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Moxonidine, a Selective Imidazoline–α_2-Adrenergic Receptor Agonist, Produces Spinal Synergistic Antihyperalgesia with Morphine in Nerve-injured Mice

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Background: **Moxonidine, a novel imidazoline–α_2-adrenergic receptor–selective analgesic, was recently identified as antinociceptive but has yet to be evaluated in neuropathic pain models. α_2-adrenergic receptor–selective analgesics, and high-efficacy opioids, effectively inhibit neuropathic pain behaviors in rodents. In contrast, morphine potency and efficacy decreases in states of neuropathic pain, both in rodents and in humans, but may be restored or enhanced by coadministration of morphine with α_2-adrenergic receptor–selective analgesics. The current experiments extend the evaluation of opioid–coadjuvant interactions in neuropathic subjects by testing the respective antihyperalgesic interactions of moxonidine and clonidine with morphine in a test of mechanical hyperalgesia.**

Methods: **Nerve-injured mice (Chung model) were spinally administered moxonidine, clonidine, morphine, and the combinations moxonidine–morphine and clonidine–morphine. Hyperalgesia was detected by von Frey monofilament stimulation (3.3 mN) to the hind paws (plantar surface). The ED_{50} values were calculated and the interactions tested by isobolographic analysis.**

Results: **In nerve-injured mice, moxonidine, clonidine, and morphine all dose-dependently inhibited mechanical hyperalgesia. Furthermore, the combinations of moxonidine–morphine and clonidine–morphine resulted in substantial leftward shifts in the dose–response curves compared with those of each agonist administered separately. The calculated ED_{50} values of the dose–response curves of these combinations were significantly lower than their corresponding theoretical additive ED_{50} values. These results confirmed that both interactions were synergistic.**

Conclusions: **Moxonidine and clonidine both synergize with morphine to inhibit paw withdrawal from nociceptive mechanical stimuli in nerve-injured mice. (Key words: Chronic pain; isobologram; spinally mediated analgesia; synergy.)**

MOXONIDINE is a member of the imidazoline–α_2-adrenergic receptor (AR) class of compounds, is a centrally active compound, and is clinically used in Europe to treat hypertension.[1] We recently described a spinal antinociceptive action of moxonidine in two strains of mice.[2] In that study, we demonstrated that the receptor requirement for the spinal antinociception of moxonidine differs dramatically from that of previously studied α_2-AR agonists. In genetically altered mice,[3] intrathecally administered norepinephrine-, dexmedetomidine- and UK-14,304–mediated analgesia showed a large dependence on α_{2A}-AR subtype[2,4]; clonidine showed an absolute requirement for activation of the α_{2A}-AR subtype to produce analgesia.[2] In contrast, spinal antinociception mediated by moxonidine requires some α_2-AR activation but is not α_{2A}-AR–dependent.[2] This spinal independence of the α_{2A}-AR subtype distinguishes moxonidine from clonidine and suggests an analgesic role for either α_{2B} or α_{2C} ARs, consistent with *in vitro* evidence indicating that moxonidine is not selective for one α_2-AR subtype over another (α_{2A} AR: 13.0 ± 4.2 nM; α_{2B} AR: 9.5 ± 4.1 nM; α_{2C} AR: 15.6 ± 9.8 nM).[5] The significance of this observation is underscored by evidence suggesting a requirement for activation of the α_{2A}-AR subtype to produce sedation.[6] Selective activation of an α_2-AR subtype other than α_{2A} AR (*e.g.*, α_{2B} or α_{2C}) might, therefore, improve α_2-AR–mediated analgesia by reducing the incidence of sedation. Furthermore, comparisons of the

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analgesic 5profile of spinally administered clonidine (α_{2A}-AR–dependent) and moxonidine (α_{2A}-AR–independent) may expand current understanding of the role of α_2-AR subtypes in spinally mediated analgesia, particularly in light of recent evidence demonstrating distinct localization of α_2-AR subtypes in spinal cord dorsal horn.[7]

To further characterize moxonidine-mediated analgesia, we also demonstrated spinal moxonidine–morphine and moxonidine–deltorphin II antinociceptive synergism in mice.[8] To expand this characterization, the current study evaluates the effects of spinally administered moxonidine (delivered alone or with morphine) on neuropathic pain behaviors[9] in mice subjected to peripheral nerve injury (Chung model).[10] For comparison with clinically used agents, the current study also characterizes the action of intrathecally administered morphine, clonidine, and their combination in this mouse model of neuropathic pain.

