Effect of the NMDA-antagonist, MK 801, on benzodiazepine-opioid interactions at the spinal and supraspinal level in rats

¹Thomas J. Luger, Ingo H. Lorenz, Christina Grabner-Weiss & *Takahiko Hayashi

Clinic of Anesthesia and General Intensive Care Medicine, University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria and *Dept. of Anesthesiology, School of Medicine, Osaka University, 2-2 Yamadaoka Suita City, Osaka, Japan

1 Benzodiazepines potentiate morphine antinociception at the spinal level via GABAergic mechanisms. At the supraspinal level, the inhibitory effect of midazolam on morphine antinociception cannot be easily explained by GABA_A receptor activation. Since excitatory amino acids play a role in central transmission, we investigated the effect of dizocilpine (MK 801) on this interaction in spinal cord and brain.

2 In rats with an intrathecal or intracerebroventricular catheter, the mechanisms of the antinociceptive effect of benzodiazepine-morphine combinations were tested during thermal nociceptive tests.

3 The principal findings of this study were that at the spinal level, midazolam potentiation of morphine antinociception can be antagonized by the NMDA antagonist, MK 801 (10 µg), as assessed by hot-plate and tail-flick tests. When drugs were administered supraspinally, midazolam inhibited morphine antinociception only in the hot-plate test, an effect also inhibited by MK 801. In the tail-flick assay, midazolam failed to influence the morphine response.

4 The NMDA antagonist significantly affected midazolam antinociception at the spinal level, but was not effective following i.c.v. administration of the drugs. MK 801 had no effect on morphine antinociception after i.t. and i.c.v. administration of the drugs.

5 The paradoxical effect of midazolam on morphine antinociception and its reversal by MK 801 might be due to modulation at various levels of the neuraxis and/or modulation of different pathways mediated via both GABA_A and NMDA receptor mechanisms.

Keywords: Benzodiazepines; opioids; NMDA antagonists; dizocilpine (MK 801); nociceptive tests; intrathecal; intracerebroventricular

Introduction

Many neurotransmitters are involved in the modulation of incoming pain information. Opioid drugs can produce analgesia at supraspinal and spinal sites by inhibiting nociceptive input (Yaksh, 1984). These two sites of opioid actions may be influenced either directly or indirectly by non-opioids in an additive or antagonistic manner (Luger *et al.*, 1992; 1993). A growing body of evidence suggests there are mechanisms whereby interactions between two major neurotransmitter systems, excitatory amino acids (EAAs) γ-aminobutyric acid (GABA), can significantly modulate signal transmission in the brain (Watkins & Evans, 1981; Wilcox, 1991).

Several lines of investigation have indicated that in laboratory animals, benzodiazepine agonists injected intrathecally (i.t.) potentiate opioid antinociception in the spinal cord (Yanez *et al.*, 1990; Luger *et al.*, 1993; 1994), while other studies indicate that benzodiazepines given intracerebroventricularly (i.c.v.) reduce the antinociceptive effects of systemic opioids (Mantegazza *et al.*, 1979; 1982; Luger *et al.*, 1993; 1994). GABA is well established as the main inhibitory neurotransmitter found at synapses throughout the brain and spinal cord (Goodchild, 1993). Benzodiazepine agonists exert their effects by potentiation of GABA at a specific receptor site on a benzodiazepine GABA_A-ionophore complex in various parts of the CNS (Haefely *et al.*, 1993). Since the inhibitory effect of midazolam on morphine antinociception at the supraspinal level (Mantegazza *et al.*, 1982) cannot be sufficiently explained by GABA_Aergic mechanisms (Lorenz *et al.*, 1993;

