPE have been demonstrated in rodents (Kovacs et al., 1988; Stewart and Hammond, 1994) and this effect can be blocked by the specific δ antagonist

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PII: S0091-3057(02)00961-9

naltrindole (Calcagnetti and Holtzman, 1991). SNC80 is a nonpeptidic, systemically active agonist selective for the δ-opioid receptor (Calderon et al., 1994; Bilsky et al., 1995; Knapp et al., 1996). SNC80 has been shown to possess antinociceptive properties in the warm-water tail flick test in mice, mediated by δ receptors alone (Bilsky et al., 1995; Kamei et al., 1995). Antinociceptive effects of SNC80 have also been reported in rhesus monkeys although behavioural effects were shown to differ markedly from those of μ agonists and pointed to reduced abuse potential (Negus et al., 1998). There is interest in the development of δ agonists due to the anticipated low side-effect profile including lack of effects on respiration and the gastrointestinal system (Porreca et al., 1984; Negus et al., 1993). NMDA antagonists could also be expected to reduce δ-mediated convulsions (Comer et al., 1993; Dykstra et al., 1993). Dextromethorphan was recently found to potentiate the antinociceptive effects of SNC80 in squirrel monkeys (Allen et al., 2002). A spinal mechanism of potentiation was suggested, since in a previous study the antinociceptive effects of intracerebroventral δ agonists were attenuated by NMDA antagonists (Bhargava and Zhao, 1996b).

Several studies have suggested a spinal localisation for the antinocieptive activity of κ receptor agonists such as U50,488H (Piercey et al., 1982; Han et al., 1984; Jhamandas et al., 1986). In addition, κ agonists may diminish the respiratory depression induced by μ agonists (Verborgh et al., 1997). However, human studies involving the κ agonist enadoline were disappointing since no reduction in experimentally induced hyperalgesia in volunteers was observed (Pande et al., 1996a) and efficacy in postoperative pain for patients undergoing knee surgery was hampered by neuropsychiatric adverse effects (Pande et al., 1996b). As with other opioids, adjunctive agents could potentially allow lower doses of κ agonists to provide adequate pain relief, thereby reducing side effects.

In the present study, the interactions of μ-, δ- and κ-opioids with two clinically available NMDA antagonists (ketamine and dextromethorphan) were compared within a single study of acute nociception. Classical morphine was selected along with fentanyl and sufentanil, which are highly selective for the μ-opioid receptor (Maguire et al., 1992). The δ agonist SNC80 and κ-opioid agonist U50,488H are well established prototypical δ and κ agonists, respectively. A hot-plate test was used as a means of assessing acute antinociceptive activity sensitive to spinal and supraspinal mechanisms.

2. Methods

2.1. Animals

Approval from the Institutional Animal Care and Use Committee was obtained to perform the described experiments.

Male NMRI mice obtained from Iffa Credo (Germany) were used. Mice were housed for 2 weeks in colonial stock cages following arrival. Animals were transferred to individual housing 24 h before experimentation. Food and water were available ad libitum at all times. The environment was temperature controlled (22 ± 1 °C) and maintained on a 12-h light/dark cycle (a.m./p.m.). All experiments were carried out during the light phase.

2.2. Hot-plate test

A metal hot-plate was maintained at 50 ± 0.5 °C. A mobile transparent colourless acrylic cylinder (diameter: 20 cm, height: 30 cm) was placed on the hot-plate to form the observation area. After each measurement, the plate was wiped in order to remove traces of urine and faeces. The temperature of the plate was monitored at all times.

2.3. Procedure

Measurements were made by placing one mouse on the hot-plate at a time and noting the response latency with a stop-watch to the nearest 0.1 s. A cut-off latency of 30 s was used. Responses were characterised by licking of either the fore- or hind-paws or by jumping. Mice were removed from the apparatus after responding or after the allocated cut-off period. Other effects such as sedation and motor impairment were noted.

A single injection was administered to each mouse. Response latencies were determined immediately before the administration of drugs to give a single preliminary (PRE) value, followed by four further measurements made at 15-min intervals until 60 min to give four postdrug measurements. For saline controls and single compounds, $n = 20$ per dose. For combinations of drugs, $n = 10$ per dose.

2.4. Drugs

Morphine–HCl was purchased from Belgopia, Louvain-La-Neuve, Belgium. Fentanyl–HCl and sufentanil–HCl were obtained from Janssen Phamaceutica, Beerse, Belgium. SNC80 and U50,488H–HCl were purchased from Tocris Cookson, Bristol, UK. Dextromethorphan–HBr and Ketamine–HCl were purchased from Sigma Aldrich, Belgium. Drugs were freshly prepared as aqueous solutions. All doses refer to base equivalents. Drugs and drug combinations were administered to mice intraperitoneally in a single 10 ml/kg volume. Control animals received an equivalent volume of physiological saline.

2.5. Statistics

Data values are expressed as mean ± S.E.M. except where otherwise stated. From the proportion of mice reaching the 30-s cut-off criterion within an experimental group,

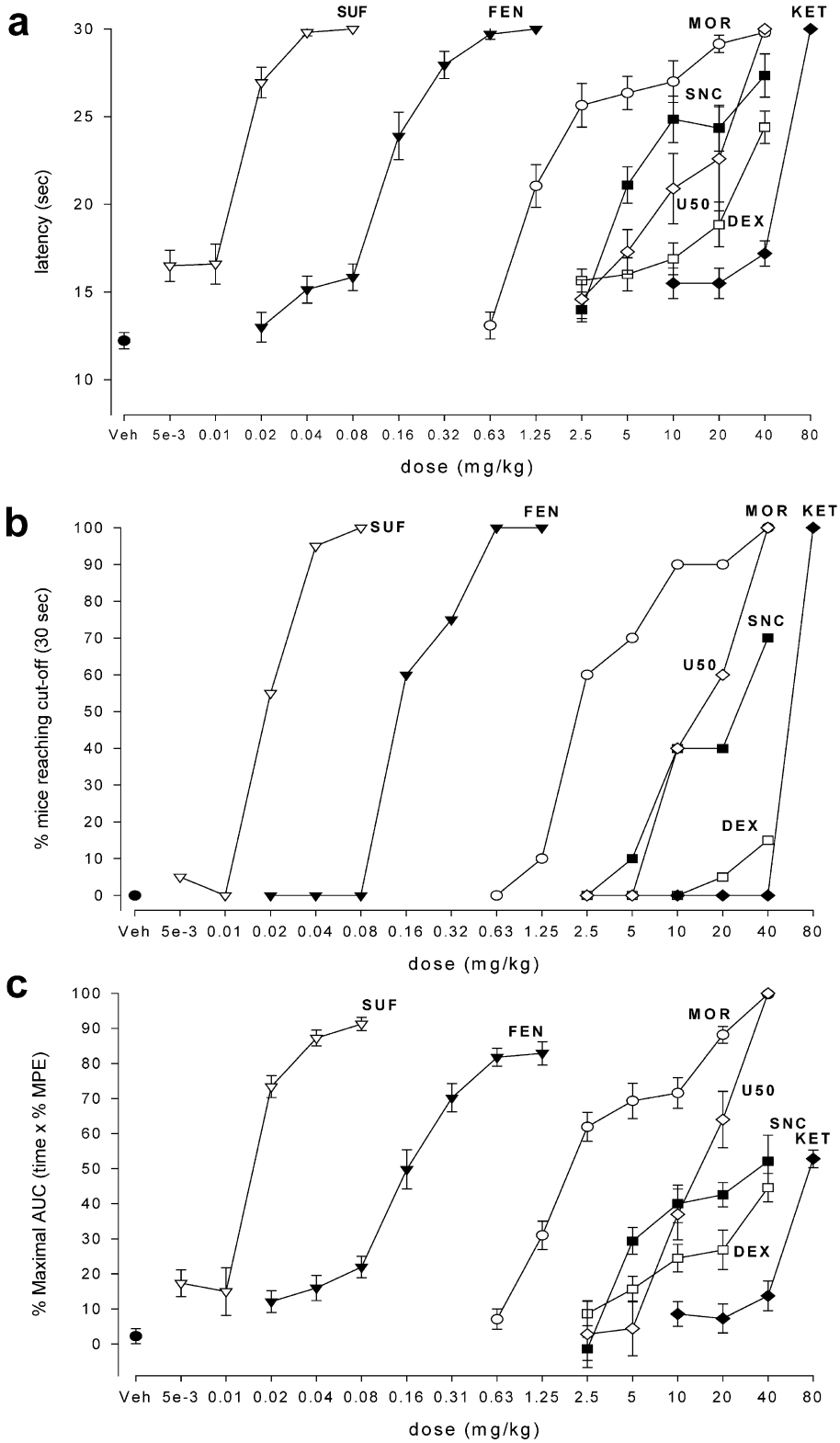

Fig. 1. Dose response relationships for single compounds after intraperionteal injection. Panel (a): mean ± S.E.M. response latencies 15 min after injection. Panel (b): percentage of subjects exhibiting at any time point, the cut-off latency of 30 s. Panel (c): mean ± S.E.M. AUC values for percentage maximum possible effect (%MPE) × time. For all groups, $n = 20$.