Materials and Methods

Animals

Experimental subjects were 25–30-g male Institute of Cancer Research mice (Harlan, Madison, WI). Subjects were housed in groups of 5–10 in a temperature- and humidity-controlled environment. Subjects were maintained on a 12-h light–dark cycle and had free access to food and water. Each animal was used only once. These experiments were approved by the Institutional Animal Care and Use Committee.

Chemicals

Moxonidine [4-chloro-5-(2imidazolin-2-ylamino)-6-methoxy-2-methylpyrimidine] chloride was a gift from Solvay Pharma (Hannover, Germany) and was dissolved in 1% acetic acid and diluted with acidified saline (pH 3.2–4). All other drugs were dissolved in 0.9% saline. Morphine was a gift from the National Institute on Drug Abuse (Bethesda, MD). Clonidine HCl (2-[2,6-dichloroaniline]-2-imidazoline) was a gift from Boehringer-Ingelheim Ltd. (Ridgefield, CT). All drugs and drug combinations were injected intrathecally by direct lumbar puncture.[11] Briefly, each mouse is gripped firmly by the pelvic girdle. A 30-gauge needle connected to a 50-μl Hamilton syringe is lowered at a 30° angle and inserted at the level of the cauda equina. Puncture of the dura is indicated by a reflexive flick of the tail.

Hyperalgesia Induction: Spinal Nerve Ligation

Hypersensitivity was induced by surgical ligation of the L5 spinal nerve in mice.[10] Mice were placed in an enclosed chamber and anesthetized by halothane and placed in a prone position before any surgery. When the animal was unresponsive to paw pinch, it was removed from the chamber, shaved from below the iliac crest to approximately halfway to the shoulders, and fitted with a facemask delivering 2 or 3% halothane, which was continuously administered to the animal throughout the surgery. Betadine was applied to the shaved area before the incision. The left paraspinal muscle was separated from the spinous processes at the L4–S2 levels and removed. Removal of this muscle does not impair mobility of the animal after surgery. A Mini-Goldstein retractor (Fine Science Tools No. 17002-02, Foster City, CA) with a 1-cm maximum spread was then inserted into the incision at the level of the iliac crest. Further removal of muscle and tissue permitted visualization of the L6 transverse process and the rostral tip of the sacrum. The L6 transverse process was then removed with use of an S&T fine forceps with a tip dimension of 0.3 × 0.25 mm (Fine Science Tools No. 00108-11). Removal of the process permitted visual identification of the L4–L5 spinal nerves. The L5 spinal nerve was tightly tied (ligated) with 6-0 silk thread distal to the dorsal root and proximal to the confluence of spinal nerves L4 and L5. After hemostasis was confirmed, the wound was sutured with 3-0 silk thread, and the skin was closed with sterile wound clips. The animal was then placed in a moderately heated oxygen-enriched plastic enclosure to facilitate recovery. The animals were fully mobile within 30 min of cessation of anesthetic. As a control, in a separate group of animals, a sham surgery identical to the aforementioned one (but without nerve ligation) was performed.

Nociceptive Testing: Tactile Sensitivity

Nociception was evaluated by responsiveness to multiple applications (10 per hind paw) of a single von Frey filament to the plantar surface of each hind paw. When the stimulus is of sufficient force, the mouse will lick, withdraw, or shake the paw; this action represents the behavioral end point. In nerve-injured mice, a von Frey filament (#3.61) exerting 3.3 mN of force elicited 66 ± 1.3% responsiveness [(number of withdrawals/10)×100] on the paw ipsilateral to the injury. This level of response is sufficient to test compounds for dose-dependent inhibition of the response to mechanical stimulation.

Table 1. L5-ligated Mice, Affected Paw: Summary of Moxonidine–Morphine Spinal Antihyperalgesic Synergy

Agonist (pmol, i.t.)	ED_{50} Morphine (95% CL)	ED_{50} Moxonidine (95% CL)
Single agonist	64 (30–135)	14 (4.1–50)
Morphine + moxonidine (4:1 ratio)		
Observed combination	1.2 (0.7–1.7)*	0.3 (0.17–0.43)*
Theoretical additive	30 (7.2–54)	7.6 (1.8–13)

* Significant difference from theoretical additive by Student *t* test ($P < 0.05$).

