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The effects of NMDA receptor antagonists on acute morphine antinociception in mice

E. Kozela and **P. Popik**

Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

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Summary. Antagonists of the *N*-methyl-D-aspartate (NMDA) receptor complex inhibit the development of tolerance to antinociceptive effects of morphine and upon acute administration, influence morphine antinociceptive activity. The analysis of numerous studies investigating acute interaction between NMDA receptor antagonists and morphine in mice indicate a variety of procedural differences and reveal that these compounds may potentiate, attenuate and produce no effect on morphine antinociception. The conditions responsible for such conflicting experimental outcome of acute interaction remain unclear. It appears that the effects of NMDA receptor antagonists on morphine tolerance are not causally related to their acute effects on morphine antinociception.

Keywords: Opiates – Morphine – Antinociception – Pain – NMDA receptor antagonists – Glutamate

Introduction

Morphine remains the primary drug treatment for severe pain, however repeated administration of this and other opioids can lead to the development of tolerance, in particular, to its antinociceptive effects (World Health Organization, 1986). The necessity of increasing morphine dosing in humans is accompanied by a number of undesirable side-effects including respiratory depression and constipation to which tolerance does not develop to the same extent as to the antinociceptive effect. The challenging aspect of prevention of tolerance to morphine had prompted a number of laboratory investigations. It is now well documented that *N*-methyl-D-aspartate (NMDA) receptor antagonists (NMDAR-A) inhibit tolerance to antinociceptive effects of morphine in laboratory animals, suggesting that activation of the NMDA receptors is critical for its development (Bisaga and Popik, 2000). The mechanism of inhibition of morphine tolerance by NMDAR-As is not fully understood, but there is a possibility that this effect results not from preventing the plastic neuronal changes leading to opioid tolerance, but rather from the inhibitory influence of the NMDAR-As on morphine actions *per se.*

Our minireview is thus focused on the effects of NMDAR-A on acute antinociceptive effects of morphine in mice. There are differences in the outcome of mice and rats studies investigating NMDAR-A and morphine interaction (Kozela et al., 2001). However, even if restricted only to mice, studies differ in many procedural details which make them difficult to compare (see Table 1). For instance, studies cited in Table 1 use various strains of mice, with morphine administered at various doses or with the use of dose-response (e.g., cumulative dose-response) paradigms. Morphine antinociception has been measured only once, or at numerous time points after its administration, and NMDAR-As were administered at various doses and at various timepoints around morphine injection. Studies differ also in respect to the route of drug administration. Antinociceptive properties of opioids can be tested in rodents with the use of two methods: the tail-flick (D'Amour and Smith, 1941) and the hot plate (Woolfe and Macdonald, 1944) tests. In studies summarized in Table 1 mainly the tail-flick test has been used. The hot plate test was used only in four out of fifteen studies (Lipa and Kavaliers, 1990; Saucier and Kavaliers, 1994; Suh et al., 1994; Bernardi et al., 1996). All these variables may be responsible for the

Table 1.

