

Complete Prevention but Stimulus-dependent Reversion of Morphine Tolerance by the Glycine/NMDA Receptor Antagonist (+)-HA966 in Neuropathic Rats

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Background: Tolerance to the analgesic effect of morphine complicates the management of chronic pain states. The authors studied the ability of the glycine/N-methyl-D-aspartate receptor antagonist (+)-HA966 to modify morphine tolerance in a rat model of neuropathic pain.

Methods: Mononeuropathy was induced by placing four ligatures around the common sciatic nerve. The 4-day pretreatment regimens with two daily subcutaneous injections of saline and saline, saline and morphine (10 mg/kg), (+)-HA966 (2.5 mg/kg) and morphine, or (+)-HA966 and saline were begun on postoperative day 12 to test the ability of (+)-HA966 to prevent the development of tolerance. Behavioral experiments were performed on postoperative day 16, when the pain-related behavior reached a stable maximum. The effect of an acute dose of morphine (1 mg/kg intravenously) was tested against both mechanical (vocalization threshold to paw pressure) and thermal (struggle latency to hind paw immersion into a 46°C hot-water bath) stimuli. In addition, to test the ability of a single injection of (+)-HA966 to reverse established morphine tolerance, groups of morphine-pretreated rats received injections of either (+)-HA966 (2.5 mg/kg subcutaneously) and morphine (1 mg/kg intravenously), saline and morphine, or (+)-HA966 and saline.

Results: Baseline vocalization thresholds and struggle latencies did not differ in the various pretreatment groups, indicating that the pretreatments had no effect on pain-related behavior. Coadministration of (+)-HA966 prevented the reduction of the effect observed with morphine alone in both the mechani-

cal test and the thermal test. (+)-HA966 reversed morphine tolerance in the thermal but not in the mechanical test.

Conclusion: (+)-HA966 prevented morphine tolerance in both mechanical and thermal tests but reversed established morphine tolerance in the thermal test only. (Key words: Analgesia; chronic constriction injury; opioid; pain; peripheral neuropathy.)

OPIOIDS are potent and widely used drugs in the treatment of persistent pain, but tolerance to the analgesic effect develops with chronic use, resulting in decreased effectiveness over time. Abundant evidence suggests that the development of morphine tolerance is mediated in part by the glutamate system. Numerous studies have shown that antagonists acting at the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors prevent the development of tolerance to the antinociceptive effect of morphine.¹⁻⁴ It is less clear whether NMDA receptor antagonists also reverse already-established tolerance. Although earlier studies found no such effect of different antagonists,^{5,6} recent reports indicate that, at least in mice, NMDA receptor antagonists inhibit both the development and expression of morphine tolerance.^{4,7,8}

Abnormal pain, referred to as neuropathic pain, often develops after damage to the peripheral nervous system. In addition to its role in morphine tolerance, there is evidence indicating that the NMDA receptor is involved also in the neuroplastic phenomena associated with neuropathic pain.⁹ This pain syndrome presents a big therapeutic challenge, and the effect of morphine remains a matter of controversy,¹⁰ which may partly be the result of an inadequate consideration of the different types of pain and stimulus modalities (mechanical *vs.* thermal, noxious *vs.* nonnoxious) under investigation and their differences in morphine sensitivity. Indeed, we have previously shown that in the chronic constriction injury to the sciatic nerve rat model of neuropathy,^{11,12} systemic morphine produces dose-dependent antinociceptive effects against mechanical¹³⁻¹⁶ and noxious hot

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(46°C) stimuli¹⁵⁻¹⁷ but is ineffective against nonnoxious cold and warm stimuli.^{16,17} We have also shown that although morphine remains effective in uninjured, healthy rats,¹⁸ tolerance to the antinociceptive effect of acute morphine against mechanical stimuli^{18,19} develops rapidly in neuropathic rats handled in the same manner. This observation, together with the finding that the antinociceptive effect of systemic morphine is enhanced in the neuropathic rat compared with the uninjured healthy rat,^{13,17} indicates that in the organism with a painful pathology, important opioid actions, such as analgesia and tolerance development, differ from that observed in the pain-free state.²⁰

The present study examined the effect of the glycine/NMDA receptor antagonist, (+)-HA966, on both the development and expression of morphine tolerance in rats with a persistent pain-related behavior. Pharmacologic modulation of the NMDA receptor at the glycine recognition site is a recent and potentially attractive therapeutic approach. In contrast to other NMDA receptor antagonists, glycine/NMDA antagonists fail to produce neurodegenerative changes^{21,22} and have been associated with less locomotor impairment.²³ We recently observed striking effects of the combination of (+)-HA966 and morphine on the pain-related behavior in our rat model of neuropathic pain¹⁶ as well as in a recently developed model of trigeminal neuropathic pain.²⁴

Materials and Methods

All experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC) as well as with Ministry of Agriculture regulations. In addition, we adhered to the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain Ethical Guidelines.²⁵ In particular, the duration of the experiments was as short as possible, and the number of animals used was kept to a minimum.