Table 1
ED_{50} values and 95% confidence limits for compounds alone and in combination

Compound	Alone	Potency	Dextromethorphan				Ketamine			
			10 mg/kg	Ratio	20 mg/kg	Ratio	10 mg/kg	Ratio	40 mg/kg	Ratio
Morphine	2.88 (2.20–3.78)	*1.00*	1.17 (0.88–1.56)*	*2.46*	1.90 (1.30–2.79)	*1.52*	3.31 (2.25–4.85)	*0.87*	1.44 (1.16–1.78)*	*2.00*
Fentanyl	0.176 (0.144–0.215)	*16.36*	0.053 (0.040–0.070)**	*3.32*	0.069 (0.047–0.104)*	*2.55*	0.170 (0.122–0.239)	*1.04*	0.105 (0.075–0.147)*	*1.68*
Sufentanil	0.0207 (0.0169–0.0254)	*139.13*	0.0057 (0.0043–0.0076)***	*3.63*	0.0100 (0.0075–0.0133)*	*2.07*	0.0264 (0.0198–0.0351)	*0.78*	0.0107 (0.0076–0.0151)*	*1.93*
SNC80	18.66 (14.22–24.49)	*0.15*	26.39 (18.79–37.06)	*0.71*	12.31 (8.77–17.29)	*1.52*	12.31 (8.38–18.08)	*1.54*	17.41 (14.05–21.58)	*1.07*
U50,488H	13.20 (9.40–18.53)	*0.22*	16.25 (13.10–20.14)	*0.81*	15.16 (11.38–20.18)	*0.87*	18.66 (15.05–23.13)	*0.71*	9.33 (6.64–13.10)	*1.42*
Ketamine	56.57 (50.81–62.98)	*0.05*								
Dextromethorphan[a]	>40	–								

The potency of the individual compounds compared to morphine and the potency ratio for each compound alone and in combination are shown in italics. Differences between ED_{50} values were evaluated by means of a Student's *t*-test for independent samples using the differences between log ED_{50} values (method of Sacks, 1982). The asterisks indicate statistical significance.

[a] ED_{50} for dextromethorphan was not calculated because only 15% of the maximum possible response was observed at the doses tested. The 80 mg/kg dose was fatal.

* $P<.05$.

** $P<.01$.

*** $P<.001$.

ED_{50} values and 95% confidence limits were calculated using linear regression.

Area under curve (AUC) values were calculated from %MPE (percentage of maximal possible effect) × time. %MPE=[postdrug response − predrug response)/((30-s cut-off duration − predrug response) × 100].

Differences between experimental conditions were evaluated using a Mann–Whitney *U*-test (two-tailed). Differences in ED_{50} values were evaluated by means of the Student's *t*-test (two-tailed) for independent samples using the differences between log ED_{50} values (method of Sacks, 1982). Asterisks indicate statistical significance: * $P<.05$, ** $P<.01$, *** $P<.001$.

3. Results

Saline-treated mice exhibited mean latencies of between 11.90 ± 0.45 and 12.85 ± 0.42 s at the various pre- and postinjection time points. No saline treated mice reached the cut-off criterion of 30 s.

All compounds when administered alone resulted in a dose-related increase in response latency after 15 min (Fig. 1a). However, SNC80 and dextromethorphan did not produce a complete block of responses in all animals at any of the doses tested. At the highest dose tested, 80 mg/kg, dextromethorphan proved lethal. Morphine (from 1.25 mg/kg), fentanyl (from 0.16 mg/kg) and sufentanil (from 0.02 mg/kg) produced μ-opioid-related behavioural changes: arched back, Straub-tail reaction and motor excitement. SNC80 produced sedation at 20 and 40 mg/kg. Ketamine caused marked motor impairment at 80 mg/kg.

Similar results were observed with regard to the percentage of animals reaching the 30-s cut-off criterion at any time point (Fig. 1b). SNC80 and dextromethorphan (40 mg/kg for both) resulted in 70% and 15%, respectively, of animals reaching the cut-off criterion of 30 s, whereas all other drugs produced the maximal effect of 100% at the highest doses

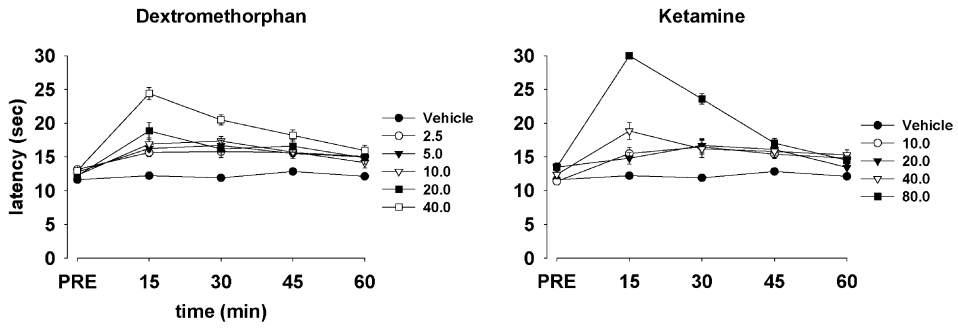

Fig. 2. Effect of dextromethorphan and ketamine on response latencies over time. Given are the mean ± S.E.M. response latencies for the different doses tested (shown in key, Mg/kg), $n=20$ per dose, $n=20$ for saline controls.

tested. An ED_{50} value was not therefore calculated for dextromethorphan. Ketamine produced a value of 0% at 40 mg/kg and a value of 100% at 80 mg/kg, which produced motor impairment. The order of potency with regard to the ED_{50} values calculated from this criterion was: sufentanil> fentanyl>morphine>U50,488H>SNC80>ketamine (Table 1).

All drugs dose-dependently increased AUC values (Fig. 1c). Morphine and U50,488H produced AUC values of 100% at the highest doses tested. Fentanyl and sufentanil

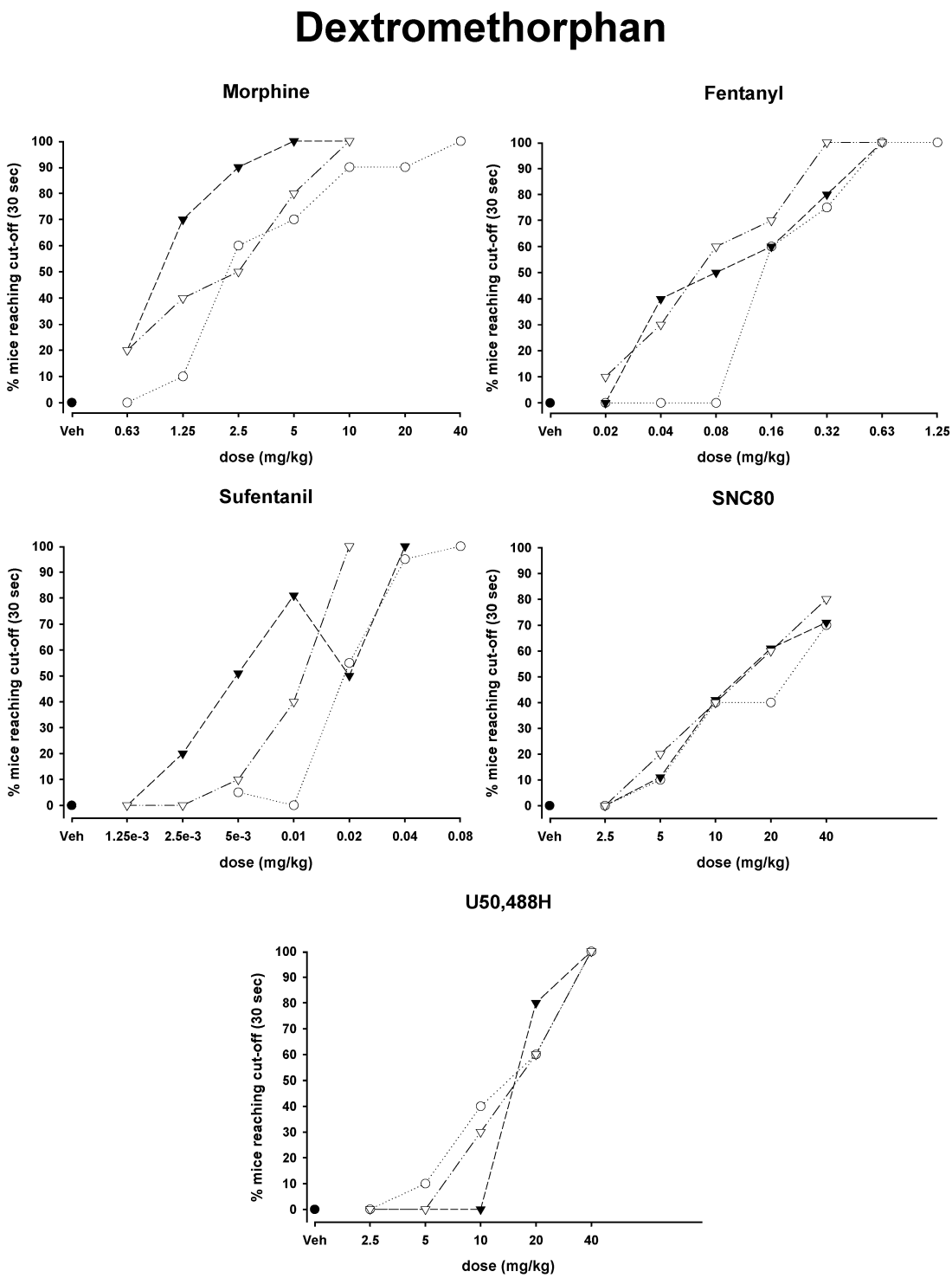

Fig. 3. Effect of dextromethorphan on opioid antinociception. Given are the percentage of subjects exhibiting at any time point, the cut-off latency of 30 s. Saline (●), vehicle (○), dextromethorphan 10 (▼) or 20 (▽) mg/kg, $n = 20$ for opioid groups, $n = 10$ for combinations.

produced near maximal AUC values of 82.84 ± 3.31% and 91.23 ± 1.89%, respectively. By contrast, SNC80, dextromethorphan and ketamine produced lower maximal values of 52.09 ± 7.42%, 44.59 ± 4.07% and 52.82 ± 2.50%, respectively, at the highest doses tested.