Effect of NMDAR-A co-administration	Strain of mice	NMDAR-A, dose, route and time of administration, if available	Dose of morphine and route of administration	Time [min] of the measurement after morphine administration	Reference
Uncompetitive NMDAR-As					
Hot plate test					
↓	Deer mice	(+) MK-801, 0.25 mg/kg i.p.	1 mg/kg, i.p[1]	30	(Lipa and Kavaliers, 1990)
↓	Deer mice	MK-801, 0.05 and 1 mg/kg, i.p.; just after the morphine injection.	10 mg/kg, i.p.[2]	0, 15, 30, 60, 90, 120	(Saucier and Kavaliers, 1994)
↓	ICR	MK-801 0.001, 0.1, 1 μg i.c.v.,	1 μg i.c.v.[3]	15	(Suh et al., 1994)
Tail-flick test					
↑	Swiss Albino	Ketamine, 0.1, 1 mg/kg, i.p.	1.5 mg/kg s.c.	30	(Dambisya and Lee, 1994)
↑	Swiss SHR	Memantine 1, 3, 10 mg/kg i.p., 90 min after morphine injection, 30 min prior to the test	5, 10, 20 mg/kg s.c.	120	(Belozertseva et al., 2000)
↑	Swiss SHR	MRZ 2/579 1, 3, 10 mg/kg i.p., 90 min after morphine injection, 30 min prior to the test	5, 10, 20 mg/kg s.c.	120	(Belozertseva et al., 2000)
–	CD-1	MK-801 0.3 mg/kg, i.p., 30 min prior to test	Cumulative dose-response curves, s.c.	30 min after each dose of morphine	(Elliott et al., 1994)
–	ICR	MK-801, 0.1 mg/kg i.p.	Dose-response studies , s.c or i.c.v.[4]	30 (s.c.), 10 (i.c.v.)	(Bilsky et al., 1996)
–	ICR	MK-801 2 μg i.t.	0.2 μg i.t.	15, 30, 45, 60	(Suh et al., 2000)
–	Swiss SHR	Memantine 1, 3, 10 mg/kg i.p., just after morphine injection, 30 min prior to the test	1, 3, 5 mg/kg s.c.	30	(Belozertseva et al., 2000)
–	Swiss SHR	MRZ 2/579 1, 3, 10 mg/kg i.p., just after morphine injection, 30 min prior to the test	1, 3, 5 mg/kg s.c.	30	(Belozertseva et al., 2000)
–	Albino Swiss	Dextromethorphan 10 mg/kg, s.c.	5 mg/kg s.c.	0, 30, 60, 90, 120	(Popik et al., 2000)
↓	Swiss Webster	MK-801, 0.1 mg/kg i.p., 30 min before morphine	7.5 mg/kg and dose-response paradigm, s.c.	45	(Lutfy et al., 1993)
↓	Swiss Albino	Memantine 10 mg/kg, s.c.	5 mg/kg, s.c.	0, 30, 60, 90, 120	(Popik et al., 2000)
↓	Swiss Albino	MRZ 2/579 10 mg/kg, s.c.	5 mg/kg, s.c.	0, 30, 60, 90, 120	(Popik et al., 2000)
↓	ICR	MK-801 0.01, 0.1, 1 μg, i.c.v., 10 min before morphine	0.2 μg i.c.v and i.t. concurrently	30	(Suh et al., 1995)
↓	ICR	MK-801 0.01, 0.1, 1 μg i.c.v	1 μg i.c.v.	30	(Suh et al., 1994)
Competitive NMDAR-As					
Hot plate test					
↑	Deer mice	NPC 12626 0.03 and 1 mg/kg, i.p., just after morphine injection.	10 mg/kg i.p.[5]	0, 15, 30, 60, 90, 120	(Saucier and Kavaliers, 1994)

Tail-flick test					
↑	Swiss-Webster	LY 235959 1, 2, 4 mg/kg, i.p., 10 min before morphine	10 mg/kg i.p.	0, 30, 60, 90 up to 360	(Bhargava, 1997)
↑	Swiss SHR	d-CPPene 0.1, 0.3, 0.56, 1 mg/kg i.p., 90 min after morphine injection, 30 min prior to the test	5, 10, 20 mg/kg s.c.	120	(Belozertseva et al., 2000)
↑	Swiss SHR	d-CPPene 0.1, 0.3, 0.56, 1 mg/kg i.p., just after morphine injection, 30 min prior to the test	1, 3, 5 mg/kg s.c.	30	(Belozertseva et al., 2000)
–	CD-1	LY 274614 6 mg/kg i.p., 30 min prior to testing	Cumulative dose-response curve	30 min after each dose of morphine	(Elliott et al., 1994)
–	CD-1	NPC 17742, 2 mg/kg i.p.	Dose-response study	30	(Kolesnikov et al., 1993)
–	Swiss Albino	NPC 17742 6 mg/kg s.c.	5 mg/kg s.c.	0, 30, 60, 90, 120	(Popik et al., 2000)
–	ICR	LY 235959 3 mg/kg i.p.,	Cumulative dose-response studies, s.c. or i.c.v.[6]	30 (s.c.), 10 (i.c.v.)	(Bilsky et al., 1996)
Gly/NMDA antagonists					
Tail-flick test					
↑	CD-1	ACEA 1328 1, 5, 10 mg/kg i.p., just before morphine	Dose-response studies	30	(Lutfy et al., 1999)
↑	Swiss SHR	MRZ 2/576 1, 3 mg/kg i.p., 105 min after morphine injection, 15 min prior to the test	5, 10, 20 mg/kg s.c.	120	(Belozertseva et al., 2000)
↑	Swiss SHR	MRZ 2/576 1, 3, 10 mg/kg i.p., 15 min after morphine injection, 15 min prior to the test	1, 3, 5 mg/kg s.c.	30	(Belozertseva et al., 2000)
↑	Swiss SHR	ACEA-1021 5, 10 mg/kg i.p., 90 min after morphine injection, 30 min prior to the test	5, 10, 20 mg/kg s.c.	120	(Belozertseva et al., 2000)
–	Swiss SHR	ACEA-1021 5, 10 mg/kg i.p., just after morphine injection, 30 min prior to the test	1, 3, 5 mg/kg s.c.	30	(Belozertseva et al., 2000)
Antagonists of polyamine modulatory site					
Hot plate test					
↑	Albino Swiss	Ifenprodil 10, 30 mg/kg i.p., probably just after morphine	5 mg/kg s.c.[7]	30, 60	(Bernardi et al., 1996)