Animals

Male Sprague-Dawley rats (Charles River, Saint-Aubin Les-Elbeuf, France; $n = 115$) that weighed 175–200 g on arrival were used. The rats were housed five in a cage on a 12-h light/12-h dark cycle. The ambient temperature was kept at 22°C, and the rats had free access to standard laboratory food and tap water. The animals were allowed to habituate to the housing facilities for at least 1 week before the experiments were begun.

Surgery

The unilateral peripheral mononeuropathy was produced on the right hind limb according to the method described by Bennett and Xie¹¹ and Attal *et al.*¹² The animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). The common sciatic nerve was exposed by blunt dissection at the level of the mid thigh, and four loose ligatures (5-0 chromic catgut, approximately 1-mm spacing) were placed around the nerve, taking care not to interrupt the epineural circulation. To minimize the discomfort and possible painful mechanical stimulation, the rats were housed in large cages with sawdust bedding after the surgery. The neuropathic rats were able to eat and drink unaided.

Behavioral Testing

All experiments were conducted in a quiet room between 8:00 AM and 12:00 PM. The animals were randomly assigned in groups of five (mechanical test) or 10 (thermal test) for a given series of tests and were not acclimatized to the test situations beforehand. The experimenter was unaware of the drug combinations used. Each animal received drugs only once and was used in only one experiment. Testing sessions lasted for approximately 2 h, and at the end of an experiment, rats were killed by an overdose of pentobarbital.

In mechanical tests, the antinociceptive action was determined by measuring the vocalization threshold elicited by pressure on the nerve-injured hind paw, using the Ugo Basile analgesymeter (Comerio, Italy). This instrument generates a linearly increasing mechanical force applied by a dome-shaped plastic tip (diameter = 1 mm) on the dorsal surface of the paw. The tip was positioned between the third and fourth metatarsus (into the sciatic nerve territory), and force was applied until the rat squeaked. This centrally integrated response is especially sensitive to analgesic compounds, particularly in this model of neuropathy.^{13,15,16,26} For each rat, a control threshold (mean of two consecutive stable thresholds expressed in grams) was determined before injecting the drugs. After drug administration, the vocalization thresholds were measured every 10 min until they had returned to the level of the control values.

Thermal nociception was tested by measuring the struggle latency elicited by immersion of the nerve-injured hind paw into a 46°C hot-water bath (Ministat MHUB 11; Bioblock Scientific, Illkirch, France) as described elsewhere.^{12,15-17} For each rat, a control latency (mean of two consecutive trials 20 min apart, expressed in seconds) was determined before injecting the drugs.

After drug administration, struggle latencies were measured every 20 min until they had returned to baseline. The long interval (20 min) between successive measurements was necessary, because in this model, abnormal reactions lasting for more than 15 min after a thermal stimulus have been reported.^{12,27}

Drugs

The following drugs were used: morphine hydrochloride (Meram, Paris, France), naloxone hydrochloride (Narcane, DuPont Pharma, Paris, France), (+)-HA966 [(+)-(1-hydroxy-3-aminopyrrolidine-2-one; Tocris Cookson, Bristol, United Kingdom), and saline (0.9% NaCl). Morphine, naloxone, and (+)-HA966 were diluted in saline and injected in a volume of 1 ml/kg. The control rats received the same volume of saline.

