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Antinociceptive interactions of ketamine with morphine or methadone in mononeuropathic rats

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Abstract

To study the antinociceptive synergy resulting from the combination of opioid receptor agonists and *N*-methyl-D-aspartate (NMDA) receptor antagonists on neuropathic pain, an isobolographic analysis of equianalgesic combinations of ketamine with methadone or morphine was performed in rats with mononeuropathy produced by placing four constrictive ligatures around the common sciatic nerve. Two weeks later, the antinociceptive effect of subcutaneous administration of the drugs alone or combined was evaluated by using the paw pressure test. Drugs and their combinations produced dose-dependent antinociception. Combinations produced synergy of a supra-additive nature in the neuropathic paw, but only additive antinociception in the normal paw. The ketamine/metadone combination was more effective to produce antinociception in the neuropathic paw than was the ketamine/morphine association, as revealed by the lower ED_{25}. The results indicate supra-additive synergy between NMDA receptor antagonists and opioids, especially methadone, to produce antinociception in experimental neuropathy.

Keywords: Morphine; Ketamine; Methadone; Pain; Mononeuropathy; Paw pressure; Isobologram; (Rat)

1. Introduction

Combinations of analgesics from different pharmacological classes are used frequently for relieving chronic pain complaints. The goal is to improve analgesia without enhancing the side effects of each drug. The effects of such combinations can be additive or synergistic and could be studied in animals by means of isobologram analysis (Tallarida, 2000). While the opioids morphine and methadone are the most widely used and most effective drugs for the treatment of many pain syndromes, they have some well-known adverse effects, including sedation, respiratory depression, nausea and constipation. Furthermore, on long-term administration, they can lose their efficacy through the development of tolerance and very high doses of the drug are necessary in these cases. A further important issue is that some chronic pain states, such as neuropathic pain, are not effectively treatable with opiates (Fields and Rowbotham, 1994). Thus, alternative strategies based on drug combinations need to be considered in order to solve these drawbacks of opioids. On the other hand, there is abundant evidence for a major role for the *N*-methyl-D-aspartate (NMDA) receptor in the generation of central sensitization, the principal factor for the development and maintenance of several forms of chronic pain (Eide, 2000). On this basis, administration of NMDA receptor antagonists could be useful, applied alone or in combination with opioids for the treatment of some forms of chronic pain. Thus, it has been shown that NMDA receptor antagonists reduce the nociceptive response and hyperalgesia in experimental models of chronic pain (Dickenson et al., 1997), as well as wind-up phenomena in dorsal horn neurons (Dickenson, 1990). Today, a number of highly specific NMDA receptor antagonists have been developed for animal pain research. Interestingly, also a number of clinically available drugs have been found to have antagonistic actions on the NMDA receptor, such as ketamine and dextromethorphan, drugs potentially useful for administration in combination with opioids. Moreover, recent data have shown potentiation of morphine antinociceptive effects by the NMDA receptor