Inhibition of Tactile Sensitivity

Varying doses of moxonidine, morphine, or clonidine, or combinations thereof, were administered to test for inhibition of tactile sensitivity. Percent inhibition was determined relative to the mean number of paw withdrawals elicited by force and according to the following equation:

% Inhibition = (no. of paw withdrawals predrug − no. of paw withdrawals postdrug)/no. of paw withdrawals predrug

Each mouse served as its own control. The ED_{50} values (the dose calculated to produce 50% inhibition) and 95% confidence limits were calculated according to the method of Tallarida and Murray.[12] To test for the antihyperalgesic effects of moxonidine and morphine over time, groups of mice injected with various doses of drug or acidified saline were concurrently tested at 5, 10, 30, 60, 90, and 120 min after intrathecal injection. ED_{50} values were calculated at the 10-min time point. To test for drug interactions, a separate group of animals (n = 126) was subjected to surgery within the same week. All behavioral testing was conducted the following week on the corresponding day 8 at 10 min after drug injection. New dose–response curves were generated for each drug given alone (morphine, moxonidine, clonidine) or given in combination (morphine–moxonidine, morphine–clonidine), and corresponding ED_{50} values were calculated (n = 4–8 mice/dose).

Statistical Analysis

Data describing antihyperalgesia are expressed as means of percent inhibition with SEM. Student *t* test comparisons were made between responses of the left and right hind paws of all animals before surgery, and left and right hind paws of nerve-injured, sham, and naive animals after surgery ($P < 0.05$). The comparison between the left (injured) hind paws of nerve-injured and the left hind paws of sham-operated and naive animals after surgery was also evaluated by analysis of variance. Drug potency comparisons are based on the calculated ED_{50} values for the dose–response curve of each drug or combination of drugs.

Isobolographic Analysis

To test for drug interactions, isobolographic analysis was applied.[12,13] When testing an interaction between two drugs given in combination for synergy, additivity, or subadditivity, a theoretical additive ED_{50} value is calculated for the combination based on the dose–response curves of each drug administered separately. This theoretical value is then compared by a *t* test ($P < 0.05$) with the observed experimental ED_{50} value for the combination. These values are based on total dose of both drugs, *i.e.*, the dose of clonidine or moxonidine plus the dose of morphine. For the purpose of comparison to the drug doses administered separately, we separated the clonidine or moxonidine and morphine components of the observed and theoretical ED_{50} values; these are presented in tables 1 and 2. An interaction is considered synergistic if the observed ED_{50} value is significantly less ($P < 0.05$) than the calculated theoretical additive ED_{50} value.[12,13] Additivity is indicated when the theoretical and experimental ED_{50} values do not differ.

Table 2. L5-ligated Mice, Affected Paw: Summary of Clonidine–Morphine Spinal Antihyperalgesic Synergy

Agonist (pmol, i.t.)	ED_{50} Morphine (95% CL)	ED_{50} Clonidine (95% CL)
Single agonist	64 (30–135)	4,600 (1,800–11,000)
Morphine + clonidine (1:44 ratio)		
Observed combination	4.0 (0.4–7.6)*	174 (16–332)*
Theoretical additive	40 (17–63)	1,740 (732–2,748)

* Significant difference from theoretical additive by Student *t* test ($P < 0.05$).

Results

Induction of Hyperalgesia

No difference was observed in baseline percent response to a force of 3.3 mN (von Frey filament #3.61, our calibration) between the left (mean = 27 ± 1.8%, n = 142) and right hind paws (mean = 27 ± 1.8%, n = 142; $P > 0.05$, Student unpaired *t* test) of mice before injury. On day 8 after surgery, a substantial increase in responsivity was observed for both hind paws (fig. 1), and the increase was significantly greater for the left hind paw (ipsilateral to the ligation, mean = 66 ± 1.3%, n = 126) than for the right hind paw (contralateral to the ligation: mean = 48 ± 1.8%, n = 126; $P < 0.01$, Student unpaired *t* test; Fig. 1). This small increase in sensitivity on the contralateral side is consistent with previous reports of contralateral effects after nerve injury.[14] Both of these responses were substantially greater than that of either hind paw of the control animals; controls included those mice that received sham surgery (left hind paw: mean = 35 ± 15%, n = 6; right hind paw, mean = 33 ± 8.4%, n = 6) and naive mice (left hind paw: mean = 30 ± 6.2%, n = 9; right hind paw: mean = 33 ± 9.9%, n = 9). These differences show that the L5 spinal nerve ligation surgery is sufficient to produce hyperalgesia in the hind paw ipsilateral to the injury.