Experimental Designs

Experiment 1: Effect of (+)-HA966 on the Development of Morphine Tolerance. Before the behavioral testing of the effect of an acute injection of morphine, rats were pretreated for 4 days with two consecutive subcutaneous injections of the following combinations of drugs: (1) saline and saline; (2) saline and morphine (10 mg/kg); (3) (+)-HA966 (2.5 mg/kg) and morphine (10 mg/kg); (4) (+)-HA966 (2.5 mg/kg) and saline. This morphine pretreatment has been shown to reliably induce tolerance to the antinociceptive effects of acute morphine in neuropathic rats.^{18,19} The 2.5-mg/kg dose of (+)-HA966 was chosen because it alone produced no antinociception but potentiated a single injection of morphine in neuropathic rats.¹⁶ The drugs were given twice daily (at 9:30 AM and 5:30 PM) for 4 days, beginning on day 12 after the surgery. The effect of an acute injection of morphine (1 mg/kg intravenously) was tested at 17 h after the last pretreatment injection, on day 16 after the surgery. At this time, the abnormal pain behavior in this model is at a stable maximum.^{11,12} Morphine was injected into the lateral tail vein. The dose of acute morphine (1 mg/kg intravenously) used in this study has repeatedly been shown to produce antinociceptive effects against both mechanical^{13,15,16} and noxious thermal¹⁵⁻¹⁷ stimuli. In an additional group of rats pretreated with (+)-HA966 and morphine, naloxone (0.1 mg/kg) was coinjected with acute morphine at a dose that has been shown to prevent the effect of 1 mg/kg morphine.^{13,19}

Experiment 2: Effect of (+)-HA966 on Established Morphine Tolerance. In this experiment, morphine tolerance was induced by two daily injections of mor-

phine (10 mg/kg subcutaneously) on postoperative days 12-15 after the same pretreatment paradigm as in experiment 1. At 17 h after the last pretreatment injection, on postoperative day 16, we examined the effect of an acute injection of the following combinations of drugs: (1) (+)-HA966 (2.5 mg/kg subcutaneously) and morphine (1 mg/kg intravenously); (2) saline (subcutaneously) and morphine (1 mg/kg intravenously); or (3) (+)-HA966 (2.5 mg/kg subcutaneously) and saline (intravenously). (+)-HA966 was administered 20 min before morphine. The latter of the drug combinations [(+)-HA966 2.5 mg/kg and saline] serves to examine whether (+)-HA966, at this dose, exhibits any analgesic properties in morphine-tolerant rats.

Statistical Analysis

Data are expressed as mean \pm SD. The overall effects of various treatments (areas under the curve [AUCs]) were calculated by use of the trapezoidal rule. Student *t* test was used to determine the difference between two means. With three or more means, analysis of variance was used. The observed significances were then confirmed with Tukey test. The statistical procedures were performed with a statistical computer program (Statgraphics Plus; Manugistics, Rockville, MD). The observed differences were regarded as significant when *P* values were < 0.05 .

Results

General Results

In agreement with numerous previous studies,^{15,16} the vocalization threshold of the nerve-injured hind paw was decreased on postoperative day 16 (216 ± 34 g *vs.* the precontraction value 330 ± 58 g; $P < 0.001$; $n = 54$).

As also reported previously,¹⁵⁻¹⁷ the struggle latency after immersion of the nerve-injured hind paw into a 46°C hot water bath was decreased (4.9 ± 1.2 s *vs.* the precontraction value 8.1 ± 1.2 s; $P < 0.001$; $n = 61$).

The mean vocalization thresholds and struggle latencies in the various pretreatment groups did not differ from the vehicle-pretreated group (table 1), which indicates that the pretreatments by themselves were devoid of effects on the pain-related behavior.

Experiment 1: Effect of (+)-HA966 on the Development of Morphine Tolerance

Effect of Morphine in Saline-pretreated Rats. In the mechanical test, morphine produced an effect that

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Table 1. Baseline Thresholds/Latencies in the Mechanical/Thermal Test after Pretreatment with (+)-HA966 and/or Morphine

Pretreatment	Mechanical Test	n	Thermal Test	n
Saline + saline	220 ± 36	12	4.9 ± 0.9	7
Saline + morphine	220 ± 36	23	5.0 ± 1.2	27
(+)-HA966 + morphine	226 ± 30	19	4.6 ± 1.1	19
(+)-HA966 + saline	194 ± 32	11	5.3 ± 1.3	9

Vocalization thresholds to paw pressure (mechanical test, in grams) or struggle latencies to paw immersion into a 46°C hot water bath (thermal test, in second) were determined on postoperative day 16 after the neuropathy-inducing surgery. Data are expressed as mean ± SD. Pretreatments consisted of two daily subcutaneous injections of (+)-HA966 (2.5 mg/kg) and/or morphine (10 mg/kg) on postoperative days 12–15. On postoperative day 12, before any pretreatment, the global mean vocalization threshold was 229 ± 36 (n = 62), and the global mean struggle latency was 4.8 ± 1.0 (n = 62).

peaked at 30 min (410 ± 30 g) and lasted for 80 min (fig. 1). In the thermal test, morphine also produced antinociceptive effects that peaked at 40 min (8.3 ± 1.2 s) and lasted for 60 min (fig. 2).

