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# GV196771A, an NMDA receptor/glycine site antagonist, attenuates mechanical allodynia in neuropathic rats and reduces tolerance induced by morphine in mice

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**Abstract**

The effects of the *N*-methyl-D-aspartate (NMDA) receptor/glycine site antagonist, GV196771A (*E*-4,6-dichloro-3-(2-oxo-1-phenyl-pyrrolidin-3-ylidenemethyl)-1*H*-indole-2-carboxylic acid sodium salt), on mechanical allodynia and on tolerance to the antinociceptive effects induced by morphine were evaluated. Its antiallodynic properties were studied in a model of chronic constriction injury applied to rat sciatic nerve. GV196771A (0.3–10 mg/kg, p.o.) dose-dependently inhibited established mechanical allodynia when tested 14 or 21 days after nerve ligation. In the formalin test in mice, GV196771A (10 or 20 mg/kg, p.o.), administered for 8 days together with morphine 10 mg/kg, i.p. inhibited morphine tolerance development in both early and late phases of the test. This finding reinforces the key role of the NMDA receptors in the plastic event, such as allodynia, which develops in some conditions of painful neuropathy. Moreover, the capability to strongly reduce morphine-induced tolerance suggests that GV196771A could be an alternative agent for the treatment of difficult pain states not only when given alone, but also in combination, in order to prolong the analgesic effects of the opiates. 

*Keywords:* NMDA receptor/glycine site antagonist; Chronic constrictive injury; Mechanical allodynia; Opioid; Tolerance; GV196771A

## 1. Introduction

Peripheral nerve injury or inflammation induces protracted activation of small afferent fibres that, in turn, can facilitate spinal sensory processing. The behavioural signs of this facilitation include a decrease in noxious response threshold to painful stimuli (hyperalgesia) and/or a noxious response evoked by normally innocuous stimuli (allodynia).

The excitatory amino-acid neurotransmitters have been shown to play a prominent role in nociceptive transmission. The excitatory effect of glutamate, mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, is involved in the development and maintenance of pain hypersensitivity (Haley et al., 1990; Davar et al., 1991; Dougherty and Willis, 1991; Ren et al., 1992; Sluka and Westlund, 1992; Malmberg and Yaksh, 1995).

The NMDA receptor complex belongs to the family of ligand-gated ion channels, but with unique features. This receptor is highly permeable to $Ca^{2+}$, is under voltage-dependent regulation of extracellular $Mg^{2+}$, and requires the simultaneous presence of two agonists to be activated: glutamate itself and glycine (Johnson and Ascher, 1987; Hollmann and Heinemann, 1994; Corsi et al., 1996). The use of agents that act at the glycine modulatory site appears to be clinically attractive because they have fewer adverse central nervous system (CNS) side effects than antagonists of the glutamate binding site or the ion channel itself (Danysz and Parsons, 1998).

Several preclinical studies showed that glycine receptor antagonists are effective to reduce nociceptive responses to formalin injection in spinal cord neurons (Dickenson and Aydar, 1991; Millan and Seguin, 1994) and to reverse the thermal hyperalgesia evoked by inflammation or peripheral neuropathy in behavioural experiments (Mao et al., 1992a; Laird et al., 1996).

To date, no clear information about an antiallodynic effect of this class of compounds is available.

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We have recently reported that a novel, potent and selective antagonist of the glycine site, GV196771A (*E*-4,6-dichloro-3-(2-oxo-1-phenyl-pyrrolidin-3-ylidenemethyl)-1*H*-indole-2-carboxylic acid sodium salt, Fig. 1; Giacobbe et al., 1998), blocks the development of thermal hyperalgesia in neuropathic rats (Quartaroli et al., 1999; Bordi and Quartaroli, 2000; Di Fabio et al., 2000). In the present work, using the same animal model, we evaluated the effect induced by GV196771A on mechanical allodynia.

Tolerance to the antinociceptive effects of μ-opioid receptor agonist, morphine, the most widely used opioid analgesic, greatly limits its therapeutic efficacy and complicates the management of chronic pain in patients. The development of tolerance to opioid antinociception is manifested as a shift to the right of the dose–response curve or as a decrease in the intensity of the response when a constant dose is administered repeatedly (Foley, 1991).

Recent data suggest that activation of the NMDA receptor may be involved in opioid tolerance. The evidence that the NMDA channel blocker dizolcipine, MK-801, prevents the development of morphine tolerance in animal models (Mao et al., 1994) supports this hypothesis. Moreover, it has been reported that NMDA receptor/glycine site antagonists, such as 5-nitro-6,7-dimethyl-1,4-dihydro-2, 3-quinoxalinedione, ACEA-1328, can block tolerance in both the formalin (Lufty et al., 1996) and tail flick tests in mice (Lufty et al., 1995), and that (+)-(1-Hydroxy-3-aminopyrrolodine-2-one), (+)-HA966, prevents morphine tolerance in neuropathic rats (Christensen et al., 2000). This evidence suggests that antagonism of the glycine coagonist site of the NMDA receptor may provide another approach to prevent opioid tolerance.

Therefore, studies of the interaction between GV-196771A and morphine are of fundamental importance to fully appreciate the clinical potential of NMDA receptor/glycine site antagonists as novel antihyperalgesic agents, not only as alternative drugs to produce analgesia without tolerance development, but also as adjunct therapeutic agent to prevent tolerance induced by continued opiate treatment in chronic pain disorders.

In the present study, the potential for GV196771A to prevent morphine-induced tolerance of its antinociceptive effect was also investigated in the formalin test in mice.

