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Sex-related differences in the enhancement of morphine antinociception by NMDA receptor antagonists in rats

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Abstract

The effect of the *N*-methyl-D-aspartate (NMDA) receptor antagonists dextromethorphan (DEX), ketamine (KET), and MK-801 on morphine (MOR)-induced antinociception has been investigated in male and female rats. DEX (7.5, 15, and 30 mg/kg), KET (0.75, 1.5, and 3 mg/kg), and MK-801 (0.075, 0.15, and 0.3 mg/kg) dose-dependently enhanced MOR-induced (3 mg/kg) analgesia in female rats. DEX and KET enhanced the peak effect, whereas MK-801 increased both magnitude and duration of analgesia. DEX also enhanced MOR-induced analgesia in male rats. However, the interaction was of less magnitude in male compared with female rats. The effects of KET and MK-801 on MOR-induced analgesia were negligible in male rats. A 3-mg/kg dose of MOR given alone produced greater analgesia in male than in female rats, but in the presence of NMDA antagonists, MOR elicited similar analgesic responses in both sexes.

Keywords: Dextromethorphan; Ketamine; MK-801; Morphine; Antinociception; Tail-flick test; Sex; Rats

1. Introduction

The opioid analgesic drugs are widely used for the treatment of moderate to severe pain. However, there are significant opioid-related side effects including sedation, respiratory depression, and constipation. In addition, the chronic use of opioids is associated with the development of tolerance to the analgesic effect. Finally, long-term opioid use can be associated with abuse and dependence. One approach to the limitations with opioid-only use for pain has been to attempt to combine other agents with opioids (see Caruso, 2000; Hewitt, 2000; Katz, 2000) in an effort to enhance analgesia, reduce side effects, and limit the development of tolerance and dependence. In this regard, the combination of drugs acting at glutamate receptors, particularly the *N*-methyl-D-aspartate (NMDA) receptor, has been of particular interest.

It has been established that the glutamate system interacts with the opioid system. There is a significant body of evidence that μ-opioids affect NMDA receptors and that NMDA receptors are involved in the development of opioid tolerance (see Bell, 1999; Mao, 1999 for review) and physical dependence (see Bisaga et al., 2001; Bisaga and Popik, 2000 for review). In addition, it has been shown that NMDA antagonists may enhance and prolong opioid analgesia in humans (Caruso, 2000; Bisaga et al., 2001; Katz, 2000) and in at least some studies in rodent models of pain (see Belozertseva et al., 2000; Kozela et al., 2001 and discussion). This combination therapy may be effective in the treatment of neuropathic pain (see Hewitt, 2000 for review), which is often poorly managed by opioids alone. NMDA antagonists alone do have some utility in treating pain, particularly neuropathic pain; however, the usefulness of these drugs is limited by a narrow therapeutic index and severe side effects (psychotomimetic, sedation, and motor effects; see Sang, 2000; Schmid et al., 1999).

It has been repeatedly shown that the analgesic responsiveness to opioid drugs is related to sex in rodent models of pain. Typically, morphine (MOR) has been found to produce greater analgesia in males compared with female rats in the tail-flick assay (Craft, 2003; Kest et al., 2000). In earlier studies, we have found that enhancement of opioid analgesia is related to sex (Holtman et al., 2003). In addition, recent preliminary data (unpublished observations) suggest that NMDA receptor mechanisms may be involved in sexual dimorphism of opioid effects in rats. This phenomenon has also been examined in mice where sex

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differences in the effect of MK-801 (dizolcipine) on MOR-induced analgesia were demonstrated (Lipa and Kavaliers, 1990).

Therefore, to further explore the possibility of the involvement of NMDA receptor mechanisms in the sexual dimorphism of MOR, we compared the effects of three noncompetitive antagonists of NMDA receptors (dextromethorphan [DEX], ketamine [KET], and MK-801) on MOR-induced analgesia in male and female rats. DEX, an opioid derivative that does not act at opioid receptors (Tortela et al., 1989), is a widely used antitussive medication. It is a moderately potent, noncompetitive/nonselective NMDA receptor antagonist (Chou et al., 1999) with activities at non-NMDA receptor sites (Church et al., 1991; Netzer et al., 1993). It has been combined with MOR for human studies (Caruso, 2000). KET is a noncompetitive/selective NMDA receptor antagonist (Yamamura et al., 1990), which also acts on σ-receptors (Hustveit et al., 1995). It is used clinically as a dissociative anesthetic and in subanesthetic doses is a potent analgesic (Fisher and Hagen, 1999). MK-801 is a prototypical, highly potent, noncompetitive/selective NMDA receptor channel blocker (Wong et al., 1986).

The aim of the present study was to compare the analgesic effects of DEX, KET, and MK-801 alone as well as their affect on MOR analgesia (tail-flick test) in both female and male rats. The results of this animal study may be of importance in further clinical studies on the effectiveness of NMDA antagonist–opioid agonist combination therapy in male and female patients experiencing acute or chronic pain.

2. Materials and methods

2.1. Animals

Ninety male (350 g) and female (250 g) age-matched (85–90 days old) Sprague–Dawley rats (Harlan) were used in the present study. The estrous cycle was not determined. The rats were housed individually in a temperature-controlled environment with a 12:12-h light/dark cycle (lights on at 6:00 am). Standard laboratory chow and tap water were available ad libitum. Rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals. The experiments were performed according to a protocol approved by the University of Kentucky Animal Care and Use Committee.