Effect of Morphine in Morphine-pretreated Rats.

As previously observed,^{18,19} the intensity and duration of the effect of morphine in this group compared with the saline-pretreated rats was either strongly reduced, as in the mechanical test (AUC decreased to one fourth of that of the saline-pretreated group; figs. 1B and 1E), or absent, as in the thermal test (figs. 2B and 2E). This indicates that tolerance has developed to the antinociceptive effect of morphine.

Effect of Morphine in Morphine- and (+)-HA966-pretreated Rats. When (+)-HA966 was administered in combination with morphine for 4 consecutive days, it prevented the development of tolerance to the antinociceptive effect of morphine on day 5. In the mechanical test, an intravenous injection of morphine resulted in antinociception that peaked at 30 min (450 ± 52 g) and lasted for 80 min (fig. 1C). Likewise, in the thermal test, morphine produced an effect that peaked at 40 min (7.6 ± 2.6 s) and lasted for 60 min (fig. 2C). The effect of morphine in the morphine and (+)-HA966-pretreated rats did not differ from the effect of morphine in the saline-pretreated rats (figs. 1E and 2E). Naloxone completely blocked the effect of morphine in both the mechanical (fig. 1C) and the thermal (fig. 2C) test.

Effect of Morphine in (+)-HA966-pretreated Rats. In this group, the effect of morphine in the mechanical test peaked at 30 min (373 ± 50 g) and lasted up to 60 min (fig. 1D). In the thermal test, the effect of morphine peaked at 40 min (9.8 ± 3.2 s) and lasted for 60 min (fig. 2D). The effect of morphine against both mechanical (fig.

1E) and thermal (fig. 2E) stimuli was similar to the effect observed in the saline-pretreated group, indicating that pretreatment with (+)-HA966 on days 1–4 does not modify the effect of morphine on day 5.

Experiment 2: Effect of (+)-HA966 on Established Morphine Tolerance in Neuropathic Rats

Effect of Saline and Morphine in Morphine-pretreated Rats. As in experiment 1, the acute injection of morphine in morphine-pretreated rats resulted in very weak overall effects (AUCs; figs. 3A and 3B) compared with the effect of morphine in saline-pretreated rats (figs. 1E and 2E). This indicates that tolerance has developed to the antinociceptive effect of morphine.

Effect of (+)-HA966 and Morphine in Morphine-pretreated Rats. In the mechanical test, acute concomitant injection of (+)-HA966 with morphine in morphine-pretreated rats produced an effect ($F_{9,60} = 4.4$, analysis of variance; $P < 0.001$) that peaked at 30 min (251 ± 35 g) and lasted for 50 min. The overall effect (AUC) of the combination of (+)-HA966 and morphine did not differ from the overall effect of morphine alone (fig. 3A; $P = 0.12$), indicating that the NMDA/glycine receptor antagonist is unable to inhibit established morphine tolerance. In the thermal test, combined administration of (+)-HA966 and morphine resulted in an antinociceptive effect ($F_{7,64} = 2.8$, analysis of variance; $P < 0.05$) that peaked (9.4 ± 2.7 s) at and lasted for 40 min. The overall effect (AUC) of the combination of (+)-HA966 and morphine was increased compared with the overall effect of morphine alone (fig. 3B; $P < 0.05$), indicating that against a noxious hot stimulus, (+)-HA966 inhibits established morphine tolerance. The effect of the combination of (+)-HA966 and morphine in morphine-pretreated rats did not differ from the effect of morphine alone in rats pretreated with (+)-HA966 and morphine ($P = 0.44$).

Effect of (+)-HA966 in Morphine-pretreated Rats. (+)-HA966 alone was devoid of any effect in the mechanical test (fig. 3A) as well as in the thermal test (fig. 3B).