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antagonists dextromethorphan and MK-801, in normal rats (Chapman and Dickenson, 1992; Grass et al., 1996; Hoffmann and Wiesenfeld-Hallin, 1996) and in mononeuropathic rats (Yamamoto and Yaksh, 1992; Nichols et al., 1997). Besides, it has been reported that combination of NMDA receptor antagonists with opioids results in marked potentiation of clinical analgesia (see review by Wiesenfeld-Hallin, 1998). However, although potentiation was clearly evidenced in these studies, the question of whether the potentiation of morphine-induced antinociception by NMDA receptor antagonists is additive or synergistic has received little attention and only two animal studies have considered this aspect using isobolographic analysis. These studies showed synergy between morphine and the NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (AP-5), in the formalin test but not in acute thermal nociception (Nishiyama, 2000; Nishiyama et al., 1998), which suggests that synergy between opioid agonists and NMDA receptor antagonists could depend on temporal aspects (i.e. tonicity) of the experimental pain induced. Since this question would be of great importance for treating chronic pain with combinations of opioids and NMDA receptor antagonists, the present study was designed to investigate the antinociceptive interactions between the NMDA receptor antagonist ketamine and the μ-opioid receptor agonists, morphine or methadone, in both normal and mononeuropathic rats, by using isobolographic analysis of paw pressure testing.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 230–280 g were used in these experiments. The experiments conformed to ethical guidelines of the International Association for the Study of Pain, for experimental pain in conscious animals (Zimmermann, 1983). In particular, the duration of the experiments was as short as possible, the number of animals involved was kept to a minimum, and the animals were killed immediately after the recording period by the administration of an anaesthetic overdose. Each animal was used only once and received only one dose of the drugs tested. Rats were housed in plastic cages with soft bedding (four per cage) with free access to food and water. They were maintained in a climate- (23 ± 1 °C) and light-controlled (12/12-h dark/light cycle with light on at 0800 h) for at least 1 week before the experiments. All observations during the assay were performed in a randomised and blind manner. Mononeuropathy was produced according to the method described by Bennett and Xie (1988). Briefly, four constrictive ligatures made of No. 4.0 chromium catgut were placed around the right common sciatic nerve of rats under halothane anesthesia. The left hind limb was left intact, since it has been claimed that sciatic nerve exposure during sham procedures does not modify the response threshold to nociceptive mechanical stimulation (Meyerson et al., 1995). Postoperatively, the animals were housed together as before surgery. Starting from the postoperative day 5, the development of hyperalgesia and allodynia was evaluated every 3 days using calibrated von Frey filaments applied to the plantar skin of both hind paws (Meyerson et al., 1995). At day 14, once maximum hyperalgesia and allodynia had developed, algesimetric evaluation was done using the paw pressure test (Randall and Selitto, 1957).

2.2. Nociceptive behavioral test

For each rat, the paw pressure threshold expressed in grams was determined using an Ugo Basile analgesiameter, by applying increasing pressure to the right hindpaw until an audible squeak was elicited. This criterion was chosen because it represents a more integrated nociceptive behavior than paw withdrawal (Kayser and Guilbaud, 1990). Algesimetric evaluation of the contralateral normal paw served for control measurement. To avoid injury, a cut-off value of 700 g was used in the normal paw, while in the neuropathic paw it was diminished to 570 g. In fact, according to our previous experience, the paw pressure threshold in the neuropathic hind paw is about 130 g less than in the normal paw.

2.3. Measurements of the antinociceptive effect

Two weeks after mononeuropathy induction, subcutaneous injections of analgesic drugs were administered. For each animal, the paw pressure threshold was measured in both the normal and the neuropathic paws before and 15, 30, 60, 120, 180 and 240 min after drug injection, and the results were calculated with the equation $[(TPP_t - TPP_{t=0}) \times 100]/TPP_{t=0}$, where TPP_t is the threshold paw pressure at the time of peak effect (at time $= t$) and $TPP_{t=0}$ is the threshold paw pressure before drug injection (at $t = 0$). The variation of the threshold at time t is then obtained as a percentage of the control value. In a first experimental series, the drugs were administered alone and dose–response curves were made using eight animals at each of four doses. For each drug, the dose that produced 25% antinociception (ED_{25}: 25% increase of the threshold paw pressure) was calculated for the normal and the neuropathic paws using standard linear regression analysis of the dose–response curve. In a second experimental series, the drugs were administered in combinations at their fixed ED_{25} ratios calculated from experiments with the normal hind paw, and the ED_{25} of the combination were obtained from the dose–response plots. Table 1 lists the different doses and ratios. To characterize the interaction between drug pairs, isobolographic analysis was performed. With this method, only equieffective doses of each drug and their combination, drawn from the dose–response curves, were considered for analysis. A theoretically additive dose of the combination in the same component ratio was computed from the equief-