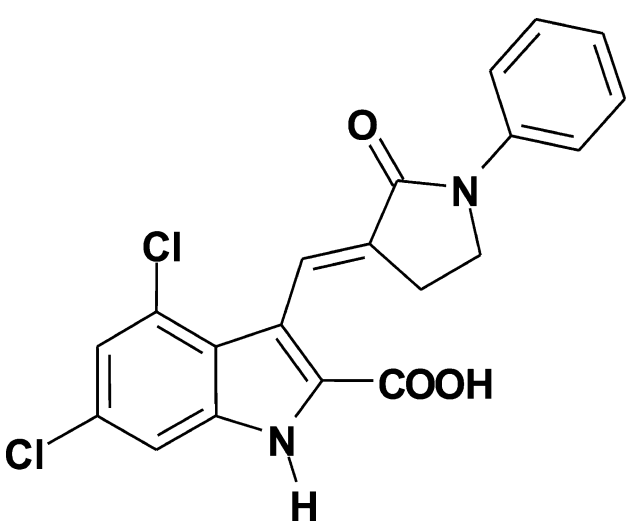

Fig. 1. Chemical structure of GV196771A.

## 2. Materials and methods

The research complied with national legislation and with company policy on the Care of Use of Animals.

### *2.1. Effect of GV196771A on mechano-allodynia in a rat model of painful mononeuropathy: chronic constriction injury model*

Male Sprague–Dawley rats (Charles River) weighing 200–300 g were used. The animals were housed in groups of 2–3 in plastic cages with sawdust bedding and fed a chow pellet diet with free access to water and a 12:12 h light/dark cycle.

Two groups of rats (10–20 per group) were used. One group was a control (naive group), whereas the other had an experimental neuropathy of the sciatic nerve (chronic constriction injury group), produced as previously described by Bennett and Xie (1988). The rats were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.). The left common sciatic nerve was exposed and, proximal to the sciatic trifurcation, about 10 mm of nerve was freed of adhering tissue and 4 ligatures (3.0 chromic gut) were tied loosely around it at 1-mm intervals. Animals with this nerve injury develop hyperalgesia, allodynia and behaviours suggestive of spontaneous pain sensation.

#### *2.1.1. Mechano-allodynia: withdrawal response from mechanical stimulus*

To quantify the sensitivity of the paw to mechanical stimuli, the rats were placed in individual plastic boxes on a mesh floor and allowed to acclimatise for 15 min. A range of calibrated von Frey filaments (exerting pressure of 0.07, 0.2, 0.4, 0.7, 1.2, 1.5, 2, 4, 6, 9, 12 and 15 g; Stoelting, Wood Dale, IL) was used to find the threshold stimulation.

Each filament, tested in order of increasing bending force, was applied perpendicularly to the plantar surface of the left paw 10 times for 3 s; in the absence of a response the next filament was applied. The filament that elicited four to six hind paw withdrawals was designated the threshold filament. This selection is reasonable, as threshold is often defined as a 50% response rate. A reduction of the response threshold in rats with sciatic nerve ligation compared to that of unoperated naive rats confirmed the presence of mechano-allodynia. The withdrawal response from mechanical stimulation was measured on days 0, 4, 11, 14, 18, 22, 27, 33, 36 and 40 after nerve ligation.

GV196771A (0.3, 1 or 10 mg/kg) or vehicle was administered orally to chronic constriction injury animals on day 14 or 21 and allodynia was tested 1 h after treatment. An operator, blind to the drug treatment, carried out the evaluation.

### 2.2. Effect of tolerance induced by morphine after chronic administration of GV196771A in the formalin test in mice

Male albino CD mice (Charles River) weighing 25–30 g were used. The animals were housed in groups of 5–6 and fed a chow pellet diet with free access to water.

Before the formalin injection, mice were placed individually into clear Perspex cages, which served as observation chambers. After 15 min of adaptation to the cage, 20 μl of 1% formalin was injected into the plantar surface of the left hind paw. The amount of time, in seconds, that the animals spent licking the injected paw within the first 5 min (early phase) and then within the period from 20 to 60 min (late phase) after formalin was used as measurement of pain intensity.

The mice, divided randomly into eight groups (10–21 mice per group), received treatment once daily for 8 days as follows: groups g1 and g2 received saline 10 ml/kg, i.p.; groups g3 and g8 received morphine 10 mg/kg, i.p.; groups g4, g6 and g7 received GV196771A 10 mg/kg, p.o. and morphine 10 mg/kg, i.p.; and group g5 received GV196771A 20 mg/kg, p.o. and morphine 10 mg/kg, i.p.

On day 9, the mice received saline i.p. (g1, g7 and g8), morphine 3 mg/kg, i.p. (g2, g3, g4 and g5), and GV196771A 3 mg/kg, p.o. (g6); the treatment protocol is also described in Table 1.

GV196771A (3 mg/kg, p.o.) was administered 1 h before formalin injection, whereas morphine (3 mg/kg, i.p.) was administered 30 min before formalin injection. An operator, blind to the drug treatment, carried out the evaluation.

