



Research papers

Mrz 2/579, a fast kinetic NMDA channel blocker, reduces the development of morphine tolerance in awake rats

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Received 12 October 1999; received in revised form 15 August 2000; accepted 30 August 2000

Abstract

The purpose of the present study was to investigate whether uncompetitive NMDA antagonists with fast channel blocking kinetics, which show fewer side effects in man than compounds such as ketamine, affect the development of tolerance to continuous exposure to morphine. Rats were trained on the Randall–Selitto apparatus before being implanted, under halothane anaesthesia, with primed mini-osmotic pumps (240 μ l/day). Six rats were implanted with a vehicle filled pump, seven with a morphine filled pump (28.8 mg/kg/day), and eight with a pair of pumps, one containing morphine and the other Mrz 2/579, a new NMDA antagonist (40 mg/kg/day). A fourth group was implanted with a morphine filled pump followed 25 h later by a Mrz 2/579 filled pump. Paw withdrawal tests were undertaken immediately before, and at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h after the first pump was implanted. Before pump implantation, withdrawal thresholds were 120 ± 7 g (mean \pm SEM, $n = 30$). Vehicle infusion had no effect on withdrawal thresholds, whereas morphine infusion increased them significantly at 2 and 4 h after pump implantation (+2 h: 208 ± 14 g; $P < 0.001$ vs. control). From 6 h the antinociception elicited by morphine declined progressively; at 10 h withdrawal thresholds were significantly lower than the 2 h post-treatment value ($P < 0.001$). In rats treated with morphine plus Mrz 2/579, thresholds remained significantly higher between 10–72 h post-implantation than with morphine alone ($P < 0.05$). In contrast, infusion of the same level of Mrz 2/579 once tolerance had developed did not reverse tolerance. These results indicate that fast NMDA channel blockers such as Mrz 2/579 may prove to be useful in enhancing analgesia to continuous morphine administration. © 2001 International Association for the Study of Pain. Published by Elsevier Science B.V. All rights reserved.

Keywords: Mrz 2/579; NMDA channel blocker; Morphine tolerance

1. Introduction

Opioid analgesics are central to the treatment of many types of chronic pain. However, the development of tolerance to opioids can become a problem (see Fundytus and Coderre, 1999), most notably in pain therapy in opioid addicts. Numerous reports indicate that in laboratory animals the co-administration of *N*-methyl-D-aspartate (NMDA) antagonists can attenuate the development of tolerance to morphine, in a range of experimental tests and with a variety of morphine administration regimes (see Manning et al., 1996; Price et al., 1997; Allen and Dykstra, 2000). Some (e.g. Manning et al., 1996; Chow et al., 1998) but not all (e.g. Nishiyama et al., 1998), investi-

gations have also shown enhancement of peak morphine analgesia by co-administration of NMDA antagonists.

The attenuation of morphine tolerance has been investigated using a range of NMDA antagonists, although most studies have been with compounds that bind uncompetitively within the channel of the NMDA receptor complex. There is a range both of binding/unbinding kinetics and of voltage dependence amongst this group of compounds (Chen et al., 1992; Parsons et al., 1999a). Those with slow unbinding kinetics and weak voltage dependence are associated with dysphoric side effects and are poorly tolerated in humans; this is exemplified by MK-801. However, most studies to date on the effects of NMDA antagonists on morphine tolerance have used such slow kinetic compounds (e.g. Marek et al., 1991a,b; Trujillo and Akil, 1991, 1994; Ben-Eliyahu et al., 1992; Bhargava and Matwyshyn, 1993; Mao et al., 1994). In comparison, NMDA channel blockers with faster kinetics and stronger voltage dependence, such as memantine, are tolerated better in man (Grossman and Schutz, 1982; Ditzler, 1991) and are undergoing clinical

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trials in various chronic pain situations (e.g. Headley, 1999). It is important to establish whether they, like the slower blockers that have more side-effects in man, are also effective at enhancing morphine analgesia across a range of durations of morphine administration. Dextromethorphan has indeed been shown to attenuate morphine tolerance (Manning et al., 1996) but this compound is weak and not selective for NMDA receptors. A more recent study with another weak, clinically available NMDA antagonist, memantine has also been shown to attenuate morphine tolerance (Popik and Kozela, 1999).