Luger *et al.*, 1994), non-GABA receptors, like N-methyl-D-aspartate (NMDA) receptors, might be involved in this interaction in the brain. EAAs are accepted as the main neurotransmitters mediating synaptic excitation in the CNS (Watkins & Evans, 1981; Wilcox, 1991). The role of EAAs in central nociceptive transmission is based first on neurochemical studies. These show that EAAs such as glutamate are present in the superficial dorsal horn C fibre central terminals, in coexistence with substance P containing C fibres (Greenmayre *et al.*, 1984). Secondly, electrophysiological studies have shown glutamate to activate superficial dorsal horn neurones in the spinal cord (Schneider & Perl, 1988). NMDA has been implicated in the phenomenon called 'wind up', which is thought to be responsible for central hyperalgesia, and which can be abolished by NMDA antagonists (Goodchild, 1993). Thirdly, behavioural studies investigating the spinal administration of NMDA or NMDA agonists show hyperalgesia in the hot-plate and tail-flick assay in mice and rats (Aanonsen & Wilcox, 1987; Raigorodsky & Ugra, 1987; Malmberg & Yaksh, 1993). However, this hyperalgesic effect of NMDA-agonists can be reversed by selective NMDA antagonists (Cahusac *et al.*, 1984; Raigorodsky & Ugra, 1987) and antagonized by muscimol (Aanonsen & Wilcox, 1989). Intrathecal NMDA antagonists at doses below those that resulted in motor dysfunction produced no alterations in the acute response to thermal nociceptive stimuli (Cahusac *et al.*, 1984; Aanonsen & Wilcox, 1987; Yaksh, 1989; Aanonsen *et al.*, 1990). In contrast, they abolish the 'wind up' phenomenon (Yaksh, 1989; Goodchild, 1993). They also inhibit the response to noxious stimuli in dorsal horn neurones in electrophysiological studies (Haley *et al.*, 1990; Woolf & Thompson, 1991). Synaptic excitation can also be antagonized by NMDA antagonists in other regions of the CNS, e.g. in the

¹ Author for correspondence.

cerebral cortex, cuneate nucleus or hippocampus (Watkins & Evans, 1981). Besides these direct effects of EAAs on nociception, NMDA agonists and antagonists may alter opioid antinociception. NMDA antagonists injected into the PAG antagonized morphine and NMDA antinociception, indicating that morphine produces analgesia in the PAG by disinhibition of neurones that contain NMDA receptors (Jacquet, 1988). Morphine antinociception, but not NMDA-induced antinociception, was antagonized by an NMDA antagonist administered in the nucleus raphe magnus (van Praag & Frenk, 1990). In the majority of reports investigating the acute effects of the noncompetitive NMDA antagonist, MK 801, on morphine antinociception, no effect was observed when it was administered i.t. and i.c.v. (Marek *et al.*, 1991a,b; Trujillo & Akil, 1991b; Yamamoto & Yaksh, 1992). The data of Lipa & Kavaliers (1990) indicate, however, that intraperitoneal administration of MK 801 reduces morphine-induced antinociceptive response in male mice. Additionally, diazepam probably exerts some effect on amino acid-induced responses in spinal motoneurons of the frog (Evans *et al.*, 1977).

Benzodiazepines potentiate morphine antinociception at the spinal level via GABAergic mechanisms. At the supraspinal level, the inhibitory effect of midazolam on morphine antinociception cannot be easily explained by GABA_A receptor activation (Lorenz *et al.*, 1993; Luger *et al.*, 1993; 1994). Since excitatory amino acids may play a role in central transmission and in the light of the above-mentioned facts, our study aimed to investigate the effect of MK 801 on midazolam-morphine interaction at both spinal cord and supraspinal level.

Methods

Animals

For these experiments we used male Sprague-Dawley rats (250–320 g; 5–8 animals/dose). All animals were housed in individual cages on a 12 h-light/12 h-dark cycle at a constant temperature of $23 \pm 1^\circ\text{C}$ with free access to food and water. All studies were performed during the light cycle. The study was approved by the 'Kommission für Tierversuchsangelegenheiten' (Tierschutzgesetz BGBl. Nr.501/1989: GZ 66.009/253-I/A/2/92) of the Federal Ministry of Science and Research, Vienna, Austria.

Intrathecal and intracerebroventricular catheter implantation

Intrathecal and intracerebroventricular catheters were implanted under halothane anaesthesia and aseptic conditions with the rats placed in a stereotaxic frame with the head flexed forward. Separate groups of rats received different catheter implantations. An intrathecal catheter (polyethylene tube PE – 10; length 8.5 cm) was inserted through a slit in the cisternal membrane and passed rostrally to the thoracolumbar level. The external part of the catheter was then tunnelled subcutaneously to exit on top of the skull (see Yaksh & Rudy, 1976). For implantation of an intracerebroventricular catheter (polyethylene tube PE – 10; length 4.5 cm), the rat was placed in a stereotaxic frame and a hole trephined in the skull at coordinates overlaying the left ventral ventricle; 1 mm from the bregma and 1 mm from the midline (as described by Paxinos & Watson, 1986). The catheter was inserted 3 mm into the lateral ventricle, fixed by attachment to a stainless steel screw placed in the skull bones, and then tunnelled subcutaneously to exit on top of the skull. Animals with normal motor function and behaviour post surgery were used 4–7 days later for the experiment. Each animal was used only once.

