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Effects of NMDA receptor antagonists on opioid-induced respiratory depression and acute antinociception in rats

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Abstract

Although exogenous opioids alter the responses of animals to tissue-damaging stimuli and therefore are the cornerstone in the treatment of acute antinociception, they have profound side effects on ventilation. To diminish ventilatory effects, combination therapies have been advocated. Recent studies reported the effectiveness of the addition of *N*-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine to morphine in the treatment of acute pain. However, NMDA receptors, together with non-NMDA receptors are known to be involved in the neurotransmission of inspiratory drive to phrenic motoneurons. Co-administration of NMDA and non-NMDA receptor antagonists has been shown to be deleterious to respiratory function. The present study investigated the hypothesis that the association of opioids and NMDA receptor antagonists may add to the impairment of respiratory parameters. In male Wistar rats, combinations of opioids (fentanyl or morphine) at antinociceptive doses and NMDA receptor antagonists (ketamine, 40 mg/kg, or dextromethorphan, 10 mg/kg) at subanesthetic doses were administered intraperitoneally. Antinociception was tested with the tail-withdrawal reaction (TWR) test, while the effect on respiratory parameters was investigated with blood-gas analysis.

We found that, in rats, co-administration of NMDA receptor antagonists and opioids may result in an increased respiratory depression as compared to the opioids alone. The effect of the NMDA receptor antagonists on opioid-induced antinociception was limited.

Keywords: NMDA receptor antagonists; Opioids; Respiratory depression; Antinociception; Tail-withdrawal reaction test

1. Introduction

Opioids have been known for a long time to alter animal and human responses to tissue-damaging stimuli by binding to specific opioid receptors. Binding to these receptors may, however, besides strong antinociceptive effects, give rise to several other central nervous system-related effect such as respiratory depression (Borison, 1977a,b). Combinations of drugs have been proposed in an effort to increase efficacy or to diminish the occurrence of these side effects (Verborgh et al., 1997; Kehlet et al., 1999).

Glutamate is the most abundant excitatory neurotransmitter in the mammalian central nervous system and its receptors are classified into *N*-methyl-D-aspartate (NMDA) and non-NMDA ionotropic and metabotropic receptors (Peoples and Weight, 1998). Several authors have demonstrated the involvement of NMDA receptors in the transmission and modulation of nociceptive information at the spinal cord level. Activation of these receptors prolongs and amplifies the response to depolarisation leading to clinical effects such as allodynia and hyperalgesia (Kim et al., 1997). Moreover, opioid treatment results in an increase in protein kinase C (PKC) that subsequently activates the NMDA receptor, in this way reducing the antinociceptive effects of the opioid (Chen and Huang, 1992). Blocking the NMDA receptor, therefore, could enhance opioid-induced antinociception. Moreover, the concurrent administration of these drugs and opioids prevents opioid tolerance from developing (Elliott et al., 1994, 1995; Mao et al., 1995). This implies that NMDA receptor antagonists are drugs that potentially may provide antinociception (Davar et al., 1991; Yamamoto and Yaksh, 1992; Coderre et al., 1993; Mao et al., 1993).

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Whereas μ receptor agonists have been shown to cause respiratory depression, this may theoretically also be the case for NMDA receptor antagonists. Both NMDA and non-NMDA receptors have been shown to play a central role in the regulation of respiration (Foutz et al., 1989, 1994; Pierrefiche et al., 1994; Chitravanshi and Sapru, 1996). Although the impact of NMDA receptor antagonism on respiratory parameters by itself may be limited, combination of NMDA and non-NMDA receptor antagonists may cause apnea (Abrahams et al., 1991; Jung et al., 1991; Connelly et al., 1992). Therefore, combination of NMDA receptor antagonists with other respiratory depressant drugs such as opioids could potentially increase respiratory depression. Previous studies already hypothesized the possible respiratory depression after combinations of NMDA receptor antagonists and opioids (Trujillo and Akil, 1991; Vanderschuren et al., 1997).

The present study investigated whether equianalgesic doses of opioids or a combination of an opioid with an NMDA receptor antagonist at subanaesthetic doses had an influence on arterial blood-gas analysis in rats. An evaluation of the possible potentiating effects of NMDA receptor antagonists on opioid antinociception was measured in the tail-withdrawal test. This test was chosen because of the existence of a good correlation between opioid potency in this test and the clinically used doses in postoperative pain (Meert and De Kock, 1994). Another interesting feature was the relatively easy setup of the experiment without unnecessary excitement of the animals.

2. Methods

2.1. Animal preparation

After approval from the local research ethics committee, male Wistar rats (Charles River, Sulzseld, Germany) weighing 250 ± 10 g were used in the experiments. Rats were housed in groups of seven in standard rodent cages for a week following arrival. They were subject to a 12-h-light/dark cycle, a room temperature of 22 °C with background noise of 60 dB provided by a transistor radio; food and water were available ad libitum.