Discussion

This study confirms our previous findings, showing that morphine tolerance develops rapidly in neuropathic rats in the mechanical test,^{18,19} and extends this observation to the noxious thermal test. Although 1 mg/kg of intravenous morphine was completely ineffective against the thermal stimulus, it remained effective

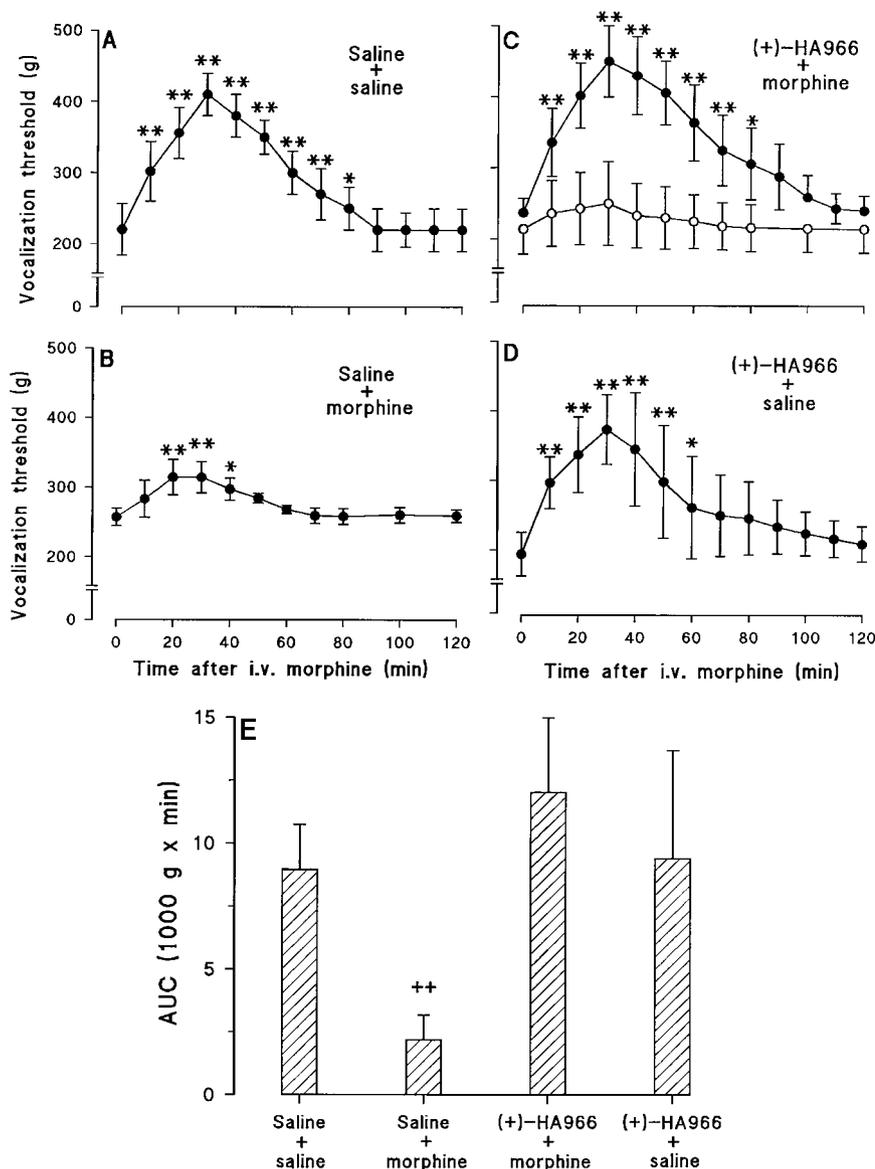


Fig. 1. Effect of morphine (1 mg/kg intravenously; solid circles, A–D) or morphine and naloxone (0.1 mg/kg; open circles, C) on the vocalization threshold to paw pressure of the nerve-injured hind paw of neuropathic rats after a 4-day pretreatment with (A) saline and saline; (B) saline and morphine (10 mg/kg); (C) (+)-HA966 (2.5 mg/kg) and morphine; or (D) (+)-HA966 and saline. (E) Areas under the curve of the respective time curves. Pretreatments consisted of two daily subcutaneous injections on postoperative days 12–15. Testing was performed at 17 h after the last pretreatment injection on postoperative day 16. Naloxone was coadministered with morphine. Each point represents the mean \pm SD of seven to nine rats. * P < 0.05, ** P < 0.01 versus control; ++ P < 0.01 versus pretreatment with saline and saline, Tukey test.

against the mechanical stimulus; however, it was reduced to approximately one fourth-fold after the morphine tolerance-inducing pretreatment. This may reflect previously observed differences in the effect of morphine depending on the stimulus modality. We have previously shown that the mechanical test exhibits a higher sensitivity to morphine than the thermal test.^{13,15}