Drug administration

In subseries 1, we compared the effects of saline, midazolam in doses not effective in nociceptive assessments (i.t.: 2.5 µg, i.c.v.: 4 µg), morphine (i.t.: 1 µg, i.c.v.: 4 µg), midazolam and morphine in combination (as fixed doses: 2.5 µg midazolam and 1 µg morphine i.t.; 4 µg midazolam and 4 µg morphine i.c.v.) and the effect of MK 801 (i.t. 10 µg, i.c.v.: 10 µg) on all combinations. All doses of morphine sulphate (Mallinckrodt), midazolam (Hoffman – La Roche) and MK 801 (Dizocilpine, Research Biochemicals Incorporated, RBI), were calculated as the free base. Drugs for i.t. administration were dissolved in 0.9% sodium chloride, so that 10 µl contained the desired dose of the agents. After each injection, the catheter was flushed by an i.t. injection of 10 µl 0.9% sodium chloride. Drugs for i.c.v. administration were dissolved in 0.9% sodium chloride to a volume of 5 µl and injected into the ventricle, followed by 5 µl of 0.9% sodium chloride to flush the catheter. All drugs were administered over a period of 20 s with a microinjector. The NMDA antagonist, (+) MK 801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohept-ten-5,10-imine hydrogenmaleate] was dissolved in 0.9% sodium chloride to provide doses of 10 µg 10 µl⁻¹, i.t. and 10 µg 5 µl⁻¹, i.c.v.

For subseries 2, saline or a fixed dose of MK 801 (i.t.: 10 µg, i.c.v.: 10 µg) were administered with various doses of midazolam and morphine for dose-response curves.

Behavioural tests

Time course The day before each study, animals were placed on an unheated hot-plate for 1–2 min for adaptation. Baseline assessments, including nociceptive and motor function tests, as well as tail skin temperature, were performed on the morning of the testing day. The NMDA antagonist or saline was administered 5 min before the agonists or saline (0 time) and each of the antinociceptive and motor function tests, as well as the tail skin temperature measurements, were repeated at 5, 15, 30, 60, 90 and 120 min. All tests were performed by one of the investigators, who was unaware of the drug or dosage administered.

Nociceptive tests For the hot-plate test, the animals were placed on a metal plate maintained at $52.5 \pm 0.5^\circ\text{C}$ within a plexiglass enclosure. The behavioural endpoint was defined as the time at which the animal jumped off the plate or licked a hindpaw. The cutoff time to prevent tissue damage was 60 s. There was one trial per test per time interval. For the tail-flick test, each animal was placed on a rectangular metal plate under an illuminated lamp (100W), and the light beam focussed on the ventral surface at 1.5 and 3 cm from the tip of the tail. The endpoint was defined as the time when the rat flicked its tail out of the beam. If an animal failed to respond, the test was terminated at 6 s to avoid tissue damage. Two trials were conducted per test per time interval. Tail-skin temperature was measured concurrently on the ventral surface at the proximal part of the tail and recorded immediately after the tail-flick occurred. Changes in tail skin temperature were ruled out as a possible confounding factor in the tail-flick test.

Motor function All rats were observed and scored for motor dysfunction (behavioral index: 0 = no change; 1 = complete loss of hindlimb function). To evaluate catalepsy, animals were placed with their front paws on a step (height 9 cm) and the time measured until the rat either climbed to the top with both hindpaws or removed both front paws from the step. Animals showing neither response within 30 s were scored maximum positive for catalepsy. Additional side effects, like spontaneous agitation and vocalization, were observed and noted.