On the morning of the experiments, rats were weighed and then anaesthetized with etomidate 0.4 mg/kg. After skin incision, the right femoral artery was cannulated with a polyethylene catheter (PE50). The catheter was anchored and the skin was closed. The rats were then placed in a Bolman cage and left undisturbed for 3 h to recover from anaesthesia. A Bolman cage is a construction holding eight adjustable longitudinal rods arranged as a cylinder, allowing liberal movement of head, paws and tail. The metal rods of the cages were adjusted as to minimize breathing impairment. Moreover, a previous study has shown that after 2 h of habituation in Bolman cages, no antinociception was observed (Verborgh et al., 1998). The femoral catheters were kept patent by regular injections of 0.25 ml of a heparin solution (100 U/ml).

Because the large number of groups, testing was spread over several days in such a way that in every test session, rats from different groups were tested side-by-side in a blinded manner for the investigator. In this way, the influence of inter-day variability was minimized across the different treatment groups.

After each test session, rats were sacrificed by an overdose of sodium pentobarbital.

2.2. Assessment of respiratory function and antinociception

After rats had recovered completely from anesthesia, arterial blood samples (0.3 ml) were taken, and a basal blood-gas analysis was performed (ABL System 610, Radiometer Medical A/S, Copenhagen, Denmark). Blood-gas analysis consisted of pH, p_aCO_2, p_aO_2 and oxygen saturation measurements. Only rats with basal pCO_2 values of less than 32 mm Hg were considered to have recovered sufficiently from anaesthesia or not to experience any negative influence of the Bolman cage. We chose to analyse blood gases because respiratory depression after exogenously administered opioids involves both reduction in tidal volume and in respiratory rate since both affect pCO_2 values (Borison, 1977a,b).

Drugs were administered intraperitoneally at $t = 0$. To evaluate the analgesic potential of the drugs, the tail-withdrawal reaction (TWR) test was used. In this test, the distal 5 cm of the tail were immersed in water of 55 ± 1 °C and the time to withdrawal was measured to the nearest 0.1 s. A cutoff time of 10 s was adopted to minimize tissue damage. This criterion is often used to evaluate deep surgical anaesthesia (Janssen, 1982; Meert and De Kock, 1994). Animals with TWR latencies of more than 3.0 s were excluded.

In this way, less than 1% of the animals with a basal TWR latency of more than 3.0 s or pCO_2 values exceeding 32 mm Hg were excluded.

Tail-withdrawal latencies and blood gases were measured every 15 min for 1 h after the administration of the medication.

In order to exclude acute effects of TWR measurements on respiratory parameters, blood samples were taken before the measurement of TWR and stored in ice before being analysed. After each sample, 1 ml of saline containing 100 U of heparin/ml was injected via the arterial catheter to prevent clotting.

2.3. Drugs

Rats were randomly allocated to receive one of the following treatments (all doses in parentheses in mg/kg):

Saline (10)
Morphine HCl (0.63; 2.5; 10 or 40)
Fentanyl HCl (0.01; 0.04; 0.16; 0.63 or 2.5)

Ketamine HCl (2.5; 10; 40 or 80)
Dextromethorphan HCl (0.16; 0.63; 2.5; 10; 20; 40; 80 or 160)
Dextromethorphan HCl (10) + Morphine HCl (0.31; 0.63; 1.25 or 2.5)
Dextromethorphan HCl (10) + Fentanyl HCl (0.01, 0.04, 0.16)
Ketamine HCl (40) + Morphine HCl (0.31; 0.63; 1.25 or 2.5)
Ketamine HCl (40) + Fentanyl HCl (0.01; 0.02 or 0.04)

All drugs were dissolved in saline. Doses are expressed as the hydrochloride salts.

In total, 36 groups were formed, each containing 7 rats.

The best doses of ketamine and dextromethorphan used in the combinations were chosen on the basis of previous tests (Baker et al., 2002).

2.4. Data analysis

Tail-withdrawal latencies were analysed using a standard 10-s cutoff criterion (Janssen, 1982; Meert and De Kock, 1994).

For each time point, the percentage of maximal possible effect (%MPE) was calculated according to the formula:

$$[\%\mathrm{MPE} = \{(\mathrm{TWL} - \mathrm{TWL\ preinjection}) / (\mathrm{cutoff\ time} - \mathrm{TWL\ preinjection})\} \times 100]$$

Based on %MPE, the areas under the curve (AUC) were calculated as follows: $[\mathrm{AUC}=\{(\%\mathrm{MPE}_{t1}+\%\mathrm{MPE}_{t2})/2\} \times (t2 - t1)]$ for each time interval of 15 min. In this way four AUCs were calculated that were summated to form total AUC.

For the 10-s criterion, ED_{50} values (median effective dose, dose at which 50% of the animals reached the 10-s cutoff) and 95% confidence limits were calculated according to Finney's iterative method (Finney, 1971).