Based on the presence of similar antinociceptive effects, the following doses of dextromethorphan and ketamine were selected for combination studies with opioids: 10 and 20 mg/kg dextromethorphan and 10 and 40 mg/kg ketamine (Fig. 2). Dextromethorphan produced mean

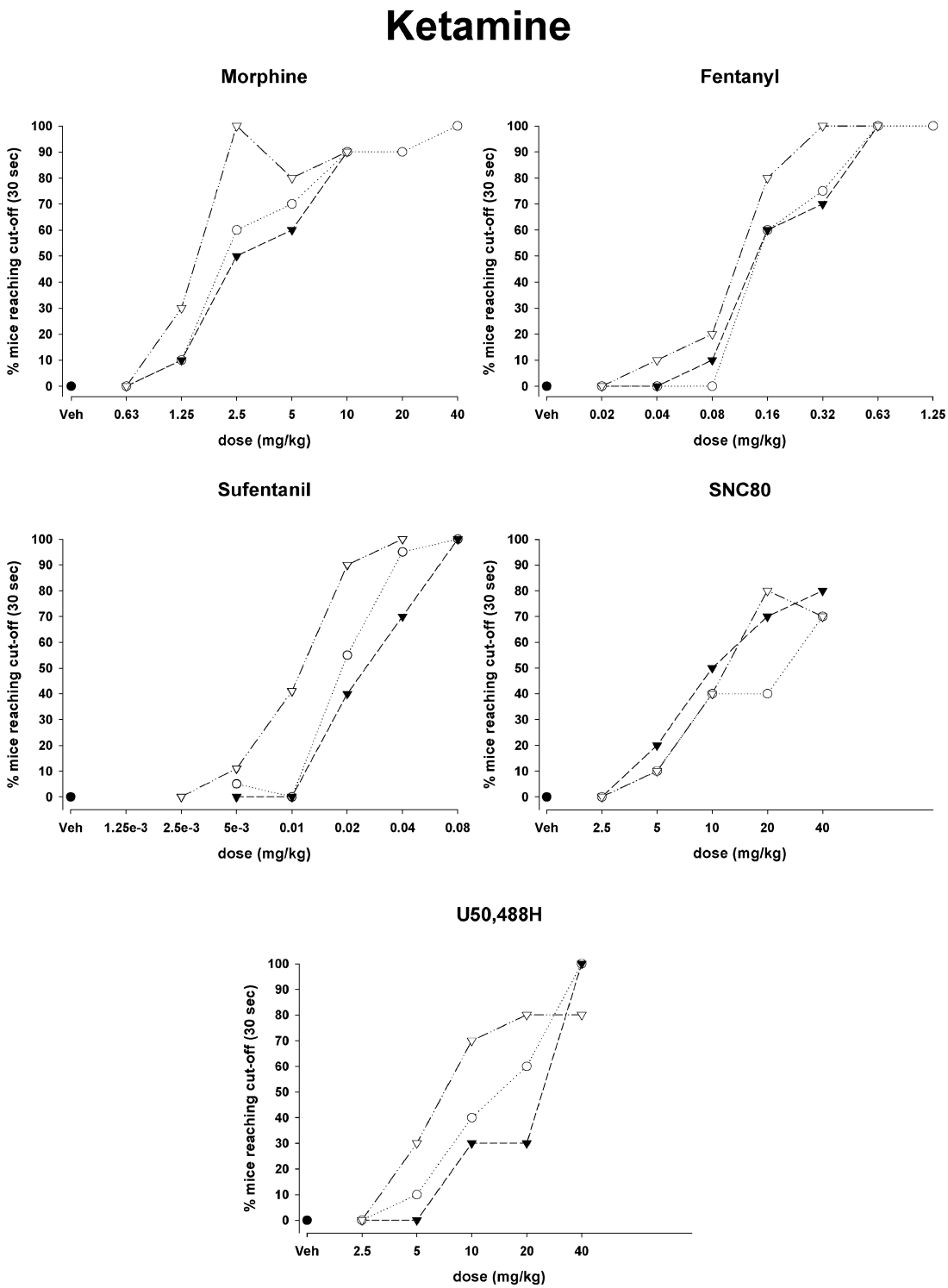

Fig. 4. Effect of ketamine on opioid antinociception. Given are the percentage of subjects exhibiting at any time point, the cut-off latency of 30 s. Saline (●), vehicle (○), ketamine 10 (▼) or 40 (▽) mg/kg, $n = 20$ for opioid groups, $n = 10$ for combinations.

response latencies of 16.90 ± 0.91 and 17.35 ± 0.69 s 15 and 30 min, respectively, following the 10 mg/kg dose. At 20 mg/kg, latencies of 18.85 ± 1.27 and 16.20 ± 1.27 s were produced after 15 and 30 min, respectively. Ketamine at 10 mg/kg produced mean response latencies of 15.50 ± 0.87 and 16.50 ± 1.10 s after 15 and 30 min, respectively. At 40 mg/kg, ketamine produced response latencies of 17.20 ± 0.72 and 15.00 ± 0.93 s after 15 and 30 min, respectively.

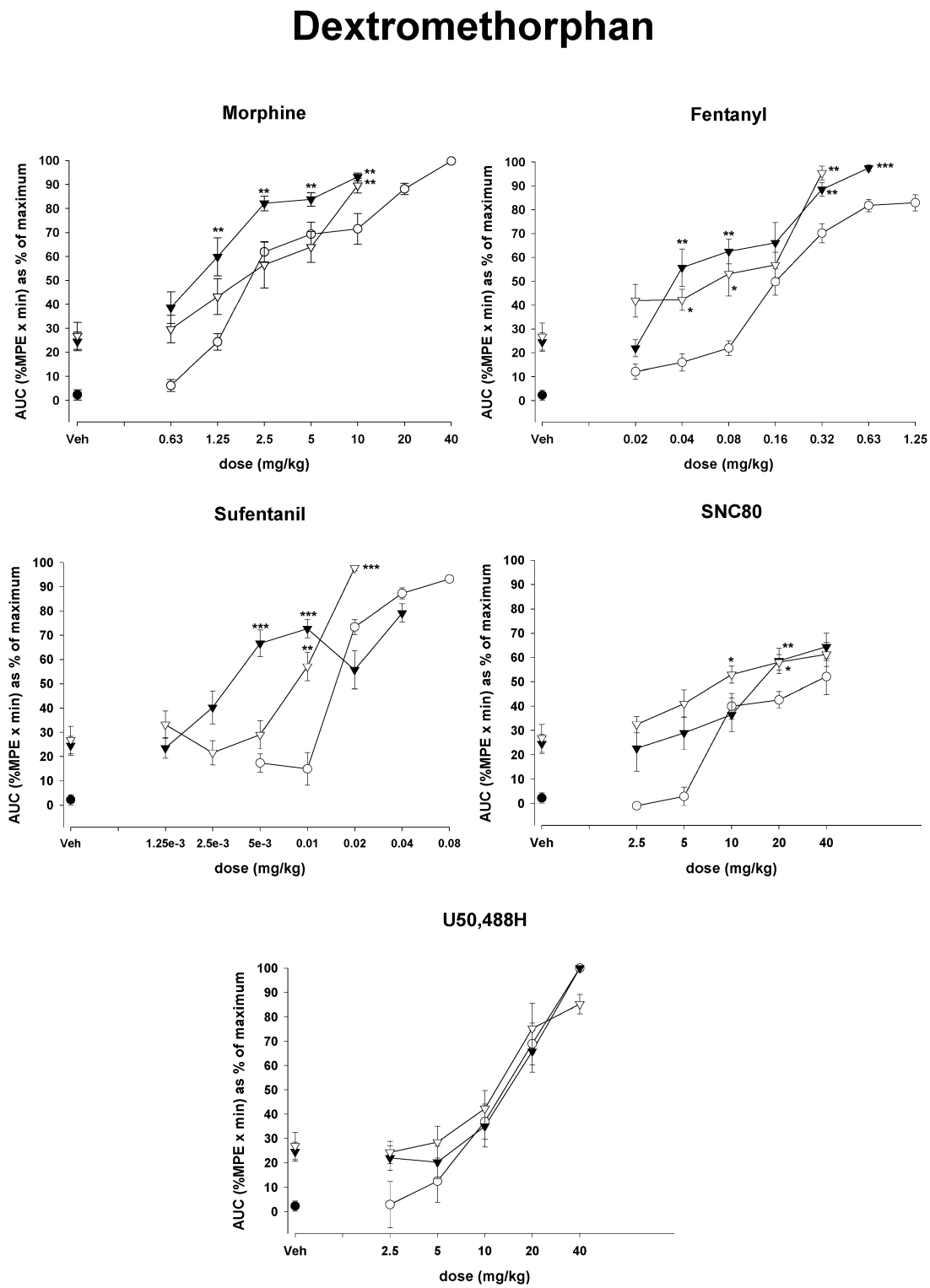

Fig. 5. Effect of dextromethorphan on opioid antinociception. Given are the mean ± S.E.M. AUC values for the various opioid doses (mg/kg; see panels), $n = 20$ for opioid groups, $n = 10$ for combinations. Saline (●), vehicle (○), dextromethorphan 10 (▼) or 20 (▽) mg/kg. The asterisks indicate statistical significance of combination groups from both opioid/vehicle groups and dextromethorphan alone. (* $P < .05$, ** $P < .01$, *** $P < .001$) Mann–Whitney U-test (two-tailed).