In the present study we have used a novel, selective, fast-kinetic uncompetitive NMDA channel blocker, Mrz 2/579 (Parsons et al., 1999a), to examine whether such an antagonist is able to attenuate tolerance development during the continuous administration of morphine via a subcutaneously implanted minipump. We examined first whether the peak antinociceptive effect of morphine could be enhanced and/or prolonged by co-infusion of Mrz 2/579, and second whether tolerance, once developed, could be reversed by later infusion of Mrz 2/579. Some of these data have been published previously in abstract form (Houghton et al., 1999).

2. Methods

All experiments were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. Male Wistar rats (180–340 g) were housed individually on soft bedding with free access to food and water.

2.1. Behavioural testing

Animals were acclimatized to the testing apparatus for three days prior to drug testing.

2.1.1. Randall–Selitto analgesy-meter test

Rats were supported manually and each hindpaw, in turn, placed on the meter (Ugo Basile). A smoothly increasing mechanical pressure (15 g/s) was applied via a foot pedal control, until the paw was withdrawn. This process was repeated twice on each hindpaw, and the four withdrawal thresholds averaged; the inter-test interval was 30 s.

2.1.2. Motor responsiveness

Motor co-ordination was assessed using a fixed speed (7 rev./min) rotarod (Ugo Basile) with a cut-off time of 180 s. If the rats failed to walk for 180 s the righting reflex and stepping/placing reflexes were assessed subjectively and scored on a 0–2 scale; 0 = absence of both reflexes, 1 = righting reflex intact but slow response to stepping/placing, and 2 = normal righting reflex and normal stepping/placing reflex. Rats were in addition observed for the degree of alertness and for any abnormal behaviour.

2.2. Pump Implantation

For the continuous delivery of drugs, minipumps (Alzet[®] 2ML01, ALZA, Palo Alto, CA) were implanted subcutaneously under halothane anaesthesia. A small incision was made into the nape of the neck and one or two pumps implanted with their spout orientated in a caudal direction.

2.3. Drugs

Morphine hydrochloride (A.C. Daniel) and Mrz 2/579 hydrochloride, a new fast kinetic NMDA channel blocker (1-amino-1,3,3,5,5-pentamethyl cyclohexane; Parsons et al., 1999a), were dissolved in sterile saline to a concentration of 30 and 40 mg/ml, respectively. Pumps were filled to full capacity (228 μ l) 12 h prior to surgery and were maintained in 37°C physiological saline, so ensuring immediate release of the drugs from the moment of implantation. Pumps were prepared for release at 28 mg/kg per day for morphine (similar to the rate used by Manning et al., 1996), and 40 mg/kg per day for Mrz 2/579 (the maximum that could be administered because of limited solubility).

Mrz 2/579 has good CNS penetration, and a plasma half-life of 140–180 min in rats (Parsons et al., 1999a). This compares with a plasma half-life of morphine in rats of 115 min (Iwamoto and Klaassen, 1977) indicating that the kinetics of distribution of the two agents should have been comparable following administration by infusion.

2.4. Experimental design

The rats were tested on the Randall–Selitto apparatus and on the rotarod before the minipumps were implanted (designated as time 0 h). Rats were divided into four treatment groups. One group was implanted with a single pump filled with morphine; a second group had two pumps implanted at time 0 h, one filled with morphine and the other with Mrz 2/579; a third group had a morphine pump implanted followed 25 h later by a pump filled with Mrz 2/579. To control for any effects pump implantation may have had on withdrawal thresholds, a fourth group was implanted with a pump filled with sterile saline. The behavioural tests were then repeated at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h after initial pump implantation. At the end of the experiment the rats were killed humanely with an overdose of pentobarbitone.

2.5. Data analysis

Values for withdrawal threshold (g) in the Randall Selitto test and time (s) on the rotarod are presented as the mean \pm standard error of the mean (SEM). Due to the small sample sizes all data were analysed using non-parametric statistics. Data for each dose over time were analyzed using the Friedman repeated measures test. Pair-wise comparisons were then made between baseline and specific time points using the Wilcoxon test. Different treatment groups were compared using the Kruskal–Wallis ANOVA. The effects

of two different drug treatments at the same post-drug time point were compared using the Mann–Whitney *U*-test.