Data analyses

Hot-plate, tail-flick and catalepsy response latencies are expressed as the area under the time-effect curve (AUC) from zero time to 120 min using the trapezoidal rule, and also as a percentage of the maximum possible effect (%MPE) calculated as follows:

$$\%MPE = \frac{(\text{postdrug time} - \text{predrug time})}{(\text{cutoff time} - \text{predrug time})} \times 100$$

The time-effect courses were analysed by analysis of variance (ANOVA) for repeated measures with the Bonferroni correction (MDPD statistical manual, procedure V4). To compare the maximum antinociceptive effect (peak effect %MPE) and area under the curve (AUC) for the various groups, factorial analysis of variance (ANOVA) was used. In order to construct dose-response curves for the effect of MK 801 on the saline/midazolam and saline/morphine curves, MK 801 dose was plotted against peak %MPE and analysed by least square linear regression analysis. From the curves, values were calculated for ED₅₀ (95% confidence intervals [CI]) and slope values (95% CI) when testing for parallelism. Statistical significance was accepted at $P \leq 0.05$.

Results

In dose-ranging studies for MK 801 given both i.t. ($n = 4-7$ /dose) and i.c.v. ($n = 4$ /dose), the highest doses of the NMDA antagonist used for these experiments were those which did not produce catalepsy, and did not show behavioural effects, such as spontaneous agitation and/or vocalization, in more

than 15% of the animals (MK 801 = $10 \mu\text{g}$ i.t. and $10 \mu\text{g}$ i.c.v., respectively) (data not shown, but described below). All other drugs administered i.t. and i.c.v. had no effect on the behavioural index except those short-lasting episodes of agitation and vocalization.

Intrathecal administration

Figure 1 shows the time course of the antinociceptive effects of i.t. administration of saline/morphine, saline/midazolam and morphine in combination and the EAA antagonist MK 801 ($10 \mu\text{g}$) injected prior to midazolam plus morphine (%MPE plotted against time). The mean baseline hot-plate (HP) and tail-flick (TF) latency for all animals measured before intrathecal injection was in the normal range and did not differ for the various groups (HP: 8.5 ± 2.2 s; TF: 2.82 ± 0.44 s). Combined administration of saline/midazolam $2.5 \mu\text{g}$ and morphine $1 \mu\text{g}$, i.t., significantly increased response latencies within 15 min in both nociceptive tests as compared to saline/morphine $1 \mu\text{g}$. Preadministration of $10 \mu\text{g}$ MK 801 i.t. significantly reduced this potentiating antinociceptive effect of the midazolam-morphine interaction

Figure 1 The antinociceptive effect, percentage maximum possible effect (%MPE), in (a) the hot plate (HP) and (b) tail flick test (TF), at different times after intrathecal administration of saline/morphine $1 \mu\text{g}$ ($\square$), saline/midazolam $2.5 \mu\text{g}$ + morphine $1 \mu\text{g}$ in combination ($\blacklozenge$) and MK 801 $10 \mu\text{g}$ /midazolam + morphine ($\blacksquare$). Mean $\pm$ s.e.mean, ANOVA: drug effects: (HP) $P = 0.001$, (TF) $P = 0.0009$; time effect: (HP) $P = 0.0001$, (TF) $P = 0.0009$, * $P < 0.05$.

Figure 2 Peak effect (peak %MPE) and area under the time-effect curve (MPE $\times$ min) of agonists given i.t. alone and in combination with MK 801 ($10 \mu\text{g}$), showing that the potentiation and prolongation of the midazolam ($2.5 \mu\text{g}$) morphine ($1 \mu\text{g}$) interaction and the effect of saline/midazolam ($2.5 \mu\text{g}$) is significantly reversed by the antagonist MK 801. Saline/saline and MK 801/saline were ineffective. Saline/midazolam + morphine (1), MK 801/midazolam + morphine (2), saline/midazolam (3), MK 801/midazolam (4), saline/morphine $1 \mu\text{g}$ (5), MK 801/morphine (6), saline/saline (7), MK 801/saline (8). Mean $\pm$ s.e.mean, ANOVA: peak %MPE (HP) $P = 0.0001$, (TF) $P = 0.0001$; AUC: (HP) $P = 0.0001$, (TF) $P = 0.0001$, * $P < 0.05$ compared to saline/midazolam + morphine; ° $P < 0.05$ compared to saline/midazolam.

