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Synergistic Antinociceptive Interactions of Morphine and Clonidine in Rats with Nerve-ligation Injury

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Background: **Ligation injury of the L5/L6 nerve roots in rats produces behavioral signs representative of clinical conditions of neuropathic pain, including tactile allodynia and thermal and mechanical hyperalgesia. In this model, intrathecal morphine shows no antiallodynic activity, as well as decreased antinociceptive potency and efficacy. This study was designed to explore the antinociceptive activity of intrathecal clonidine alone or in combination with intrathecal morphine (1:3 fixed ratio) in nerve-injured rats. The aims, with this study, were to use nerve-injured animals to determine: (1) whether the antinociceptive potency and efficacy of intrathecal clonidine was altered, and (2) whether the combination of intrathecal morphine and clonidine would act synergistically to produce antinociception.**

Methods: **Unilateral nerve injury was produced by ligation of the L5 and L6 spinal roots of male Sprague-Dawley rats. Sham-operated rats underwent a similar surgical procedure but without nerve ligation. Morphine and clonidine were given intrathecally through implanted catheters alone or in a 1:3 fixed ratio. Nociceptive responses were measured by recording tail withdrawal latency from a 55° C water bath, and data were calculated as % maximal possible effect (%MPE).**

Results: **Morphine produced a dose-dependent antinociceptive effect in both sham-operated and nerve-injured rats. The doses calculated to produce a 50 %MPE (*i.e.*, A_{50}) (±95% confidence intervals [CI]) were 15 ± 4.9 μg and 30 ± 18 μg, respectively. Though morphine was able to produce a maximal response (100%) in sham-operated rats, the maximal response achieved in nerve-injured animals was only 69 ± 21.9 %MPE. Clonidine produced a dose-dependent effect, with an A_{50} (±95% CI) of 120 ± 24 μg in sham-operated rats. In nerve-ligated rats, clonidine produced a maximal effect that reached a plateau of 55 ± 10.9 %MPE and 49 ± 10.2 %MPE at 100 and 200 μg, respectively, preventing the calculation of an A_{50}. In sham-operated rats, a morphine–clonidine mixture produced maximal efficacy, with an A_{50} (±95% CI) of 15 ± 9.2 μg (total dose), significantly less than the theoretical additive A_{50} of 44 ± 10 μg. In L5/L6 nerve-ligated rats, the morphine–clonidine combination produced maximal efficacy, with an A_{50} (±95% CI) of 11 ± 5.4 μg (total dose), which was significantly less than the theoretical additive A_{50} of 118 ± 73 μg, indicating a synergistic antinociceptive interaction. The intrathecal injection of [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAMGO) produced A_{50} values of 0.23 μg (range, 0.09–0.6) and 0.97 μg (range, 0.34–2.7) in sham-operated and ligated rats, respectively. Phentolamine (4 mg/kg, intraperitoneally) produced no antinociceptive effect alone and attenuated, rather than enhanced, the effect of morphine in both groups of rats.**

Conclusions: **These data show that: (1) clonidine, like morphine, loses antinociceptive potency and efficacy after nerve ligation injury, and (2) strongly suggest that a spinal combination of morphine and clonidine synergize under conditions of nerve injury to elicit a significant antinociceptive action when either drug alone may be lacking in efficacy. It is unlikely that the synergy of morphine with clonidine is due to an attenuation of spinal sympathetic outflow by clonidine, because the sympatholytic agent phentolamine produced an opposing effect on morphine antinociception. The data suggest that combinations of morphine and clonidine may prove useful in controlling pain in patients with neuropathic conditions. (Key words: morphine-clonidine synergy, neuropathic pain, antinociception, rat, intrathecal.)**

UNILATERAL ligation of the L5 and L6 nerve roots has been shown to reliably produce signs that appear representative of clinical neuropathic pain.[1] Such signs include mechanical allodynia and hyperalgesia to both mechanical and thermal stimuli.[1] The efficacy of morphine in neuropathic pain states is somewhat controversial. Some investigators have suggested that morphine is ineffective against neuropathic pain in both clinical[2–4] and animal studies,[5,6] whereas others have found that opioids may alleviate neuropathic pain, but at "higher than normal doses."[7–9] Recent studies by our laboratory[10] demon-

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strated that morphine administered intracerebroventricularly or intraperitoneally was efficacious against mechanical allodynia in rats with ligation of the L5/L6 nerve roots, whereas intrathecal morphine was not.[10,11] In addition, in recent studies, researchers revealed the surprising finding that ligation of the L5/L6 nerve roots significantly diminishes the efficacy of intrathecal morphine to block an acute pain stimulus,[12] and that such efficacy may be restored by intrathecal pretreatment with MK-801 or by local application of bupivacaine at the injury site.[13]