2.2. Administration of drugs

Fresh solutions of MOR sulfate (Mallinckrodt, St. Louis, MO) and DEX hydrobromide, (±) KET hydrochloride, and (+) MK-801 hydrogen maleate (Sigma, St. Louis, MO) were prepared in saline (SAL) on the day of use. Rats were injected by the intraperitoneal route (0.5 ml/kg) with DEX (7.5, 15, and 30 mg/kg), KET (0.75, 1.5, and 3 mg/kg), MK-801 (0.075, 0.15, and 0.3 mg/kg), or SAL followed by a fixed dose (3 mg/kg and 0.5 ml/kg) of MOR at weekly intervals. This dose of MOR has been shown to provide a consistent but less than maximum antinociceptive effect (Holtman et al., 2003). A Latin square design was used to balance the order of doses of DEX, KET, and MK-801. Additional rats (controls) were injected with DEX (7.5, 15, and 30 mg/kg), KET (0.75, 1.5, and 3 mg/kg), MK-801 (0.075, 0.15, and 0.3 mg/kg), or vehicle (SAL). The doses refer to the salt forms.

2.3. Experimental procedure

Analgesia was assessed using a standard tail-flick apparatus (EMDIE Instrument, Roanoke, VA). The intensity of the thermal stimulus was adjusted to provide average baseline tail-flick latency (TFL) of 2–3 s. A cutoff time of 10 s was used to avoid any injury to the tail. All rats were handled and trained in the test situation before the initiation of the tail-flick test. During the experiments, TFL was measured twice (15 min apart) before and at 15, 30, 60, and 120 min following administration of the drug(s).

2.4. Data analysis

For each rat and at each time point, TFL was normalized for preinjection baseline measured on the day of the experiment (postinjection TFL − baseline TFL). The time–action curve for the normalized TFL was generated. The areas under the time–action curve for experimental data ($AUC_{0-120\ min}$) and theoretic data (maximum $AUC_{0-120\ min}$ [assuming TFL = cutoff at each postinjection time point]) were then calculated by the trapezoidal rule. Percentage of the maximal possible effect (%MPE) was obtained according to the formula: $\%MPE=(AUC_{0-120\ min}/\text{maximum-}AUC_{0-120\ min})\times 100$. Data were analyzed by two-way repeated-measures (RM) AVOVA (factors: Treatment × Sex and Treatment × Time) using post hoc Student's–Newman–Keuls' (SNK) and Bonferroni tests and one-way RM ANOVA (factor: Treatment) using post hoc SNK test (SigmaStat computer software). The level of significant difference was $P \leq .05$.

3. Results

Table 1 summarizes baseline sensitivity to thermally induced pain (preinjection TFL) in male and female rats, which were repeatedly challenged with either MOR alone or in combinations with NMDA antagonists: DEX, KET, or MK-801. The data indicate that the baseline (preinjection) responsiveness to radiant heat did not significantly change across time with treatment within each group of male or female rats.

Table 1
Baseline (preinjection) tail-flick response (TFL, s) in rats treated with MOR (3 mg/kg) alone and MOR + DEX (7.5, 15, and 30 mg/kg), MOR + KET (0.75, 1.5, and 3 mg/kg), and MOR + MK-801 (MK, 0.075. 0.15, and 0.3 mg/kg)

Treatment dose	Number of rats	Male	Female
MOR	12/sex	2.91 ± 0.183	2.42 ± 0.134
MOR + DEX (7.5 mg/kg)		3.00 ± 0.213	2.35 ± 0.139
MOR + DEX (15 mg/kg)		2.92 ± 0.184	2.39 ± 0.161
MOR + DEX (30 mg/kg)		2.65 ± 0.118	2.17 ± 0.117
MOR	12/sex	2.79 ± 0.204	2.17 ± 0.119
MOR + KET (0.75 mg/kg)		2.97 ± 0.269	2.30 ± 0.162
MOR + KET (1.5 mg/kg)		3.00 ± 0.210	2.25 ± 0.126
MOR + KET (3 mg/kg)		2.80 ± 0.147	1.89 ± 0.122
MOR	8/sex	2.73 ± 0.156	2.87 ± 0.109
MOR + MK (0.075 mg/kg)		2.78 ± 0.245	2.64 ± 0.181
MOR + MK (0.15 mg/kg)		2.70 ± 0.190	2.62 ± 0.183
MOR + MK (0.3 mg/kg)		2.81 ± 0.255	2.49 ± 0.204

Drugs were administered in weekly intervals (randomized doses). Data are means ± S.E.M. of *n* rats/sex/group.

Fig. 1A–D present time–action curves (TFL) for SAL (1 ml/kg), DEX (30 mg/kg), KET (3 mg/kg), and MK-801 (0.3 mg/kg) in male and female rats. The data show that neither SAL nor the NMDA receptor antagonists alone produce significant analgesia in male or female rats.

Fig. 2A and B show time–action curves (TFL) for MOR alone (3 mg/kg) and MOR + DEX (7.5, 15, and 30 mg/kg) in male and female rats. The data indicate that DEX enhanced the magnitude and had some effect on the duration of MOR analgesia. The analgesic effect in female rats was significantly related to Treatment and Time [$F(3,191) = 6.7$, $P < .0025$, $F(3,191) = 12.2$, $P < .0001$, respectively]; however, there was no statistically significant interaction between treatment and time (two-way RM ANOVA). Analgesia was related to time [$F(3,191) = 20.3$, $P < .0001$] but not to treatment in male rats.

Fig. 3 presents the dose–response relationship of the analgesic effect (%MPE) produced by DEX alone, MOR alone, and MOR + DEX. In the tested dose range, DEX alone (7.5–30 mg/kg) did not produce analgesia. MOR alone, as previously shown (Cicero et al., 1996), produced a greater analgesic effect in male compared with female rats. MOR (3 mg/kg) analgesia was progressively enhanced by increasing doses of DEX (7.5, 15, and 30 mg/kg) in female rats [$F(3,47) = 5.6$, $P < .005$, one-way RM ANOVA] while the dose–effect relationship was below the level of statistical significance in male rats. A two-way RM ANOVA indicated significant between-sex and between-treatment differences [$F(3,95) = 12.5$, $P < .025$ and $F(3,95) = 6.3$, $P < .001$] with no significant Sex × Treatment interaction. The extent of enhancement of MOR-induced analgesia tended to be greater in female than in male rats. In female rats, MOR alone (3 mg/kg) produced 15.2 ± 2.9% MPE and MOR + DEX (30 mg/kg) produced 56.6 ± 10.3% MPE. This represents a 2.7-fold increase in analgesic effect. In male rats, MOR alone produced 38.9 ± 8.3% MPE and MOR + DEX (30 mg/kg) produced 62.9 ± 9.4% MPE. This represents only 0.6-fold increase in analgesic effect.