Table 1
Chronic treatment with saline, morphine (M) or GV196771A (GV): study design

| GROUP | Treatment (1× daily) | Formalin test treatment |
|---|---|---|
| | Days 0–8 | Day 9 |
| g1 | saline i.p. | saline i.p. |
| g2 | saline i.p. | M 3 mg/kg, i.p. |
| g3 | M 10 mg/kg, i.p. | M 3 mg/kg, i.p. |
| g4 | GV 10 mg/kg, p.o. + M 10 mg/kg, i.p. | M 3 mg/kg, i.p. |
| g5 | GV 20 mg/kg, p.o. + M 10mg/kg, i.p. | M 3 mg/kg, i.p. |
| g6 | GV 10 mg/kg, p.o. + M 10 mg/kg, i.p. | GV 3 mg/kg, p.o. |
| g7 | GV 10 mg/kg, p.o. + M 10 mg/kg, i.p. | saline i.p. |
| g8 | M 10 mg/kg, i.p. | saline i.p. |

### 2.3. Drugs and solution

#### 2.3.1. Mechano-allodynia

GV196771A was prepared as a stock solution of 1 mg/ml in 0.5% methylcellulose (methocel); further dilutions were prepared in 0.5% methocel. GV196771A was administered orally (p.o.) in a volume of 10 ml/kg.

#### 2.3.2. Formalin test

GV196771A was prepared as a stock solution of 2 mg/ml in 0.5% methocel. Further dilutions were prepared in 0.5% methocel. GV196771A (10 or 20 mg/kg) was administered orally in a volume of 10 ml/kg. Morphine was prepared as a stock solution of 10 mg/ml in saline. Further dilutions were prepared in saline. Morphine 3 and 10 mg/kg or vehicle (saline) was administered intraperitoneally (i.p.) in a volume of 10 ml/kg.

### 2.4. Statistical analysis

#### 2.4.1. Mechano-allodynia

Nonparametric analysis (Dunnett's test) was used to compare the mechanical threshold (median values) calculated at each time point following ligation with basal values prior to surgery.

The Wilcoxon-signed ranks matched pairs test was used to compare, within each group, the threshold changes detected before and after GV196771A or vehicle treatment. Significance was accepted at a $P < 0.05$ level.

#### 2.4.2. Formalin test

The data are expressed as means ± S.E.M. Statistical analysis was performed to the compare vehicle-treated group (g1) with the GV196771A- and morphine-treated groups (g2–g8) using a one-way analysis of variance (ANOVA) followed by Dunnett's test where $P < 0.05$ was considered significant.

## 3. Results

### 3.1. GV196771A reduced mechano-allodynia in chronic constriction injury model

Prior to surgery, no significant difference in withdrawal response induced by mechanical stimulation was detected between naive and chronic constriction injury groups. Stimulation with von Frey filaments, exerting a pressure of 9–15 g, evoked a brisk, very brief withdrawal-like response in all rats; the median value of the bending force was 12 g for both groups (Fig. 2). After placement of loose ligatures around the sciatic nerve, the animals developed mechanical allodynia, since there was a significant ($P < 0.05$) change in the response threshold. Stimulation with

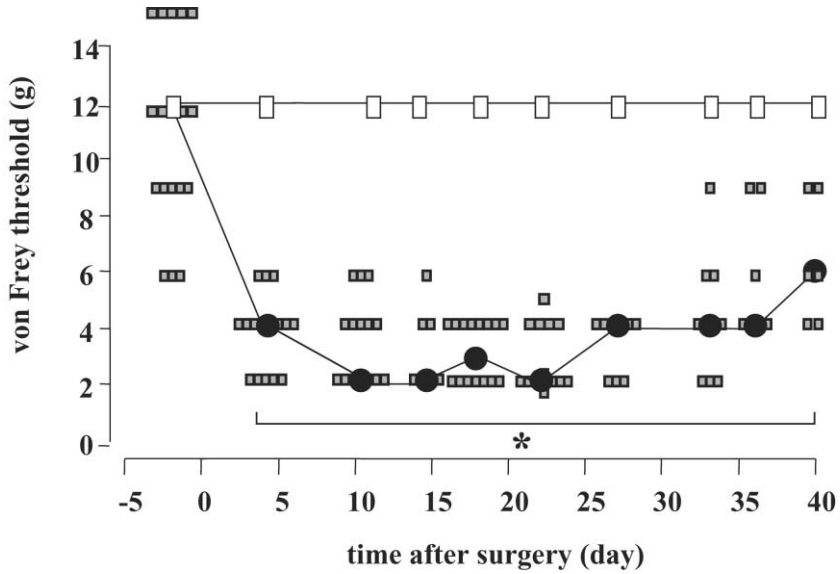


Fig. 2. Time course of mechanical allodynia expressed as median values, in nonoperated animals (□) and in rats with sciatic nerve ligation (●). The mechanical thresholds of all ligated animals are plotted as scattered grey squares. In unoperated (naive) animals no significant variation in bending force threshold response was observed during the entire experiment. In ligated animals, a significant reduction in the response threshold (from 12 g to 4–2 g) was detected from days 4 to 40. $n = 10–20$ each group; * $P < 0.05$ vs. preoperative value.

von Frey filaments, exerting a pressure of 0.07–4 g, a force to which the rats rarely if ever responded before nerve injury, now evoked clear pain-related withdrawal reflexes. The mechano-allodynia was significant by the fourth day and lasted for at least 40 days ($P < 0.05$); the median value of the bending force ranged from 2 to 4 g. In contrast, no changes in bending force values were observed throughout the experiment in the naive group (Fig. 2).

GV196771A administered orally to animals on day 14 or 21 after ligation, produced a dose-related reversal of mechanical allodynia.

A significant dose-related decrease ($P < 0.05$) of mechanical allodynia was observed at 1 and 10 mg/kg (Fig. 3). The median values for threshold bending force measured before and after treatment were 4 and 6 g, respectively, at 1 mg/kg, and 4 and 9 g, respectively at 10 mg/kg. No changes in threshold response were observed at 0.3 mg/kg.