to near saline/morphine values over the observation period (ANOVA for repeated measures: drug-effect: (HP) $P = 0.0001$, (TF) $P = 0.0009$; time-effect: (HP) $P = 0.0001$, (TF) $P = 0.0009$; Figure 1). Maximum antinociceptive effects in tail-flick and hot-plate tests (peak %MPE) independent of the time of peak effect and AUC for both tests and for each drug treatment also demonstrate that i.t. coadministration of a minimally effective (effect in less than 15%MPE) dose of midazolam (2.5 μg) with 1 μg morphine potentiated the opioid effects in nociceptive tests (Figure 2). Spinal injection of MK 801 (10 μg) before the respective agonists significantly blocked potentiation by midazolam and prolongation of morphine antinociception in tail-flick and hot-plate tests (ANOVA: peak %MPE (HP) $P = 0.0001$, (TF) $P = 0.0001$; AUC: (HP) $P = 0.0001$, (TF) $P = 0.0001$; Figure 2). This reversal occurred at doses of MK 801 which did not alter response latency when injected alone. It should be noted that the nociceptive tests employed were not designed and not able to detect any significant reduction in latency (e.g. hyperalgesic effect). A lack of effect of MK 801 administered alone was reported previously (Trujillo & Akil, 1991a,b; Malmberg & Yaksh, 1992). Results of subseries 2 are shown in Figure 3. The dose-response curve for peak effect (peak %MPE) of MK 801/midazolam shifted to the right of the saline/midazolam curve in nociceptive tests. The area under the curve (AUC) was decreased by MK 801 as compared to saline/midazolam (Figure 3). There was no shift in the MK

801/morphine dose-response curve in comparison to the saline/morphine curve, showing that MK 801 had no influence on morphine antinociception. It should be noted that in 14.2% of the animals, injection of 10 μg MK 801 caused spontaneous agitation and vocalization for 3 to 5 min. These short-lasting behavioural changes ended before the administration of the agonists and did not influence the catalepsy tests. Single injections of 10 μg MK 801/saline and saline/saline did not produce antinociception (Figure 2).

Intracerebroventricular administration

The time course of antinociception following injection of midazolam and morphine in combination and the influence of the NMDA antagonist MK 801 on effects of the agonist combination are presented in Figure 4. Baseline measurements for the hot-plate and tail-flick test were in normal range for all animals, and there was no between-groups difference (HP: 7.9 ± 3.5 s; TF 2.84 ± 0.46 s). Over the observation period, the administration of saline/morphine (4 μg) resulted in an increase in %MPE, that was significantly reduced by coadministration of midazolam (4 μg) in the hot-plate assay only. This inhibitory effect of midazolam on morphine antinociception was abolished by pretreatment with MK 801 at the dose given (ANOVA for repeated measures: (HP) $P = 0.0044$, (TF) $P = 0.0029$; time-effect: (HP) $P = 0.0001$, (TF) $P = 0.0001$; Figure 4). The maximum antinociceptive effect (peak %MPE), AUCs of the midazolam-morphine treatment and the influence of MK 801 are shown in Figure 5. MK 801 (10 μg), administered prior to the agonists, significantly abolished the inhibition produced by saline/midazolam + morphine at the supraspinal level in the hot-plate test only. In the tail-flick assay, MK 801 was

Figure 3 Dose-response curves (peak %MPE) and AUC (MPE $\times$ min) for antinociceptive effects in hot plate (a,b) and tail flick (c,d) tests and the incidence of catalepsy (e) after i.t. administration of saline/morphine ($\bullet$), saline/midazolam ($\blacksquare$), MK 801/morphine ($\circ$) and MK 801/midazolam ($\square$) demonstrating a right shift of the saline/midazolam curve by MK 801 without affecting saline/morphine.

Figure 4 The antinociceptive effect (%MPE) on (a) hot plate and (b) tail flick tests across time after i.c.v. administration of saline/morphine 4 μg ($\square$), saline/morphine 4 μg + midazolam 4 μg ($\blacklozenge$), MK 801 (10 μg)/midazolam + morphine ($\blacksquare$) in rats. Mean $\pm$ s.e.mean, ANOVA: drugs effect: (HP) $P = 0.044$, (TF) $P = 0.0029$; time effect: (HP) $P = 0.0001$, (TF) $P = 0.0001$, * $P < 0.05$. NS, not significant.

not effective at the dose used in altering the midazolam-morphine interaction (Figures 4 and 5). In subseries 2, the dose-response curve for peak %MPE and AUC of saline/midazolam (1–4 μ g) was not affected by MK 801 (Figure 6). The dose-response curve for saline/morphine (1–4 μ g) was not shifted by MK 801 10 μ g (Figure 6), indicating that MK 801 at the dose administered does not influence morphine antinociception in the brain. Injection of 10 μ g MK 801 alone was without effect when compared to saline controls in both antinociceptive tests. Rats given the maximum dose of morphine i.c.v. and midazolam i.c.v. in combination with MK 801 showed no motor dysfunction, although 6.25% of the rats produced short-lasting spontaneous agitation and vocalization over 3 to 5 min after i.c.v. administration of the NMDA antagonist. This did not affect the catalepsy test results.