Table 1
Doses and ratios used for drug combinations

Drug combination (drug 1/drug 2)	N	Ratio (ED_{25} drug 1/ ED_{25} drug 2)[a]	Equieffective dose (mg/kg)		Combination dose (mg/kg)
			Drug 1	Drug 2	Drug 1 + drug 2
Ketamine/ morphine	8	28.98	5.65	0.19	5.84
	8		11.30	0.39	11.69
	8		22.61	0.78	23.39
	8		45.21	1.56	46.77
Ketamine/ methadone	8	22.49	5.65	0.25	5.90
	8		11.30	0.50	11.80
	8		22.61	1.01	23.62
	8		45.21	2.01	47.22

[a] ED_{25} values from experiments with the normal hind paw (see Fig. 1).

fective doses of the single drugs, according to the method described by Tallarida et al. (1989). The comparison of both doses of the combination—experimentally and theoretically additive—allowed us to define the nature of the interaction (synergy or antagonism) or to conclude that there was no interaction (additivity).

2.4. Drugs and statistics

The following drugs and doses were given alone: morphine hydrochloride, from Laboratorio Biosano, Santiago, Chile (0.625, 1.25, 2.5 and 5.0 mg/kg s.c.), methadone hydrochloride from Laboratorio Biosano (1.25, 2.5, 5.0 and 10.0 mg/kg s.c), and ketamine hydrochloride from Merial, Lyon, France (12.5, 25.0, 50.0 and 100.0 mg/kg s.c.). For combinations (see Table 1), the drugs were not mixed in the same syringe and two subcutaneous injections were performed successively. The results are presented as mean values ± S.E.M. or as ED_{25} values and 95% confidence intervals. To compare ED_{25} values for the same drug or combination on the normal and neuropathic paws, the results were examined using Student's *t*-test for unpaired data; to compare effects of the two combinations on both normal and neuropathic paws, the results were examined using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparisons test. Significance was accepted at the 0.05 level. For all the drugs, the ED_{25} values were used instead of the ED_{50} values because, in the case of ketamine, the ED_{50} dose is sedative in addition to analgesic, interfering with behavioral measures of nociception.

3. Results

3.1. Individual drug responses

The four doses of ketamine (12.5, 25, 50, 100 mg/kg), morphine (0.625, 1.25, 2.5, 5 mg/kg), and methadone (1.25, 2.5, 5, 10 mg/kg) produced a dose-dependent antinociceptive effect in both the normal and the neuropathic paws. While the ED_{25} for ketamine was markedly higher than the ED_{25} for morphine and methadone (see Fig. 1), no significant differences were found on comparison of the