### *3.2. Chronic treatment with GV196771A-inhibited tolerance induced by morphine in the mouse paw formalin test*

In control mice (g1; saline–saline), subcutaneous injection of formalin at day 9 induced marked spontaneous nociceptive behaviour. The licking times measured during the early and the late phases were 145.5 ± 7.1 and 416.7 ± 28.8 s, respectively (Fig. 4). Animals receiving 3 mg/kg,

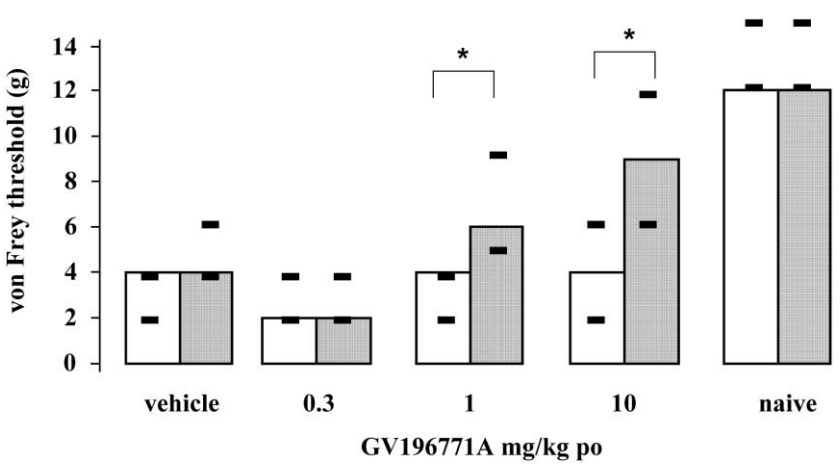


Fig. 3. The graph illustrates the mechanical allodynia in chronic constriction injury animals before (□) and 1 h after treatment (▨) with vehicle and GV196771A 0.3, 1 or 10 mg/kg, p.o.; the threshold response of the nonoperated (naive) animals is also shown. $n = 7–13$ each group; the results are expressed as medians and the 25 and 75 percentile values (▬) are reported; * $P < 0.05$ vs. pretreatment value.

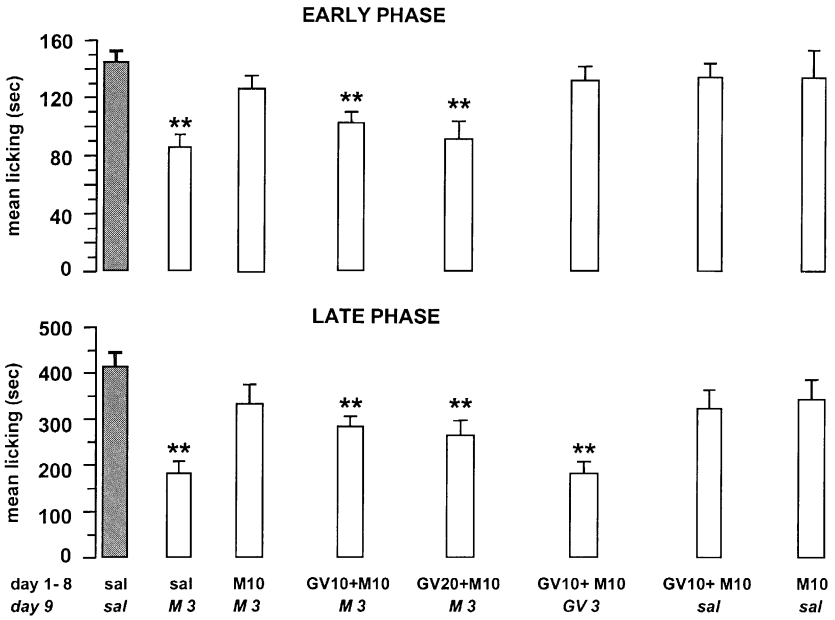


Fig. 4. In the control group (g1; sal–sal), subcutaneous injection of formalin at day 9 induced marked spontaneous nociceptive behaviour in both early and late phases. Animals receiving morphine 3 mg/kg, i.p. at day 9, after chronic treatment with saline (g2; sal–M3), showed significant attenuation of basal nociceptive responses in both phases. Administration of morphine 3 mg/kg, i.p. at day 9, after chronic morphine treatment (10 mg/kg, i.p.), was ineffective in both phases (g3; M10–M3). GV196771A (10 and 20 mg/kg, p.o.), administered for eight days together with morphine 10 mg/kg, i.p. inhibited morphine tolerance in both early and late phases (g4; GV10 + M10–M3 and g5; GV20 + M10–M3). Chronic coadministration of GV196771A 10 mg/kg, p.o. and morphine 10 mg/kg, i.p. did not alter the antihyperalgesic effect on late phase of GV196771A 3 mg/kg, p.o. administered at day 9 (g6; GV10 + M10–GV3). Chronic treatment with GV196771A 10 mg/kg, p.o. in combination with morphine 10 mg/kg, i.p. did not induce a significant effect on basal nociception measured 24 h after the last treatment (g7; GV10 + M10–sal). Chronic treatment with morphine 10 mg/kg, i.p. did not induce significant changes in basal nociception measured 24 h after the last treatment (g8; M10–sal). Saline (sal); morphine (M); GV196771A (GV). $n = 10–21$ for each group. ** Denotes $P < 0.01$ vs. g1 (sal–sal).

i.p. of morphine at day 9 (g2; saline–M3) showed significant attenuation of basal nociceptive responses in both phases ($P < 0.01$ vs. g1; Fig. 4). The licking times were $86.2 \pm 8.5$ and $184.5 \pm 23.3$ s in the early and the late phases, respectively.