Likewise, pCO_2–ED_{50} calculations were made for CO_2 values of 40 mm Hg (i.e., the dose at which 50% of the animals reached a pCO_2 of 40 mm Hg), indicating the occurrence of a significant change in respiratory depression (this is an increase of pCO_2 of at least 25% as compared to baseline values).

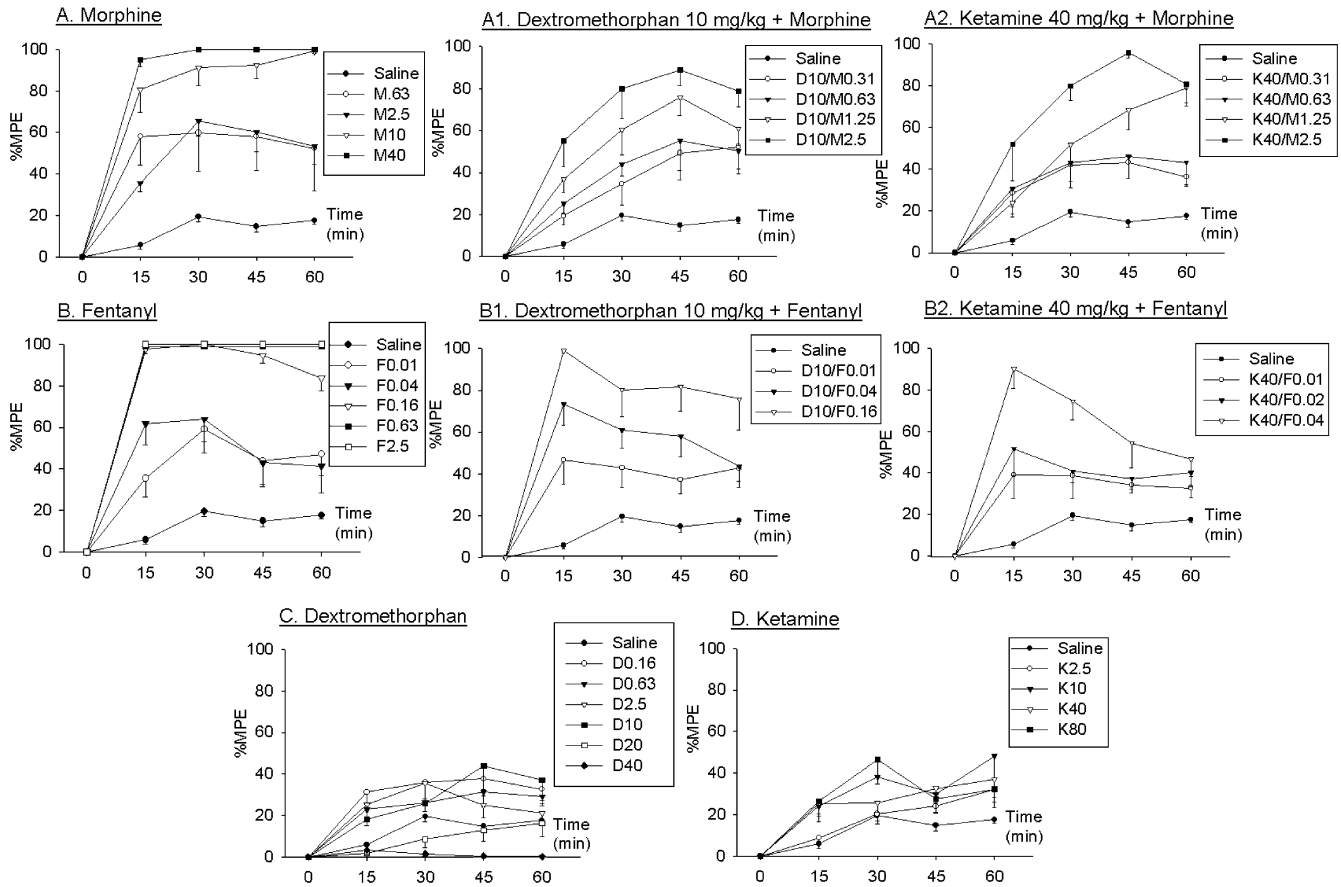

Fig. 1. Dose–response curves of the percentage of maximal possible effect (%MPE) of TWR after morphine, fentanyl, ketamine, and dextromethorphan and their combinations. The same dose–response curve for saline is included in each graph as a reference.

2.5. Statistical analysis

A one-way ANOVA for repeated measurements was used to compare %MPE and pCO_2. Comparison of AUC-TWR and AUC-pCO_2 was done with a one-way ANOVA. The Tukey test was used for pairwise multiple comparisons.

Differences between ED_{50} values were evaluated using the Student's *t* test for independent samples on differences of log ED_{50} (two-tailed). The standard errors on the log ED_{50} values were obtained from the 95% confidence limits (Sacks, 1982).

P values $<.05$ were considered statistically significant.