Moxonidine-mediated Antihyperalgesia

Moxonidine inhibition of mechanical hyperalgesia is represented in figure 2 and expressed as percent inhibition of the percent response to mechanical stimulation. Moxonidine at 0.1- and 1-nmol doses significantly attenuated the hyperalgesia for 10 and 90 min, respectively, whereas 0.03 nmol moxonidine and acidified saline had minimal effect on hyperalgesia. Moxonidine appeared to have a longer duration of action in the ipsilateral hind paw relative to the contralateral hind paw. The calculated ED_{50} values of moxonidine at the 10-min time point were comparable between the ipsilateral and contralateral hind paws (ipsilateral: 0.12 nmol, 0.058–0.24; contralateral: 0.12 nmol, 0.037–0.39). We evaluated the doses at the 10-min time point because that time represents the peak analgesic effect at a time most likely involving a selectively spinal effect.[11]

Morphine-mediated Antihyperalgesia

Morphine inhibition of mechanical hyperalgesia is represented in figure 3. Morphine at 3- and 10-nmol doses significantly attenuated the hyperalgesia for the duration of the test period (120 min) in both the ipsilateral and

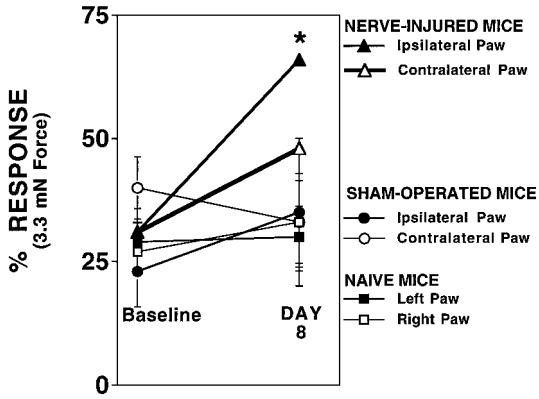

Fig. 1. In nerve-injured mice, a substantial increase in responsivity was observed for both hind paws; the increase was significantly greater for the paw ipsilateral to the ligation (closed triangles) than for the paw contralateral to the ligation (open diamonds; $P < 0.01$, Student unpaired *t* test). Control animals include sham-operated mice (ipsilateral paw, closed circles; contralateral paw, open circles) and naive mice (left paw, closed squares; right paw, open squares). *Indicates statistical significance (ANOVA, $P < 0.05$).

contralateral hind paws. The calculated ED_{50} values for morphine at the 10-min time point were comparable between the ipsilateral and contralateral hind paws (ipsilateral: 1.1 nmol, 0.5–2.4; contralateral: 2.4 nmol, 0.88–6.4, not significantly different). Morphine appeared to have comparable duration of action in both the ipsilateral and contralateral hind paws.

Moxonidine–Morphine Synergy (Hind Paw Ipsilateral to the Injury)

Intrathecally administered moxonidine (ED_{50}: 14 pmol, 4.1–50) and morphine (ED_{50}: 64 pmol, 30–135) both inhibited mechanical hyperalgesia (fig. 4A). Based on these ED_{50} values, the moxonidine–morphine equieffective dose ratio used was 1:4. Combination of moxonidine and morphine at this dose ratio resulted in significant leftward shifts in the dose–response curves (*i.e.*, increased potency) compared with those of each agonist administered separately (fig 4A and table 1). The coadministration of moxonidine–morphine combinations in mice resulted in antihyperalgesic dose–response curves with ED_{50} values significantly less than the calculated theoretical additive values (fig. 4B and table 1). This result indicates a synergistic interaction.