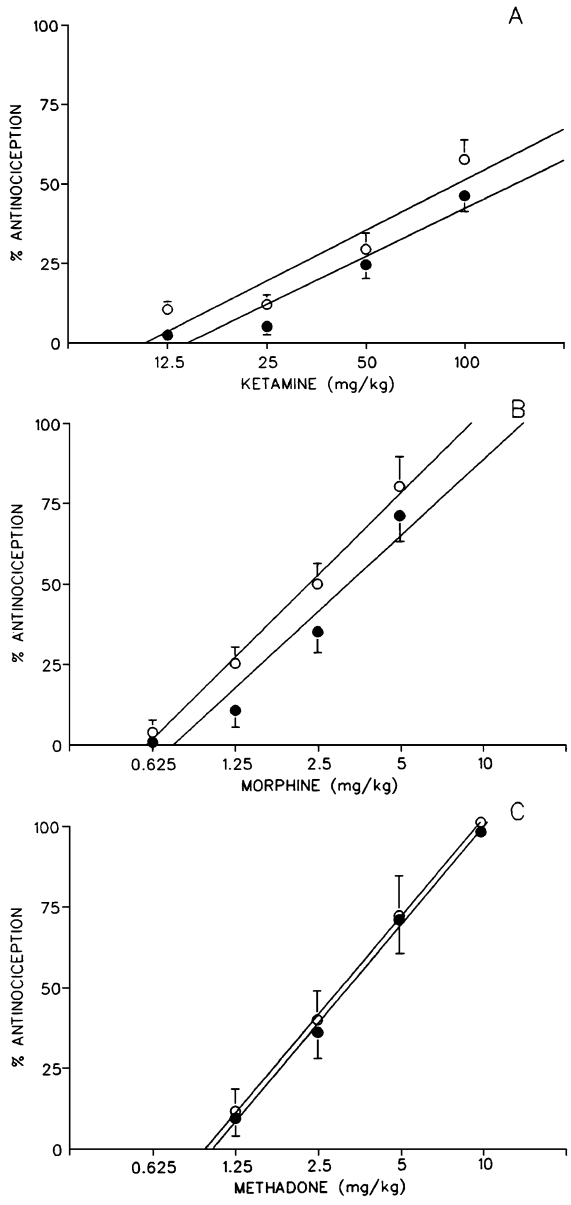

Fig. 1. Dose–response curves for the antinociceptive effect of subcutaneous administration of 12.5, 25, 50 and 100 mg/kg of ketamine (A), 0.625, 1.25, 2.5 and 5 mg/kg of morphine (B) and 1.25, 2.5, 5 and 10 mg/kg of methadone (C) on the vocalization threshold (as a percentage) in response to graded paw pressure in both the normal (closed circles) and the neuropathic (open circles) paws. Values are means ± S.E.M. $N = 8$ in each group. (A) The ED_{25} and the 95% confidence limit (in parentheses) for ketamine when the normal and the neuropathic paws were tested were 45.2 (30.5–66.9) and 31.8 (23.9–42.2) mg/kg, respectively. (B) The ED_{25} and the 95% confidence limit (in parentheses) for morphine when the normal and the neuropathic paws were tested were 1.6 (1.1–2.2) and 1.2 (1.1–1.3) mg/kg, respectively. (C) The ED_{25} and the 95% confidence limit (in parentheses) for methadone when the normal and the neuropathic paws were tested were 2.0 (1.4–2.9) and 1.7 (1.1–2.6) mg/kg, respectively. No significant differences were found on comparison of the drug ED_{25} obtained by testing the normal and the neuropathic paws (unpaired Student's *t*-test).

ED_{25} of each drug in the normal and in the neuropathic paw (Fig. 1).

3.2. Drug combination responses

The association of ketamine with morphine and ketamine with methadone at equieffective doses (see Table 1) resulted in dose-dependent antinociceptive effects in both the normal (Fig. 2A) and the neuropathic (Fig. 2B) paws. The statistical analysis demonstrated that the ED_{25} of the ketamine/methadone combination in the neuropathic paw was significantly lower than the ED_{25} of the same combination in the normal paw or the ED_{25} of the ketamine/morphine combination in both paws.

Isobolographic analysis of drug interactions showed that co-administration of the combinations ketamine/morphine and ketamine/methadone produced antinociceptive effects greater than simple additivity in the neuropathic paw, since the ED_{25} points for each combination were significantly different from the corresponding theoretical ED_{25} and they were located in the region of the isobologram that denotes superadditivity (Fig. 3), the ED_{25} for the ketamine/metadone combination being lower than that for the ketamine/morphine association. For both associations, however, the effect on the normal paw was only additive because the ED_{25} point for each combination was not statistically different from the theoretical ED_{25} (Fig. 3).