3. Results

3.1. Effects on antinociception

For all drugs, the intrinsic antinociceptive properties were tested at various doses (Fig. 1). With both fentanyl and morphine, we observed a clear dose–response relationship. Increasing doses led to an increasing number of animals reaching the cutoff latency of 10 s (Fig. 1A,B). The ED_{50} values of fentanyl and morphine were 0.065 (0.039–0.109) and 3.38 (1.50–7.60) mg/kg, respectively (Table 1).

With dextromethorphan, except for 0.16 mg/kg, no significant antinociceptive effect was seen as compared to saline (Fig. 1C). In contrast, increasing doses of dextromethorphan decreased tail-withdrawal latencies over time. Tail-withdrawal latencies after dextromethorphan 20 and 40 mg/kg were significantly decreased as compared to those after lower doses of dextromethorphan ($P<.05$). After a dose of 160 mg/kg dextromethorphan, five out of seven animals died within 5 min. Therefore, an additional group (Dex 80 mg/kg) was included in the study. However, also in this group, five out of seven animals died. This happened due to respiratory arrest as seen by bradypnea. Cutoff values were never reached with any dose of dextromethorphan. An ED_{50} could not be calculated.

Ketamine was administered in increasing doses from 2.5 to 80 mg/kg. Statistically significant differences as compared to saline were found for doses of 10, 40, and 80 mg/kg ketamine (P=.002, P=.017, and P=.005, respectively) (Fig. 1D). No statistical differences were found between the different ketamine doses. No ED_{50} could be calculated since TWR latencies of 10 s in more than three animals were never observed.

A dose of ketamine 40 mg/kg potentiated antinociceptive effects of 2.5 mg/kg morphine in %MPE as compared to morphine, 2.5 mg/kg, alone (P=.013). In contrast, ketamine did not potentiate morphine, 0.63 mg/kg. The combination of ketamine with morphine, 1.25 mg/kg, was equal to morphine, 2.5 mg/kg, alone (Fig. 1A2). The ED_{50} of morphine tended to decrease, although statistically not significant, from 3.38 to 1.45 (0.96–2.18) mg/kg after adding ketamine, 40 mg/kg. Dextromethorphan in a dose of 10 mg/kg did not potentiate the different doses of morphine tested (Fig. 1A1). Likewise, the addition of 10 mg/kg of dextromethorphan did not significantly decrease the ED_{50} of morphine (3.38 versus 1.45 [0.91–2.29] mg/kg) (Table 1). Both NMDA receptor antagonists did not cause a significant leftward shift of the AUC curve of morphine ($P>.05$) (Fig. 3A).

Fentanyl in combination with ketamine, 40 mg/kg, did not result in improved %MPE as compared to corresponding doses of fentanyl alone (Fig. 1B2) ($P>.05$). Likewise, combinations of dextromethorphan, 10 mg/kg, with doses of fentanyl from 0.01 to 0.16 mg/kg did not result in improved antinociception as compared to corresponding doses of fentanyl alone (Fig. 1B1). In terms of ED_{50} for fentanyl, no effect was seen for the combination with dextromethorphan ($P>.05$), whereas with ketamine, a statistically significant decrease of the ED_{50} of fentanyl was found from 0.065 (0.039–0.109) to 0.023 (0.015–0.035) mg/kg ($P<.01$) (Table 1). Neither 40 mg/kg ketamine nor 10 mg/kg dextromethorphan significantly potentiated the effects of corresponding doses of fentanyl over time (Fig. 3B1).

Table 1
ED_{50} of each drug and the combinations of TWR latencies and pCO_2 (CI)

Result/drug	ED_{50}–TWR (CI) (mg/kg)	ED_{50}–pCO_2 (CI) (mg/kg)	Ratio $ED_{50}CO_2$/ ED_{50} TWR
Morphine (M)	3.38 (1.50–7.60)	24.4 (14.4–40.7)	7.2
Fentanyl (F)	0.065 (0.039–0.109)	0.21 (0.13–0.35)	3.2
Dextrometorphan (D)	–	–	–
Ketamine (K)	–	–	–
M+K 40	1.45 (0.96–2.18)	1.76 (1.17–2.65)#	1.2
M+D 10	1.45 (0.91–2.29)	–	–
F+K 40	0.023 (0.015–0.035)*	0.031 (0.024–0.040)**	1.3
F+D 10	0.044 (0.020–0.099)	0.08 (0.04–0.16)*	1.8

The calculated ratios indicate the potential of a drug or combination of drugs to increase pCO_2 relatively to its analgesic effect.

* Different from fentanyl ($P<.05$).

** Different from fentanyl ($P<.01$).

\# Different from morphine ($P<.05$).