Morphine–Clonidine Synergy (Hind Paw Ipsilateral to the Injury)

Intrathecally administered clonidine (ED_{50}: 4,600 pmol, 1,800–11,000) and morphine (ED_{50}: 64 pmol,

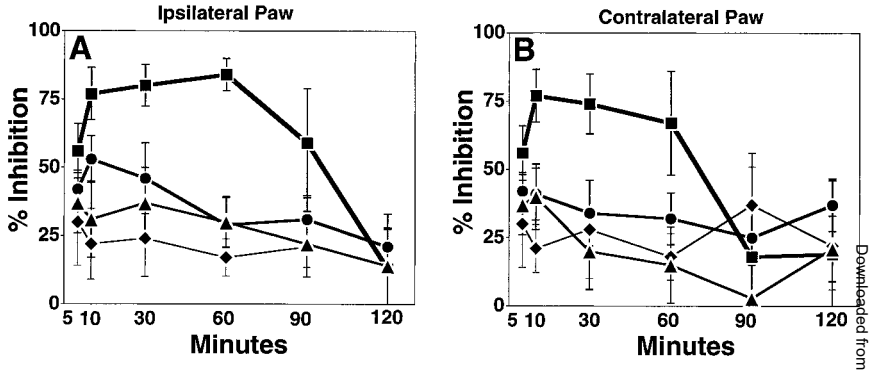

Fig. 2. Moxonidine dose-dependently attenuated mechanical hyperalgesia. (*A*) Ipsilateral (injured) paw. Moxonidine at 1-nmol (squares) and 0.1-nmol (circles) doses significantly attenuated mechanical hyperalgesia for 90 and 10 min, respectively, whereas 0.03 nmol (triangles) moxonidine moderately decreased hyperalgesia. (*B*) Contralateral paw. Moxonidine at 1-nmol (squares) and 0.1-nmol (circles) doses significantly attenuated the hyperalgesia for 60 and 10 min, respectively, whereas 0.03 nmol (triangles) moxonidine moderately decreased hyperalgesia. For both (*A*) and (*B*) statistical significance of the dose-dependent effect of moxonidine at the 10–60-min time points was shown by repeated-measures analysis of variance followed by Bonferroni *post hoc* test. A dose of 0.01 nmol (data not shown) did not have an effect greater than that of acidified saline (diamonds), which had minimal effect on hyperalgesia. Before administration of moxonidine, confirmation of induction of hyperalgesia (similar to that shown in fig. 1) was conducted for this time-course study (data not shown).

30–135) both inhibited mechanical hyperalgesia (fig. 5A). The morphine–clonidine equi-effective dose ratio used was 1:44. Combination of clonidine and morphine at this dose ratio resulted in significant leftward shifts in the dose–response curves compared with those of each agonist administered separately (fig. 5A and table 2). The coadministration of clonidine–morphine combinations in mice resulted in antihyperalgesic dose–response curves with ED_{50} values significantly less than the calculated theoretical additive values (fig. 5B and table 2). This result confirms a synergistic interaction.

Side Effects

We did not detect obvious motor or sedative side effects with use of these doses of moxonidine, morphine, clonidine, and the combinations; however, we have not conducted systematic evaluation of these effects through use of the rotorod or righting reflex assays.

Discussion

The current study introduces a new antihyperalgesic agent: the imidazoline–α_2-AR agonist moxonidine. The study also shows that both the imidazoline–α_2-AR agonists moxonidine and clonidine combined with morphine produce spinal antihyperalgesic synergy in nerve-injured mice.

The ability of α_2-AR agonists to produce antihyperalgesia in the mechanical von Frey monofilament stimulation test has been previously observed.[15,16] Spinal administration of dexmedetomidine, oxymetazoline, and guanfacine resulted in a dose-dependent reversal of the hyperalgesia induced by L5–L6 spinal nerve ligation in rats.[15,16] We have now shown that, like these other α_2-AR agonists, moxonidine also dose-dependently decreased hyperalgesic paw withdrawals with a potency comparable to that of morphine and greater than that of