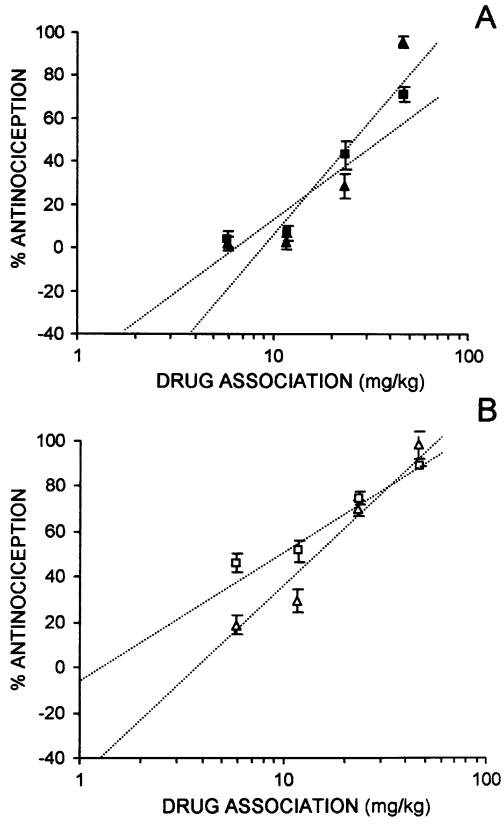

Fig. 2. Dose–response curves for the antinociceptive effect of subcutaneous administration of ketamine/morphine combination (triangles) and ketamine/methadone combination (squares) on the vocalization threshold (as a percentage) in response to graded paw pressure in both the normal (closed symbols) and the neuropathic (open symbols) paws. Values are means ± S.E.M. for eight rats in each group. (A) The ED_{25} and the 95% confidence limit (in parentheses) for the ketamine/morphine and the ketamine/methadone combinations were 14.1[a] (10.9–18.1) and 13.9[a] (9.4–20.5) mg/kg, respectively, when the normal paw was tested. (B) The ED_{25} and the 95% confidence interval (in parentheses) for the ketamine/morphine and the ketamine/methadone combinations were 8.0[a] (6.1–10.6) and 2.7[b] (2.0–3.6) mg/kg, respectively, when the neuropathic paw was tested. ED_{25} values with different superscripts are significantly different ($P<0.05$, Tukey–Kramer post hoc test).

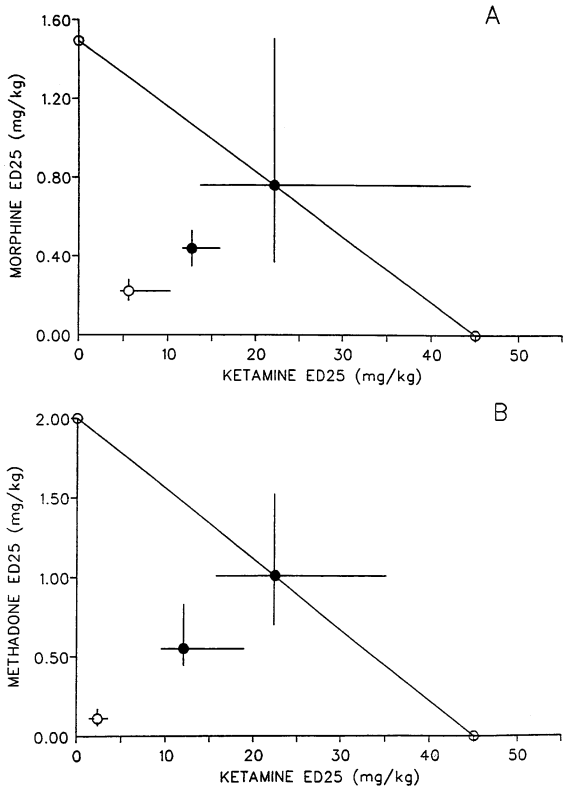

Fig. 3. Isobolograms for the co-administration of ketamine/morphine (A) and ketamine/methadone (B) at fixed-ratio combinations (shown in Table 1). For each combination, the circle above the straight line corresponds to the theoretically calculated point with 95% confidence limits, while the circles under the straight line correspond to the experimental points with 95% confidence limits for the normal (closed circle) and the neuropathic (open circle) paws.