Fig. 4A and B show the time–action curves (TFL) for MOR alone (3 mg/kg) and MOR + KET (0.75, 1.5, and 3 mg/kg) in male and female rats. KET increased the peak effect of MOR but did not prolong its action in female rats. The analgesic effect was significantly related to Treatment and Time [$F(3,191) = 12.8$ and $F(3,191) = 15.8$, respectively, $P < .0001$], but there was no significant Treatment × Time interaction in female rats (two-way RM ANOVA). The effect of KET on MOR was less prominent in male rats.

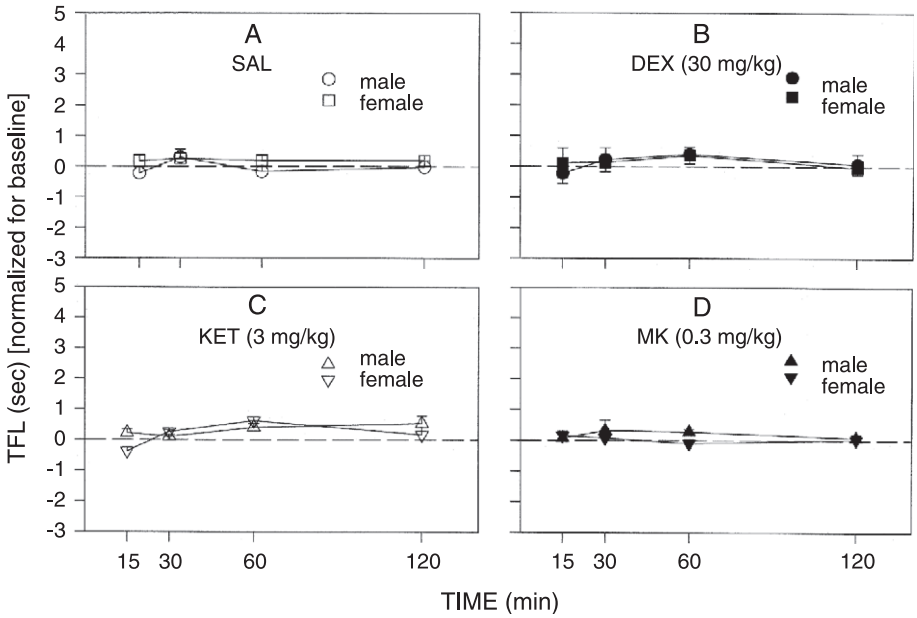

Fig. 1. Time–action curves for SAL (1 ml/kg) (A), DEX (30 mg/kg) (B), KET (3 mg/kg) (C), and MK-801 (MK, 0.3 mg/kg) (D) in male and female rats. Analgesia is presented as TFL (s, normalized for baseline). Data are means ± S.E.M. for 6 SAL and 4 DEX, KET, and MK rats/sex.

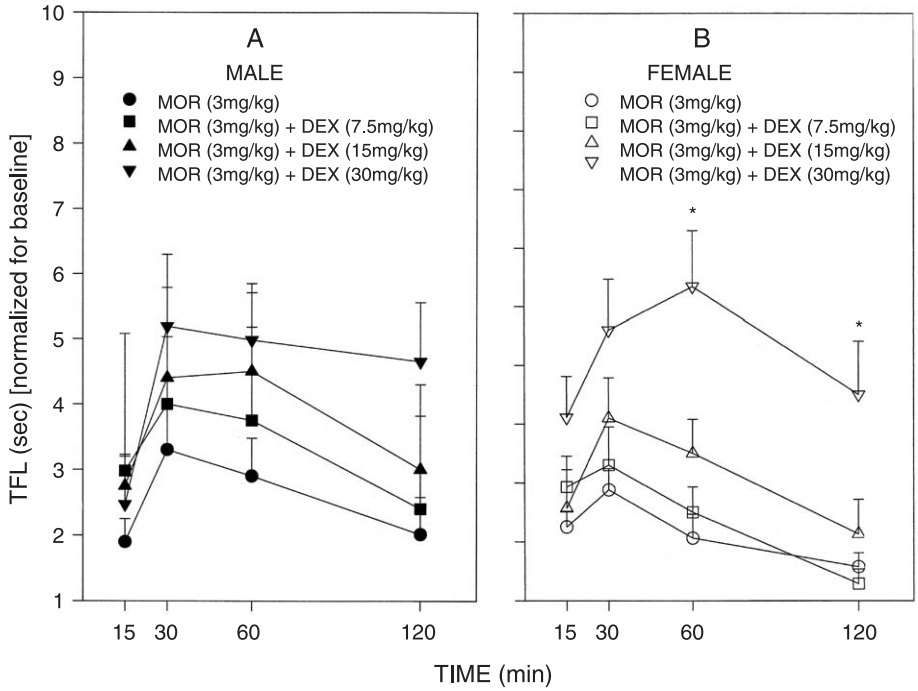

Fig. 2. Time–action curves for MOR (3 mg/kg) alone and MOR + DEX (7.5, 15, and 30 mg/kg) in male (A) and female (B) rats. Analgesia is presented as TFL (s, normalized for baseline). Data are means ± S.E.M. for 12 rats/sex. * Significantly different from MOR alone ($P < .05$, post hoc Bonferroni method).