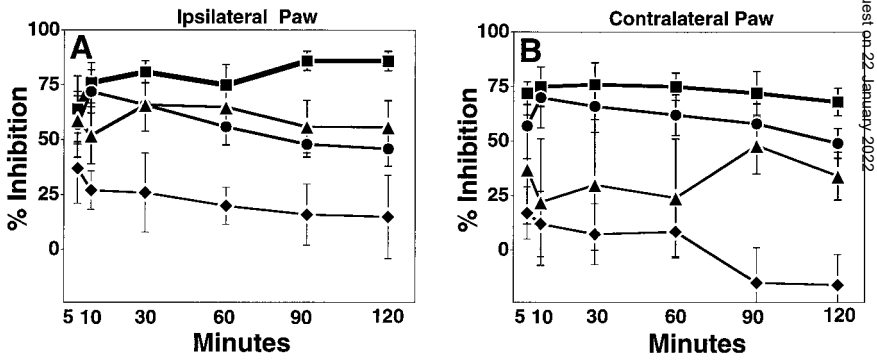

Fig. 3. Morphine dose-dependently attenuated mechanical hyperalgesia. (*A*) Ipsilateral (injured) paw. Morphine at 10-nmol (squares) and 3-nmol (circles) doses significantly attenuated the hyperalgesia for the duration of the study (120 min); 1 nmol (triangles) morphine moderately attenuated hyperalgesia. (*B*) Contralateral paw. Morphine at 10-nmol (squares) and 3-nmol (circles) doses significantly attenuated the hyperalgesia for the duration of the study (120 min); 1 nmol (triangles) morphine moderately attenuated hyperalgesia. Intrathecal administration of 0.3 nmol (diamonds) morphine had minimal effect on hyperalgesia. The significance of the dose-dependent effect of morphine was shown by repeated-measures analysis of variance followed by Bonferroni *post hoc* test. Before administration of morphine, confirmation of induction of hyperalgesia (similar to that shown in fig. 1) was conducted for this time-course study (data not shown).

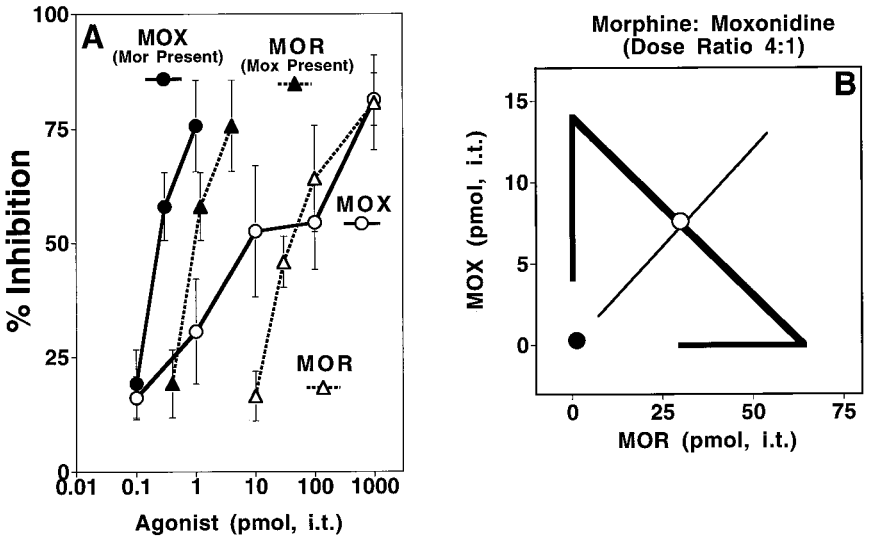

Fig. 4. Moxonidine and morphine synergize to alleviate mechanical hyperalgesia. Dose–response curves for moxonidine, morphine, and moxonidine–morphine administered intrathecally separately and in combination. (*A*) Dose–response curves of the spinal antihyperalgesic effect of moxonidine (open circles, solid lines, ED_{50}: 14 pmol, 4.1–50), morphine (open triangles, dashed lines, ED_{50}: 64 pmol, 30–135), moxonidine in the presence of morphine (closed circles, solid lines, ED_{50}: 0.3 pmol, 0.17–0.43), and morphine in the presence of moxonidine (closed triangles, dashed lines, ED_{50}: 1.2 pmol, 0.7–1.7). (*B*) Isobolographic representation of the antihyperalgesic (percent inhibition) effect of the combination of moxonidine–morphine in nerve-injured mice. Drug interactions may be illustrated through construction of such isobolograms. The ED_{50} values of clonidine or moxonidine and morphine are respectively plotted as the *y*- and *x*-axis intercepts. The thicker lines directed from each ED_{50} value toward zero represent the respective lower confidence limits of each ED_{50} value. The straight line connecting these two points is the theoretical additive line. The open circle that lies on or near the theoretical additive line represents the calculated theoretical ED_{50} value of the combination where the interaction is additive. The closed circle represents the experimentally observed ED_{50} value of the combination of clonidine–morphine. If the interaction is synergistic, the closed circle will be plotted significantly below the theoretical additive line and outside the lower confidence limits of ED_{50} values of clonidine and morphine. In this isobologram, the ED_{50} value of the combination of clonidine–morphine is significantly lower than that of the theoretical additive ED_{50} value and is synergistic.