3. Results

The mean baseline response values in the absence of any treatment were as follows: the mean force at which rats withdrew their paws from the Randall–Sellitto device was 120 ± 7 g ($n = 32$) and the mean time the rats walked on the rotarod was 167 ± 5 s ($n = 30$). Baseline withdrawal thresholds were not significantly different between the drug treatment groups, but the vehicle group had a significantly higher baseline threshold compared to the drug treatment groups.

3.1. Saline treated rats

Infusion of saline (240 μ l/day) did not affect the mean withdrawal threshold (Fig. 1A; Friedman’s ANOVA, $P > 0.35$); 2 h after the pump was implanted the mean with-

drawal threshold was 163 ± 22 ($n = 6$) compared to 156 ± 17 g before pump implantation. Similarly there was no effect on the time the rats walked on the rotarod (Fig. 1B; Friedman’s ANOVA, $P > 0.15$). These results demonstrate the stability of the test conditions.

3.2. Morphine treated rats

Infusion of morphine (28 mg/kg per day) resulted in a significant increase in withdrawal threshold (Friedman’s ANOVA, $P < 0.0001$; $n = 8$). This increase had peaked by 2 h (Fig. 2A), at which time the withdrawal threshold had increased significantly from 94 ± 6 g before morphine infusion to 207 ± 12 g (Wilcoxon test, $P < 0.008$). This increase in withdrawal threshold was, as expected, transient; by 10 h the withdrawal threshold was not significantly different to that measured before pump implantation (Wilcoxon test, $P > 0.05$). Furthermore, withdrawal thresholds recorded at 6 h and beyond were significantly different

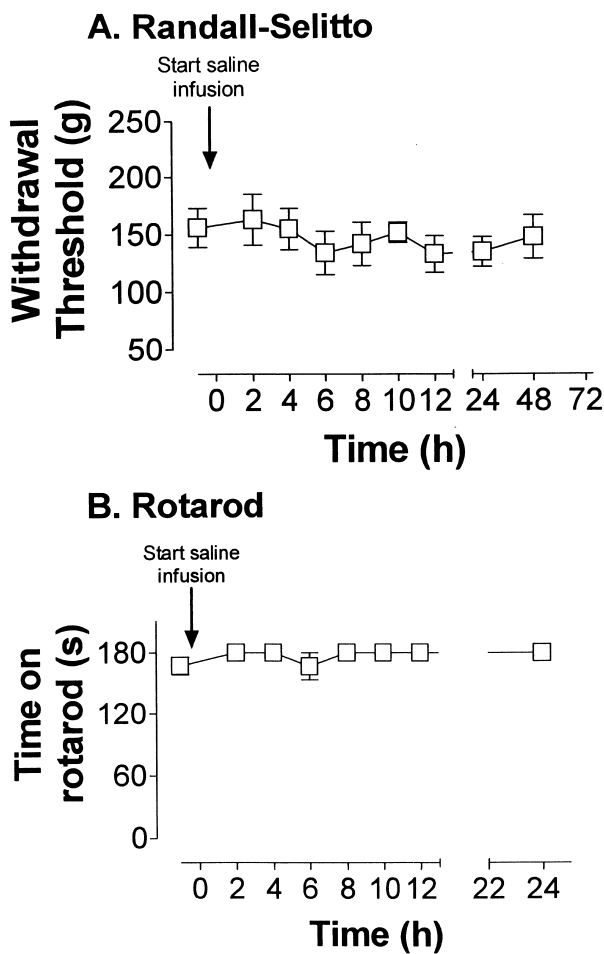


Fig. 1. Lack of effect of saline infusion (\square , 240 μ l/day) on (A) the withdrawal threshold (g) when an increasing force was applied to the hindpaw (Randall–Sellitto test); or (B) the time the rats walked on a rod rotating at 7 rev./min ($n = 6$). In this and subsequent figures error bars are SEM; where not shown error bars fall within the symbols.