Fig. 5 shows the dose–response relationship of the analgesic effect (%MPE) produced by KET alone (0.75, 1.5, and 3 mg/kg), MOR alone (3 mg/kg), and MOR + KET (0.75, 1.5, and 3 mg/kg). As can be seen, the analgesic effect of KET alone was negligible. KET produced a dose-related enhancement of MOR analgesia in female rats [$F(3,47) = 12.0$, $P < .00025$, one-way RM ANOVA], while male rats were less sensitive. A two-way RM ANOVA revealed that between-treatment differences were statistically significant [$F(3,95) = 3.3$, $P < .025$], while the effect of sex was just under significance [$F(3,95) = 3.9$, $P = .058$]. In female rats, KET (3 mg/kg) produced a threefold increase

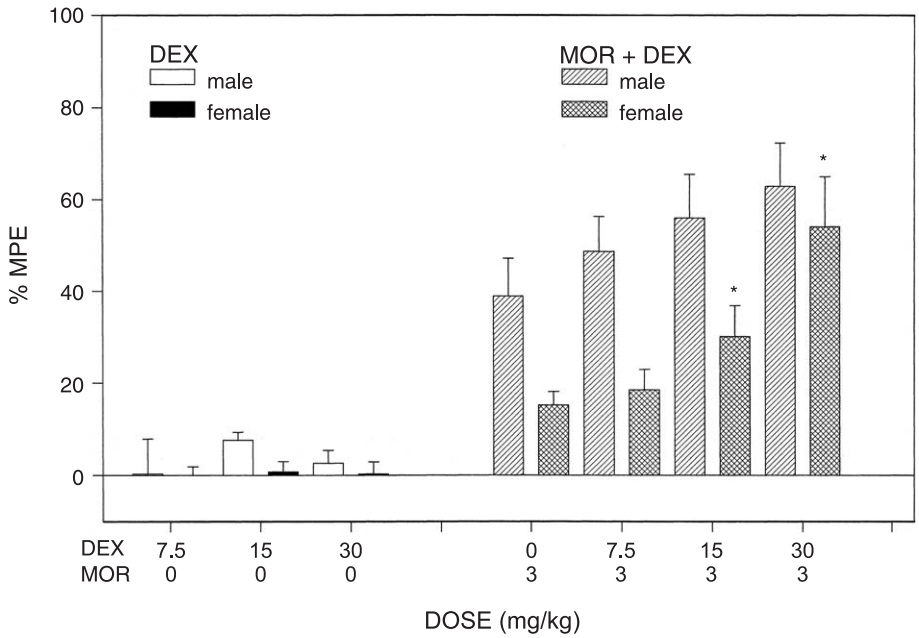

Fig. 3. The dose–response relationships of the analgesic effect produced by DEX alone, MOR alone, and MOR + DEX in male and female rats. Analgesia is presented as %MPE. Data are the means ± S.E.M. for 4 DEX and 12 MOR + DEX rats/sex. * Significantly different from MOR alone ($P < .05$, post hoc SNK test).

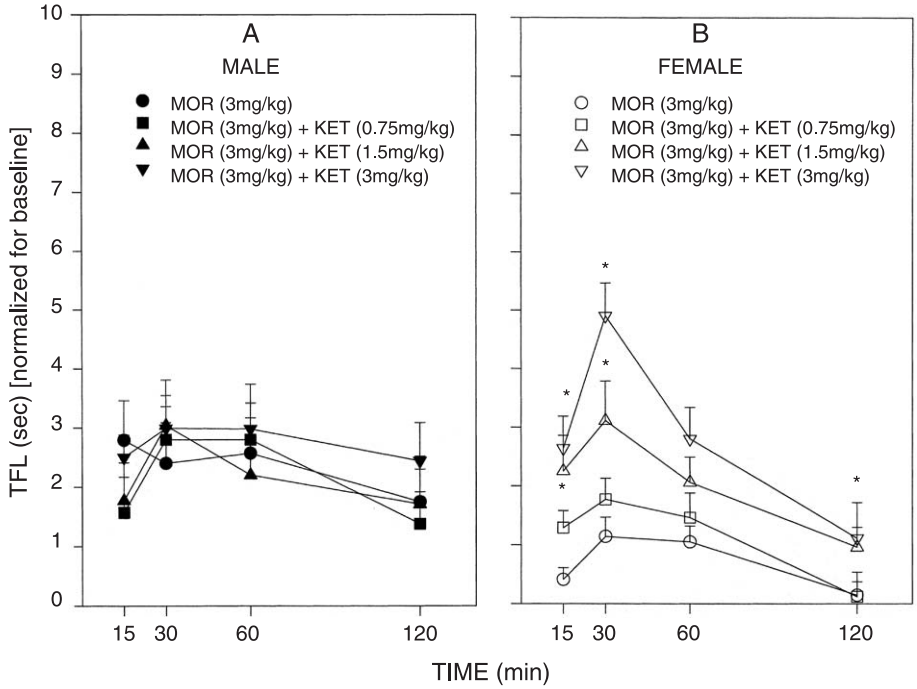

Fig. 4. Time–action curves for MOR (3 mg/kg) alone and MOR + KET (0.75, 1.5, and 3 mg/kg) in male (A) and female (B) rats. Analgesia is presented as TFL (s, normalized for baseline). Data are means ± S.E.M. for 12 rats/sex. * Significantly different from MOR alone ($P < .05$, post hoc Bonferroni method).

in MOR (3 mg/kg) analgesia (45 ± 5.7% MPE vs. 11.4 ± 2.5% MPE), while in male rats the increase in MOR analgesia was negligible (38.2 ± 6.9% MPE vs. 35.4 ± 8.27% MPE).

Fig. 6A and B show the time–action curves (TFL) for MOR alone (3 mg/kg) and MOR + MK-801 (0.075, 0.15, and 0.3 mg/kg) in male and female rats. MK-801 affected both peak action and duration of MOR analgesia in female rats. The analgesic effect was significantly related to Treatment [$F(3,127) = 4.3$, $P < .025$] and Time [$F(3,127) = 3.1$, $P < .0001$], with a significant Treatment × Time interaction [$F(9,127) = 2.4$, $P < .025$] in female rats. Analgesia was related to Time [$F(3,127) = 11.3$, $P < .0001$] but not to treatment in male rats (two-way RM ANOVA).