Animals chronically treated with morphine 10 mg/kg, i.p. (g3; M10–M3) showed that both early and the late phases were unaffected by 3 mg/kg, i.p. morphine injected at day 9. The licking times during the early and the late phases ($127.8 \pm 8.8$ and $334.6 \pm 40.5$ s, respectively) were not significantly different from the values detected in the control group (g1).

When morphine 3 mg/kg, i.p. was administered at day 9, after chronic coadministration for 8 days of GV196771A 10 mg/kg, p.o. and morphine 10 mg/kg, i.p. (g4; GV10 + M10–M3) or with GV196771A 20 mg/kg, p.o. and morphine 10 mg/kg, i.p. (g5; GV20 + M10–M3), its antihyperalgesic activity was significantly maintained ($P < 0.01$ vs. g1).

In g4, the licking times during the early and the late phases were $103.4 \pm 6.7$ and $286.5 \pm 19.3$ s, respectively, whereas in g5, the values for the early and the late phases were $92.4 \pm 11.35$ and $267.5 \pm 30.5$ s, respectively.

GV196771A 3 mg/kg, administered p.o. at day 9, after chronic coadministration of GV196771A 10 mg/kg, p.o. and morphine 10 mg/kg, i.p. (g6; GV10 + M10–GV3) had no effect on the early phase ($130.7 \pm 9.0$ s). However, a significant ($P < 0.01$) reduction of the hyperalgesic response in the late phase was observed ($188.7 \pm 28.5$ s).

In the group receiving saline i.p. at day 9, after chronic coadministration of GV196771A 10 mg/kg, p.o. and morphine 10 mg/kg, i.p. (g7; GV10 + M10–saline) subcutaneous injection of formalin induced marked spontaneous nociceptive behaviour. The licking times during the early and the late phases were $133.1 \pm 8.2$ and $328.9 \pm 28.5$ s, respectively (Fig. 4).

When saline was administered i.p. at day 9, after chronic morphine, 10 mg/kg, i.p. (g8; M10–saline) subcutaneous injection of formalin induced marked spontaneous nociceptive behaviour. The licking times during the early and the late phases were $134.2 \pm 13.8$ and $353.2 \pm 53.8$ s, respectively (Fig. 4).

## 4. Discussion

The present study assessed the ability of GV196771A to attenuate mechanical allodynia in neuropathic rats and to prevent the development of morphine tolerance in the formalin test in mice.

The results indicate that GV196771A suppressed neuropathic mechano-allodynia; furthermore, it was able to reduce tolerance induced by morphine.

It is well established that glutamate receptors are involved in nociceptive processes, and that both NMDA and non-NMDA receptors are involved in the development and maintenance of pain hypersensitivity (Haley et al., 1990; Davar et al., 1991; Dougherty and Willis, 1991; Ren et al., 1992; Sluka and Westlund, 1992; Malmberg and Yaksh, 1995). Antagonists of the NMDA glycine site receptor could represent a new analgesic class devoid of a marked influence on motor coordination (Millan and Seguin, 1994).

Peripheral nerve injury often leads to chronic pain states, whose characteristic features include hyperalgesia and allodynia. These sensory abnormalities, which are commonly observed in patients with a clinical neuropathy (Thomas, 1984), are also characteristic of the rat model of peripheral neuropathy (Bennett and Xie, 1988).

We recently reported that the novel selective antagonist of the glycine site, GV196771A, blocks the development of thermal hyperalgesia in rats with an experimental neuropathy (Quartaroli et al., 1999; Bordi and Quartaroli, 2000; Di Fabio et al., 2000). In the present work, we demonstrated that in the same animal model, the compound also inhibits the manifestation of mechanical allodynia. The decreased withdrawal response to mechanical stimulation was dose-dependent and represented a 70% recovery to physiological levels of response at 10 mg/kg. Moreover, as in a previous study (Quartaroli et al., 1999), this effect was obtained at doses that did not induce motor dysfunction or any other behavioural side effects as measured with an Irwin test (Irwin, 1968). In this battery of tests, the rats were observed for changes in behaviour, skeletal muscle tone, reflex response and overt gastrointestinal, as well as neurological and autonomic effects. GV196771A appears to be well tolerated up to 600 mg/kg, p.o. (data not shown). These results suggest a clean profile for the compound in comparison to that of typical NMDA receptor antagonists, which induce side effects, such as ataxia or myorelaxation. In addition, we excluded that its analgesic activity, as assessed from a reflex response induced by the application of exogenous stimuli, could be interpreted as false positive effects, as the evidence described in the present work and in the previous ones (Quartaroli et al., 1999; Bordi and Quartaroli, 2000) demonstrated that GV196771A blocked the expression of both mechanical allodynia and thermal hyperlagesia in the neuropathic paw without modifying the physiological response in the nonoperated paw.

This interpretation is also supported by findings from the formalin test in mice, where the acute response induced by this chemical algogenic agent in the early phase was not modified by pretreatment with GV196771A and was not different from the vehicle effect.

Interestingly, by comparing the antiallodynic efficacy of GV196771A with its antihyperalgesic effect reported previously (Quartaroli et al., 1999), we can conclude that in neuropathic animals, GV196771A inhibits both mechanical allodynia and thermal hyperalgesia with similar potencies in a range between 1 and 10 mg/kg. In contrast, a previous study published by Tal and Bennet (1994) showed that the NMDA receptor antagonist, dextrorphan, blocked heat hyperalgesia, but not mechano-allodynia in rats with experimental painful peripheral neuropathy.