4. Discussion

The foregoing results indicate that the antinociceptive efficacy of the NMDA receptor antagonist, ketamine, in both the normal and the neuropathic paw, was clearly less than that of those drugs having agonistic properties on μ-opioid receptors, such as morphine and methadone, as revealed by a ketamine ED_{25} higher than 30 mg/kg. This is consistent with previous data showing ineffectiveness of

10 mg/kg of ketamine to inhibit mechanical cutaneous nociception in both normal (Olivar and Laird, 1999) and fentanyl-induced hyperalgesic (Laulin et al., 2002) rats.

Combination of equieffective doses of ketamine with the two opioids tested, however, resulted in a superadditive synergic antinociceptive action in the neuropathic paw, while in the normal paw the effect was only additive. Theoretically, superadditivity of the effects of two simultaneously administered antinociceptive drugs implies that the combined molecules act on anatomically and/or functionally different neuronal substrates for nociceptive processing, which is consistent with the well-known different mechanisms of action for opioid receptor agonists and NMDA receptor antagonists. Moreover, it is known that the NMDA receptor and its associated transduction pathway do not play a significant role in acute pain but only in the development and maintenance of chronic pain, where noxious inputs are tonically active and generate hyperexcitability in pain-transmitting neurons of the spinal cord dorsal horn (Haley et al., 1990; Dickenson and Aydar, 1991), a notion that further supports the presence of superadditivity only in the neuropathic paw of the rat. The present observations also fully agree with results of other studies showing synergy between morphine and the NMDA receptor antagonist, AP-5, in the second phase of the formalin test but not in acute thermal nociception (Nishiyama, 2000; Nishiyama et al., 1998), indicating that superadditivity between opioid agonists and NMDA receptor antagonists may only occur when dorsal horn nociresponsive neurons receive a tonic nociceptive input.

Interestingly, the ketamine/methadone combination was significantly more effective than the ketamine/morphine combination to produce antinociceptive effects, in both the normal and the mononeuropathic paws. It is difficult to reconcile higher synergy with the ketamine/methadone combination, since this is just the combination that seems to be redundant at the NMDA receptor level. A plausible explanation for this behavior could be that some μ-opioids such as morphine (Crain and Shen, 2001) and fentanyl (Laulin et al., 2002) may produce acute hyperalgesic effects when low doses are utilized, which seem be the result of either hyperactivation of NMDA receptors (Bespalov et al., 2001) or excitation of Gs protein-coupled opioid receptors (Shen and Crain, 2001). Possible mechanisms and experimental conditions under which μ-opioids could enhance NMDA receptor-mediated currents, thereby contributing to hyperalgesia, have been well delineated by Mao (1999). As a cause of these hyperalgesic effects, the Y intercept for the ketamine/morphine dose–effect plot combination could be displaced down the *Y*-axis, resulting in a reduced ED_{25}. Similar excitatory effects have not been reported so far for the mixed μ-opioid receptor agonist/NMDA receptor antagonist methadone.

Knowledge of how superadditive is the combination of a certain opioid agonist with a NMDA receptor antagonist against neuropathic pain may have potential clinical applications, because it would allow a significant decrease of the dose prescribed for both antinociceptive drugs together with the respective associated side-effects, while maintaining the antinociceptive action. This is consistent with the recommendation of Mao (1999) that opioids and clinically available NMDA receptor antagonists should be combined because these two classes of agents would complement each other for a well-balanced pain treatment.

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References

Bennett, G.J., Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33, 87–107.

Bespalov, A.Y., Zvartau, E.E., Beardsley, P.M., 2001. Opioid–NMDA receptor interactions may clarify conditioned (associative) components of opioid analgesic tolerance. Neurosci. Biobehav. Rev. 25, 343–353.

Chapman, V., Dickenson, A.H., 1992. The combination of NMDA antagonism and morphine produces profound antinociception in the rat dorsal horn. Brain Res. 573, 321–323.

Crain, S.M., Shen, K., 2001. Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low-dose naltrexone, unmasking potent opioid analgesia. Brain Res. 888, 75–82.