clonidine. Morphine remains the standard with which other analgesics are compared, and clonidine is the prototypic analgesic α_2-AR agonist. Our comparisons of moxonidine to clonidine and morphine in neuropathic pain in mice suggest that the performance of moxonidine in humans as an analgesic and antihyperalgesic agent may compare favorably with that of morphine and clonidine.

The ability of opioid receptor agonists to inhibit hyperalgesia in nerve-injured animals has also been previously evaluated. Two studies[17,18] report that systemically and intracerebroventricularly (but not intrathecally) administered morphine inhibited mechanical hyperalgesia in nerve-injured rats. Additionally, intrathecally administered deltorphin II, a δ opioid receptor–selective agonist, showed decreased antihyperalgesic potency and efficacy in nerve-injured rats.[19] Other studies with use of thermal stimulation of the tail as the nociceptive stimulus showed that the intrathecal antinociceptive potency of morphine was decreased approximately twofold[20] or fourfold[21] in the nerve-injured rats relative to their sham-operated controls. Collectively, these data paralleled the clinical observations that neuropathic pain may be less sensitive to opioids than is nociceptive pain.[22–26]

However, there remains disagreement in the clinical literature over opioid resistance in patients with neuropathic pain.[27,28] Some reports have shown success with use of opioids to treat neuropathic pain.[27–30] Opioids delivered spinally have been shown to be effective in human patients with neuropathic pain.[31–33] Consistent with this clinical experience, at least one study showed that the higher efficacy μ opioid receptor–selective agonist, [D-ala(2),N-MePhe(4),Gly-ol(5)] enkephalin (DAMGO), produced full dose-related antihyperalgesia when given intrathecally to nerve-injured rats.[19] Additionally, the intrathecally administered combinations of morphine–deltorphin[19] and morphine–clonidine[20] produced antihyperalgesia and antinociceptive synergy, respectively, in nerve-injured rats.

Unlike the comparable rat studies,[17,18] we observed that intrathecal morphine produces antihyperalgesia in nerve-injured mice at doses comparable to those that are effective in sham-operated and naive controls (data not shown). Furthermore, we observed that morphine synergizes with other antihyperalgesic agents in nerve-injured mice, consistent with other studies showing morphine-coadjuvant synergy (morphine–deltorphin,[19] morphine–clonidine[20]) in nerve-injured rat. Retention of opioid sensitivity during conditions of neuropathic pain agrees with other clinical reports,[28,34,35] that opioids are effective as therapeutic agents for neuropathic pain, albeit with higher dose and/or coadjuvant requirements.