Several neuroplastic changes in the spinal cord occur after peripheral nerve injury, including upregulation of cholecystokinin and dynorphin and downregulation of substance P and calcitonin gene-related peptide.[14-16] Other changes, such as anomalous sympathetic innervation of the dorsal root ganglia[14,17] and stimulation of mechanoreceptor nerve endings by sprouting sympathetic efferents may contribute to sympathetically maintained pain after peripheral nerve injury.[14,18,19] Sprouting of Aβ fibers may form novel synapses with second-order neurons associated with nociceptive input such that normally innocuous stimuli may be perceived as being nociceptive.[14] In addition, continuous spontaneous discharges from ectopic foci may elicit hypersensitivity of wide dynamic range neurons, which respond to both low (innocuous) and high threshold (nociceptive) stimuli, such that light touch further activates the wide dynamic range neurons, producing allodynia. These events make it likely that the spinal pharmacology after the never injury state is different from the normal condition.

The intrathecal administration of the α_2 adrenergic agonist clonidine was reported recently to be fully effective against mechanical allodynia in the L5/L6 ligation model.[6] This effect has been attributed to a reduction of spinal sympathetic outflow mediated at presynaptic sympathetic ganglionic sites. The intrathecal administration of clonidine was shown to produce antinociception in mice, rats, sheep,[20-22] and humans.[23] It was demonstrated, by nerve transection studies in mice and rats, that α_2-mediated antinociception occurs at the level of the spinal cord.[24,25] For example, transection of the spinal cord of mice permitted the nociceptive tail-flick reflex to occur independently of descending inhibitory controls. It was demonstrated that, in these animals, the dose-effect curve of systemically administered morphine was shifted, whereas that of clonidine remained unchanged.[24] Clonidine is inactive in producing antinociception when given into the periaqueductal gray, lateral reticular nucleus, or locus coeruleus—supraspinal sites known to be activated by opioids.[26-28] In this respect, morphine differs from clonidine, in that a synergistic interaction between morphine administered spinally and supraspinally has been established.[29]

It has been well established, however, that the μ-opioid morphine and the α_2-adrenergic agonist clonidine act in a synergistic fashion in the modulation of acute nociception. Fielding and colleagues[30] described a positive interaction between systematically administered morphine and clonidine in the mouse. The effect of morphine administered systematically[31] or spinally to mice[32] or rats[21,33,34] produced a definitive synergistic antinociceptive interaction with spinally administered α_2 agonists clonidine or medetomidine.

Although intrathecal clonidine has attenuated tactile allodynia,[6] there is little evidence to demonstrate whether the antinociceptive efficacy of intrathecal clonidine is maintained in the nerve injured animal, or whether intrathecal clonidine would act synergistically with morphine in such animals, as it does in rats without nerve injury. For these reasons, the effect of intrathecal clonidine on the nociceptive tail-flick reflex was explored alone or in combination with morphine in rats with L5/L6 nerve root ligation and compared with the effects in sham-operated animals. Although it was demonstrated that tactile allodynia induced by nerve root ligation injury may be maintained sympathetically, a sympathetic component has not been demonstrated with regard to acute nociception. Therefore, the effects of phentolamine were studied alone or together with morphine to investigate this possibility. In light of the significant loss of efficacy of intrathecal morphine against allodynia and acute nociception in nerve-injured animals, it was of interest to determine the generality of the changes resulting from nerve injury with regard to adrenergic, as well as opioid, receptors. In addition, it would be of potential clinical significance if a mixture of clonidine and morphine would act synergistically under these conditions to elicit antinociceptive effects.

Methods

Nerve Ligation Surgery

Male Sprague-Dawley rats, weighing 200-350 g, were used in all experiments. These experiments were approved by the Institutional Animal Care and Use Committee. Rats were anesthetized with 2% halothane in oxygen delivered at 2 l/min. The L5/L6 nerve ligation was performed according to the method described by

Kim and Chung.[1] The skin was incised over the caudal lumbar region, and the muscles were retracted. The L5 and L6 branches were identified and carefully isolated from the surrounding fascia. A "finger tight" ligature was made with 4-0 silk suture around each branch proximal to the confluence into the left common sciatic nerve. Hemostasis was ensured, and the incision was sutured. Sham control rats were treated the same way, except the nerves were not ligated.