Dickenson, A.H., 1990. A cure for wind up: NMDA receptor antagonists as potential analgesics. Trends Pharmacol. Sci. 11, 307–309.

Dickenson, A.H., Aydar, E., 1991. Antagonism at the glycine site on the NMDA receptor reduces spinal nociception in the rat. Neurosci. Lett. 121, 263–266.

Dickenson, A.H., Chapman, V., Green, G.M., 1997. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. Gen. Pharmacol. 28, 633–638.

Eide, P.K., 2000. Wind-up and the NMDA receptor complex from a clinical perspective. Eur. J. Pain 4, 5–15.

Fields, H.L., Rowbotham, M.C., 1994. Multiple mechanisms of neuropathic pain: a clinical perspective. In: Gebhart, G.F., Hammond, D.L., Jensen, T.S. (Eds.), Progress in Pain Research and Management, vol. 2. IASP Press, Seattle, pp. 437–454.

Grass, S., Hoffmann, O., Xu, X.J., Wiesenfeld-Hallin, Z., 1996. *N*-methyl-D-aspartate receptor antagonists potentiate morphine's antinociceptive effect in the rat. Acta Physiol. Scand. 158, 269–273.

Haley, J.E., Sullivan, A.F., Dickenson, A.H., 1990. Evidence for spinal *N*-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Res. 518, 218–226.

Hoffmann, O., Wiesenfeld-Hallin, Z., 1996. Dextromethorphan potentiates morphine antinociception, but does not reverse tolerance in rats. NeuroReport 7, 838–840.

Kayser, V., Guilbaud, G., 1990. Differential effects of various doses of morphine and naloxone on two nociceptive test thresholds in arthritic and normal rats. Pain 41, 353–363.

Laulin, J.P., Maurette, P., Corcuff, J.B., Rivat, C., Chauvin, M., Simonnet, G., 2002. The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. Anesth. Analg. 94, 1263–1269.

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

Meyerson, B.A., Ren, B., Herregodts, P., Linderoth, B., 1995. Spinal cord stimulation in animal models of mononeuropathy: effects on the withdrawal response and the flexor reflex. Pain 61, 229–243.

Nichols, M.L., Lopez, Y., Ossipov, M.H., Bian, D., Porreca, F., 1997. Enhancement of the antiallodynic and antinociceptive efficacy of spinal morphine by antisera to dynorphin A (1–13) or MK-801 in a nerve-ligation model of peripheral neuropathy. Pain 69, 317–322.

Nishiyama, T., 2000. Interaction between intrathecal morphine and glutamate receptor antagonists in formalin test. Eur. J. Pharmacol. 395, 203–210.

Nishiyama, T., Yaksh, T.L., Weber, E., 1998. Effects of intrathecal NMDA and non-NMDA antagonists on acute thermal nociception and their interaction with morphine. Anesthesiology 89, 715–722.

Olivar, T., Laird, J.M., 1999. Differential effects of *N*-methyl-D-aspartate receptor blockade on nociceptive somatic and visceral reflexes. Pain 79, 67–73.

Randall, P.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. 111, 409–419.

Shen, K.F., Crain, S.M., 2001. Cholera toxin-B subunit blocks excitatory opioid receptor-mediated hyperalgesic effects in mice, thereby unmasking potent opiod analgesia and attenuating opiod tolerance/dependence. Brain Res. 919, 20–30.

Tallarida, R.J., 2000. Drug synergism and dose–effect data analysis. Chapman & Hall/CRC Press, Boca Raton, pp. 57–75.

Tallarida, R.J., Porreca, F., Cowan, A., 1989. Statistical analysis of drug–drug and site–site interactions with isobolograms. Life Sci. 45, 947–961.

Wiesenfeld-Hallin, Z., 1998. Combined opioid-NMDA antagonist therapies. What advantages do they offer for the control of pain syndromes? Drugs 55, 1–4.

Yamamoto, T., Yaksh, T.L., 1992. Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. Anesthesiology 77, 757–763.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.