In combination with the different opioids, NMDA antagonists did not produce any obvious changes in morphine, fentanyl and sufentanil-induced excitation or SNC80 and U50,488H-induced sedation. Dextromethorphan at the 10 mg/kg dose level produced reductions in the ED_{50} values for morphine, fentanyl and sufentanil from 2.88 (2.20–3.78), 0.176 (0.144–0.215) and 0.207 (0.0169–0.0254) mg/kg to 1.17 (0.88–1.56), 0.053 (0.040–0.070) and 0.0057 (0.0043–0.0076) mg/kg, respectively (Table 1 and Fig. 3). However, the 20 mg/kg dose did not potentiate the antinociceptive effects of morphine. Ketamine did not produce any change in ED_{50} values for the opioids at 10 mg/kg

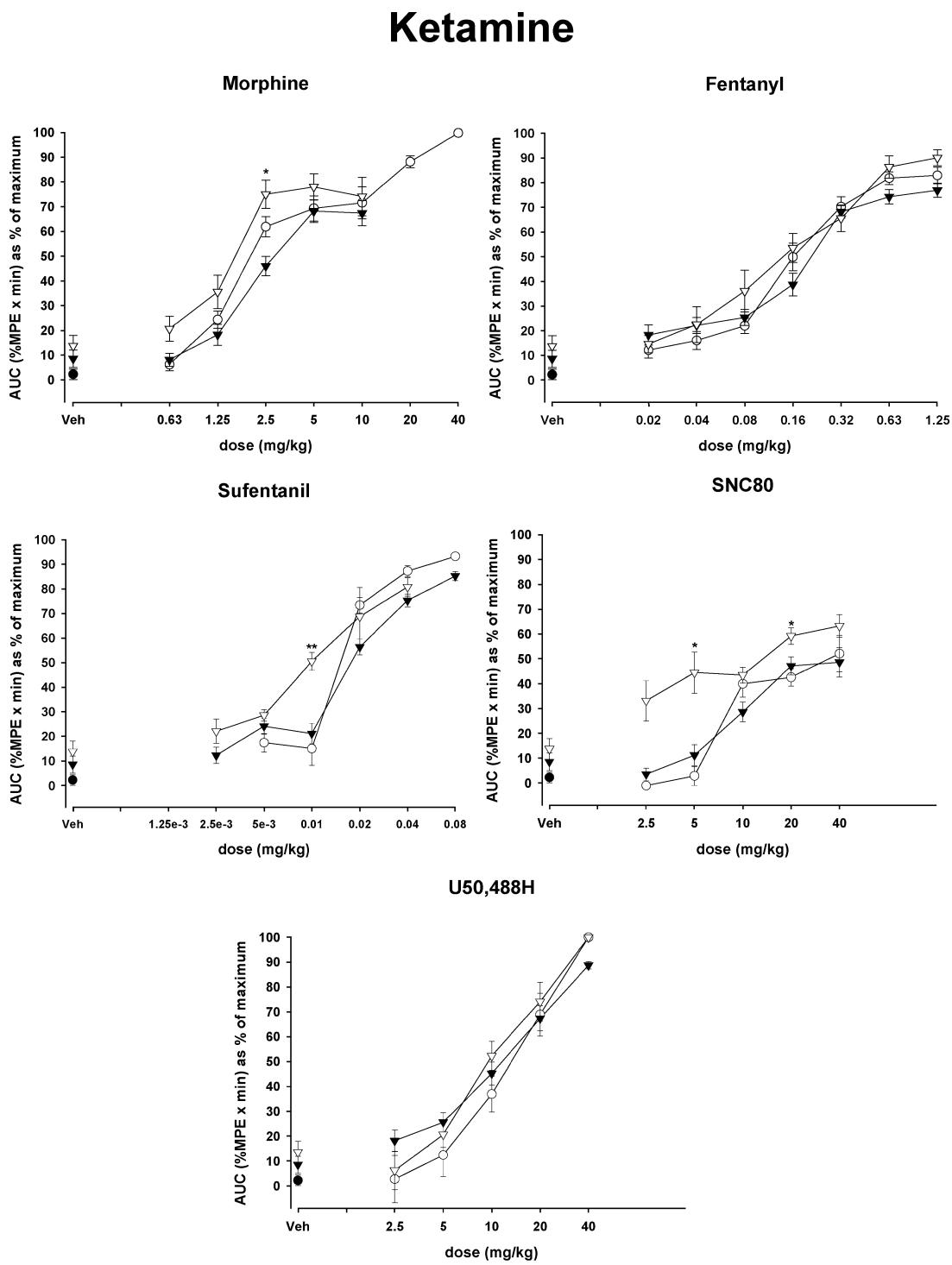

Fig. 6. Effect of ketamine on opioid antinociception. Given are the mean ± S.E.M. AUC values for the various opioid doses (mg/kg; see panels), $n = 20$ for opioid groups, $n = 10$ for combinations. Saline (●), vehicle (○), ketamine 10 (▼) or 40 (▽) mg/kg. The asterisks indicate statistical significance of combination groups from both opioid/vehicle groups and ketamine alone. ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$) Mann–Whitney *U*-test (two-tailed).

(Table 1 and Fig. 4). At 40 mg/kg ketamine, ED_{50} values of morphine, fentanyl and sufentanil decreased to 1.44 (1.16–1.78), 0.105 (0.075–0.147) and 0.0107 (0.0076–0.0151) mg/kg, respectively. Neither dose of ketamine or dextromethorphan affected the ED_{50} values of SNC80 and U50,488H.

Analysis of AUC values revealed differences between dextromethorphan and ketamine in their interactions with opioids. For morphine, fentanyl and sufentanil, the number of animals reaching the fixed cut-off criteria was reflected by the AUC values for the different doses of the opioids in combination with dextromethorphan (Fig. 5). The AUC values for of 10 mg/kg morphine, 0.32 mg/kg fentanyl and 0.01 mg/kg sufentanil were increased by 10 and 20 mg/kg dextromethorphan similarly. These doses of the opioids alone produced AUC values of 71.54 ± 6.45%, 70.19 ± 4.04% and 14.95 ± 6.78%, respectively. With 10 mg/kg dextromethorphan these values were 93.18 ± 1.64%, 88.46 ± 2.88% and 72.65 ± 3.70%, respectively. Dextromethorphan at 20 mg/kg gave rise to values of 89.53 ± 3.12%, 95.31 ± 2.92% and 57.1 ± 5.86%, respectively. However, lower doses of these opioids were potentiated more strongly by 10 mg/kg dextromethorphan than by the higher 20 mg/kg dose. In addition, the AUC for 10 and 20 mg/kg SNC80 was increased slightly by dextromethorphan. At 20 mg/kg dextromethorphan, the AUC values for 10 and 20 mg/kg SNC80 were increased from 39.96 ± 5.37 and 42.57 ± 3.48% to 52.93 ± 3.59% and 58.00 ± 3.17%, respectively. No effect on U50,488H was observed. In contrast with the effect of dextromethorphan, ketamine produced less effect on AUC values for morphine and sufentanil and no effect on any dose of fentanyl was measured (Fig. 6). Ketamine at 40 mg/kg raised the AUC value only for 2.5 mg/kg morphine (from 61.90 ± 4.11% to 75.00 ± 5.73%) and 0.01 mg/kg sufentanil (14.95 ± 6.78% to 50.49 ± 3.61%). Increases in the AUC values for 5 and 20 mg/kg SNC80 were observed with 40 mg/kg ketamine from 2.89 ± 3.82% and 42.57 ± 3.48% for the two doses of SNC80 alone to 44.49 ± 8.31 and 59.17 ± 3.29 in combination. No effect of ketamine of U50,488H was found.