Intrathecal Drug Injection

During anesthesia, catheters were implanted intrathecally for administration of drugs into the region of the lumbar cord, according to the method described by Yaksh and Rudy.[35] An 8-cm length of PE-10 polyethylene tubing was inserted through an incision made in the atlanto-occipital membrane to the level of the lumbar enlargement. The catheter was secured to the musculature at the incision, which was then closed. The rats received 4.4 mg/kg gentamycin intramuscularly and were allowed 5 days recovery before experimentation began. Intrathecally administered drugs were given in a volume of 5 μl, followed by a 9-μl flush. Rats received increasing doses of either morphine, clonidine, or a 1:3 fixed ratio of morphine to clonidine by intrathecal injection (n = 5-10 rats per group). Separate groups of nerve-ligated and sham-operated rats received intrathecal doses of [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAMGO) to explore the possible loss of antinociceptive potency of a high-efficacy opioid μ agonist in nerve-injured rats.

Nociceptive Testing

Nociceptive testing was performed by the 55° C hot water tail-flick test. The latency to withdrawal of the tail from a water bath maintained at 55° C was determined once before and at 15-min intervals after intrathecal drug administration. A cut-off latency of 10 s was used to prevent tissue injury. Increasing doses of morphine were administered to construct dose-response curves from data gathered at the time of peak effect; each animal received only a single dose of morphine. Naloxone (5 mg/kg, intraperitoneally) was administered 1 h after drug administration to determine whether the effects were mediated *via* opioid receptors. Data were converted to %MPE (maximal possible effect) by the formula: %MPE = 100 × (test latency − control latency)/(10 − control latency). The observer could not be blinded to the condition of the rats (nerve-ligated or sham-operated) because rats with the nerve-ligated revealed their condition by guarding the injured paw. Although this situation may allow some bias on the part of the experimenter, this was guarded against by only measuring one tail-flick response at each time point. The tail-flick response is a clear, nonarbitrary response on the part of the rat to the thermal stimulus.

Data Analysis

The interaction between clonidine and morphine was examined by isobolographic analysis, described in detail by Tallarida and colleagues.[36-38] Dose-effect curves were constructed for intrathecal morphine, clonidine, and a 1:3 fixed ratio of morphine to clonidine for L5/L6 nerve-ligated and sham-operated rats. For each drug or drug combination, the A_{50} (*i.e.*, dose calculated to produce 50 %MPE) and its associated variances and 95% confidence intervals (95% CI) in terms of total dose were calculated from the log-dose-response curves. The confidence intervals were then arithmetically arranged around the A_{50} by the formula $\ln(10) \times A_{50} \times$ standard error of log A_{50}. This transformation is necessary for subsequent statistical calculations of the isobolographic analysis, and the derivation of these calculations are described in detail by Tallarida and colleagues.[36,38] Where only a single drug was given, the "total dose" was the dose of the drug alone; otherwise, it represented the amount of clonidine plus morphine given. A theoretical additive A_{50} is calculated for the drug combination. If the relative potency of the additive A_{50} relative to the experimentally derived A_{50} for the mixture is significantly ($P \leq 0.05$) greater than 1, a synergistic interaction is indicated. An additive interaction is indicated when the theoretical and experimental A_{50} values were not significantly different from each other. When clonidine was administered intrathecally to nerve-ligated rats, no A_{50} value could be calculated. In that instance, the isobolographic method described by Porreca and colleagues[39] to determine synergistic interaction when one component of the drug mixture is inactive was applied.

Posttreatment means for each time-effect curve were compared with baseline tail-flick latencies by analysis of variance, followed by *post hoc* analysis (least significance difference test). A probability level of 0.05 indicated significance.

Phentolamine

Separate groups of sham-operated and nerve-ligated animals received 4 mg/kg phentolamine intraperitone-

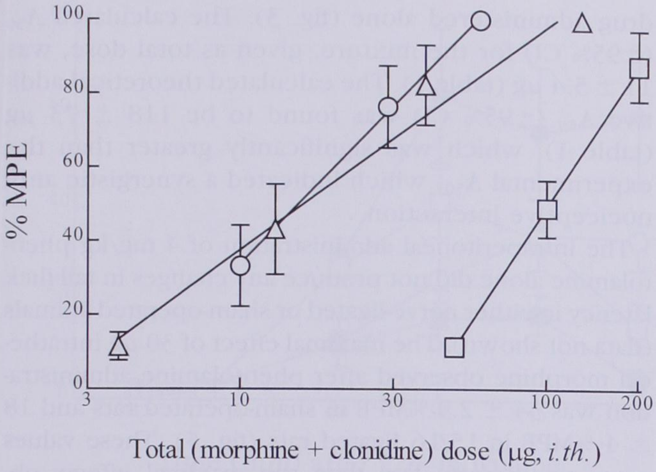

Fig. 1. Dose–effect curves for the antinociceptive effects of morphine, clonidine, and a 1:3 fixed ratio of morphine to clonidine injected intrathecally to sham-operated rats are shown. Morphine, clonidine, and the morphine–clonidine mixture are represented by open circles, open squares, and open triangles, respectively, administered to sham-operated rats. Error bars represent ± SEM. Dose is expressed as the total (*i.e.*, morphine plus clonidine) administered. n = 5–10 rats per group.

ally 20 min before the intrathecal administration of 30 μg morphine.