Discussion

The principal findings of this study show that (1) the (potentiating) midazolam-morphine interaction can be antagonized by the NMDA antagonist, MK 801, at the spinal level as assessed by hot-plate and tail-flick nociceptive tests. (2) When the drugs were administered supraspinally MK 801 blocked

this (inhibitory) interaction in the hot-plate test only. (3) MK 801 had no effect on morphine antinociception when given i.t. and i.c.v. and (4) MK 801 significantly affected midazolam antinociception at the spinal level, but had a lack of effect following i.c.v. administration of the drugs.

Several recent findings suggest that interactions between the two major neurotransmitter systems, EAAs and GABA, are involved in nociceptive transmission and processing in the spinal cord (Watkins & Evans, 1981; Cahusac *et al.*, 1984; Aanonsen & Wilcox, 1987; Wilcox, 1991; Woolf & Thompson, 1991). EAAs are co-released with substance P in response to noxious stimuli (Battaglia *et al.*, 1986; Wilcox, 1991). There is no direct effect of glutamate, the endogenous ligand of the EAA receptor subtype NMDA (Wilcox, 1991) on binding to the benzodiazepine recognition site on the GABA_A receptor ionophore complex (Schatzki *et al.*, 1990). Therefore it is unlikely that MK 801 affected midazolam antinociception at the spinal level due to direct interaction at the GABA_A receptor. It thus appears reasonable to suggest that the influence of MK 801 on midazolam antinociception in the spinal cord might be due to indirect alteration at the GABA_A ionophore complex. So far, it is unclear through what system (peptidergic) this interaction might take place. In the mouse spinal cord NMDA-induced hyperalgesia is dose-dependently inhibited by muscimol in the tail-flick test (Aanonsen & Wilcox, 1989). In primary cultures of striatal neurones the NMDA receptor system was coupled to the evoked release of GABA (Weiss, 1990). These observations

Figure 5 Peak effect of %MPE and area under the time-effect curve (MPE $\times$ min) of agonists and MK 801 injected i.c.v., showing that the inhibitory effect midazolam on morphine antinociception is reversed by MK 801 in the hot plate test. Saline/midazolom + morphine (1), MK801/midazolom + morphine (2), saline/midazolom (3), MK 801/midazolom (4), saline/morphine 1 μ g (5), MK 801/morphine (6), saline/saline (7), MK 801/saline (8). Mean $\pm$ s.e.mean, ANOVA: peak %MPE (HP) $P = 0.0001$, (TF) $P = 0.0001$; AUC: (HP) $P = 0.0001$, (TF) $P = 0.0001$, * $P < 0.05$ compared to saline/midazolom + morphine; ** $P < 0.05$ compared to saline/midazolom.

Figure 6 Dose-response curves (peak %MPE) and AUC (MPE $\times$ min) for antinociceptive effects in hot plate (a,b) and tail flick (c,d) and the incidence of catalepsy (e) after i.c.v. administration of saline/morphine (■), saline/midazolom (●), MK 801/morphine (□) and MK 801/midazolom (○).

in different areas of the central nervous system indicate that GABA_A receptors may modulate EAA-induced behaviour. Furthermore, NMDA antagonist neurotoxicity studies in animals demonstrate that blockade of the NMDA receptor results in cessation of GABA release (Olney *et al.*, 1991). Benzodiazepine agonists exert their action by increasing the opening frequency of the chloride ion channel at the GABA_A receptor complex (Haefely, 1985). MK 801 acts at the NMDA receptor-operated ion channel as an open channel blocker (Wilcox, 1991). Independent postsynaptic selective modulation of the ion channel of the different receptors may be an explanation for our finding that the antinociceptive effects of midazolam alone were diminished by MK 801 in both nociceptive tests at the spinal level. MK 801 had no effect on midazolam-induced changes in thermal nociceptive latencies following i.c.v. administration of the drugs. This lack of supraspinal effect by MK 801 on midazolam justifies the assumption that this interaction takes place postsynaptically only in the spinal cord. It is conceivable that higher doses of MK 801 would have affected midazolam at the supraspinal level in both acute nociceptive tests. However, this does not seem to be the case, because the doses used were already the highest that did not appear to produce motor dysfunction.