Owing to the large amount of data, full time courses are only shown graphically (Figs. 7–10) for selected interactions for submaximal doses of opioids, which cannot be sufficiently described by Figs. 3–6. Analysis of latencies over time revealed that the peak effect of 10 mg/kg dextromethorphan on 1.25 mg/kg morphine occurred after 30 min, producing an increase in latency from 18.13 ± 0.77 to 26.80 ± 1.48 s (Fig. 7). The maximal effect of 20 mg/kg dextromethorphan on 1.25 mg/kg morphine occurred 15 min later, represented by an increase from 17.41 ± 1.07 to 23.13 ± 2.53 s, which was comparable to 10 mg/kg dextromethorphan (24.47 ± 1.18 s). Ketamine exerted a significant effect on 2.5 mg/kg morphine at 40 but not at 10 mg/kg, producing the maximal latency after 30 min of 30.0 s in all animals although no significant effects were evidenced at other time points. As with 1.25 mg/kg morphine, the effect of 10 mg/kg dextromethorphan on 0.04 mg/kg fentanyl was seen earlier than with 20 mg/kg dextromethorphan; after 15 min from 15.01 ± 0.85 to 20.97 ± 1.65 s (Fig. 8). A significant effect on 0.04 mg/kg fentanyl seen with 20 mg/kg dextromethorphan was not observed until 45 min, with a latency of 21.41 ± 2.04 s compared to 15.17 ± 0.76 s with

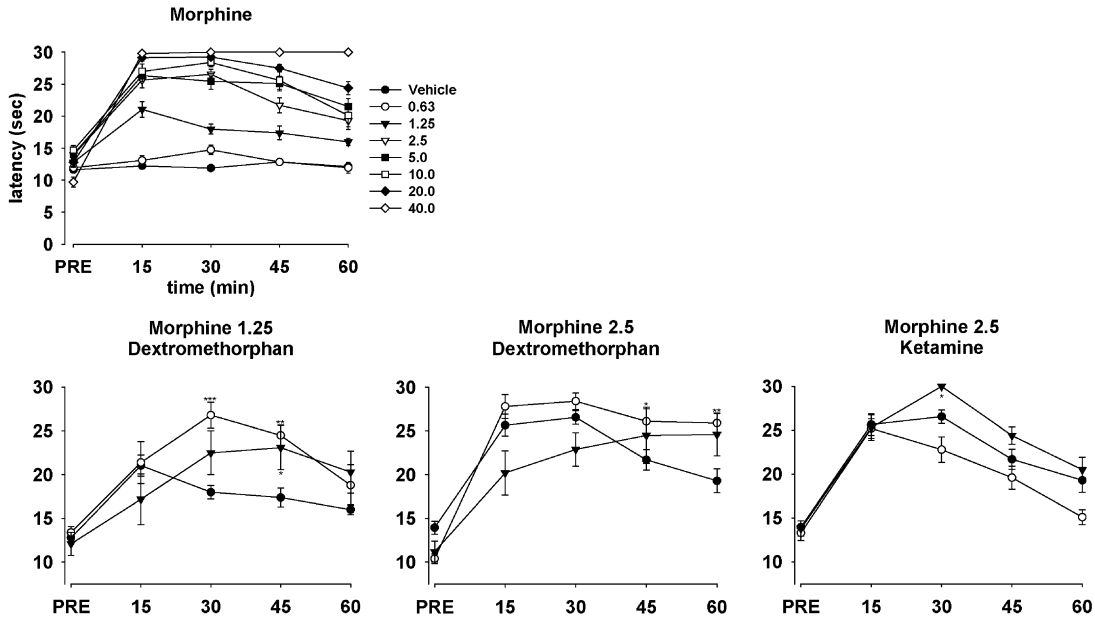

Fig. 7. Effect of dextromethorphan or ketamine on opioid antinociception over time. Given are the mean ± S.E.M. response latencies over time for the various doses of opioids alone (top plot) or for fixed doses of opioids in combination with 10 mg/kg dextromethorphan or ketamine (○) $n = 10$, or in combination with 20 mg/kg dextromethorphan or 40 mg/kg ketamine (▼) $n = 10$. Opioid vehicle controls are also shown (●) $n = 20$. The asterisks indicate statistical significance of combination groups from opioid vehicle groups. ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$) Mann–Whitney U-test (two-tailed).

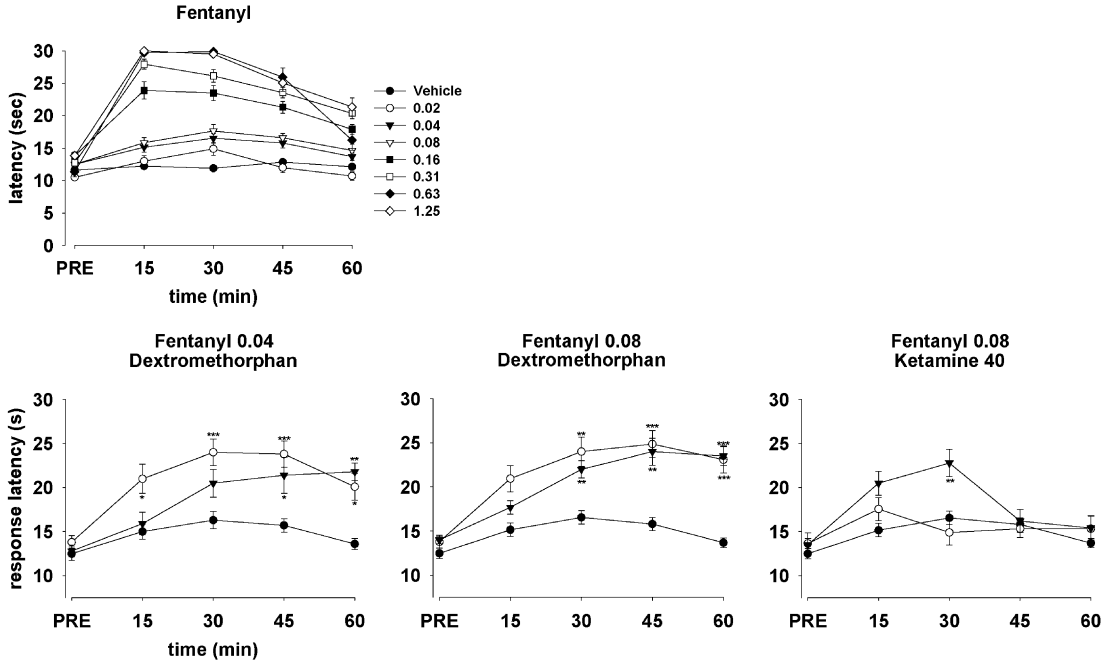

Fig. 8. Effect of dextromethorphan or ketamine on opioid antinociception over time. Given are the mean ± S.E.M. response latencies over time for the various doses of opioids alone (top plot) or for fixed doses of opioids in combination with 10 mg/kg dextromethorphan or ketamine (○) $n = 10$, or in combination with 20 mg/kg dextromethorphan or 40 mg/kg ketamine (▼) $n = 10$. Opioid vehicle controls are also shown (●) $n = 20$. The asterisks indicate statistical significance of combination groups from opioid vehicle groups. (* $P < .05$, ** $P < .01$, *** $P < .001$) Mann–Whitney U-test (two-tailed).

0.04 mg/kg fentanyl alone, which was comparable to that seen with 10 mg/kg dextromethorphan (23.81 ± 1.48 s). There were no differences between the effects of 10 and 20 mg/kg dextromethorphan on 0.08 mg/kg fentanyl, with peak effects occurring after 45 min for both doses of dextromethorphan. Only 40 mg/kg ketamine produced a significant effect on 0.08 mg/kg fentanyl, occurring after 30 min, from 15.80 ± 0.99 to 22.79 ± 1.52 s. Similarly to morphine and fentanyl, significant effects of 10 mg/kg dextromethorphan on 0.005 mg/kg sufentanil occurred earl-

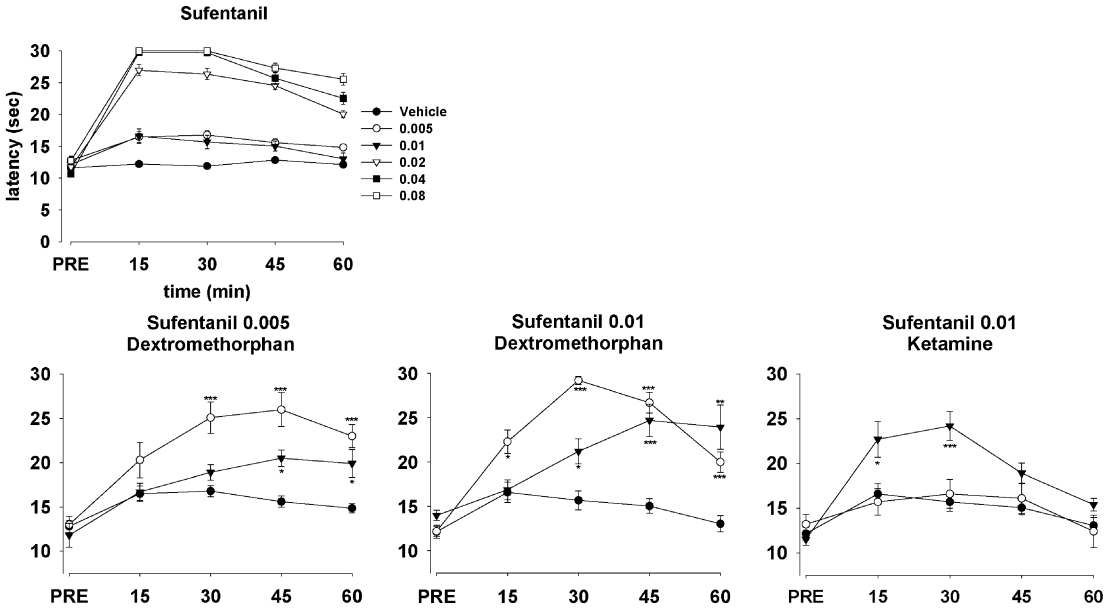

Fig. 9. Effect of dextromethorphan or ketamine on opioid antinociception over time. Given are the mean ± S.E.M. response latencies over time for the various doses of opioids alone (top plot) or for fixed doses of opioids in combination with 10 mg/kg dextromethorphan or ketamine (○) $n = 10$, or in combination with 20 mg/kg dextromethorphan or 40 mg/kg ketamine (▼) $n = 10$. Opioid vehicle controls are also shown (●) $n = 20$. The asterisks indicate statistical significance of combination groups from opioid vehicle groups. (* $P < .05$, ** $P < .01$, *** $P < .001$) Mann–Whitney U-test (two-tailed).