Results

The mean baseline tail-flick latency of sham-operated rats was 2.93 ± 0.10 s, and that of nerve ligated rats was 3.15 ± 0.09 s. These baseline latencies did not differ between the groups. Under the conditions of the current experiments, it was not possible to detect hyperalgesia of the tail.

Sham-operated Rats

Both morphine and clonidine produced dose-dependent antinociception. Morphine produced a maximal effect of 100 ± 0 %MPE at the largest dose tested, 60 μg (fig. 1). The A_{50} of morphine was 15 ± 4.9 μg (table 1). No behavioral deficits were observed at these doses of morphine. Clonidine produced a maximal effect of 88 ± 9.5 %MPE (fig. 1). Animals that received the highest dose of clonidine, 200 μg, appeared mildly sedated, but there was no impairment of the ability of the animal to produce a vigorous tail-flick when the nociceptive stimulus was applied. The calculated A_{50} of clonidine was 120 ± 24 μg (table 1). Administration of a 1:3 fixed ratio of morphine to clonidine produced dose-dependent antinociception (fig. 2), with a maximal effect of 100 ± 0 %MPE at the largest dose tested (130 μg total dose; fig. 1). The A_{50} (±95% CI) of the mixture was 15 ± 9.2 μg (table 1). This value was significantly less than the calculated theoretical additive A_{50} (±95% CI) of 44 ± 10 μg, which indicated a synergistic antinociceptive interaction between morphine and clonidine.

Table 1. Summary of Actual and Theoretical Additive A_{50} (±95% CL) Values for a 1:3 Fixed Ratio of Morphine to Clonidine in the Tail-flick Test in Sham-operated and Nerve-injured Rats

Drug Combination	L5/L6 Ligated A_{50} (±95% CL) (μg)	Sham-operated A_{50} (±95% CL) (μg)
Morphine	30 (±18)	15 (±4.9)
Clonidine	Not calculated	120 (±24)
Morphine + clonidine		
(1:3 ratio; total dose)	11 (±5.4)*	15 (±9.2)*
Additive A_{50} (calculated)	118 (±73)	44 (±10)

The A_{50} [±95% confidence limits (CL)] values are derived from dose-effect curves depicted in figs. 1 and 3, and calculated as described in the text. Each data point represents the mean of 5 to 10 rats/group. The A_{50} for morphine + clonidine is the value actually derived from the dose-effect curve for the drug combination and is represented as the total dose of administered drug. The additive A_{50} is the value derived from the dose-effect curves for morphine and for clonidine, and represents the total dose A_{50} that would be obtained if the drug interaction was additive.

*A significant ($P \leq 0.05$) difference in A_{50} between the actual A_{50} for the mixture and theoretical additive A_{50}; a synergistic interaction.

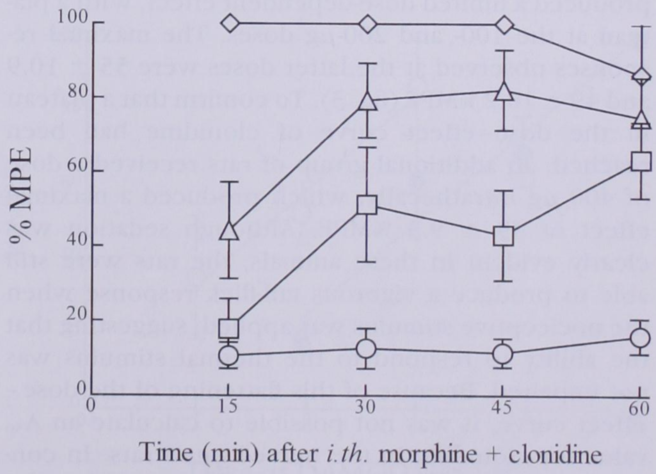

Fig. 2. Time–effect curves for a 1:3 fixed ratio of morphine to clonidine administered to sham-operated rats are shown. Circles, squares, triangles, and diamonds represent 1 μg morphine + 3 μg clonidine, 3 μg morphine + 10 μg clonidine, 10 μg morphine + 30 μg clonidine, and 30 μg morphine + 100 μg clonidine, respectively. Error bars represent ± SEM.