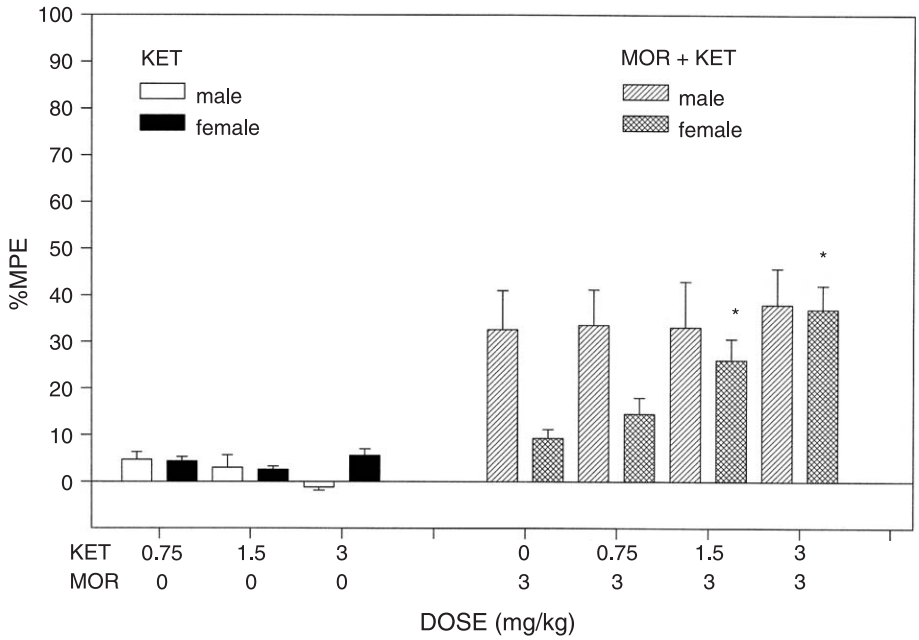

Fig. 5. The dose–response relationship of the analgesic effect produced by MOR alone and MOR + KET in male and female rats. Analgesia is presented as %MPE. Data are the means ± S.E.M. for 4 KET and 12 MOR + KET rats/sex. * Significantly different from MOR alone ($P < .05$, post hoc SNK test).

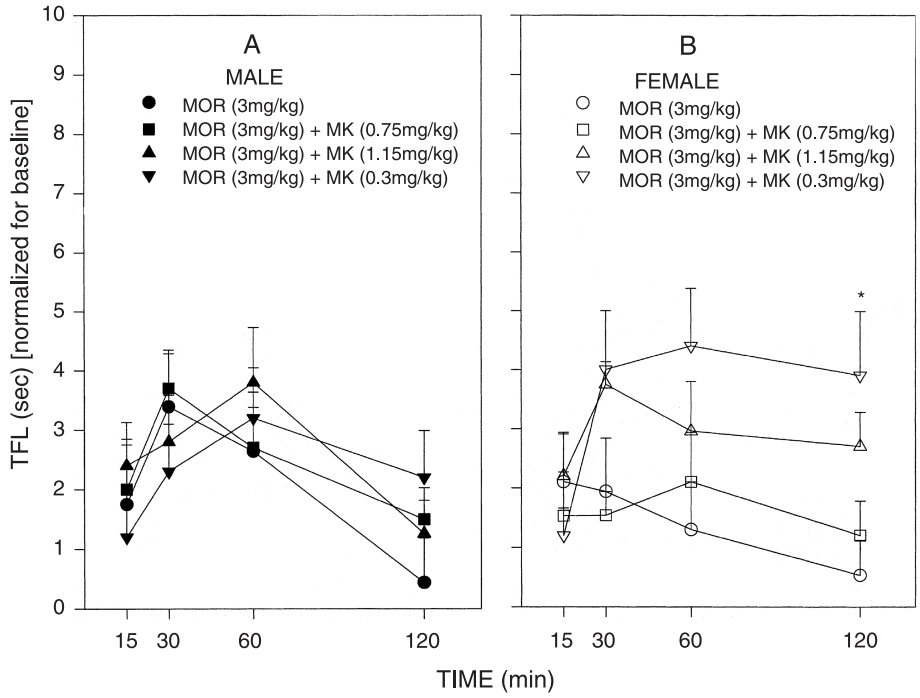

Fig. 6. Time–action curves for MOR (3 mg/kg) alone and MOR + MK-801 (MK, 0.075, 0.15, and 0.3 mg/kg) in male (A) and female (B) rats. Analgesia is presented as TFL (s, normalized for baseline). Data are means ± S.E.M. for 8 rats/sex. * Significantly different from MOR alone ($P < .05$, post hoc Bonferroni method).

Fig. 7 illustrates dose–response relationships (%MPE) for MK-801 alone (0.075, 0.15, and 0.3 mg/kg), MOR alone (3 mg/kg), and MOR + MK-801 (0.075, 0.15, and 0.3 mg/kg). The data show that MK-801, at doses that by themselves did not cause analgesia, dose-dependently enhanced the analgesic effect of MOR in female rats [$F(3,31) = 3.3$, $P < .01$, one-way RM ANOVA]. The effect of MK-801 on MOR-induced analgesia was less pronounced in male rats. The addition of MK-801 (0.3 mg/kg) caused a 1.7-fold increase in MOR (3 mg/kg) analgesia in female rats