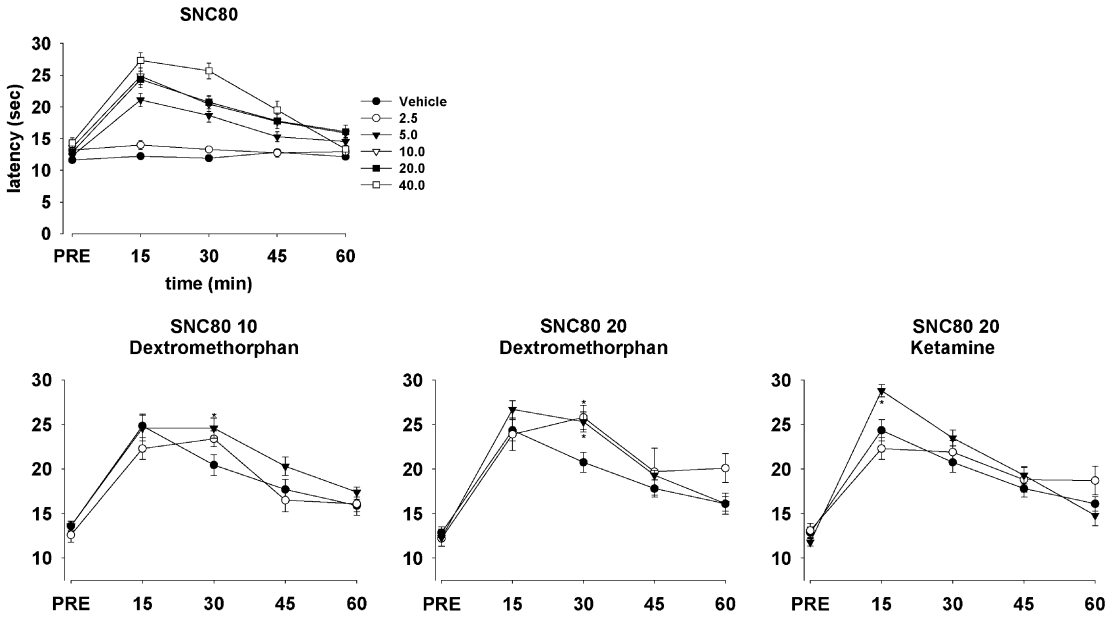

Fig. 10. Effect of dextromethorphan or ketamine on opioid antinociception over time. Given are the mean ± S.E.M. response latencies over time for the various doses of opioids alone (top plot) or for fixed doses of opioids in combination with 10 mg/kg dextromethorphan or ketamine (○) $n = 10$, or in combination with 20 mg/kg dextromethorphan or 40 mg/kg ketamine (▼) $n = 10$. Opioid vehicle controls are also shown (●) $n = 20$. The asterisks indicate statistical significance of combination groups from opioid vehicle groups. (* $P < .05$, ** $P < .01$, *** $P < .001$) Mann–Whitney U-test (two-tailed).

ier (after 30 min) than 20 mg/kg dextromethorphan (after 45 min), although the peak effect of both doses occurred after 45 min with values of 25.96 ± 1.51 and 20.50 ± 0.93 s for 10 and 20 mg/kg dextromethorphan respectively compared to 15.60 ± 0.63 s for sufentanil alone (Fig. 9). The effect of 10 mg/kg dextromethorphan on 0.01 mg/kg sufentanil occurred after 15 min with a latency of 22.33 ± 1.33 s compared to 16.62 ± 1.14 s for 0.01 mg/kg sufentanil alone. At 30 min, the effect of 10 mg/kg dextromethorphan peaked with a latency of 29.20 ± 0.47 s compared to 21.19 ± 1.40 s for 20 mg/kg dextromethorphan. By comparison, at 45 and 60 min the effect of the two doses of dextromethorphan were similar (Fig. 9). The effect of 40 mg/kg ketamine on 0.01 mg/kg sufentanil began after 15 min and peaked after 45 min although no differences were seen at later time points. Dextromethorphan co-administration did not elevate the peak effect of SNC80 although the duration of action of 10 mg/kg SNC80 was longer with 20 mg/kg dextromethorphan, as was 20 mg/kg SNC80 co-administered with 10 or 20 mg/kg dextromethorphan, evidenced by elevated latencies after 30 min (Fig. 10). The peak effect of 40 mg/kg ketamine on 20 mg/kg SNC80 after 30 min was a latency of 28.80 ± 0.68 s compared to 24.35 ± 1.19 s for SNC80 alone. No effects of NMDA antagonists on U50,488H were seen.

4. Discussion

Although many previous studies have investigated interactions of NMDA antagonists with opioids, they have tended to focus on one or two opioid receptor types and so the specific methods used in each case have clouded the direct comparison of the different effects of μ, δ and κ receptors. In the comparison presented here, we found that dextromethorphan and ketamnine did not change the potency of SNC80 or U50,488H under conditions which clearly potentiate the antinociceptive effects of μ agonists.

In the mouse hot-plate test described, morphine, fentanyl, sufentanil and U50,488H all produced dose-related antinociception with full efficacy at sufficient doses. SNC80 also produced dose-related antinociception although the maximum observed effect at the highest dose, was complete blockade of a behavioural response in only 70% of subjects. Dextromethorphan produced complete blockade of a response in only 15% of subjects at 40 mg/kg. The activity of dextromethorphan was too weak to calculate an ED_{50} value. At the highest dose tested, 80 mg/kg, dextromethorphan proved lethal. Ketamine was shown to produce short acting activity at the highest dose tested, although this dose produced marked motor effects and therefore it cannot be concluded that full antinociceptive activity occurs in this test. These findings observed for individual agents closely resemble the preclininical and clinical literature (see Section 1). Clinical data have indicated that κ agonists do not provide sufficient efficacy or are associated with unacceptable adverse effects (Pande et al., 1996a,b). There are currently no published clinical data available regarding the clinical efficacy of δ-opioid agonists.

However, the major finding is that in combination with opioids, the NMDA antagonists increased the potency of μ agonists, but not δ or κ agonists, as evidenced by the peak activities. The theory of acute opioid tolerance and the effect

of NMDA antagonists was born from the finding that the antinociceptive action of morphine lasts for a much shorter time than the serum and brain concentrations would suggest (Kissin et al., 1991). Opioid receptor stimulation may enhance glutamatergic transmission through activation of protein kinase C (Chen and Huang, 1991) and decreased expression of mRNA for NMDA receptor subunits (Le Greves et al., 1998), which subsequently reduces opioid action, at least at the spinal level. Anatomically, μ-opioid receptors and NMDA receptors are co-localized on synaptic membranes in the dorsal horn and periaqueductal grey matter (PAG) (Commons et al., 1999). The effect of NMDA antagonists on opioids may be different at the different sites within the nociceptive pathway, with inhibition occurring after NMDA antagonist administration into the PAG (Jacquet, 1988). Attenuation of the effect of systemic morphine in a tail-flick assay by MK801 was also demonstrated (Lufty et al., 1993). This could imply that NMDA antagonists exert bidirectional effects on opioid antinociception. Specific nociceptive pathways could interact differently with NMDA antagonists as shown by recordings from the tail and the hind paw (Kozela et al., 2001). Spinal and supraspinal mechanisms have also been suggested previously for the interaction of NMDA antagonists with δ-opioid agonists (Bhargava and Zhao, 1996b; Suzuki et al., 2000; Allen et al., 2002).

In accordance with this idea, the difference seen in the present study between the two doses of dextromethorphan should be addressed. However, it is not possible to conclude that 10 mg/kg dextromethorphan was more effective than the 20 mg/kg dose in potentiating the potency of morphine, fentanyl and sufentanil, since the ED_{50} values for each of the μ-opioids combined with the two doses of this agent are not different from each other. However, significant effects of 20 mg/kg dextromethorphan on morphine, fentanyl and sufentanil antinociception occurred later than with 10 mg/kg dextromethorphan, although effects after 45 or 60 min tended to be similar. In addition, area under the curve values for morphine, fentanyl and sufentanil reveal a smaller effect of 20 compared to 10 mg/kg dextromethorphan with lower doses of opioids, but a similar or greater effect of the higher dose with higher doses of opioids. Taken together, these findings may point to the presence of an inhibitory and synergistic action of dextromethorphan on μ-opioids. It has already been suggested that the action which predominates may depend on the nature of the nociceptive stimulus, but here we have shown that the relative opioid and NMDA receptor occupation may also be important. The injection interval may be critical, since some NMDA antagonists only affected morphine tolerance 120 but not 30 min after morphine (Belozertseva et al., 2000). This raises the issue of the pharmacokinetic properties of the NMDA antagonists used, since relatively higher or lower plasma concentrations could be achieved by administering at different time points. Ketamine is metabolised more rapidly than dextromethorphan and this alone could account for the differences between the two NMDA antagonists in AUC values in combination with opioids.