The authors suggested that different kinds of abnormal pain sensation might be caused by different pathophysiological mechanisms that are differentially affected by drug therapy.

However, there are recent reports that mechanical and thermal allodynia, produced by partial denervation of the rat tail, are sensitive to NMDA receptor antagonism by MK-801 (Kim et al., 1997).

Furthermore, other studies have demonstrated that the NMDA receptor antagonist, memantine, relieved thermal or mechanical hyperalgesia as well as mechanical allodynia in neuropathic rats (Carlton and Hargett, 1995; Eisenberg et al., 1995). All these studies support the hypothesis that similar mechanisms affect different modalities of neuropathic pain.

In addition, a clinical study with ketamine, another noncompetitive NMDA receptor antagonist, demonstrated that both mechanical allodynia and ongoing pain in human patients were reduced by the treatment (Felsby et al., 1995) and, therefore, spontaneous pain and allodynia are likely to be related and mediated by the NMDA receptor.

It has been shown that GV196771A was able to inhibit selectively the late phase of the inflammatory pain induced by formalin without any activity on the early phase, supporting the concept that NMDA receptors are involved mainly in chronic nociceptive transmission (Quartaroli et al., 1999).

In the same test, the μ-opioid receptor agonist, morphine, inhibited both the early and the late phases with similar potency, suggesting the participation of opioid receptors in acute as well as in chronic nociceptive transmission. It has also been demonstrated that GV196771A, in contrast to morphine, does not induce tolerance to its antihyperalgesic activity after chronic treatment for 8 days (Quartaroli et al., 1999).

In the present study, the ability of GV196771A to prevent morphine-induced tolerance to the latter's antinociceptive effect was investigated in the formalin test in mice.

Eight days' chronic treatment with morphine strongly reduced the ability of the compound, administered at day 9, to block the early and late phases of pain induced by formalin. The reduced effectiveness of morphine reflects the development of tolerance and confirms our previous results (Quartaroli et al., 1999).

GV196771A administered for 8 days, together with morphine, inhibited morphine tolerance in both early and the late phases. Furthermore, tolerance to morphine was blocked in a dose-dependent manner.

Chronic coadministration of GV196771A and morphine did not alter the antihyperalgesic effect of GV196771A administered at day 9. In fact, the reduction of the control pain response observed in the late phase was not different from the reduction observed in previous experiments, when GV196771A 3 mg/kg was administered at day 9 after 8 days' chronic treatment with vehicle (Quartaroli et al., 1999).

The inhibition of morphine tolerance in the early phase by GV196771A is an interesting phenomenon because the glycine antagonist does not itself modify the acute phase of pain following formalin injection. However, GV196771A seems to be able to modulate the intracellular events induced by μ-opioid receptor occupation by morphine, presumably through NMDA receptor antagonism.

One possible explanation could be that, as reported recently, NMDA receptor antagonists potentiate morphine antinociception (Lufty et al., 1999; Nishiyama, 2000).

We also examined the possibility that GV196771A might have blocked morphine tolerance because of a long-lasting antinociceptive effect, as GV196771A produces antinociception in the formalin test and shows long-lasting activity in mononeuropathy models. Our results demonstrate that chronic treatment with GV196771A in combination with morphine did not induce a significant effect on basal nociception measured 24 h after the last treatment. The nociceptive responses for both early and the late phases observed in group g7 (GV10 + M10–saline) were not significantly different from the nociceptive responses measured in group g1 (saline–saline).

The search for alternative agents that produce analgesia without development of tolerance, or as adjunct therapeutic means to block tolerance development, is currently an active area of research.

The involvement of the NMDA receptor in opioid tolerance was suggested by the evidence that synaptic and intracellular events similar to those which follow tissue injury occur during the development of tolerance to morphine analgesia (Marek et al., 1991; Trujillo and Akil, 1991; Mao et al., 1994; Mayer et al., 1995). Indeed, a high density of receptor binding sites of both opiate and excitatory amino-acid receptors has been found in the superficial lamina (I and II) of the mammalian spinal cord dorsal horn (Seybold, 1986; Mitchell and Anderson, 1991). The same spinal cord locus has been shown to be involved in the mechanism of both hyperalgesia and morphine tolerance.

It has recently been suggested that NMDA receptors contribute to the development of opioid tolerance through protein kinase C activation (Smart and Lambert, 1996).

The increase in intracellular protein kinase C activity that occurs in response to NMDA receptor activation has been implicated in spinal cord mechanisms of neurogenic and inflammatory hyperalgesia. An increase in membrane-bound protein kinase C in the lumbar spinal cord was observed in neuropathic pain models, concurrent with thermal hyperalgesia and spontaneous pain-related behaviour induced by sciatic nerve injury (Mao et al., 1992b, 1993).

Immunocytochemical studies, in which the development of tolerance was associated with increases in immunoreactivity of a specific protein kinase C isoform γ in lamina I–II dorsal horn neurons, showed that intrathecal administration of the noncompetitive NMDA receptor antagonist, MK-801, was able to prevent both increases protein kinase C isoform γ immunoreactivity and behavioural manifestation of morphine tolerance (Mao et al., 1995a).

Together, these results suggest that NMDA receptor-mediated intracellular protein kinase C translocation may be associated with the development of both neurogenic-inflammatory hyperalgesia and morphine tolerance.

Given the interaction between opioid and NMDA receptors and the involvement of NMDA receptors through protein kinase C in morphine tolerance, Mao et al. (1995b) have proposed that the development of tolerance to the analgesic effects of morphine is a consequence of a series of cellular and intracellular events initiated by opiate administration, in the superficial laminae of the spinal cord dorsal horn.