3.2. Effects on respiratory parameters

3.2.1. pCO_2

Respiratory depression in the rat is particularly reflected in an initial increase in pCO_2. In the present study, a clear dose–response relationship between the opioid dose (of both morphine and fentanyl) and the area under the curve of the measured pCO_2 was observed. In general, the higher

the opioid dose, the more animals reaching a pCO_2 of more than 40 mm Hg (Fig. 2A,B). The ED_{50} for reaching a pCO_2 of 40 mm Hg (pCO_2–ED_{50}) was 0.21 (0.13–0.35) and 24.4 (14.4–40.7) mg/kg for fentanyl and morphine, respectively (Table 1). Also, for ketamine, there was a tendency to slowly increasing pCO_2 values with increasing doses with significantly higher pCO_2 values for ketamine, 80 mg/kg, than the lower doses of ketamine ($P<.05$) or saline ($P<.001$) (Fig. 2D). A pCO_2–ED_{50} could not be calculated because after a dose of 80 mg/kg, only two out of seven animals reached 40 mm Hg. Increasing doses of dextromethorphan decreased pCO_2 values significantly (Fig. 2C). At higher doses (80 and 160 mg/kg), five out of seven animals died within 5–10 min because of respiratory arrest.

The combinations of morphine with 10 mg/kg dextromethorphan and 40 mg/kg ketamine, respectively, showed increases in pCO_2 with increasing doses of the opioid (Fig. 2A1,A2). In comparison with morphine alone, the combinations with dextromethorphan and ketamine significantly increased pCO_2 values to the same extent ($P<.05$). Adding ketamine to morphine increased the number of animals reaching 40 mm Hg pCO_2 which resulted in a significant drop of the pCO_2–ED_{50} from 24.4 (14.4–40.7) to 1.76 (1.17–2.65) mg/kg (Table 1). For the combination with dextromethorphan, we were not able to calculate an ED_{50}–CO_2, although the number of animals reaching 40 mm Hg pCO_2 was higher than with morphine alone. In terms of AUC, higher pCO_2 levels over time were measured in the combinations with ketamine ($P<.01$) and dextromethorphan ($P<.01$) as compared to the corresponding doses of morphine alone (Fig. 3A).

The combinations of fentanyl with 40 mg/kg ketamine and 10 mg/kg dextromethorphan, respectively, showed increases in pCO_2 with increasing doses of the opioid (Fig. 2B2,B1). The combinations with fentanyl showed an important increase in the number of animals reaching the level of 40 mm Hg pCO_2. This resulted in a significant decrease of pCO_2–ED_{50} for fentanyl from 0.21 (0.13–0.35) to 0.031 (0.024–0.040) and to 0.08 (0.04–0.16) mg/kg when associated with 40 mg/kg ketamine ($P<.01$) and 10 mg/kg dextrometorphan ($P<.05$), respectively (Table 1). In terms of AUC, higher levels of pCO_2 were measured over time in the combination with ketamine ($P<.01$), whereas for dextromethorphan, no statistically significant differences were found ($P>.05$) as compared to the corresponding doses of fentanyl alone (Fig. 3B).