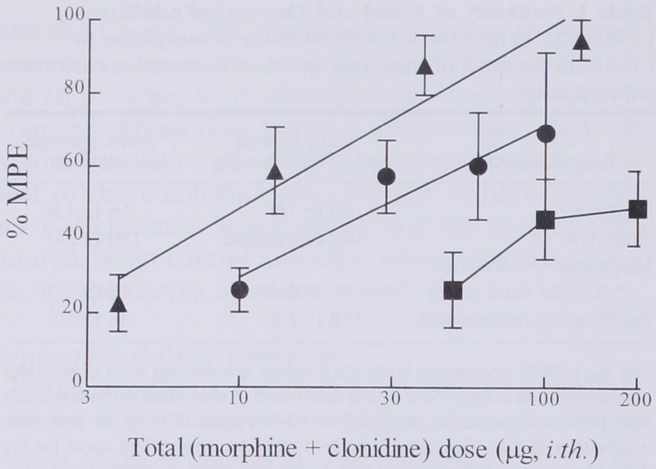

Fig. 3: Dose–effect curves for the antinociceptive effects of morphine, clonidine, and a 1:3 fixed ratio of morphine to clonidine injected intrathecally to L5/L6 ligated rats are shown. Morphine, clonidine, and the morphine–clonidine mixture are represented by filled circles, filled squares, and filled triangles, respectively. Error bars represent ± SEM. Dose is expressed as the total (*i.e.*, morphine plus clonidine) administered. n = 5–10 rats per group.

Nerve-injured Rats

In L5/L6 nerve-ligated rats, morphine also produced a dose-dependent antinociceptive effect, although to a lesser extent than in the sham-operated animals. The largest dose tested, 100 μg, produced a maximal effect of 69 ± 21.9 %MPE (fig. 3). The A_{50} (±95% CI) for morphine was 30 ± 18 μg (table 1). Clonidine produced a limited dose-dependent effect, with a plateau at the 100- and 200-μg doses. The maximal responses observed at the latter doses were 55 ± 10.9 and 49 ± 10.2 %MPE (fig. 3). To confirm that a plateau in the dose-effect curve of clonidine had been reached, an additional group of rats received a dose of 400 μg intrathecally, which produced a maximal effect of 49 ± 9.3 %MPE. Although sedation was clearly evident in these animals, the rats were still able to produce a vigorous tail-flick response when the nociceptive stimulus was applied, suggesting that the ability to respond to the thermal stimulus was not impaired. Because of this flattening of the dose-effect curve, it was not possible to calculate an A_{50} value for clonidine in the nerve-ligated rats. In contrast to morphine or clonidine alone, the 1:3 mixture of morphine and clonidine produced definitive dose-dependent antinociceptive effects (fig. 4). The dose-effect curve of the morphine-clonidine mixture was shifted to the left of the dose-effect curves for either drug administered alone (fig. 3). The calculated A_{50} (±95% CI) for the mixture, given as total dose, was 11 ± 5.4 μg (table 1). The calculated theoretical additive A_{50} (±95% CI) was found to be 118 ± 73 μg (table 1), which was significantly greater than the experimental A_{50}, which indicated a synergistic antinociceptive interaction.

The intraperitoneal administration of 4 mg/kg phentolamine alone did not produce any changes in tail-flick latency in either nerve-ligated or sham-operated animals (data not shown). The maximal effect of 30 μg intrathecal morphine observed after phentolamine administration was 34 ± 2.9 %MPE in sham-operated rats and 18 ± 4 %MPE in L5/L6 ligated rats (fig. 5). These values were significantly less than the maximal effects observed after morphine alone, 60 ± 14.4 %MPE and 35 ± 7.5 %MPE for sham-operated and nerve-ligated rats, respectively (fig. 5). Like morphine, intrathecal DAMGO also produced dose-dependent antinociception in the sham-operated and nerve-injured rats, along with a significant fourfold shift to the right of the dose-response curve (fig. 6). The A_{50} values (95% CI) for DAMGO in the sham-operated and ligated groups were 0.23 μg (range, 0.088-0.60) and 0.97 μg (range, 0.34-2.7), respectively. Unlike morphine, however, there was no loss of efficacy for intrathecal DAMGO in the nerve-injured rats; a full effect of 100 ± 0 %MPE was observed with 10 μg DAMGO (fig. 6).