This hypothesis suggests that opioids induce an abnormal presynaptic excitatory amino-acid release and postsynaptic NMDA receptor activation. The resultant intracellular biochemical consequences operate in concert, leading to the development of morphine tolerance.

This possibility is supported by the evidence that the NMDA channel blocker, MK-801, and the NMDA receptor/glycine site antagonist, ACEA1328, prevent the development of morphine tolerance in animal models (Mao et al., 1994; Lufty et al., 1995; Lufty et al., 1996). Recently, the glycine antagonist, (+)-HA966, has been shown to prevent morphine tolerance in neuropathic rats (Christensen et al., 2000). All these data suggest that antagonism of the glycine coagonist site of the NMDA receptor is another approach to block the development of opioid tolerance.

These findings suggest that the inhibition of opiate tolerance induced by NMDA receptor antagonists is probably due to specific inhibition of the neuronal plasticity implicated in this phenomenon rather than a nonspecific pharmacological effect.

In conclusion, in a rat model of traumatic nerve injury that mimics some conditions of painful neuropathy in humans, the NMDA receptor/glycine antagonist, GV196771A, produced a therapeutic effect on mechanical allodynia. Furthermore, it was able to strongly reduce morphine-induced tolerance in the mouse formalin test. Both the antiallodynic effect and the reduction of morphine-induced tolerance were observed at doses that did not induce motor dysfunction or any other detectable side effects. All these findings support the clean profile of the compound with respect to typical NMDA receptor antagonist side effects, such as ataxia and/or myorelaxation.

Finally, GV196771A could be an alternative agent for the treatment of difficult pain states (*prolonged pain state*) not only when given alone, but also in combination.

Indeed, the combination with opioids may be helpful as adjunct therapeutic agent where an inappropriate opiate treatment schedule of pain management may precipitate an unexpected hyperalgesic response to preexisting pain conditions and could be a source of the clinical complexity inherent in the responsiveness to opiate treatment (Portenoy, 1994).

## References


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

Bordi, F., Quartaroli, M., 2000. Modulation of nociceptive transmission by NMDA/glycine site receptor in the ventroposterolateral nucleus of the thalamus. Pain 84, 213–224.

Carlton, S.M., Hargett, G.L., 1995. Treatment with the NMDA antagonist memantine attenuates nociceptive responses to mechanical stimulation in neuropathic rats. Neurosci. Lett. 198, 115–118.

Christensen, D., Guilbaud, G., Kayser, V., 2000. Complete prevention but stimulus-dependent reversion of morphine tolerance by the glycine/NMDA receptor antagonist (+)-HA966 in neuropathic rats. Anesthesiology 92, 786–794.

Corsi, M., Fina, P., Trist, D.G., 1996. Co-agonism in drug-receptor activation: illustrated by the NMDA receptor. Trends Pharmacol. Sci. 17, 220–222.

Danysz, W., Parsons, C.G., 1998. Glycine and NMDA receptors—physiological significance and possible therapeutic applications. Pharmacol. Rev. 50, 597–664.

Davar, G., Hama, A., Deykin, A., Vos, B., Maciewicz, R., 1991. MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. Brain Res. 553, 327–330.

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.

Di Fabio, R., Conti, N., Corsi, M., Donati, D., Gastaldi, P., Gaviraghi, G., Giacobbe, S., Pentassuglia, G., Quartaroli, M., Ratti, E., Trist, D.G., Ugolini, A., 2000. GV196771. NMDA glycine antagonist non opioid analgesic. Drugs Future 25 (2), 137–145.

Dougherty, P.M., Willis, W.D., 1991. Modification of the responses of primate spinothalamic neurons to mechanical stimulation by excitatory amino acids and an *N*-methyl-D-aspartate antagonist. Brain Res. 542, 15–22.

Eisenberg, E., LaCross, S., Strassman, A.M., 1995. The clinically tested *N*-methyl-D-aspartate receptor antagonist memantine blocks and reverses thermal hyperlagesia in a rat model of painful mononeuropathy. Neurosci. Lett. 187, 17–20.

Felsby, S., Nielsen, J., Arendt-Nielsen, L., Jensen, T.S., 1995. NMDA receptor blockade in chronic neuropathic pain: a comparison of ketamine and magnesium chloride. Pain 64, 283–291.

Foley, K.M., 1991. Clinical tolerance to opioids. In: Basbaum, A.I., Besson, J.M. (Eds.), Towards a New Pharmacotherapy of Pain: Report of the Dahlem Workshop: Beyond Morphine. Wiley, New York, pp. 181–204.

Giacobbe, S.A., Baraldi, D., Di Fabio, R., 1998. Unusual synthesis of new glycine antagonists via sequential aldol condensation–lactonization–elimination reaction. Bioorg. Med. Chem. Lett. 8, 1689–1692.

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.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Irwin, S., 1968. Comprehensive observational assessment: 1A. A systematic, quantitative procedure for assessing the behavioural and physiologic state of the mouse. Psychopharmacologia 13, 222–257.

Johnson, J.W., Ascher, P., 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325, 529–531.

Kim, Y.I., Na, H.S., Yoon, Y.W., Han, H.C., Ko, K.H., Hong, S.K., 1997. NMDA receptors are important for both mechanical and thermal allodynia from peripheral nerve injury in rats. NeuroReport 8, 2149–2153.