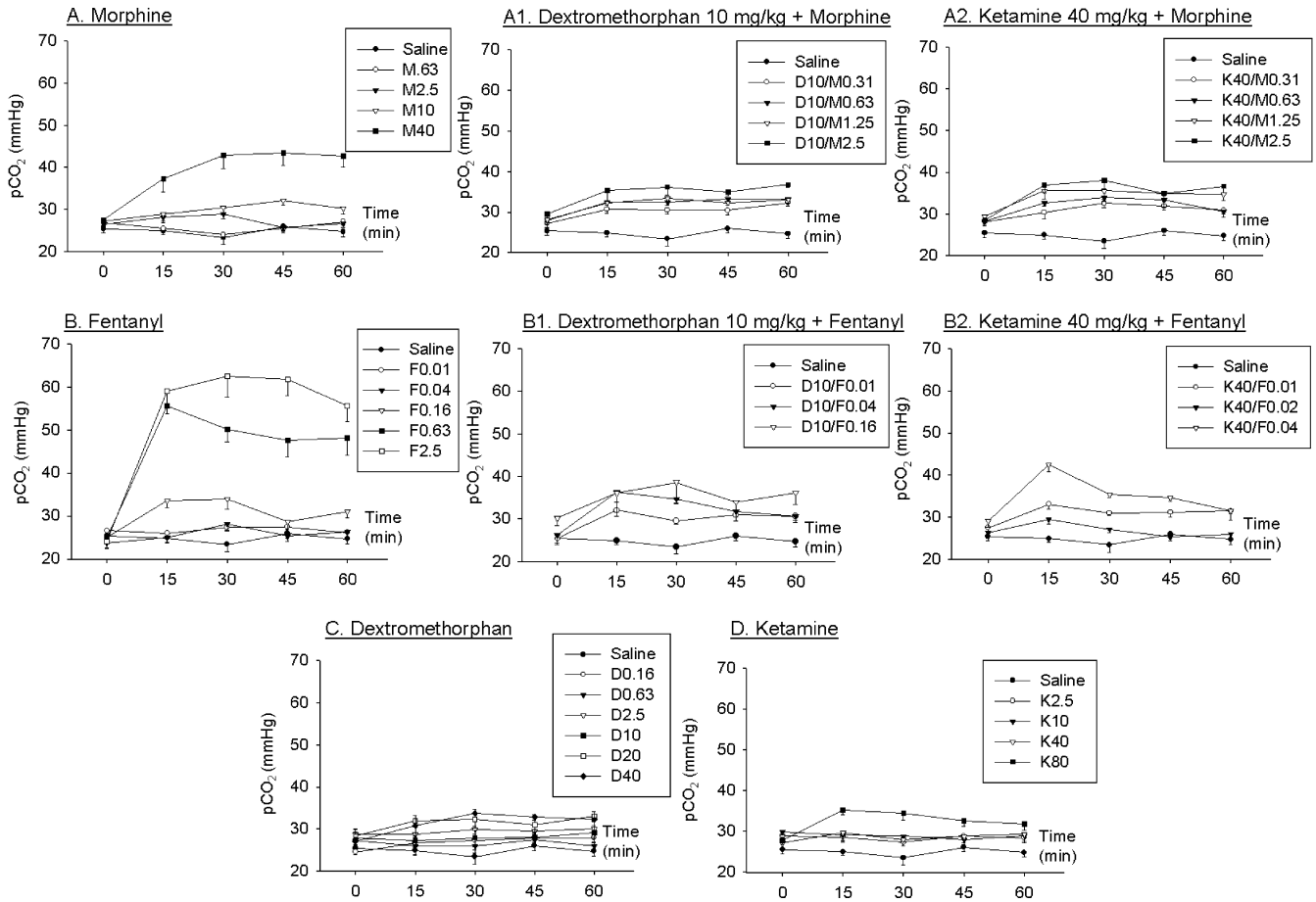

Fig. 2. Dose–response curves of the percentage of maximal possible effect (%MPE) of pCO_2 after morphine, fentanyl, ketamine, and dextromethorphan and their combinations. The same dose–response curve for saline is included in each graph as a reference.

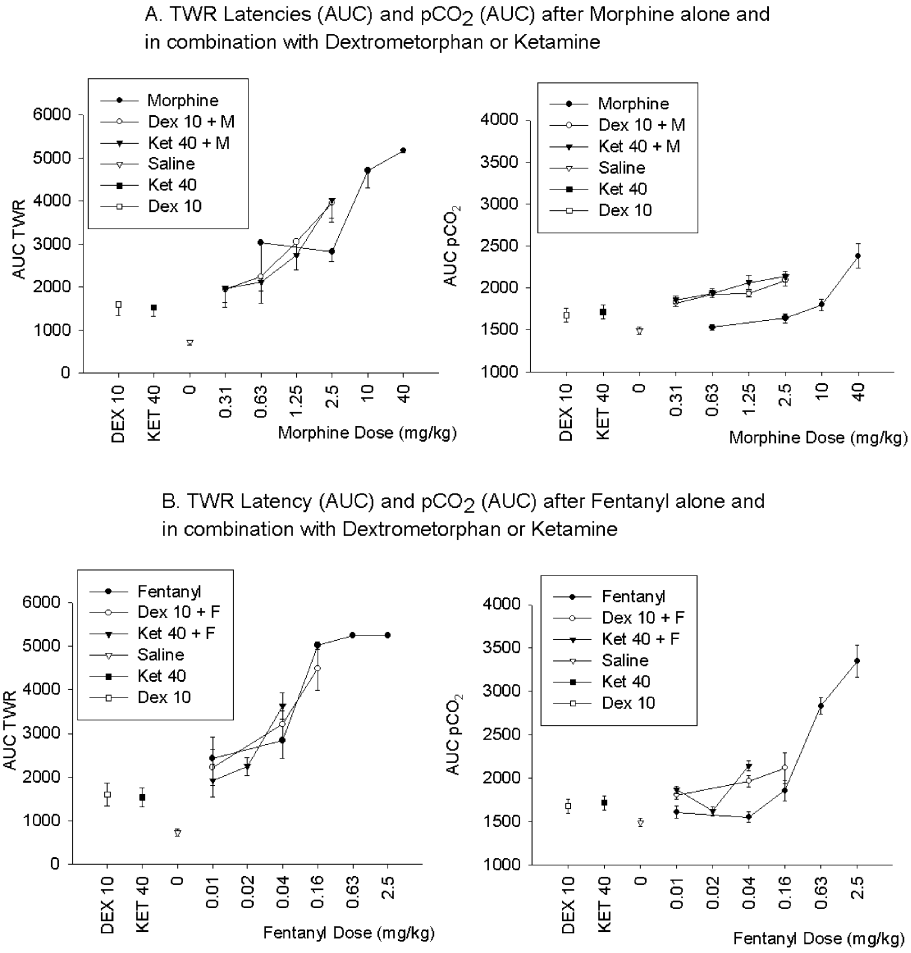

Fig. 3. Effect of morphine and fentanyl and their combinations with dextromethorphan and ketamine over time on TWR latencies and pCO_2 as expressed by AUC.