There were no marked differences between morphine and the other μ-opioids fentanyl and sufentanil. It is not clear whether the mechanisms of acute tolerance share any similarities with tolerance after repeated administration. Chronic tolerance induced by repeated injections of intracerebroventricular or subcutaneous morphine could be blocked by concomitant NMDA antagonism but this was not seen with the high efficacy μ selective agonists fentanyl or DAMGO (Bilsky et al., 1996). Present findings may suggest that acute tolerance involves markedly different mechanisms compared to chronic tolerance, apparently unaffected by the intrinsic efficacy or selectivity of the specific opioid used.

There are currently no published clinical data comparing the effectiveness of different opioids in combination with NMDA antagoinists in a single study. Preoperatively administered intravenous dextromethorphan was shown to diminish postoperative morphine needs after laparotomy as compared with postoperative injection (Chia et al., 1999). In addition, oral dextromethorphan has been found to result in an immediate, but modest and short lasting reduction of postoperative morphine consumption after knee surgery and abdominal hysterectomy respectively (Ilkjaer et al., 2000; Wadhwa et al., 2001). In chronic pain management, the combination of morphine and dextromethorphan significantly reduced the daily morphine dose in patients with moderate to severe chronic pain (Chevlen, 2000). Few clinical reports describe the effects of NMDA receptor antagonists on fentanyl-like opioids. A human experimental study revealed no advantage of the combination of alfentanil and ketamine over either drug alone in relieving pain caused by intradermal capsaicin (Sethna et al., 1998). Ketamine has been investigated in clinical practice via several routes of administration. Pretreatment with epidural ketamine potentiated morphine induced analgesia, whereas ketamine itself in monotherapy did not produce significant analgesia (Wong et al., 1996). An additional value of ketamine in acute pain may be the inhibition of secondary hyperalgesia (Warncke et al., 2000). Differences between these NMDA antagonists therefore exist although further studies are required to compare the clinical effectiveness of ketamine and dextromethorphan in combination with opioids. Since these clinical experiences appear to match preclinical data, one might expect no advantage from combining NMDA antagonists with δ- or κ-opioid agonists, based on extrapolation of the present findings.

Co-administration of NMDA antagonists with δ- and κ-opioids has not been studied clinically. Our preclinical data suggest that dextromethorphan or ketamine produce no changes in the peak antinociceptive effects of SNC80 or U50,488H, although some increases over time were evidenced with SNC80. Therefore differences not only exist between the NMDA antagonists but also between the different opioid receptor types. In one study, intracerebro-

ventricular administration of NMDA antagonists and δ agonists produced attenuation in rat hot-plate and tail-flick assays (Bhargava and Zhao, 1996b; Suzuki et al., 2000). However, spinal administration in squirel monkeys revealed potentiation of SNC80 by dextromethorphan (Allen et al., 2002). This may highlight a spinal mechanism of action, counteracted by a supraspinal one. Alternatively, there may exist species differences in responsiveness to δ agonists either generally, or in specific test procedures. Together, these studies predict that systemic δ agonists with NMDA antagonists will not be any more useful clinically than either alone.

No effects were observed on the κ agonist U50,488H with NMDA antagonists. The κ-opioid receptor is draws less attention than the μ and δ types due to the anticipated high side-effect profile including cognitive disturbance and dysphoria (Pande et al., 1996a,b). However, previous work showed an attenuation by MK801 on U50,488H but not morphine (Kest et al., 1992). The effects are likely to be test specific and may also involve routes of administration. For example, one might expect different result in the cord and the brain. Systemic co-administration, however, has shown a lack of any interaction.

In conclusion, we have found that dextromethorphan and ketamine resulted in potentiation of the antinociceptive potency of the μ-opioids morphine, fentanyl and sufentanil but not the δ agonist SNC80 or the κ agonist U50,488H. The different μ-opioids were potentiated by these NMDA antagonists to a similar degree. These findings are in agreement with preclinical and clinical data, and predict a lack of clinical advantage for NMDA antagonist combinations with δ- or κ-opioids administered systemically for management of acute pain.

References

Adriaenssens G, Vermeyen KM, Hoffmann VL, Mertens E, Adriaensen HF. Postoperative analgesia with i.v. patient-controlled morphine: effect of adding ketamine. Br J Anaesth 1999;83:393–6.

Advokat C, Rhein FQ. Potentiation of morphine-induced antinociception in acute spinal rats by the NMDA antagonist dextrorphan. Brain Res 1995;699:157–60.

Allen RM, Dykstra LA. Role of morphine maintenance dose in the development of tolerance and its attenuation by an NMDA receptor antagonist. Psychopharmacology 2000;148:59–65.

Allen RM, Dykstra LA. *N*-Methyl-D-aspartate receptor antagonists potentiate the antinociceptive effects of morphine in squirrel monkeys. J Pharmacol Exp Ther 2001;298:288–97.

Allen RM, Granger AL, Dykstra LA. Dextromethorphan potentiates the antinociceptive effects of morphine and the delta-opioid agonist SNC80 in squirrel monkeys. J Pharmacol Exp Ther 2002;300:435–41.

Belozertseva IV, Dravolina OA, Neznanova ON, Danysz W, Bespalov AY. Antinociceptive activity of combination of morphine and NMDA receptor antagonists depends on the inter-injection interval. Eur J Pharmacol 2000;396:77–83.

Bhargava HN, Thorat SN. Effect of dizocilpine (MK-801) on analgesia and tolerance induced by U-50,488H, a kappa-opioid receptor agonist, in the mouse. Brain Res 1994;649:111–6.

Bhargava HN, Zhao GM. Effects of *N*-methyl-D-aspartate receptor antagonists on the analgesia and tolerance to D-Ala-2,Glu-4 deltorphin II, a delta2-opioid receptor agonist in mice. Brain Res 1996a;719:56–61.

Bhargava HN, Zhao GM. Effects of competitive and noncompetitive antagonists of the *N*-methyl-D-aspartate receptor on the analgesic action of delta1- and delta2-opioid receptor agonists in mice. Br J Pharmacol 1996b;119:1586–90.

Bilsky EJ, Calderon SN, Wang T, Bernstein RN, Davis P, Hruby VJ, McNutt RW, Rothman RB, Rice KC, Porreca F. SNC 80, a selective, nonpeptidic and systemically active opioid delta agonist. J Pharmacol Exp Ther 1995;273:359–66.

Bilsky EJ, Inturrisi CE, Sadee W, Hruby VJ, Porreca F. Competitive and non-competitive NMDA antagonists block the development of antinociceptive tolerance to morphine, but not to selective μ or δ opioid agonists in mice. Pain 1996;68:229–37.

Bristow A, Orlikowski C. Subcutaneous ketamine analgesia: postoperative analgesia using subcutaneous infusions of ketamine and morphine. Ann R Coll Surg Engl 1989;71:64–6.

Calcagnetti DJ, Holtzman SG. Delta opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine. Pharmacol Biochem Behav 1991;38:185–90.

Calderon SN, Rothman RB, Porreca F, Flippen-Anderson JL, McNutt RW, Xu H, Smith LE, Bilsky EJ, Davis P, Rice KC. Probes for narcotic receptor mediated phenomena: 19. Synthesis of (+)-4-[(alpha *R*)-alpha-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC 80): a highly selective, nonpeptide delta opioid receptor agonist. J Med Chem 1994;37:2125–8.

Chapman V, Dickenson AH. The combination of NMDA antagonism and morphine produces profound antinociception in the rat dorsal horn. Brain Res 1992;573:321–3.

Chen L, Huang LYM. Sustained potentiation of NMDA receptor-mediated glutamate responses through activation of protein kinase C by a mu opioid. Neuron 1991;7:319–26.

Chevlen E. Morphine with dextromethorphan: conversion from other opioid analgesics. J Pain Symptom Manage 2000;19:S42–9.

Chia YY, Liu K, Chow LH, Lee TY. The preoperative administration of intravenous dextromethorphan reduces postoperative morphine consumption. Anesth Analg 1999;89:748–52.

Comer SD, Hoenicke EM, Sable AI, McNutt RW, Chang KJ, De Costa BR, Mosberg HI, Woods JH. Convulsive effects of systemic administration of the delta opioid agonist BW373U86 in mice. J Pharmacol Exp Ther 1993;267:888–95.

Commons KG, van Bockstaele EJ, Pfaff DW. Frequent colocalization of mu opioid and NMDA-type glutamate receptors at postsynaptic sites in periaqueductal gray neurons. J Comp Neurol 1999;408:549–59.

