

Effects of Intrathecal NMDA and Non-NMDA Antagonists on Acute Thermal Nociception and Their Interaction with Morphine

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Background: N-methyl-D-aspartate (NMDA) antagonists have minimal effects on acute nociception but block facilitated states of processing. In contrast, the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) antagonists decrease acute noxious responses. Morphine (a μ -opioid agonist) can also decrease acute nociceptive processing. The authors hypothesized that the interaction between morphine and AMPA receptor antagonists would be synergistic, whereas morphine and NMDA antagonists show no such interaction in acute nociception.

Methods: Sprague-Dawley rats (weight, 250–300 g) were implanted with chronic lumbar intrathecal catheters and were assigned to receive one of several doses of morphine—ACEA 1021 (NMDA glycine site antagonist), ACEA 2085 (AMPA antagonist), AP-5 (NMDA antagonist), saline or vehicle—and were tested for their effect on the response latency using a 52.5°C hot plate. The combinations of morphine and other agents also were tested.

Results: Intrathecal morphine (ED_{50} : 2 μ g/95% confidence interval, 1–4 μ g) and ACEA 2085 (6 ng/2–15 ng), but not AP-5 or ACEA 1021, yielded a dose-dependent increase in the thermal escape latency. A systematic isobolographic analysis was carried out between intrathecal morphine and ACEA 2085 using the ED_{50} dose ratio of 357:1. A potent synergy was observed with decreased side effects. Morphine dose–response curves were carried out for morphine and fixed doses of ACEA 1021 (12 μ g) or AP-5 (10 μ g). No synergistic interactions were noted.

Conclusions: Spinal μ -receptor activation and AMPA receptor antagonism showed a synergistic antinociception in response to an acute thermal stimulus. NMDA or NMDA glycine site antagonism had no effect alone nor did they display synergy with morphine. These results suggest an important direction for

development of acute pain strategies may focus on the AMPA receptor. (Key words: Analgesia; glutamate receptor; opioid receptor; spinal cord.)

GLUTAMATE, the excitatory amino acid, is contained in and released from primary afferents as well as from interneurons.¹ The excitatory effect of glutamate may be mediated by at least two distinct classes of receptors: those classified as being activated by N-methyl-D-aspartate (NMDA)² and those by alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA).^{3,4} Dorsal horn populations of spinal NMDA sites are believed to mediate a polysynaptic excitation, whereas AMPA receptors mediate the monosynaptic excitation of second order neurons.^{5,6}

The likely role of these receptor systems in nociceptive processing is supported by the observation that the iontophoretic delivery of the agonists can enhance the responses of spinal neurons to mechanical stimulation,⁷ and their intrathecal delivery will yield a potent pain-producing activity.⁸ The use of selective antagonists has suggested that these receptors may differ in the role they play in the processing of nociceptive input. NMDA antagonists have typically been shown to have minimal effects on acute nociceptive input but appear to block facilitated states of processing.^{9,10} In contrast, the AMPA receptor may alter acute afferent evoked excitation, and AMPA antagonists decrease the animal's response to an acute noxious stimulus.^{11,12} In the formalin test in rats, antagonists of the NMDA and of the non-strychnine-sensitive glycine site attenuated only the tonic second phase, whereas AMPA antagonists inhibited the acute phase.¹²

Using several selective agents, we sought to characterize the effects of antagonizing the two receptors on acute nociceptive processing in the rat. In addition, morphine, a μ -opioid agonist, can (1) reduce transmitter release from primary afferents, (2) hyperpolarize dorsal horn nociceptors, and (3) produce a powerful antinoci-

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ceptive effect when examined in models of acute thermal nociception. Given the different functional organizations, we hypothesized that intrathecal morphine might show a potent interaction with spinal glutamate receptor antagonists. There has, however, been no specific study on the nature of the interaction concerning acute nociceptive processing. On the basis of the differences in the organization of the NMDA and AMPA receptors, we hypothesized that the spinal interaction between morphine and AMPA receptor antagonists would be synergistic, whereas there would be no such interaction between morphine and NMDA antagonists in a model of acute nociception.