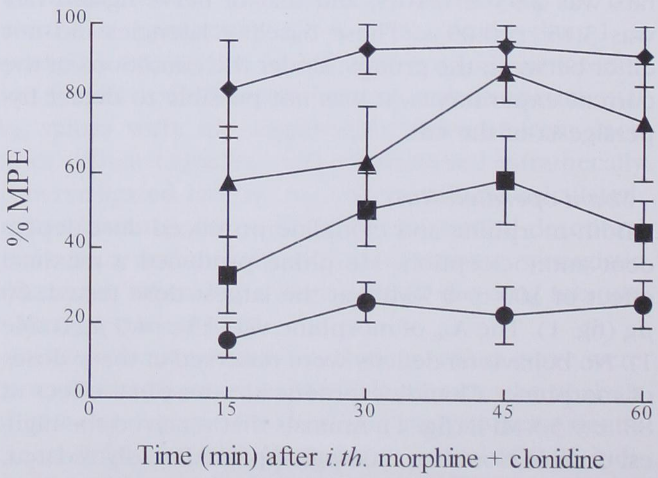

Fig. 4. Time–effect curves for a 1:3 fixed ratio of morphine to clonidine administered to L5/L6 ligated rats are shown. Circles, squares, triangles, and diamonds represent 1 μg morphine + 3 μg clonidine, 3 μg morphine + 10 μg clonidine, 10 μg morphine + 30 μg clonidine, and 30 μg morphine + 100 μg clonidine, respectively. Error bars represent ± SEM.

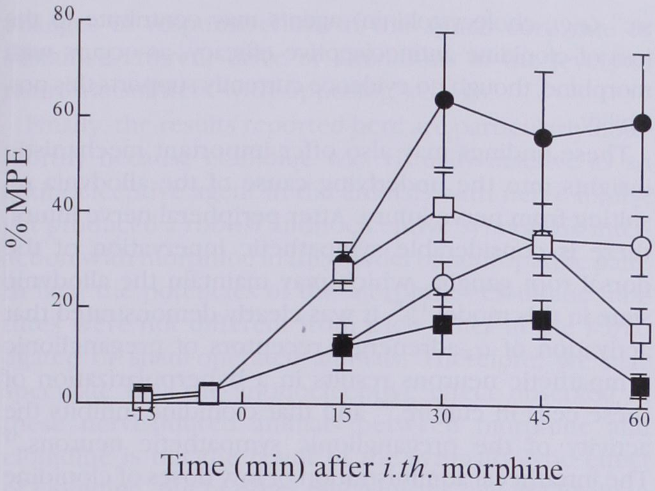

Fig. 5. Time–effect curves for 4 mg/kg phentolamine intraperitoneally administered 20 min before 30 μg intrathecal morphine to sham-operated (open symbols) and L5/L6 ligated rats (filled symbols). Morphine alone is indicated by circles, and pretreatment with phentolamine is indicated by squares. Error bars represent ± SEM.

Discussion

The observation that the intrathecal administration of morphine and clonidine produced a synergistic effect with regard to a spinally mediated nociceptive response, the tail-flick reflex, in sham-operated rats is in accordance with the established literature. A supra-additivity was described between morphine and clonidine in several species and after both systemic and intrathecal routes of administration.[21,31-34] Therefore, this observation establishes the validity of the methods used in the current study.

The acute antinociceptive action of clonidine was clearly diminished in potency and efficacy after nerve ligation injury. Noteworthy, however, is the unexpected loss of antinociceptive activity of clonidine, despite the observed and established antiallodynic action of lower (*i.e.*, 20-60 μg) doses of this compound.[6,11] The test dose of 400 μg intrathecally confirmed that a plateau in the dose-effect curve of clonidine occurred. Although sedation was evident in these animals, the rats still produced a vigorous tail-flick response when the nociceptive stimulus was applied, indicating that the sedation did not interfere with the animals' ability to respond to the thermal stimulus. In other studies, 100 μg intrathecal clonidine produced a maximal effect in the hot plate test at 52° C,[40] and 750 μg/kg clonidine subcutaneously produced a near maximal effect in a radiant heat tail-flick test.[41] The doses used and responses obtained for sham-operated animals in this study were well within the equivalent range indicated by these studies.