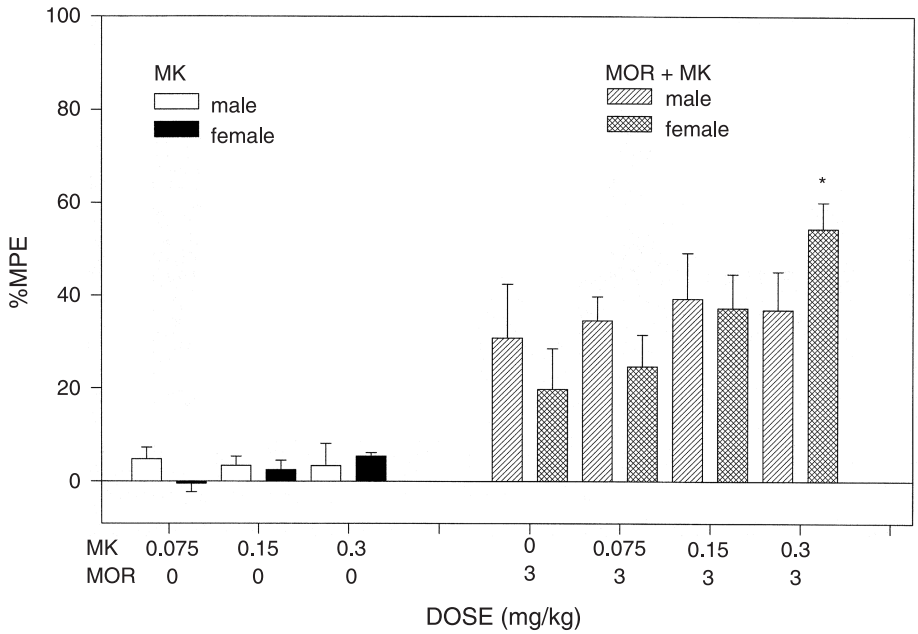

Fig. 7. The dose–response relationship of the analgesic effect produced by MOR alone and MOR + MK-801 (MK) in male and female rats. Analgesia is presented as %MPE. Data are the means ± S.E.M. for 4 MK and 8 MOR + MK rats/sex. * Significantly different from MOR alone ($P < .05$, post hoc SNK test).

(54.5 ± 5.6% MPE vs. 19.8 ± 8.7% MPE) but only a 0.2-fold increase in analgesia in male rats (36.9 ± 8.3% MPE vs. 30.8 ± 11.7% MPE).

4. Discussion

The present data indicate that NMDA receptor antagonists such as DEX, KET, and MK-801, in doses that do not produce antinociceptive effects alone, significantly and dose-dependently potentiate the antinociceptive effects of MOR in female rats when tested by the tail-flick analgesic assay. There was a trend for enhancement of MOR analgesia by DEX in male rats; however, the magnitude of this effect was lesser than in female rats. KET and MK-801 had only a marginal effect on MOR analgesia in male rats. These findings support previous studies showing a stronger effect with DEX than with KET or MK-801 in male rats (Grass et al., 1996; Kozela et al., 2001; Plesan et al., 1998).

There is a volume of literature showing potentiation, attenuation, or no effect of the NMDA antagonists on acute MOR analgesia (see Belozertseva et al., 2000; Kozela et al., 2001) For example, DEX enhanced MOR analgesia (hot-plate test) in male rats (Grass et al., 1996; Hoffman and Wiesenfeld-Hallin, 1996; Plesan et al., 1998) and in male mice (Baker et al., 2002) but not in mice tested by the tail-flick test (Popik et al., 2000). Others have shown that the DEX enhancement of MOR analgesia is related to the site of recording (tail vs. paw) (Kozela et al., 2001) and strain in male rats (Bulka et al., 2002; Plesan et al., 1999). KET enhanced MOR analgesia (hot plate) in male mice (Baker et al., 2002) but had a less pronounced effect in male rats (Plesan et al., 1998). MK-801 either reduced (hot-plate test) (Lipa and Kavaliers, 1990; Saucier and Kavaliers, 1994) or did not affect MOR analgesia (tail-flick test) in male mice (Bilsky et al., 1996; Elliott et al., 1994a; Lufty et al., 1993) and male rats (Trujillo and Akil, 1991). However, Kest et al. (1992) showed that MK-801 enhanced the analgesic effect of a low (1 mg/kg) but not a high (5 mg/kg) dose of MOR (tail-flick test) in male rats. These conflicting data indicate that the effect of NMDA antagonists on opioid-induced analgesia is a complex phenomenon and likely depends on several factors such as type of antagonist, dose, pain test, species, and strain. Our data, which appear to agree with a lack of significant enhancement of MOR analgesia in male rats (tail-flick test), additionally revealed that the effect of DEX, KET, and MK-801 on MOR is related to sex (female>male). In the present study, the more pronounced enhancement of MOR antinociception by the NMDA receptor antagonists in female than in male rats is an interesting observation because the analgesic effect of MOR is generally well accepted to be less in female than in male rats (Cicero et al., 1996). This observation was also confirmed in the current study; however, the addition of the NMDA receptor antagonists diminished the difference in MOR-induced analgesia between the sexes. Whether sex differences are maintained for equipotent doses of MOR in male and female rats remains to be tested.

In the present study, the coadministration of KET enhanced the peak effect of MOR but did not prolong its analgesic action. DEX tended to affect the peak effect and duration of MOR-induced analgesia. MK-801 enhanced both the magnitude and the duration of MOR analgesia in female rats. Enhancement of analgesia by concomitant administration of NMDA receptor antagonists and MOR may suggest an analgesic synergy between these drugs. This hypothesis can be further tested by isobolographic analysis. Alternatively, NMDA receptor antagonists seem to lengthen MOR analgesia; thus, it has been proposed that blockade of the NMDA receptor(s) attenuates acute tolerance to MOR (Ben-Eliyahu et al., 1992; Belozertseva et al., 2000; Grass et al., 1996; Plesan et al., 1998). The abilities of NMDA receptor antagonists to inhibit and reverse analgesic tolerance to chronically administered MOR has been repeatedly shown (Bilsky et al., 1996; Elliott et al., 1994a,b; Lufty et al., 1993; Manning et al., 1996; Mao et al., 1996; Popik et al., 2000; Trujillo and Akil, 1991). The mechanism of action is not entirely clear. For instance, it has been proposed that the opiate-related activation of NMDA receptors through G-proteins associated with μ-opioid receptor may affect protein kinase C and nitric oxide. Further, interaction of NMDA antagonists and MOR may occur in the signal transduction system as a change in the adenyl cyclase activity or Ca^{2+} influx (Bilsky et al., 1996; Chen and Huang, 1991; Lufty et al., 1993; Mao et al., 1995, 1996). The greater effect of DEX, KET, and MK-801 on MOR antinociception in female versus male rats indicates a sexual dimorphism in the NMDA–opioid interaction. In this context, the lesser analgesic effect of MOR as well as the greater effectiveness of NMDA antagonists suggests that during opiate-induced analgesia NMDA receptors may be activated to a greater extent in female than in male rats.