Materials and Methods

Animal Preparations

Experiments were carried out according to a protocol approved by the Institutional Animal Care Committee of the University of California, San Diego. Sprague-Dawley rats (weight, 250–300 g; Harlan Industries, Indianapolis, IN) were implanted with chronic lumbar intrathecal catheters during halothane (2%) anesthesia according to a modification of the method described by Yaksh and Rudy.¹³ An 8.5-cm polyethylene (PE-10; Clay Adams, Parsippany, NJ) catheter was advanced caudally through an incision in the atlanto-occipital membrane to the thoracolumbar level of the spinal cord. The external part of the catheter was tunneled subcutaneously to exit on top of the skull and plugged with a steel wire. Rats with normal motor function and behavior were used 5–7 days after surgery. Every rat with normal motor function was used three times at an interval of 5–7 days.

Drugs and Injection

Drugs for intrathecal injection were dissolved in solvent such that 10 μ l contained the desired quantity of the agent. Morphine (morphine sulfate, opioid agonist; Merck, Sharpe and Dohme, West Point, PA), ACEA 2085 (competitive AMPA antagonist; CoCensys, Inc., Irvine, CA), and AP-5 ((\pm)-2-amino-5-phosphonopentanoic acid, NMDA antagonist; Research Biochemical International, Natick, MA) were dissolved in normal saline, and ACEA 1021 (5-nitro-6,7-dichloro-2,3-quinoxaline dione, non-strychnine-sensitive NMDA glycine site antagonist; Eagle-Picher Industries, Lenexa, KS) was dissolved in vehicle (tris buffer). After intrathecal drug injection, the catheter was flushed by the subsequent injection of 10 μ l of normal saline (saline, morphine, ACEA 2085, and AP-5

groups) or vehicle (vehicle and ACEA 1021 groups). A microinjector syringe was used for all injections. In each dose group, 10 rats randomly received one of these doses of morphine (1 μ g, 3 μ g, 10 μ g, or 30 μ g), ACEA 1021 (2.4 μ g, 8 μ g, 12 μ g, or 24 μ g), ACEA 2085 (0.1 ng, 10 ng, 500 ng, or 750 ng), AP-5 (1 μ g, 3 μ g, 10 μ g, or 30 μ g), saline or vehicle. The saline and vehicle groups were the control groups.

Nociceptive Test

All animals were tested for their acute nociceptive response using a hot-plate test. The rats were placed on a surface maintained at $52.0 \pm 0.5^\circ\text{C}$ and enclosed by Plexiglass walls. The behavioral endpoint was taken as licking of one hind paw or less frequently, the jumping off of the plate. The cut-off time in the absence of a response was 60 s to prevent tissue injury.

Behavioral and Motor Function Test

The general behavior (including agitation and allodynia), motor function, flaccidity, pinna reflex, and corneal reflex were examined. The former two were scored as follows: 0, normal; 1, slight deficit; 2, moderate deficit; 3, severe deficit. The reflexes were judged as presence or absence. The presence of allodynia was examined by looking for agitation (escape or vocalization) evoked by lightly stroking the flank of the rat with a small probe. The stimulus was sufficient to move hair but not dent the skin. Motor function was evaluated by the placing/stepping reflex and the righting reflex. The former was evoked by drawing the dorsum of either hind paw across the edge of the table. The latter was assessed by placing the rat horizontally with its back on the table, which normally gives rise to an immediate, coordinated twisting of the body to an upright position. Flaccidity was judged as a muscle weakness. Pinna and corneal reflexes were examined with a paper string.

Experimental Paradigm

Dose Effect Relationships. The first series of experiments was performed to determine the dose-dependency and time course of the analgesic actions of intrathecally administered opioid agonist, NMDA antagonist, NMDA glycine site antagonist, and AMPA antagonist on acute thermal nociception. The hot-plate test, behavioral test, and motor function test were performed before and at intervals of 15 min, 30 min, 60 min, 90 min, 120 min, and 180 min after the injection.

To investigate the interaction between the spinal opioid agonist and spinal agents, two paradigms were used.