Suggestions of considerable overlap between mechanisms involved in the development of hyperalgesia subsequent to nerve injury and mechanisms observed after development of morphine tolerance have been made.[42,43] Both hyperalgesia and morphine tolerance, which may be associated with activation of N-methyl-D-aspartate receptors and increased activity of intracellular protein kinase C, were shown to be prevented by pretreatment with the N-methyl-D-aspartate antagonist MK-801.[42-44] These observations were taken to indicate that common neural substrates exist between morphine tolerance and loss of morphine activity in the presence of nerve injury,[42,43] and may explain the loss of antinociceptive efficacy of morphine after nerve injury seen in the current study and reported previously.[13]

Although similar mechanisms may be invoked, we must consider the possibility that morphine tolerance and loss of morphine activity after nerve injury are not identical phenomena. In the current study, both morphine, an opioid μ receptor agonist, and clonidine, an α_2 adrenergic receptor agonist, demonstrated significant loss of activity after nerve ligation injury. However, there is little evidence of antinociceptive cross-tolerance between morphine and clonidine. The antinociceptive effect of systemically or intrathecally administered morphine was virtually abolished in mice made

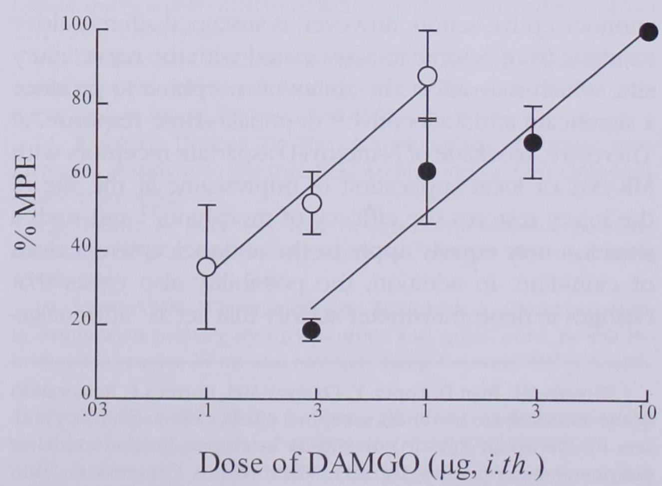

Fig. 6. Dose–effect curves for the antinociceptive effect of intrathecal [D-Al[2], NmePhe[4], Gly-ol]enkephalin (DAMGO) are shown. Open circles represent data from sham-operated rats, and filled circles represent data from nerve-ligated rats. Error bars represent ± SEM. n = 5–10 rats per group.

tolerant to morphine, whereas that of clonidine administered systematically or intrathecally was not altered,[20,30] a finding consistent with actions of these compounds at opioid μ and adrenergic α_2 receptors, respectively. In addition, it was reported that a single administration of MK-801 does not reverse morphine tolerance,[45] whereas it restores the antinociceptive efficacy of intrathecal morphine in the nerve-ligated rat.[12] Likewise, a single pretreatment with MK-801 also restores the antiallodynic activity of morphine, even though neither compound demonstrates antiallodynic activity alone in this ligation model.§,#

The reasons for the loss of antinociceptive activity of morphine and clonidine in the nerve-ligated rat are unclear and will require further investigation, but speculation about the mechanism is possible. Nerve ligation injury may hypersensitize the spinal cord to nociceptive stimuli, such that nociceptive processing is augmented as if the intensity of the stimuli were greater than in the nonligated rat, thereby increasing the opioid receptor occupancy requirement. However, it was shown recently that even when low levels of stimulus intensities were applied (*i.e.*, 48° C warm water or low-intensity radiant heat tail-flick tests), there was no evidence of hyperalgesia in the nerve-ligated rat.# In addition, the observation that DAMGO, a high-efficacy opioid μ agonist that consequently has a lower receptor occupancy requirement than morphine,[46,47] also lost potency after nerve ligation injury also argues against a greater receptor occupancy requirement as a mechanism by which intrathecal opioids lose antinociceptive activity after peripheral nerve injury. An important consideration in terms of an antinociceptive action, however, is sustained afferent drive resulting from ectopic foci associated with the nerve injury site, which may affect the ability of morphine to produce a significant antinociceptive[12] or antiallodynic response.*,# Therefore, blockade of N-methyl-D-aspartate receptors with MK-801 or local application of bupivacaine at the site of the injury restores the efficacy of morphine,[12] and such a situation may equally apply to the antinociceptive actions of clonidine. In addition, the possibility also exists that changes in neurotransmitter activity that act as "anti-analgesic" (*e.g.*, cholecystokinin) agents may contribute to the loss of clonidine antinociceptive efficacy, as occurs with morphine, though no evidence currently supports this possibility.