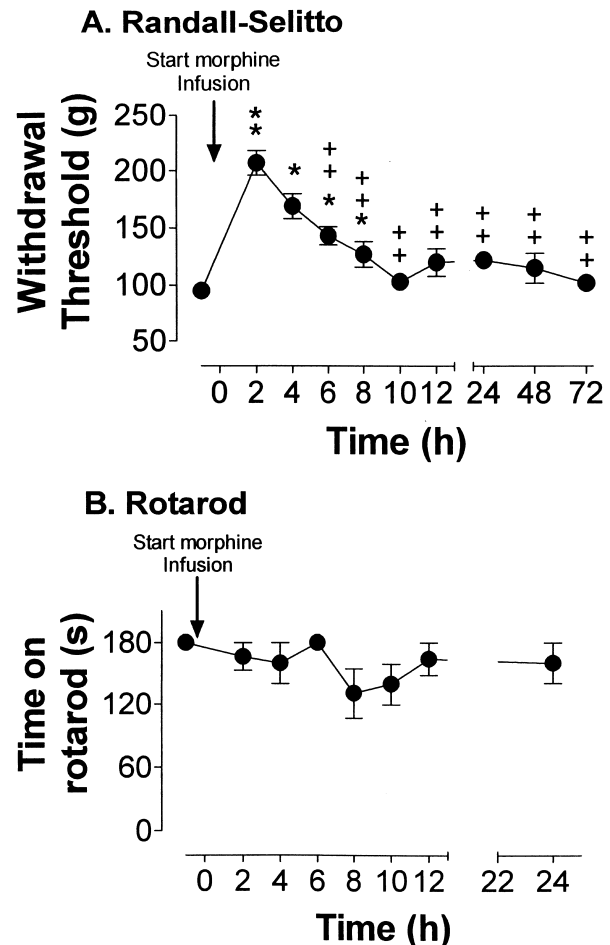


Fig. 2. Effects of morphine infusion (\bullet , 28 mg/kg/day) on Randall–Sellitto and rotarod tests. (A) Morphine significantly increased the withdrawal threshold (g) $**P < 0.01$, compared to baseline values ($n = 8$). However this increase was attenuated with increasing time, $^{++}P < 0.01$, $^{+}P < 0.05$ (Wilcoxon test) compared to 2 h (i.e. peak increase). (B) Morphine infusion did not affect the time the rats walked on a rod rotating at 7 rev./min ($n = 6$).

to the peak effect recorded at 2 h ($P < 0.01$ at all time points).

In contrast, this dose of morphine had no effect on rotarod performance (Friedman's ANOVA, $P > 0.3$, $n = 6$; Fig. 2B) nor on subjectively assessed behaviour, such as alertness.

3.3. Concurrent administration of morphine and Mrz 2/579

Concurrent infusion of morphine and Mrz 2/579 resulted in a significant increase in withdrawal threshold (Friedman's ANOVA, $P < 0.0001$; $n = 8$). This increase again peaked at 2 h (Fig. 3A) at which time the withdrawal threshold had risen from 107 ± 7 to 203 ± 11 g (Wilcoxon test, $P < 0.01$). This 2 h value was not different to that measured at 2 h in rats treated with morphine only (Fig. 3B; Mann-Whitney U -test, $P > 0.8$). The increase in withdrawal threshold decreased beyond the 2 h time point, but for 48 h remained significantly higher than that measured before pump implantation (Fig. 3A; Wilcoxon test, $P < 0.05$ for 4–48 h time points). Only the withdrawal thresholds measured at 24 and 72 h were significantly lower than those measured at 2 h.

Comparison of morphine alone with morphine plus Mrz 2/579 data indicates that in the group that received the combination treatment there was a significant prolongation of morphine effectiveness (Fig. 3B). For example, 12 h post pump implantation the mean withdrawal threshold in rats that received the combined treatment was 169 ± 12 g ($n = 8$), which was significantly greater ($P < 0.01$) than the withdrawal threshold in rats that had morphine alone (120 ± 10 g; $n = 8$). This represents a 61% decrease in the degree of tolerance. This difference between the two groups was maintained for the full 72 h of the infusion.

Morphine combined with Mrz 2/579 also led to a significant decrease in the rats' ability to walk on the rotating rod (Friedman's ANOVA, $P < 0.008$; Fig. 3C). Concomitantly, the rats failed to make any attempt to move away when the experimenter picked them from their cages. In contrast, the righting reflex and placing reactions in these rats were not detectably affected.