Dykstra LA, Schoenbaum GM, Yarbrough J, McNutt R, Chang KJ. A novel delta opioid agonist, BW373U86, in squirrel monkeys responding under a schedule of shock titration. J Pharmacol Exp Ther 1993;267:875–82.

Edwards ND, Fletcher A, Cole JR, Peacock JE. Combined infusions of morphine and ketamine for postoperative pain in elderly patients. Anaesthesia 1993;48:124–7.

Grass S, Hoffmann O, Xu XJ, Wiesenfeld Hallin Z. *N*-methyl-D-aspartate receptor antagonists potentiate morphine's antinociceptive effect in the rat. Acta Physiol Scand 1996;158:269–73.

Han JS, Xie GX, Goldstein A. Analgesia induced by intrathecal injection of dynorphin B in the rat. Life Sci 1984;34:1573–9.

Helmy SAK, Bali A. The effects of the preemptive use of the NMDA receptor antagonist dextromethorphan on postoperative analgesic requirements. Anesth Analg 2001;92:739–44.

Ilkjaer S, Dirks J, Brennum J, Wernberg M, Dahl JB. Effect of systemic *N*-methyl-D-aspartate receptor antagonist (dextromethorphan) on primary and secondary hyperalgesia in humans. Br J Anaesth 1997;79:600–5.

Ilkjaer S, Bach LF, Nielsen PA, Wernberg M, Dahl JB. Effect of preoperative oral dextromethorphan on immediate and late postoperative pain and hyperalgesia after total abdominal hysterectomy. Pain 2000;86:19–24.

Jacquet YF. The NMDA receptor: Central role in pain inhibition in rat periaqueductal gray. Eur J Pharmacol 1988;154:271–76.

Javery KB, Ussery TW, Steger HG, Colclough GW. Comparison of morphine and morphine with ketamine for postoperative analgesia. Can J Anaesth 1996;43:212–5.

Jhamandas K, Sutak M, Lemaire S. Comparative spinal analgesic action of dynorphin1-8, dynorphin1-13, and a kappa-receptor agonist U50,488. Can J Physiol Pharmacol 1986;64:263–8.

Kamei J, Saitoh A, Ohsawa M, Suzuki T, Misawa M, Nagase H, Kasuya Y. Antinociceptive effects of the selective non-peptidic delta-opioid receptor agonist TAN-67 in diabetic mice. Eur J Pharmacol 1995;276: 131–5.

Kest B, Marek P, Liebeskind JC. The specific *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 blocks U-50,488, but not morphine antnociception. Brain Res 1992;589:139–52.

Kinnman E, Nygards EB, Hansson P. Effects of dextromethorphan in clinical doses on capsaicin-induced ongoing pain and mechanical hypersensitivity. J Pain Symptom Manage 1997;14:195–201.

Kissin I, Brown PT, Robinson CA, Bradley ELJ. Acute tolerance in morphine analgesia: continuous and single injection in rats. 1991; 74: 166–171.

Knapp RJ, Santoro G, De Leon IA, Lee KB, Edsall SA, Waite S, Malatynska E, Varga E, Calderon SN, Rice KC, Rothman RB, Porreca F, Roeske WR, Yamamura HI. Structure–activity relationships for SNC80 and related compounds at cloned human delta and mu opioid receptors. J Pharmacol Exp Ther 1996;277:1284–91.

Kovacs GL, Nyolczas N, Krivan M, Gulya K. Analgesic and tolerance-inducing effects of the highly selective delta opioid agonist [D-Pen2,D-Pen5]enkephalin in mice. Eur J Pharmacol 1988;150:347–53.

Kozela E, Danysz W, Popik P. Uncompetitive, NMDA receptor antagonists potentiate morphine antinociception recorded from the tail but not from the hind paw in rats. Eur J Pharmacol 2001;423:17–26.

Le Greves P, Huang W, Zhou Q, Thornwall M, Nyberg F. Acute effects of morphine on the expression of mRNAs for NMDA receptor subunits in the rat hippocampus, hypothalamus and spinal cord. Eur J Pharmacol 1998;341:161–4.

Lipton SA. Prospects for clinically tolerated NMDA antagonists: open channel blockers and alternative redox states of nitric oxide. Trends Pharmacol Sci 1993;16:527–32.

Lufty K, Hurlbut DE, Weber E. Blockade of morphine-induced analgesia and tolerance in mice by MK-801. Brain Res 1993;616:83–8.

Maguire P, Tsai N, Kamal J, Cometta-Morini C, Upton C, Loew G. Pharmacological profiles of fentanyl analogues at mu, delta and kappa opiate receptors. Eur J Pharmacol 1992;213:219–25.

McQuay HJ, Carroll D, Jadad AR, Glynn CJ, Jack T, Moore RA, Wiffeh PJ. Dextromethorphan for the treatment of neuropathic pain: a double-blind randomised controlled crossover trial with integral *n*-of-1 design. Pain 1994;59:127–33.

Negus SS, Henriksen SJ, Mattox A, Pasternak GW, Portoghese PS, Takemori AE, Weinger MB, Koob GF. Effect of antagonists selective for mu, delta and kappa opioid receptors on the reinforcing effects of heroin in rats. J Pharmacol Exp Ther 1993;265:1245–52.

Negus SS, Gatch MB, Mello NK, Zhang X, Rice K. Behavioral effects of the delta-selective opioid agonist SNC80 and related compounds in rhesus monkeys. J Pharmacol Exp Ther 1998;286:362–75.

Pande AC, Pyke RE, Greiner M, Cooper SA, Benjamin R, Pierce MW. Analgesic efficacy of the kappa-receptor agonist, enadoline, in dental surgery pain. Clin Neuropharmacol 1996a;19:92–7.

Pande AC, Pyke RE, Greiner M, Wideman GL, Benjamin R, Pierce MW. Analgesic efficacy of enadoline versus placebo or morphine in postsurgical pain. Clin Neuropharmacol 1996b;19:451–6.

Parkhouse J, Marriott G. Postoperative analgesia with ketamine and pethidine. Anaesthesia 1977;32:285–9.

Piercey MF, Lahti RA, Schroeder LA, Einspahr FJ, Barsuhn C. U-50488H, a pure kappa receptor agonist with spinal analgesic loci in the mouse. Life Sci 1982;31:1197–200.

Plesan A, Hedman U, Xu XJ, Wiesenfeld-Hallin Z. Comparison of ketamine and dextromethorphan in potentiating the antinociceptive effect of morphine in rats. Anesth Analg 1998;86:825–9.

Plesan A, Sollevi A, Segerdahl M. The *N*-methyl-D-aspartate-receptor antagonist dextromethorphan lacks analgesic effect in a human experimental ischemic pain model. Acta Anaesthesiol Scand 2000;44: 924–8.

Porreca F, Mosberg HI, Hurst R, Hruby VJ, Burks TF. Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. J Pharmacol Exp Ther 1984;230:341–8.

Price DD, Mao J, Frenk H, Mayer DJ. The *N*-methyl-D-aspartate receptor antagonist dextromethorphan selectively reduces temporal summation of second pain in man. Pain 1994;59:165–74.

Sacks L. Applied Statistics. New York: Springer-Verlag, 1982. p. 36.

Sethna NF, Liu M, Gracely R, Bennett GJ, Max MB. Analgesic and cognitive effects of intravenous ketamine-alfentanil combinations versus either drug alone after intradermal capsaicin in normal subjects. Anesth Analg 1998;86:1250–6.

Stewart PE, Hammond DL. Activation of spinal delta-1 or delta-2 opioid receptors reduces carrageenan-induced hyperalgesia in the rat. J Pharmacol Exp Ther 1994;268:701–8.

Suzuki T, Aoki T, Ohnishi O, Nagase H, Narita M. Different effects of NMDA/group I metabotropic glutamate receptor agents in delta- and mu-opioid receptor agonist-induced supraspinal antinociception. Eur J Pharmacol 2000;396:23–8.

Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist NM-801. Science 1991;251:85–7.

Verborgh CM, Camu F, Meert TF. Interaction between sufentanil and U-50488H with respect to antinociception and respiratory depression in rats. Acta Anaesthesiol Scand 1997;41:895–902.

Wadhwa A, Clarke D, Goodchild CS, Young D. Large-dose oral dextromethorphan as an adjunct to patient-controlled analgesia with morphine after knee surgery. Anesth Analg 2001;92:448–54.

Warncke T, Stubhaug A, Jorum E. Preinjury treatment with morphine or ketamine inhibits the development of experimentally induced secondary hyperalgesia in man. Pain 2000;86:293–303.

White PF, Way WL, Trevor AJ. Ketamine—its pharmacology and therapeutic uses. Anesthesiology 1982;56:119–36.

Wong CS, Liaw WJ, Tung CS, Su YF, Ho ST. Ketamine potentiates analgesic effect of morphine in postoperative epidural pain control. Reg Anesth 1996;21:534–41.

Yamamoto T, Yaksh TL. Studies on the spinal interaction of morphine and the NMDA antagonist MK-801 on the hyperesthesia observed in a rat model of sciatic mononeuropathy. Neurosci Lett 1992;135:67–70.

Zhao GM, Bhargava HN. Effect of antagonism of the NMDA receptor on tolerance to [D-Pen2,D-Pen5]enkephalin, a mu-1-opioid receptor agonist. Peptides 1996;17:233–6.