The pharmacological properties of NMDA and opioid receptors are defined by composition of subunits; thus, concomitant treatment with NMDA antagonists and MOR may cause different compensatory adaptations in receptor function in male and female rats. Sex differences in rat brain structure have been recognized for many years. Numerous neuronal targets are probably systematically affected by steroid hormones, which may alter either temporarily or permanently the way the brain functions. Sexual dimorphisms include differences in neuroanatomical function, neuroendocrine physiology, regional neurotransmission and neurotransmitter densities, and many physiological responses influenced by steroids. In this regard, it has been shown that NMDA receptor densities differ with respect to sex and the estrous cycle in rats (Palomero-Gallagher et al., 2003) and that ovarian steroids modulate NMDA receptors (Weiland, 1992; Gazzaley et al., 1996). Further, sex-related pharmacokinetic interactions cannot be ruled out; however, this is unlikely because sexual dimorphism in the analgesic effect of MOR could not be explained by differences in

plasma and brain drug levels (Cicero et al., 1997). The analgesic effects induced by DEX, KET, and MK-801 were negligible and not related to dose and sex (present study); in addition, MK-801 has been shown to affect MOR analgesia without affecting MOR pharmacokinetics in mice (Maeda et al., 2002). However, it should be mentioned that the distribution of KET into the brain was shown to be increased by another opioid agonist, alfentanil (Edwards et al., 2002).

The present data show that DEX enhances MOR analgesia in both sexes, whereas the actions of KET and MK-801 (at least in the doses tested) seem to be selective for female rats. Both KET and MK-801 are considered to be selective antagonists of the NMDA receptor (KET may also act on σ-receptors (Hustveit et al., 1995; Nakao et al., 2002)), while it has been suggested that the action of DEX is nonselective for the NMDA receptor (Carpenter et al., 1988; Chou et al., 1999; Elliott et al., 1994a). In the latter respect, it has been thought that DEX reduces intracellular Ca^{2+} both through NMDA-gated and non-NMDA (voltage-dependent) calcium and sodium channels while KET has a more profound effect on NMDA-gated Ca^{2+} current than on K^{+}-evoked Ca^{2+} influx (see Elliott et al., 1994a). In female rats, the effect of DEX on MOR-induced analgesia resembles the effects of KET and MK-801, suggesting action through the NMDA receptor. In male rats, the more pronounced effect of DEX (compared with KET and MK-801) can be due to a non-NMDA mechanism of action.

Acknowledgements

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References

Baker AK, Hoffman VL, Meert TF. Interaction of NMDA antagonists and α2 agonist with μ, δ, and κ opioids in an acute nociception assay. Acta Anesthesiol Belg 2002;53:203–12.

Bell RF. Low-dose subcutaneous ketamine infusion and morphine tolerance. Pain 1999;83:101–3.

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.

Ben-Eliyahu S, Marek P, Vaccarino AL, Mogil JS, Sternberg WF, Liebeskind JC. The NMDA receptor antagonist MK-801 prevents long-lasting non-associative morphine tolerance in the rat. Brain Res 1992;575:304–8.

Bilsky EJ, Inturrisi CE, Sadée 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.

Bisaga A, Popik P. In search of a new pharmacological treatment for drug and alcohol addiction: *N*-methyl-D-aspartate (NMDA) antagonists. Drug Alcohol Depend 2000;59:1–15.

Bisaga A, Comer SD, Ward AS, Popik P, Kleber HD, Fischman MW. The NMDA antagonist memantine attenuates the expression of opioid physical dependence in humans. Psychopharmacology 2001;157:1–10.

Bulka A, Wiesenfeld-Hallin Z, Xu X-J. Differential antinociception by morphine and methadone in two sub-strains of Sprague–Dawley rats and its potentiation by dextromethorphan. Brain Res 2002;942:95–100.

Carpenter CL, Marks SS, Watson DL, Greenberg DA. Dextromethorphan and dextrorphan as calcium channel antagonists. Brain Res 1988;439:372–5.

Caruso FS. MorphiDex pharmacokinetic studies and single-dose analgesic efficacy studies in patients with postoperative pain. J Pain Symptom Manage 2000;19(Suppl. 1):S31–6.

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

Chou Y-C, Liao J-F, Chang W-Y, Lin M-F, Chen C-F. Binding of dimemorfan to σ-1 receptor and its anticonvulsant and locomotor effects in mice, compared with dextromethorphan and dextrorphan. Brain Res 1999;821:516–9.

Church J, Shacklock JA, Baimbridge KG. Dextromethorphan and phencyclidine receptor ligands: differential effects on K(+)- and NMDA-evoked increases in cytosolic free Ca^{2+} concentration. Neurosci Lett 1991;124:232–4.

Cicero TJ, Nock B, Meyer ER. Gender-related differences in the antinociceptive properties of morphine. J Pharmacol Exp Ther 1996;279:767–73.

Cicero TJ, Nock B, Meyer ER. Sex-related difference in morphine antinociceptive activity: relationship to serum and brain morphine concentrations. J Pharmacol Exp Ther 1997;282(2):939–44.

Craft RM. Sex differences in drug- and non-drug-induced analgesia. Life Sci 2003;72:2675–88.