3.4. Morphine followed after 25 h by Mrz 2/579

In a separate group of rats, morphine infusion (28 mg/kg per day) resulted in a significant tolerance over 24 h as described above (Fig. 2A). In this second group of rats (Fig. 4) a second pump was implanted at 25 h for the infusion of Mrz 2/579 at the same rate as that used in the previous study. Under these conditions, however, continuous infusion of Mrz 2/579 did not increase withdrawal thresholds when the 48 and 72 h measurements were compared to those at 24 h. Furthermore there was no significant difference between withdrawal thresholds in the morphine followed by Mrz 2/579 treatment group and those in the group that had the same dose of morphine alone (Mann-Whitney U -test, $P > 0.25$ and > 0.5 for the

24 and 48 h time points, respectively). For example, 48 h into morphine infusion, and 23 h into Mrz 2/579 infusion, the withdrawal threshold was 132 ± 12 g ($n = 8$) in the combination group compared to 116 ± 8 g ($n = 8$) in the rats that received morphine only.

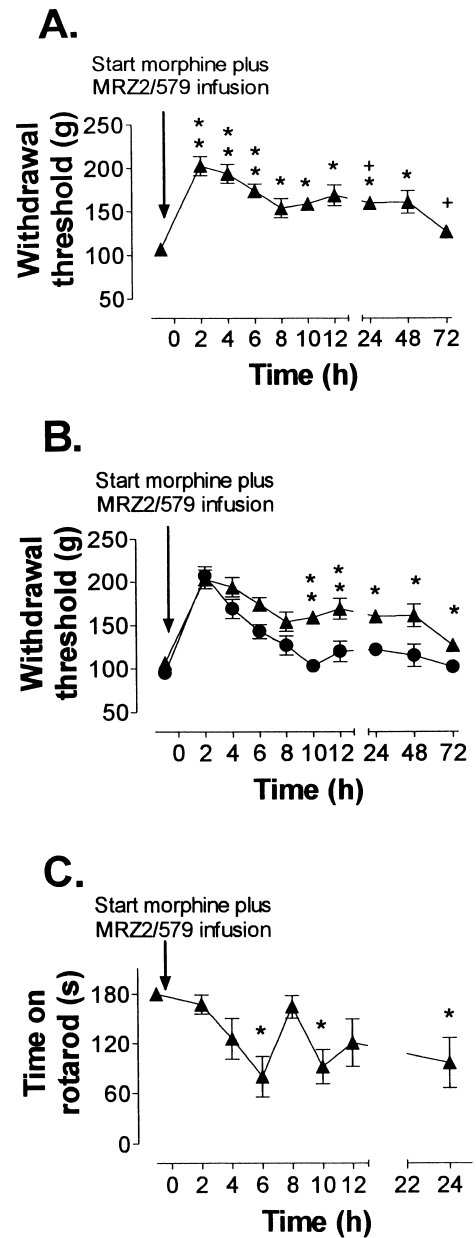


Fig. 3. Reduced morphine tolerance when tested on Randall–Selitto and rotarod tests ($n = 7$) following combined infusion of morphine (28 mg/kg/day) and Mrz 2/579 (40 mg/kg/day). (A) The combination (▲) significantly increased the withdrawal threshold (g); $**P < 0.01$, $*P < 0.05$, compared to baseline values. Subsequent reduction of morphine efficacy occurred only after 24 h ($^+P < 0.05$, compared to 2 h values; Wilcoxon test, $n = 8$). (B) Comparison of morphine alone (●, as in Fig. 2A) vs. morphine plus Mrz 2/579 (▲, as in Fig. 3A) to show significant differences ($**P < 0.01$, $*P < 0.05$; Mann-Whitney U -test). (C) The combination reduced the time the rats walked on a rod rotating at 7 rev./min ($*P < 0.05$, compared to baseline values; Wilcoxon test).