Experiment 1 found that the glycine/NMDA receptor antagonist (+)-HA966 prevents the development of morphine tolerance in neuropathic rats. The result is in accordance with previous studies performed in uninjured healthy rats that showed that NMDA receptor an-

tagonist in general,^{1,3,4} and antagonist acting at the glycine site in particular,^{8,28,29} prevents morphine tolerance in rodents. To our knowledge, this is the first study to demonstrate an inhibitory effect of a NMDA receptor antagonist on the development of morphine tolerance in a model of neuropathic pain. However, it must be noted that these results have been obtained using one drug dose, and the uncertainty of the applicability of these observations to other doses must be underlined. The effect of morphine in the (+)-HA966-pretreated group did not differ from the effect of morphine in the saline-pretreated group, indicating that

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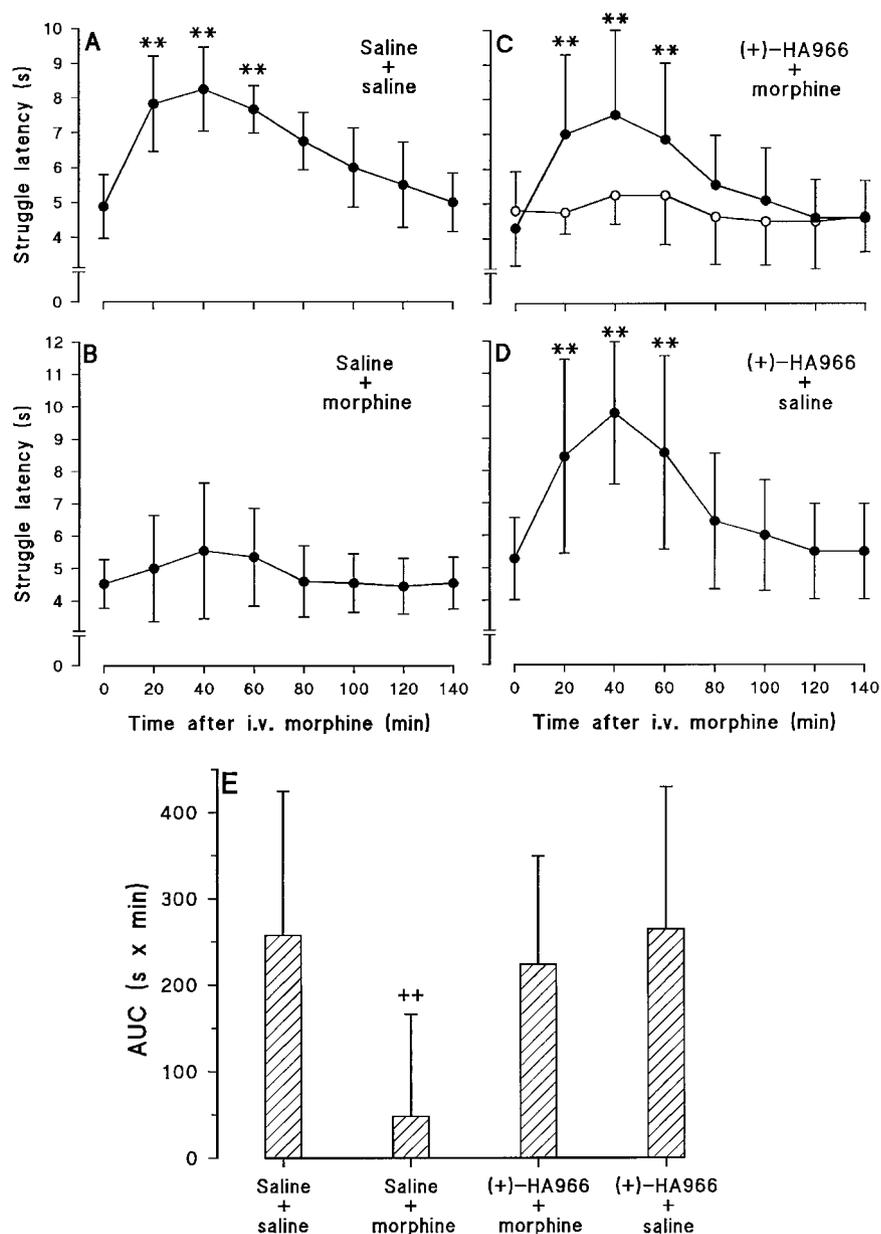