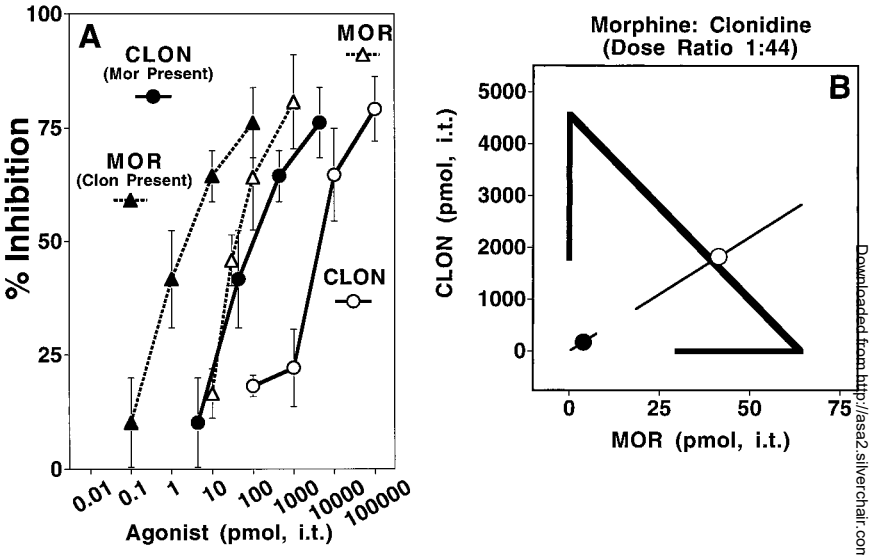

Fig. 5. Clonidine and morphine synergize to alleviate mechanical hyperalgesia. Dose–response curves for clonidine, morphine, and clonidine–morphine administered intrathecally separately and in combination. (*A*) Dose–response curves of the spinal antihyperalgesic effect of clonidine (open circles, solid lines, ED_{50}: 4,600 pmol, 1,800–11,000), morphine (open triangles, dashed lines, ED_{50}: 64 pmol, 30–135), clonidine in the presence of morphine (closed circles, solid lines, ED_{50}: 174 pmol, 16–332), and morphine in the presence of clonidine (closed triangles, dashed lines, ED_{50}: 4.0 pmol, 0.4–7.6). (*B*) Isobolographic representation of the antihyperalgesic (percent inhibition) effect of the combination of clonidine–morphine in nerve-injured mice. In this isobologram, the ED_{50} value of the combination of clonidine–morphine is significantly lower than that of theoretical additive ED_{50} value and is synergistic.

Intrathecal coadministration of morphine with moxonidine produced a synergistic antihyperalgesic effect. The observation of moxonidine–morphine synergy concurs with our previous study that showed antinociceptive synergy between intrathecally coadministered moxonidine and morphine.[8] This observation shows that the moxonidine–morphine combination alleviates neuropathic pain responses arising from nerve injury.

Originally, we expected that the morphine–clonidine interaction would not be synergistic in neuropathic mice based on three previous observations: (1) clonidine-mediated spinal analgesia requires the α_{2A} AR in mice[2]; (2) α_{2A}-AR immunoreactivity decreased in rat spinal cord dorsal horn after nerve injury[36]; and (3) clonidine antinociceptive effectiveness decreased in nerve-injured rats.[20] However, the current study shows that the clonidine–morphine combination produces antihyperalgesic synergy in nerve-injured mice. Similarly, despite decreases in effectiveness of both drugs when given alone, the clonidine–morphine combination produced antinociceptive synergy in nerve-injured rats[20]; these results suggest that, despite decreases in α_{2A}-AR immunoreactivity in rat dorsal horn after nerve-injury, sufficient receptor numbers remain functional to participate in this interaction with morphine. Recent evidence provides support for this assertion by showing increased α_{2A}-AR mRNA[37] and α_{2A}-AR immunoreactivity[38] in dorsal root ganglia of rats subjected to sciatic nerve transections. These results in dorsal root ganglia together with a previous report[36] raise the possibility of altered splicing or trafficking of α_{2A} AR in the neuropathic state. Alternatively, nerve injury may unmask a latent clonidine effect at upregulated α_{2C} AR.[36] This second possibility is supported by *in vitro* studies that indicate that clonidine shows comparable affinity for human α_{2A}- and α_{2C}-AR subtypes.[5] Regardless, the current data support the use of clonidine as a coadjuvant for morphine for the treatment of neuropathic pain.

In summary, the current results show that both moxonidine and clonidine produce spinal antihyperalgesic synergy with morphine in nerve-injured mice. These results concur with previous evaluations of adrenergic agonists in neuropathic pain[15,16] and of morphine–clonidine interactions in normal rodents[39–41] and nerve-injured rats.[20] This is the first study to show an antihyperalgesic property of the imidazoline–α_2-AR agonist moxonidine. It is noteworthy that prior clinical trials of systemically administered moxonidine as an antihypertensive agent show that moxonidine is well-tolerated.[42–46] Furthermore, moxonidine presents an improved side-effect profile over clonidine in terms of reduced sedation and dry mouth,[42,43] rebound withdrawal syndrome,[1,45,47] and hypotensive effects in normotensive subjects.[48] The data presented here would predict that moxonidine may prove effective as a spinal antihyperalgesic agent or coadjuvant to morphine for the treatment of neuropathic pain in humans.

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