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As will be discussed, neither the NMDA antagonist nor the NMDA-glycine site antagonist produced a greater than 50% effect; accordingly, the interaction studies examined the maximum usable dose of AP-5 or ACEA 1021 coadministered with various doses of morphine. For the interaction between morphine and the AMPA antagonist, ACEA 2085, an isobolographic analysis¹⁴ was used. The method is based on comparisons of dose ratios that are determined to be equieffective. From the dose-response curves of the agents alone, the respective ED₅₀ values are determined. Subsequently, a dose-response curve is obtained by coadministration of the two drugs in a constant dose ratio based on the ED₅₀ values of the single agents. From the dose-response curve of the combined drugs, the ED₅₀ value of the total dose of the mixture was calculated. To determine whether the effects of the combined administration of morphine and ACEA 2085 depend on the μ receptor, naloxone, 90 μ g, in 300 μ l saline was injected intraperitoneally 5 min before the intrathecal administration.

Data Analysis and Statistics

Response latency (s) data from hot plate measurements were converted to a percentage of the maximum possible effect (%MPE) according to the formula: %MPE = [(postdrug latency - baseline latency)/(cut-off time - baseline latency)] \times 100. ED₅₀ was calculated by a computer program, which is made in our laboratory as a dose that produces a value of 50% MPE.

Data were expressed as mean \pm SEM. The differences between doses were analyzed with one-way analysis of variance (ANOVA) followed by a Fisher's Protected Least Significant Difference test.

To obtain a value for describing the magnitude of the interaction between morphine and ACEA 2085, a total dose fraction value was calculated as follows¹⁵: [(ED₅₀ dose in combination of drug 1)/(ED₅₀ value for drug 1 given alone)] + [(ED₅₀ dose in combination of drug 2)/(ED₅₀ value for drug 2 given alone)]. Fractional values indicate what portion of the single ED₅₀ value was accounted for by the corresponding ED₅₀ value for the combination. Values near 1 indicate an additive interaction; values greater than 1 imply an antagonistic interaction, whereas values less than 1 indicate a synergistic interaction. To compare the theoretical additive point with experimentally derived ED₅₀, isobolographic analysis using the ED₅₀ for maximal effect was used. A *P* value less than 0.05 was considered statistically significant.

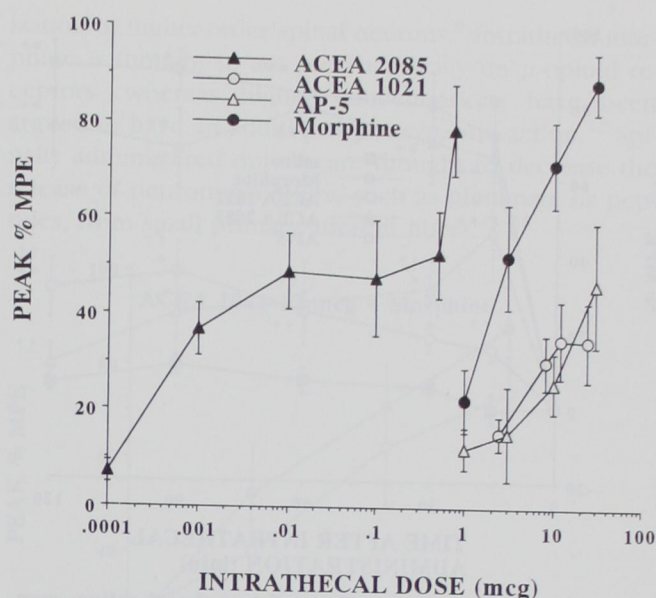


Fig. 1. Dose-response curves of peak %MPE for intrathecal morphine (opioid agonist), AP-5 (NMDA antagonist), ACEA 1021 (glycine site antagonist), and ACEA 2085 (AMPA antagonist). Each point represents the mean \pm SEM of 10 animals.