Fig. 2. Effect of morphine (1 mg/kg intravenously; solid circles, *A–D*) or morphine and naloxone (0.1 mg/kg; open circles, *C*) on the struggle latency to immersion of the nerve-injured hind paw of neuropathic rats into a hot (46°C) water bath after a 4-day pretreatment with (*A*) saline and saline; (*B*) saline and morphine (10 mg/kg); (*C*) (+)-HA966 (2.5 mg/kg) and morphine; or (*D*) (+)-HA966 and saline. (*E*) Areas under the curve of the respective time curves. Pretreatments consisted of two daily subcutaneous injections on postoperative days 12–16. Testing was performed at 17 h after the last pretreatment injection on postoperative day 16. Naloxone was co-injected with morphine. Each point represents the mean \pm SD of seven to nine rats. ** $P < 0.01$ versus control; *** $P < 0.01$ versus pretreatment with saline and saline, Tukey test.

the repeated administration of (+)-HA966 on days 1–4 does not modulate the antinociceptive effect of morphine on day 5. The observation minimizes the possibility that a residue of (+)-HA966 injected on days 1–4 may have unmasked an effect of morphine in the morphine-tolerant rats rather than prevented the development of morphine tolerance. This is of particular importance because of the previously demonstrated synergy between (+)-HA966 and morphine in nociceptive tests.^{16,24} The (+)-HA966-induced prevention of mor-

phine tolerance was completely reversed by naloxone, suggesting that opioid receptors mediate not only the acute effect of (+)-HA966 and morphine interaction as previously observed,¹⁶ but also events after chronic concomitant exposure of the μ -opioid and NMDA receptor.

Experiment 2 showed that a single injection of (+)-HA966 at 2.5 mg/kg reverses established morphine tolerance in the thermal test. Although we cannot completely exclude that the effect of (+)-HA966 and morphine in the morphine-pretreated rats reflects a sim-

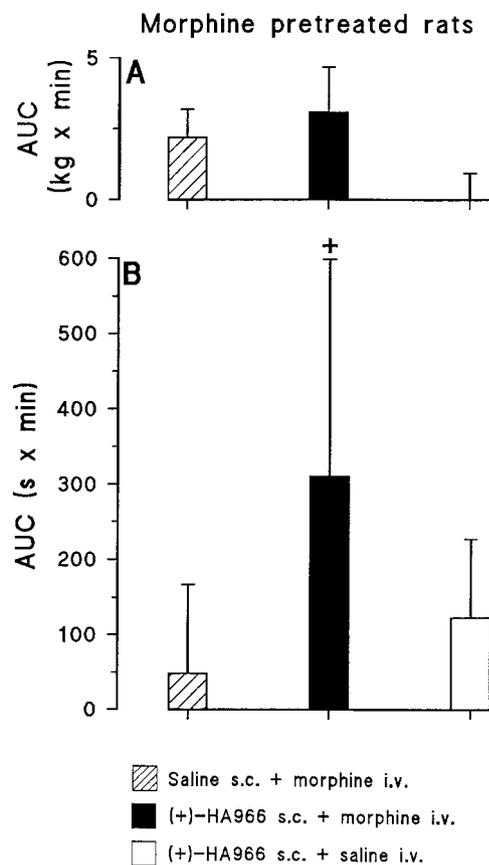


Fig. 3. Overall effect (area under the curve) of morphine (1 mg/kg intravenously; hatched bars), (+)-HA966 (2.5 mg/kg subcutaneously; open bars), or (+)-HA966 and morphine (solid bars) on the vocalization threshold to paw pressure (A) or the struggle latency to paw immersion into a hot (46°C) water bath (B) of neuropathic, morphine-tolerant rats. Tolerance was produced by two daily subcutaneous injections of morphine (10 mg/kg) on postoperative days 12–15. Testing performed at 17 h after the last pretreatment injection on postoperative day 16. (+)-HA966 was injected 20 min before morphine. Each bar represents the mean \pm SD of seven to nine rats. $^+P < 0.05$ versus saline and morphine, Tukey test.