3.2.2. pO_2 and O_2 saturation

pO_2 values lower than 90 mm Hg were only found at different time points in the high-dose opioid groups (morphine, 40 mg/kg; fentanyl, 0.63 and 2.50 mg/kg), and in the groups of 0.16 mg/kg fentanyl at $t=30$ min ($pO_2=87$), ketamine, 40 mg/kg, plus morphine, 2.5 mg/kg, again at $t=30$ min ($pO_2=89.1$) and ketamine, 40 mg/kg, plus fentanyl, 0.04 mg/kg, at $t=15$ min ($pO_2=87.1$).

The same applies for the measurements of oxygen saturation. Again, in the groups of high-dose opioids and in the combinations of ketamine 40 mg/kg/morphine 2.5 mg/kg at $t=30$ min (O_2 sat = 89.1) and ketamine 40 mg/kg plus fentanyl 0.04 mg/kg at $t=15$ min (O_2 sat = 87.8), a saturation $<90\%$ was found.

3.3. Ratio of antinociception and respiratory depression

The ED_{50} values of each drug and the combinations of drugs for antinociception and pCO_2 are listed in Table 1. All drugs show a lower ED_{50} for antinociception than for CO_2, which implies that analgesia is induced at lower doses than those which induce important respiratory depression. Only for the combination of dextromethorphan and morphine, no ED_{50}–pCO_2 could be calculated.

Ratios of ED_{50} for antinociception and pCO_2 were calculated to make comparisons possible between the effects on respiration and antinociception. The higher the pCO_2–ED_{50}/TWR ratio, the better the antinociceptive effect relatively to respiratory depression. The results show that the ED_{50} ratios are lower in the combination groups as compared to the opioids alone, which means that respiratory depression is proportionally greater in the combination groups relative to the increase in analgesic potential. Comparing the ratio of morphine with the ratio of the combination with ketamine, there is a sixfold difference. A similar trend is seen for fentanyl and its combination with dextromethorphan and ketamine, although the changes are less pronounced (a 1.8- and 2.5-fold change, respectively).

The different dose ranges that we used in the opioid and combination groups may account for some of the mild shift in ED_{50} for changes in antinociception that we observed. However, the shift in ED_{50}–CO_2 is much larger, and it therefore seems unlikely that this shift can be explained by missing data points.

4. Discussion

The present study investigated the effects of two NMDA receptor antagonists (dextromethorphan and ketamine) on acute antinociceptive and respiratory effects of two opioids (fentanyl or morphine) in rats. Both tests are not mediated by the same mechanism and therefore measure different pathophysiological systems. Whereas the TWR test is a spinally mediated reflex, this is not the case for respiratory depression (Kozela et al., 2001).

Our results demonstrate that, when administered intraperitoneally at subanesthetic doses, neither dextromethorphan nor ketamine showed a strong antinociceptive effect in the TWR test. On the other hand, both fentanyl and morphine demonstrated a clear dose–response relationship with ED_{50} of 0.065 and 3.38 mg/kg for fentanyl and morphine, respectively. These results are well in accordance with those of previous authors (Dambisya and Lee, 1994; Kozela et al., 2001).

When added to submaximal doses of morphine, dextromethorphan did not demonstrate a potentiating effect, whereas ketamine only marginally potentiated the antinociceptive effects of morphine. Other authors reported similar results (Luger et al., 1995; Joo et al., 2000; Kozela et al., 2001). Dextromethorphan did not increase the duration of antinociceptive effect or the potency of fentanyl. With ketamine, a significant decrease in the ED_{50} of fentanyl was found, mainly due to the potentiating effect of ketamine on one particular dose of fentanyl. Our results are in accordance with those of Smith et al. (1985) who described an analgesic effect of ketamine in the tail-flick test only with anaesthetic doses (>160 mg/kg). They also reported an additive effect on morphine analgesia (Smith et al., 1985). Similarly to our results, Lutfy et al. (1997) did not find any antinociceptive properties of NMDA receptor antagonists in the tail flick test in mice. On the other hand, other authors reported increased morphine-induced tail-flick latencies after the administration of NMDA receptor antagonists (Advokat and Rhein, 1995; Grass et al., 1996; Mao et al., 1996; Plesan et al., 1998). This variability in effect of NMDA receptor antagonists on μ antinociception in several studies may be due to methodological differences or animal strains (Kozela et al., 2001). However, more specifically, several mechanisms may explain the inefficiency of NMDA receptor antagonists in the TWR test. Firstly, NMDA receptor antagonists may induce spontaneous movements of the tail in the tail-flick test by blocking NMDA receptors in the nucleus accumbens (Millan et al., 2000). Secondly, the tail withdrawal response is a spinally mediated reflex with a high specificity for opioids (Meert and De Kock, 1994). Although ketamine has been shown to interact with various receptors (Hirota and Lambert, 1996) including the μ receptor (Smith et al., 1980, 1987), the affinity of ketamine for this opioid receptor is low (Finck and Ngai, 1982; Klepstad et al., 1990). In several studies, ketamine increased tail-flick latencies only in anesthetic doses (Pekoe and Smith, 1982; Smith et al., 1987). Thirdly, activation of opioid receptors by morphine induces phosphorylation of mitogen-activated protein kinase (MAPK) (Trapaidze et al., 2000). Application of ketamine alone did not increase the level of MAPK phosphorylation, whereas the combination of ketamine and morphine led to a fourfold increase in MAPK phosphorylation (Gomes et al., 2000). As such, there is some evidence indicating that NMDA receptor antagonists, and especially ketamine, do not exert their effects by the same route as opioids.