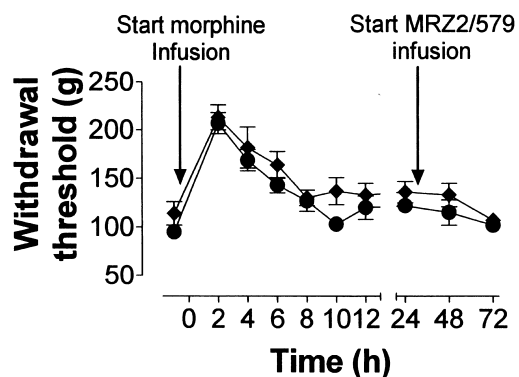


Fig. 4. Lack of reversal of established morphine tolerance by Mrz 2/579. Combined infusion (◆) of Mrz 2/579 (40 mg/kg per day) starting 25 h after infusion of morphine (28 mg/kg per day) did not increase withdrawal thresholds when compared to morphine infusion alone (●; data from Fig. 2A).

This treatment had no effect on rotarod performance (data not shown) or on righting reflexes. No other side effects were noted in this group of rats.

4. Discussion

Mrz 2/579 is a new uncompetitive NMDA antagonist that is selective for NMDA *in vitro*, has fast channel blocking kinetics, and *in vivo* has a plasma half life of 140–180 min (Parsons et al., 1999a), which is considerably longer than the plasma half life of ketamine in rats (about 10 min; Cohen et al., 1973). By systemic administration Mrz 2/579 is also selective between iontophoretically-administered NMDA and AMPA *in vivo* (M McClean and PM Headley, unpublished observations). Its preclinical profile is consistent with a better side-effect profile in man than currently licensed NMDA antagonists such as ketamine (see Parsons et al., 1999b). The present study indicates that co-infusion of this new NMDA antagonist can delay the development of tolerance to continuous administration of morphine. In this experimental model it did not, however, reverse tolerance once it had become established.

4.1. Acute antinociception

The increase in withdrawal threshold 2 h into morphine infusion is consistent with morphine's known analgesic actions. Since the rats that had vehicle infusion showed no change in withdrawal threshold at this (or any other) time point, the increase can be ascribed to the actions of morphine, rather than to an effect of the anaesthesia or surgery required to implant the minipumps. Comparison with other data from this laboratory (M. McClean, unpublished observations) indicates that the peak antinociceptive effect seen here was not supramaximal; a single 5 mg/kg dose of morphine raised PWL using the same apparatus to

235 ± 7 g ($n = 12$), i.e. more than the peak seen here of 207 ± 12 g.

Since the antinociceptive test used in the current study, the Randall–Selitto test, depends on the withdrawal of a hindlimb from the apparatus, it is important to establish whether an increase in withdrawal threshold could be secondary to motor impairment; we therefore carried out rotarod tests. As no impairment of rotarod performance was observed after morphine administration, we can conclude that the action of morphine was due to a change in the nociceptive response threshold rather than to an effect on motor ability.

Work in our laboratory has shown that 30 min after a single systemic dose of 10 mg/kg Mrz 2/579, a dose that selectively reduces responses to iontophoretic NMDA, there was no change of withdrawal thresholds tested with the Randall–Selitto apparatus (M. McClean, unpublished observations). With a plasma half-life of 140–180 min, the prediction is that a single 10 mg/kg dose would cause higher plasma levels than infusion of 1.67 mg/kg per h, the rate used here. Therefore, although infusion of Mrz 2/579 alone was not examined in this study, there is no reason to suppose that it would affect acute nociceptive thresholds. This is consistent with the lack of enhancement by Mrz 2/579 of the peak morphine antinociception recorded 2 h after the start of morphine infusion. The latter finding is consistent with those of Nishiyama et al. (1998), who found that intrathecal administration of an NMDA antagonist did not potentiate morphine antinociception to an acute thermal stimulus; in addition Lutfy et al. (1999) have reported that longer term administration of an NMDA antagonist is less likely than acute administration to enhance morphine antinociception. On the other hand some evidence does suggest that co-administration of morphine and sub-threshold doses of NMDA antagonists can result in enhanced acute antinociception. For instance, Manning et al. (1996) demonstrated that although dextromethorphan (32 mg/kg per day) did not produce significant antinociception when administered alone, it did potentiate the antinociceptive effects of morphine infusion (24 mg/kg per day). Co-administration of ketamine with morphine has also been shown to increase acute analgesia when compared to morphine administration alone (Chow et al., 1998). It seems likely that differences in the rate of rise of brain concentrations of morphine and the NMDA antagonist following their administration influence the degree of potentiation of morphine antinociception; this has not been controlled in this or in previous studies, and in the absence of direct monitoring this aspect cannot be resolved.