The NMDA antagonist MK 801 showed a lack of effect on morphine antinociception when administered i.t. and i.c.v. The absence of an effect agrees with observations reported previously in rats (Marek *et al.*, 1991a; Trujillo & Akil, 1991a,b; Yamamoto & Yaksh, 1992). In fact, low doses of MK 801, like other NMDA antagonists, did not affect acute morphine antinociception, but do alter the 'wind up' phenomenon and tolerance development to the antinociceptive action of morphine (Yaksh, 1989; Marek *et al.*, 1991b; Trujillo & Akil, 1991b; Goodchild, 1993). These findings stand in contrast to the recent data of Lipa & Kavaliers (1990) where intraperitoneal administration of MK 801 reduced morphine-induced antinociceptive responses in male mice, indicating that NMDA receptors are involved in the mediation of opioid analgesia. This divergence from our observation might be due to the different species used for these experiments (mice versus cats), the high dose used by these authors, and/or the different means of administering the test drugs.

Since morphine antinociception is not influenced by MK 801 at both spinal and supraspinal level, and in light of the proposed mechanism of the noncompetitive NMDA antagonist on benzodiazepine antinociception, this study demonstrates that the effect of the benzodiazepines on morphine antinociception can be modulated by MK 801. Most investigators have found that the two major neurotransmitter systems, EAAs and GABA, may be involved in nociception in the spinal cord (Watkins & Evans, 1981; Cahusac *et al.*, 1984; Aanonsen & Wilcox, 1987; Wilcox, 1991; Woolf & Thompson, 1991) and that the two receptor systems may operate in concert to amplify signals in various regions of the brain (Aanonsen & Wilcox, 1988; Weiss, 1989; Walden *et al.*,

1990). Thus, the assumption is justified that the paradoxical effect of midazolam on morphine antinociception and its reversal by MK 801 might be due to modulation at various levels of the neuraxis and/or modulation of different pathways, signifying a general physiological rather than a pharmacological receptor-receptor interaction. Thus, preadministration of MK 801 might influence the midazolam-morphine interaction postsynaptically, thereby altering the effect of the benzodiazepine on morphine at a spinal and probably supraspinal level. NMDA receptors play an important role in descending pain inhibition from the brainstem (Jacquet, 1988; Aanonsen *et al.*, 1990; Van Praag & Frenk, 1990) and it appears that changes in this system may contribute to alteration of the morphine-midazolam interaction in the spinal cord.

Even the highest doses of morphine, midazolam and their combination with 10 µg MK 801 did not affect catalepsy. This is in agreement with the data of Trujillo & Akil (1991a) who reported that MK 801 potentiated morphine catalepsy and lethality only when high doses were administered systemically. Moreover, the present observation of an immediate and short-lasting spontaneous vocalization after i.t. and i.c.v. administration of 10 µg MK 801 may demonstrate that the neuroprotective potency of delayed application of MK 801, seen in rats and mice after various excitatory stimuli (Turski *et al.*, 1990; Kehne *et al.*, 1991), occurs following initial activation of the receptor (Marek *et al.*, 1991a). Therefore, 10 µg MK 801 administered before an agonist, may initially result in short-lasting motor dysfunction seen as agitation and vocalization followed by a neuroprotective effect in both spinal cord and brain. Other investigators have observed similar behavioural effects (Yamamoto & Yaksh, 1992; Malmberg & Yaksh, 1992), and found that higher doses of MK 801 result in a higher incidence of these behavioural effects. The noncompetitive NMDA antagonist was thus injected i.t. and i.c.v. at the selected dose level in our study to give an incidence of behavioural signs in ≤15%MPE of the rats. As 10 µg MK 801 did not affect catalepsy, it appears that our findings were not influenced by these short-lasting spontaneous behavioural episodes in such a way as to cause false positive interpretations.

In conclusion, this study shows that midazolam-morphine interaction can be modulated by the NMDA antagonist, MK 801, at the spinal level and partly also supraspinally. Furthermore, we observed that MK 801 dose-dependently affected midazolam antinociception at the spinal level, but was without effect on morphine antinociception in either the spinal cord or the brain.

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