Results

Effects of Morphine and Glutamate Antagonists

Baseline latency was 6.8 ± 0.1 s (SEM; range, 5.5–8.0 s). Intrathecal administration of morphine, AP-5 (NMDA antagonist), ACEA 1021 (NMDA glycine site antagonist), and ACEA 2085 (competitive AMPA antagonist) resulted in dose-dependent increases in the thermal response latency (fig. 1). The ED₅₀ of morphine was 2.0 μ g (95% confidence interval [CI], 1.0–4.2 μ g), and that of ACEA 2085 was 5.6 ng (95% CI, 2.1–15.4 ng). The equivalent dose ratio was 357:1 for morphine and ACEA 2085. The ED₅₀ of AP-5 and ACEA 1021 could not be obtained at the maximum usable dose. The rank order of potency was ACEA 2085 > morphine > ACEA 1021 = AP-5 (fig. 1). Figure 2 shows the time-effect curves of each drug (usable maximum dose).

Interaction between Morphine and AMPA Antagonist

Coadministration of morphine and ACEA 2085 intrathecally showed a significant increase in the thermal response latency compared with morphine or ACEA 2085 alone (fig. 3). The experimentally obtained ED₅₀ of the combination of morphine and ACEA 2085 was morphine, 0.4 μ g, with ACEA 2085, 1.1 ng. These doses were significantly lower than the theoretical additive

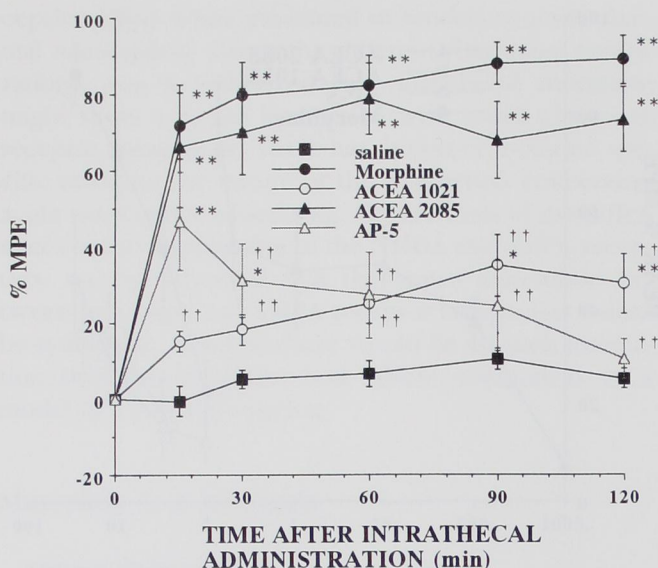


Fig. 2. Time course of the effects of intrathecal saline; morphine, 30 μ g (opioid agonist); AP-5, 30 μ g (NMDA antagonist); ACEA 1021, 24 μ g (glycine site antagonist); and ACEA 2085, 750 ng (AMPA antagonist). These doses were the usable maximum doses. Each point represents the mean \pm SEM of 10 animals. * P < 0.05 versus saline, ** P < 0.01 versus saline, † P < 0.05 versus morphine, †† P < 0.01 versus morphine.

doses (morphine, 1 μ g, with ACEA 2085, 2.8 ng). The total fractional dose value was calculated to be 0.39, which indicates a synergistic interaction. Pretreatment with intraperitoneal naloxone inhibited the increase in the thermal response latency by morphine plus ACEA 2085 (fig. 4).

Interaction between Morphine and NMDA Antagonists

Isobologram could not be used for the interaction study between morphine and ACEA 1021 or AP-5 because these two NMDA antagonists did not show the analgesic effects of more than 50%.

ACEA 1021 (NMDA glycine site antagonist) and AP-5 (NMDA antagonist) showed no shift in the morphine dose-effect curve, indicating no interaction with morphine (fig. 5).

Behavior and Motor Function

In all drug-treated groups, agitation or allodynia and motor disturbance (tested by the placing/stepping reflex and the righting reflex) increased with higher doses (table 1). Flaccidity occurred in rats that received ACEA 2085 at 10 ng or more and in those with AP-5, 30 μ g. Intrathecal morphine, 30 μ g, and ACEA 1021, 12 μ g or

more, induced the loss of pinna reflex. No rats showed loss of corneal reflex in the present study.

In the combination drug studies, dose combinations of morphine and ACEA 2085 displayed fewer side effects than when equianalgesic doses of the agents were administered alone (fig. 6).