Edwards SR, Minto CF, Mather LE. Concurrent ketamine and alfentanil administration: pharmacokinetic considerations. Br J Anesth 2002;88(1):94–100.

Elliott K, Hynansky A, Inturrisi CE. Dextromethorphan attenuates and reverses analgesic tolerance to morphine. Pain 1994a;59:361–8.

Elliot K, Minami N, Kolesnikov YA, Pasternak GW, Inturrisi CE. The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, N^{G}-nitro-L-arginine, attenuate analgesic tolerance to the μ-opioid morphine but not to κ opioids. Pain 1994b;56:69–75.

Fisher K, Hagen NA. Analgesic effect of oral ketamine in chronic neuropathic pain of spinal origin: a case report. J Pain Symptom Manage 1999;18(1):61–6.

Gazzaley AH, Weiland NG, McEwen BS, Morrison JH. Differential regulation of NMDAR1 mRNA and protein by estradiol in the rat hippocampus. J Neurosci 1996;16:6830–8.

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

Hewitt DJ. The use of NMDA-receptor antagonists in the treatment of chronic pain. Clin J Pain 2000;16:S73–9.

Hoffman O, Wiesenfeld-Hallin Z. Dextromethorphan potentiates morphine antinociception, but does not reverse tolerance in rats. NeuroReport 1996;7:838–40.

Holtman Jr JR, Sloan JW, Wala EP. Modification of morphine analgesia and tolerance by flumazenil in male and female rats. Eur J Pharmacol 2003;470:149–56.

Hustveit O, Maurset A, Oye I. Interaction of chiral forms of ketamine with opioid, phencyclidine, σ and muscarinic receptor. Pharmacol Toxicol 1995;77:355–9.

Katz NP. MorphiDex (MS:DM) double-blind, multiple-dose studies in chronic pain patients. J Pain Symptom Manage 2000;19(Suppl. 1):S37–41.

Kest B, Marek P, Liebeskind JC. The specific *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 blocks U-50,488, but not morphine antinociception. Brain Res 1992;589:139–42.

Kest B, Sarton E, Dahan A. Gender differences in opioid-mediated analgesia. Anesthesiology 2000;93:539–47.

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.

Lipa SA, Kavaliers M. Sex differences in the inhibitory effects of the NMDA antagonists, MK-801, on morphine and stress-induced analgesia. Brain Res Bull 1990;24:627–30.

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

Maeda T, Kishioka S, Fan X, Fukazawa Y, Shimizu N, Ozaki M, et al. Effects of diltiazem and MK-801 on morphine analgesia and pharmacokinetics in mice. Neurosci Lett 2002;326(3):216–8.

Manning BH, Mao J, Frenk H, Price DD, Mayer DJ. Continuous co-administration of dextromethorphan or MK-801 with morphine: attenuation of morphine dependence and naloxone-reversible attenuation of morphine tolerance. Pain 1996;67:79–88.

Mao J. NMDA and opioid receptors: their interactions in antinociception, tolerance and neuroplasticity. Brain Res Rev 1999;30:289–304.

Mao J, Price DD, Mayer DJ. Mechanism of hyperalgesia and morphine tolerance: a current view of their possible interactions. Pain 1995;62: 259–74.

Mao J, Price DD, Caruso FS, Mayer DJ. Oral administration of dextromethorphan prevents the development of morphine tolerance and dependence in rats. Pain 1996;67:361–8.

Nakao S, Miyamoto E, Masuzawa M, Kambara T, Shingu K. Ketamine-induced c-Fos expression in the mouse posterior cingulate and retrosplenial cortices is mediated not only via NMDA receptors but also via σ receptors. Brain Res 2002;926:191–6.

Netzer R, Pflimlin P, Trube G. Dextromethorphan blocks *N*-methyl-D-aspartate-induced currents and voltage-operated inward currents in cultured cortical neurons. Eur J Pharmacol 1993;238:209–16.

Palomero-Gallagher N, Bidmon H-J, Zilles K. AMPA, kainate, and NMDA receptor densities in the hippocampus of untreated male rats and females in estrus and diestrus. J Comp Neurol 2003;459:468–74.

Plesan A, Hedman U, Xu X-J, 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, Hoffmann O, Xu X-J, Wiesenfeld-Hallin Z. Genetic differences in the antinociceptive effect of morphine and its potentiation by dextramethorpan in rats. Neurosci Lett 1999;263:53–6.

Popik P, Kozela E, Danysz W. Clinically available NMDA receptor antagonist memantine and dextromethorphan reverse existing tolerance to the antinociceptive effects of morphine in mice. Naunyn-Schmiedeberg's Arch Pharmacol 2000;361:425–32.

Sang CN. NMDA-receptor antagonists in neuropathic pain: experimental methods to clinical trials. J Pain Symptom Manage 2000;19(Suppl. 1): S21–5.

Saucier DM, Kavaliers M. Antagonistic effects of the selective, competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, NPC 12626, on κ opiate-induced analgesia in male deer mice. Brain Res 1994;637: 292–6.

Schmid RL, Sandler AN, Katz J. Use and efficacy of low-dose ketamine in the management of acute postoperative pain: a review of current techniques and outcomes. Pain 1999;82:11–125.

Tortela FC, Pellicano M, Bowery NG. Dextromethorphan and neuromodulation: old drug coughs up new activities. Trends Pharmacol Sci 1989; 10:501–7.

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

Weiland NG. Estradiol selectively regulates agonist binding sites on the *N*-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. Endocrinology 1992;131:662–8.

Wong EHF, Kemp JA, Priest T, Knight AP, Woodruff GN, Iversen LL. The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. Proc Natl Acad Sci U S A 1986;83:7104–8.

Yamamura T, Harada K, Okamura A, Kemmotsu O. Is the site of action of ketamine anesthesia the *N*-methyl-D-aspartate receptor? Anesthesiology 1990;72:704–10.