[1] Hot-plate temperature: 50 ± 0.5°C, foot-lifting response
[2] Hot-plate temperature: 50 ± 0.5°C, foot-licking or jumping response
[3] Hot-plate temperature: 55°C licking or shaking the front paws
[4] warm water tail-flick, temp: 55 ± 0.5°C
[5] Hot-plate temperature: 50 ± 0.5°C, foot-licking or jumping response
[6] warm water tail-flick, temp: 55 ± 0.5°C
[7] Hot-plate temperature: 50 ± 0.5°C, paw licking

dissimilarities among results reported in studies presented in Table 1, as the inhibition, no effect or potentiation of morphine antinociception by NMDAR-As were reported. The other possible reasons of such discrepancies will be discussed further in this article, based, in part on our own data.

The function of the NMDA receptor complex may be inhibited by a number of antagonists acting at various binding sites. The competitive (glutamate antagonists), uncompetitive (receptor channel blockers) and Gly/NMDA antagonists (binding at glycine modulatory site) as well as compounds inhibiting other modulatory sites of NMDA receptor complex (like polyamine site antagonists) affect differently the characteristics of receptor blockade. Moreover, NMDAR-As acting at various sites at the NMDA receptor may have different effects because of a different subunit composition specificity and different localization of subunits in the brain (Porter and Greenamyre, 1995).

Uncompetitive NMDA receptor antagonists

With respect to the uncompetitive NMDAR-As, their effects on morphine antinociception were tested predominantly in the tail-flick test, and the lack of effect, potentiation or inhibition of morphine antinociception were reported. This includes high affinity channel blockers like MK-801 (dizocilpine) as well as relatively low affinity and fast kinetic antagonists like dextromethorphan and memantine.

In some studies, MK-801 produced no effect on morphine antinociception (Elliott et al., 1994; Bilsky et al., 1996; Suh et al., 2000). Similarly, in the report of Belozertseva et al. (2000), neither memantine nor MRZ 2/579 influenced the antinociceptive effect of morphine used at low (1–5 mg/kg) doses and tested 30 min after administration of both drugs. However, the antinociception produced by morphine used at relatively high (5–20 mg/kg) doses was potentiated when memantine and MRZ 2/579 were administered long time (90 min) after morphine and short time (30 min) before the tail-flick test (Belozertseva et al., 2000). These findings suggest that both the dose of morphine, the sequence of injections and the time of test may play a crucial role in the experimental outcome. The potentiation of morphine antinociceptive effect was also demonstrated for ketamine (Dambisya and Lee, 1994), however ketamine exerted its effect only at the dose which by itself was active in tail-flick test. Other studies demonstrate an inhibition of morphine antinociception by MK-801 (Lutfy et al., 1993; Suh et al., 1994; Suh et al., 1995). Similarly, the relatively low affinity antagonists used in our experiments (memantine and MRZ 2/579) significantly attenuated morphine antinociception (Popik et al., 2000). In the same study (Popik et al., 2000), dextromethorphan appeared to diminish morphine antinociception, but this effect was not significant perhaps due to the insufficient dosing. We found three studies with the use of the hot plate test, and in all these cases, MK-801 inhibited morphine antinociception (Lipa and Kavaliers, 1990; Saucier and Kavaliers, 1994; Suh et al., 1994).

Competitive NMDA receptor antagonists

No effect of NPC 17742 pretreatment described by Kolesnikov et al. could be due to the use of low dose of antagonist and only one time point of test after morphine administration (Kolesnikov et al., 1993). Although in our experiments with NPC 17742 we observed a trend to prolong the morphine effect, it did not reach statistical significance (Popik et al., 2000). This agrees well with other reports (Kolesnikov et al., 1993; Elliott et al., 1994; Bilsky et al., 1996) where no effect of competitive NMDAR-As were observed. However, some authors reported the potentiation of morphine-induced antinociception in tail-flick (Bhargava, 1997; Belozertseva et al., 2000) and hot plate test (Saucier and Kavaliers, 1994) by competitive NMDAR-As. We did not find data suggesting inhibitory action of competitive NMDAR-As on morphine antinociceptive activity.

Gly/NMDA receptor antagonists

Antagonists of glycine site on NMDA receptor complex also produce no effect (Belozertseva et al., 2000) or potentiate (Lutfy et al., 1999; Belozertseva et al., 2000) morphine-induced antinociception in tail-flick studies.