4.2. Tolerance to morphine

Continuous subcutaneous infusion of morphine (28 mg/kg per day) resulted in early antinociception that within 4 h of the start of infusion began to wane; this finding is consistent with those of Manning et al. (1996), who used an infu-

sion rate of 24 mg/kg per day. Co-infusion of Mrz 2/579 from the start of morphine administration significantly reduced the degree of tolerance over the 3 days of infusion examined. Manning et al. (1996) reported similar findings when they co-administered morphine and dextromethorphan, a much less selective fast NMDA channel blocker. It is possible that a higher infusion rate of Mrz 2/579 would have reduced the development of tolerance to a greater extent, but this was not examined because the combined infusion rates tested were already sufficient to cause some abnormal motor behaviours. The lack of increase of antinociception seen in the current study 2 h after starting the infusion, together with the fact that the 2 h time point was not supramaximal antinociception (see above), suggests that the reduced tolerance seen subsequently was indeed an effect on tolerance development rather than simply an additive effect of the two agents.

Some, but not all, previous studies have demonstrated reversal of tolerance with NMDA antagonists. For example, administration of a single dose of an NMDA antagonist was effective in re-establishing antinociceptive effects of morphine in tolerant animals in some studies (Shimoyama et al., 1996; Popik et al., 2000) but not in others (Trujillo and Akil, 1991; Tiseo and Inturrisi, 1993). In a study in which a single dose of an NMDA antagonist failed to reverse tolerance, repeated administration of the same antagonist did produce reversal of tolerance (Tiseo and Inturrisi, 1993). Lower doses of an NMDA antagonist (in this case the competitive NMDA receptor antagonist LY235959) were able to prevent the induction of morphine tolerance than to reverse established tolerance (Allen and Dykstra, 1999), which is equivalent to the results obtained here. Furthermore reversal of morphine tolerance can be stimulus dependent, with the glycine/NMDA receptor antagonist (+)-HA966 reversing established morphine tolerance in a thermal but not in a mechanical test of antinociception (Christensen et al., 2000). Since in the current study mechanical but not thermal tests were utilised, this may have motivated against our seeing effective reversal. It is also one possible explanation for the finding that in mouse tail flick tests Mrz 2/579 did cause reversal of morphine tolerance (Popik et al., 2000). It therefore appears that the specific experimental conditions are critical in influencing the degree to which reversal of tolerance is observed. The relevant factors are not at present known, although as well as the modality of the test stimulus used, as discussed above, the maintenance dose of morphine appears to be one (Allen and Dykstra, 2000). In the current co-infusion study, the infusions rate that reduced the onset of tolerance failed to reverse established tolerance. Interestingly, there was also no evidence of altered motor behaviours when the NMDA antagonist infusion started 24 h after morphine infusion. This aspect appears not to have been examined in most comparable studies.

These observations indicate that the co-presence of an NMDA antagonist is more effective in the early stages of morphine administration than after prolonged periods of

morphine administration. This in turn implies that NMDA receptors are more involved in the initiation than in the maintenance of the tolerant state. From a clinical point of view this is not the preferred outcome, given that additional therapy is likely to be wanted after rather than before standard opiate use.

In conclusion, the selective fast kinetic NMDA channel blockers Mrz 2/579, at a dose that neither has effects itself nor enhanced peak morphine antinociception, can reduce the development of morphine tolerance in a manner similar to that reported for slow channel blockers such as MK-801. However, Mrz 2/579 at the dose tested in this co-infusion paradigm did not reverse morphine tolerance once developed. Since fast kinetic NMDA channel blockers have a better side effect profile than slow channel blockers (Parsons et al., 1999b), and since they are effective in some forms of neuropathic pain (Headley, 1999), we hypothesize that the use of compounds such as Mrz 2/579 may prove to be useful adjunct therapy in the management of some forms of clinical pain by μ -opioid agonists.

Acknowledgements

This work was supported by NIH grant no GM35523.

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