In the present study, doses of up to 40 mg/kg of dextromethorphan lead to a decrease in TWR latencies, possibly indicating hyperalgesia. However, a further increase of the doses caused death in five out of seven animals. In humans, high doses of dextromethorphan have been shown to induce central nervous system toxicity in children. Symptoms may include hyperexcitability, hyperreflexia, increased muscle tone, ataxia and even death (Pender and Parks, 1991).

Respiratory depression after the administration of opioids may become apparent by both a decrease in tidal volume and in respiratory rate (Borison, 1977a,b). Opioids act directly on brainstem respiratory centers resulting in a significant reduction of the CO_2 responsiveness. They also interfere with pontine and medullary respiratory centers that regulate respiratory rhythmicity. This results in a decrease of the slope and a displacement to the right of the CO_2 intercept that corresponds with the hypercapnea seen at rest. The fact that both rhythmicity and tidal volume are affected after the administration of opioids is an argument to use measurements of pCO_2 as parameter to detect respiratory depression. Theoretically, hypercapnea may also be a reflection of an increase in metabolic rate. Ketamine can increase muscle tone and sympathetic tone or generate muscle spasms and spontaneous movements (White et al., 1982). Moreover, it is known to increase cerebral metabolism (White et al., 1982). In the present study, high doses of ketamine only marginally increased p_aCO_2 levels. Moreover, the effects of dextromethorphan on opioids were comparable to those of ketamine. These observations do not support the argument that an increase in metabolic rate was responsible for the hypercapnea seen after combinations of ketamine and opioids. Therefore, our results indicate that NMDA receptor antagonists can enhance opioid-induced respiratory depression in rats.

Respiration in mammals depends on a neuronal network, the respiratory central pattern generator (CPG), which is located in the brainstem that controls the timing of inspiration and expiration. Structures outside the brainstem can modulate respiratory rhythm and pattern according to physiological changes (Bianchi et al., 1995). The role of both non-NMDA and NMDA receptors in the respiratory regulation control nuclei in the medulla has been investigated recently (Foutz et al., 1988a,b; McManigle et al., 1994; Chitravanshi and Sapru, 1996; Borday et al., 1998; Anderson and Speck, 1999; Krolo et al., 1999). NMDA receptors are involved in inspiratory termination and there-

fore in the precise timing of inspiratory actions. Non-NMDA receptors and, to a lesser extent NMDA receptors, determine phrenic nerve activity (Liu et al., 1990; Bianchi et al., 1995). Administration of both NMDA and AMPA receptor antagonists synergistically depress respiration both in adult and in neonate mammals (Foutz et al., 1994; McManigle et al., 1994; Borday et al., 1998; Anderson and Speck, 1999; Krolo et al., 1999). Since activation of μ receptors induces a reduction in tidal volume through reduction of glutamate-induced depolarisation (Zieglgansberger and Bayerl, 1976), application of combinations of NMDA receptor antagonists and opioids could theoretically aggravate each other's respiratory depressant effect. Although rats in the present study did not die after the combination of NMDA receptor antagonists and opioids, our results confirm the observations by previous authors. Both Trujillo and Vanderschuren hypothesized that the increased lethality after the combination of MK-801 and morphine occurred due to an increase of respiratory depression. Trujillo and Akil (1991) described a similar behavior after high doses of morphine and after the combination of MK-801 and morphine, whereas Vanderschuren et al. (1997) hypothesized that MK-801 not only prevented tolerance to the antinociceptive activity of opioids, but also to the sedative and respiratory depressant effects.

In the present study, the ratio of antinociception and respiratory depression was lower for the combinations of NMDA receptor antagonists and opioids as compared to the opioids alone. Since the TWR test is a spinally mediated reflex and respiration is not, the differential effect of the combinations on both systems may be due to the interaction of the drugs in different neuronal structures. However, the observation itself that NMDA receptor antagonists may aggravate opioid-induced respiratory depression does not fit with the general idea that combinations of analgesic drugs in acute pain management should improve analgesia relatively to the occurrence of side effects (Kehlet and Dahl, 1993; Kehlet et al., 1999).

In conclusion, NMDA receptor antagonists only offered minor additive effects to the acute antinociceptive actions of opioids in rats. In contrast, the opioid-induced respiratory depression was more pronounced when combined with dextromethorphan or ketamine.

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