Laird, J.M., Mason, G.S., Webb, J., Hill, R.G., Hargreaves, R.J., 1996. Effects of a partial agonist and a full antagonist acting at the glycine site of the NMDA receptor on inflammation-induced mechanical hyperalgesia in rats. Br. J. Pharmacol. 117, 1487–1492.

Lufty, K., Shen, K.-Z., Kwon, I.-K., Cai, S.X., Woodward, R.M., Keana, J.F.W., Weber, E., 1995. Blockade of morphine tolerance by ACEA-1328, a novel NMDA receptor antagonist/glycine antagonist. Eur. J. Pharmacol. 273, 187–189.

Lufty, K., Shen, K.-Z., Woodward, R.M., Weber, E., 1996. Inhibition of morphine tolerance by NMDA receptor antagonist in the formalin test. Brain Res. 731, 171–181.

Lufty, K., Doan, P., Weber, E., 1999. ACEA-1328, a NMDA receptor/glycine site antagonist, acutely potentiates antinociception and chronically attenuates tolerance induced by morphine. Pharmacol. Res. 40, 435–442.

Malmberg, A.B., Yaksh, T.L., 1995. Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: a microdialysis study in unanesthetized rats. J. Neurosci. 15, 2768–2776.

Mao, J., Price, D.D., Mayer, D.J., Lu, J., Hayes, R.L., 1992a. Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviors in rats with experimental peripheral mononeuropathy. Brain Res. 576, 254–262.

Mao, J., Price, D.D., Mayer, D.J., Hayes, R.L., 1992b. Pain-related increases in spinal cord membrane-bound protein kinase C following peripheral nerve injury. Brain Res. 588, 144–149.

Mao, J., Mayer, D.J., Hayes, R.L., Price, D.D., 1993. Spatial patterns of increased spinal cord membrane-bound protein kinase C and their relation too increases in $^{14}$C-2-deoxyglucose metabolic activity in rats with painful peripheral mononeuropathy. J. Neurophysiol. 70, 470–481.

Mao, J., Price, D.D., Mayer, D.J., 1994. Thermal hyperalgesia in association with the development of morphine tolerance in rats: role of excitatory amino acid receptors and protein kinase C. J. Neurosci. 14, 2301–2312.

Mao, J., Price, D.D., Phillips, L.L., Lu, J., Mayer, D.J., 1995a. Increases in protein kinase C gamma immunoreactivity in the spinal cord of rats associated with tolerance to the analgesic effects of morphine. Brain Res. 677, 257–267.

Mao, J., Price, D.D., Mayer, D.J., 1995b. Mechanism of hyperalgesia and morphine tolerance in rats: a current view of their possible interactions. Pain 62, 259–274.

Marek, P., Ben Eliyahu, S., Gold, M., Liebeskin, J.C., 1991. Excitatory amino acid antagonists (kynurenic acid and MK-801) attenuate the development of morphine tolerance in the rat. Brain Res. 547, 77–81.

Mayer, D.J., Mao, J., Price, D.D., 1995. The development of morphine tolerance and dependence is associated with the translocation of protein kinase C. Pain 61, 365–374.

Millan, M.J., Seguin, L., 1994. Chemically-diverse ligands at the glycine B site coupled to *N*-methyl-D-aspartate (NMDA) receptors selectively block the late phase of formalin-induced pain in mice. Neurosci. Lett. 178, 139–143.

Mitchell, J.J., Anderson, K.J., 1991. Quantitative autoradiographic analysis of excitatory amino acid receptor in the cat spinal cords. Neuroscience 254, 115–118.

Nishiyama, T., 2000. Interaction between intrathecal morphine and gluta-

mate receptor antagonists in formalin test. Eur. J. Pharmacol. 395, 203–210.

Portenoy, R.K., 1994. Opioid tolerance and responsiveness: research findings and clinical observation. In: Gebhart, G.F., Hammond, D.L., Jensen, T.S. (Eds.), Proc. 7th World Congress on Pain. IASP Press, Seattle, WA, pp. 595–619.

Quartaroli, M., Carignani, C., Dal Forno, G., Mugnaini, M., Ugolini, A., Arban, R., Bettelini, L., Maraia, F., Belardetti, F., Trist, D., Ratti, E., Di Fabio, R., Corsi, M., 1999. Potent antihyperalgesic activity without tolerance produced by the glycine site antagonist of the NMDA receptor GV196771A. J. Pharmacol. Exp. Ther. 290, 158–169.

Ren, K., Hylden, J.L.K., Williams, G., Ruda, M.A., Dubner, R., 1992. The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioural hyperalgesia in rats. Eur. J. Pharmacol. 219, 235–243.

Seybold, V.S., 1986. Neurotransmitter receptor site in the spinal cord. In: Yaksh, T.L. (Ed.), Spinal Afferent Processing. Plenum, New York, pp. 117–139.

Sluka, K.A., Westlund, K.N., 1992. An experimental arthritis in rats: dorsal horn aspartate and glutamate increases. Neurosci. Lett. 145, 141–144.

Smart, D., Lambert, D.G., 1996. The stimulatory effects of opioids and their possible role in the development of tolerance. Trends Pharmacol. Sci. 17, 264–269.

Tal, M., Bennet, G.J., 1994. Neuropathic pain sensation are differentially sensitive to dextrorphan. NeuroReport 5, 1438–1441.

Thomas, M., 1984. Clinical features and differential diagnosis of peripheral neuropathy. In: Dyck, P.J., Thomas, P.K., Lambert, E.H., Bungle, R. (Eds.), Peripheral Neuropathy. Sanders, Philadelphia, PA, pp. 1169–1190.

Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by NMDA receptor antagonist MK-801. Science 251, 85–87.