Polyamine NMDA receptor antagonists

We found only one study investigating the effect of polyamine antagonist (ifenprodil) pretreatment on morphine antinociception tested in hot plate test (Bernardi et al., 1996). Ifenprodil potentiated and prolonged morphine activity. However, the dose of ifenprodil enhancing morphine effect was producing

antinociceptive effect in the same test on its own. Ifenprodil antinociception was inhibited by naloxone, suggesting the involvement of opioid receptors and, perhaps, an additive effect with morphine.

Discussion

It is believed that the tail-flick response (tail withdrawal) is predominantly spinally mediated and that in the hot plate response (paw lifting, licking or jumping) the supraspinal sites play a crucial role. However, morphine also produces antinociception in the tail-flick test after intracerebroventricular administration and is active in hot plate test when is given intrathecally (Suh et al., 1995; Luger et al., 1995b). Both tests have different pharmacological characteristics (Luger et al., 1995a; Luger et al., 1995b). Prevailing usage of the tail-flick appears to limit the investigations on the discussed topic to the spinal interactions between NMDA and opioid receptors. On the other hand, the parameters of the tail-flick paradigm are similar in almost all tail-flick studies (baseline ~3 sec and cut-off = 10 sec) and the criterion of nociceptive response is always the same (tail withdrawal). Similar baseline and cut-off times indicate that similar intensities of nociceptive stimuli were applied. This is in contrast to the hot plate test where authors often use different temperature of the metal plate and choose various criteria of antinociceptive response (paw licking, lifting or jumping) (Lipa and Kavaliers, 1990; Saucier and Kavaliers, 1994). In several studies, the effect of NMDAR-A and opioid coadministration was tested at only one time point. This approach allows to observe only potentiation or inhibition, but not prolongation of the morphine effect.

With the exception of ketamine and ifenprodil, none of the tested NMDAR-As in the studies listed in Table 1 produced its own activity in acute nociceptive paradigms (although not all studies report direct effects of NMDAR-As on nociception).

The inconsistencies in modulation of antinociceptive effects of morphine by NMDAR-As may result from different pharmacological characteristics and different interaction with the NMDA receptor (Parsons et al., 1998; Dingledine et al., 1999). Furthermore, even NMDAR-As belonging to the same group and characterized by relatively similar interaction with the NMDA channel complex, kinetics and voltage dependency, may differ in selectivity for NMDA receptor subunit types as it is the case for dextromethorphan, memantine and MRZ 2/579 (Avenet et al., 1997; Parsons et al., 1999a). The various NMDAR2 subunits confer the functional, pharmacological and physiological variability of the NMDA receptors in different brain regions. These differences are probably responsible for dissimilar modulation of morphine effect not only among NMDAR-As binding to the different sites but also within a given group of NMDAR-As.

It seems that glycine as well as competitive NMDAR-As enhance or have no effect on morphine antinociception in the tail-flick test in mice and we found no data showing inhibitory influence of these compounds on morphine antinociception. More diverse effects on morphine antinociception were reported for uncompetitive NMDAR-As (channel blockers).

The analysis of Table 1 suggests that modulation of morphine effect by NMDAR-As may be due to a number of variables including neuroanatomical, neurochemical and inter-injection interval. In particular, data reported by Belozertseva et al. (2000) suggest that the potentiation of morphine antinociceptive effect by NMDAR-As is easier to demonstrate when NMDAR-As are administered long time after morphine and short time before the test, indicating that the effects of NMDAR-As on morphine antinociception may depend on the phase of morphine action. Since there are within-species differences in the metabolism of drugs, it is possible that in animals that metabolize opiates faster, the augmentation of morphine antinociception could be more difficult to demonstrate. In addition, it should be kept in mind that NMDAR-As may be less specific for the NMDA receptor than previously thought. For example, at behaviorally relevant doses, memantine may also act at nicotinic (Parsons et al., 1999b) and 5-HT3 (Rammes et al., 2001) receptors and dextromethorphan binds to sigma receptors (Chou et al., 1999); such effects should also be taken into account in considering differences among effects of NMDAR-As on morphine antinociception.

Both in mice and rats, NMDAR-As are able to inhibit the development of tolerance (Bisaga and Popik, 2000), however, these compounds appear to exert opposite effects on acute morphine antinociception, since in rats, the potentiation of morphine effect has been more frequently reported (Kozela et al., 2001). However, despite the inter-species differences in both rats and mice NMDAR-As inhibit morphine tolerance, suggesting that their effects on tolerance

are not related to their acute effects on morphine antinociception.

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Authors' address: Ewa Kozela, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Kraków, Poland, Fax: (+48-12)-637-45-00, E-mail: kozela@if-pan.krakow.pl