ple synergic antinociceptive effect of the two drugs, this seems unlikely. We have previously shown that a single injection of low doses of morphine and (+)-HA966 results in profound antinociceptive effects that are more pronounced in mechanical than in thermal tests.¹⁶ Thus, it would be difficult to explain why the current study demonstrates an effect of combined administration of (+)-HA966 and morphine in morphine-pretreated rats in the thermal but not the mechanical test if it was the result of a simple synergy between the two drug systems. The result contrasts with studies in the uninjured healthy rat that demonstrated that a single injection of different noncompetitive NMDA receptor antagonist

was devoid of effects on established morphine tolerance.^{5,6,30} However, more recent studies have shown a pattern of gradual reversal of tolerance by treatment with the NMDA receptor antagonist over a period of 2–4 days.^{4,7,31,32} Our study differs from previous studies in several aspects. First, we used a noxious thermal test to assess analgesia that differs from the often-used tail-flick or hot-plate test. In comparison to these tests, immersing the hind paw into a hot-water bath may stimulate a larger skin area and activate a greater number of receptors. Second, we used a glycine/NMDA receptor antagonist, whereas previous studies have used noncompetitive or competitive antagonists. The pharmacologic differences among NMDA receptors in different regions of the central nervous system may explain divergent results between studies using different antagonists. It was recently demonstrated that, although (+)-HA966 exhibits a preference of binding to the spinal cord, competitive antagonists conversely has a higher relative potency in the cerebral cortex.³³ Furthermore, the study found that the generation of a chronic constriction injury had no effect on the density of NMDA receptors in the spinal cord, nor on the relative potencies of the different antagonist. The relative spinal cord selectivity of (+)-HA966 compared with other antagonists is interesting because the spinal cord is probably a major site of action of NMDA receptor antagonists in attenuating morphine tolerance.³⁴ Finally, our study is performed in neuropathic rats, in which the spinal NMDA receptors are supposed to be already in an activated state before any drug pretreatment.⁹ Increased spontaneous activity of the injured nerves is believed to result in sustained activation of the NMDA receptor and to be partly responsible for the phenomenon of central sensitization, resulting in allodynia and hyperalgesia.³⁵ Woolf and Thompson³⁵ showed that both competitive and noncompetitive NMDA antagonists cannot only prevent but also reverse the central sensitization state once it is established, an observation that parallels our results, demonstrating that (+)-HA966 prevents and reverses tolerance in the thermal test. Our results join those of previous studies, suggesting that the reorganization of the nervous system during continued opioid exposure leading to a reduction in opioid responsiveness is mediated mainly by the activation of the NMDA receptor. Because the NMDA receptor seems to be implicated in the events leading to both neuropathic pain and morphine tolerance, it has been suggested that the development of these phenomena shares common intracellular events, of which an important step is an increased intracellular calcium concentration.³⁶ Thus, one might ex-

pect a relatively high level of intracellular calcium because of prolonged NMDA receptor activation in the animal exposed to both morphine tolerance and nerve injury. It may therefore seem unexpected that the glycine/NMDA receptor antagonist is able to reverse the adaptive processes, leading to morphine tolerance in the nerve-injured rat and not in the uninjured healthy rats.^{5,6}

(+)-HA966 reversed established morphine tolerance in the thermal but not in the mechanical test. This may suggest that the expression of morphine tolerance in the neuropathic pain state is related to the modality and/or the intensity of the nociceptive stimulus. Although the analgesymeter used in the mechanical test generates a punctiform stimulus, the thermal stimulus is applied to a much larger surface. Phenomena such as spatial summation and spatial convergence are major features of the central encoding of noxious heat messages.³⁷ The test-depending ability to reverse morphine tolerance may also rely on a difference in afferent fibers that are stimulated in the mechanical and the thermal test of the nerve-injured animal.³⁸⁻⁴⁰ Finally, primary afferent fibers contain several neuropeptides and excitatory amino acids, and the spinal release of putative neurotransmitters, such as substance P, seems to vary in animals subjected to different nociceptive stimuli.⁴¹ Further studies are needed to confirm if the ability of NMDA receptor antagonists to reverse established morphine tolerance vary with the applied stimuli.

Whatever the precise mechanisms underlying the effect of (+)-HA966 on morphine tolerance, the design of the present study mimics the clinical situation in which morphine administration is started only after the manifestation of a painful disorder. By adding an NMDA receptor antagonist, more efficient antinociception and inhibition of morphine tolerance is obtained in animal studies. However, the clinical utility of these antagonists is diminished by their adverse side effects. Antagonists acting at the glycine site of the NMDA receptor complex represent a subclass that may have the potential of exerting beneficial therapeutic effects without side effects, properties that merit further exploration also in clinical trials.

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