These findings may also offer important mechanistic insights into the underlying cause of the allodynia resulting from nerve injury. After peripheral nerve injury, there is considerable sympathetic innervation of the dorsal root ganglia, which may maintain the allodynic state in this model.[14,17] It was clearly demonstrated that activation of α_2-adrenergic receptors of preganglionic sympathetic neurons results in a hyperpolarization of these cells in culture,[48] and that clonidine inhibits the activity of the preganglionic sympathetic neurons.[49] The intrathecal administration of low doses of clonidine to rats produced significant reductions in firing rates of neuronal activity of the lumbar sympathetic chain and also altered thermoregulation[50] and reduced resting blood pressure and heart rate,[51] which indicates a reduction in efferent sympathetic activity. Clonidine, administered intrathecally, at lower doses than used in these studies, attenuated tactile allodynia in the L5/L6 nerve ligation model, and this effect was attributed to reduced spinal sympathetic outflow from preganglionic sympathetic neurons.[6] In addition, it also was shown that bilateral lumbar sympathectomy attenuated allodynia in rats with L5/L6 nerve ligation injury, and that the allodynia was rekindled by the administration of norepinephrine to the previously allodynic site, strongly suggesting that a sympathetic component exists in this model of neuropathic pain.[19] Finally, clinically, a sympathetic component of neuropathic pain is also suggested by the observations that chemical sympathectomy, by administration of clonidine[52] or phentolamine,[53] effectively diminishes painful neuropathy in humans. For this reason, the antiallodynic actions of clonidine have been thought to be possibly related to an effect on afferent input or, alternatively, to be the result of a sympatholytic action. The data presented here that show that clonidine is only partially active in inhibiting the tail-flick response at doses that are fully active against allodynia strongly suggest that the observed antiallodynic action[6] occurs by blocking spinal sympathetic outflow and not by acting to limit afferent nociceptive input. For these reasons, clonidine can be demonstrated to be fully antiallodynic, but only partially effective in suppressing acute nociceptive stimuli, as shown in the tail-flick test in nerve-injured animals. Again, the partial effectiveness of clonidine as an antinociceptive agent in nerve-injured animals may reflect

§ Nichols ML, Bian D, Lopez Y, Ossipov MH, Porreca F: Restoration of the antiallodynic and antinociceptive efficacy of morphine by antisera to dynorphin A(1-13) or MK801 in a nerve-ligation model of peripheral neuropathy (abstract). VIIIth World Congress on Pain 1996; 351.

Bian D, Wgert S, Ossipov MH, Vanderah T, Nichols ML, Porreca F: Differential sensitivities of hyperesthesias after L5/L6 nerve ligation injury to spinal pharmacologic manipulations (abstract). VIIIth World Congress on Pain 1996; 350.

changes in responsiveness in the spinal cord due to sustained afferent drive or alterations in the levels of neurotransmitters with opposing actions.

Finally, the results reported here are particularly noteworthy because clonidine was virtually inactive as an antinociceptive agent in the animals with nerve injury, yet produced a *robust* antinociceptive synergistic interaction with morphine in this model of neuropathic pain. In fact, the potencies of the morphine-clonidine mixtures were not different from each other in the L5/L6 ligated or sham-operated animals. Therefore, we may speculate that the antinociceptive effect observed in these nerve-injured animals between morphine and clonidine is not the result of the sympatholytic effect of clonidine, but of other mechanisms that are likely to be occurring in the spinal cord. It might be suggested that the potentiation of morphine antinociception was, in fact, dependent on a blockade sympathetic outflow elicited by intrathecal clonidine, possibly resulting in a reduction of spontaneous pain secondary to the nerve ligation injury. In that case, however, phentolamine, which produces a sympatholytic effect equivalent to sympathetic ganglion block by local anesthesia,[54] would be expected to augment the antinociceptive effect of morphine in the current study. However, the observation that phentolamine appeared to diminish the antinociceptive effect of morphine in both the nerve-ligated and sham-operated rats suggests that thermal hyperalgesia, unlike tactile allodynia, may not be sympathetically dependent. Also note that phentolamine was demonstrated to attenuate the antinociceptive effect of morphine administered either systemically or centrally in healthy animals.[54,55]

In summary, the data presented in this study strongly indicate that the antinociceptive effect of both morphine and clonidine are significantly diminished after peripheral nerve injury. This loss of efficacy may be attributed to neuroplastic changes, including changes in levels of endogenous antianalgesic neurotransmitters as well as sustained afferent drive. Noteworhty, however, is the observation that the effective doses of the combination of morphine and clonidine were significantly reduced, yet were fully efficacious as antinociceptive agents in this model of neuropathic pain. Combination therapy involving spinal administration of mixtures of clonidine and morphine, known to produce relief against acute pain clinically, may be of significant clinical importance in the treatment of patients suffering from neuropathic states.

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