Discussion

In the present study, the opioid agonist (morphine) and AMPA antagonist (ACEA 2085), but not NMDA antagonist (AP-5) or NMDA glycine site antagonist (ACEA 1021), produced a dose-dependent increase in the thermal escape latency. Morphine and the AMPA antagonist, but not the NMDA antagonists, showed a potent synergistic antinociception, with the combination dosage showing a decreased side effect profile compared with equianociceptive doses of either agent given alone.

Acute thermal nociception, measured by such techniques as the hot-plate test, is believed to be mediated by the monosynaptic excitation in the spinal cord evoked by the stimulation of primary afferents.¹⁶ Consistent with the belief that the NMDA receptor is not located postsynaptic to primary afferent input,¹⁶ NMDA antago-

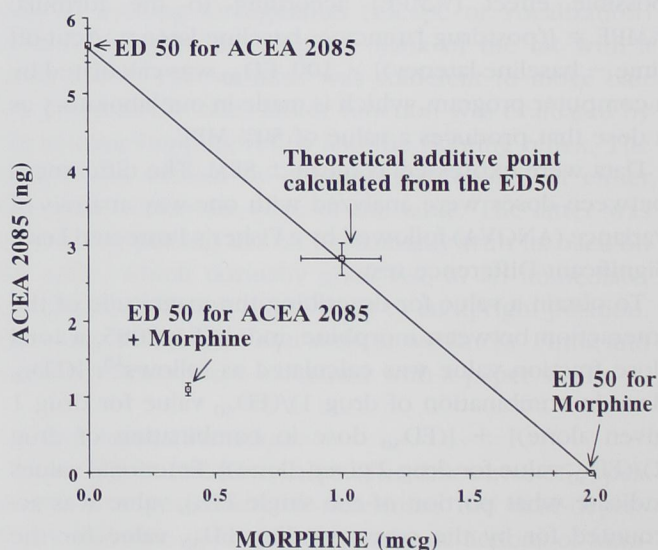


Fig. 3. Isobolograms for the interaction of intrathecal morphine and ACEA 2085. The ED₅₀ values for the single agents are plotted on the x and y axes. Horizontal and vertical bars indicate SEM. The oblique line between the x axis and y axis is the theoretical additive line. The point in the middle of this line is the theoretical additive point calculated from the ED₅₀ values and their variance. The experimental point lies interior to the line of additivity line, indicating a significant synergy. Calculation of the fractional dose value of the combination gave a value of 0.39, indicating a significant synergy. (See text for details.)

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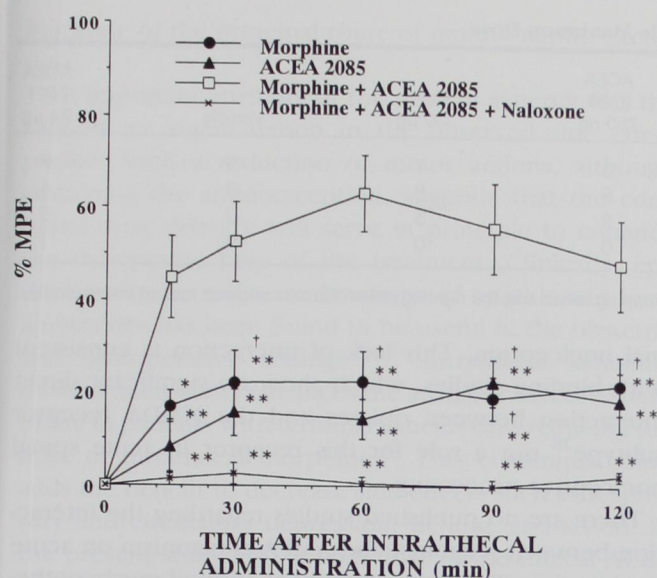


Fig. 4. Time course of the effects on hot-plate response latency (expressed as the % of the maximum possible effect) of intrathecal morphine (1 μ g), ACEA2085 (2.8 ng), morphine (1 μ g) + ACEA 2085 (2.8 ng), and morphine (1 μ g) + ACEA 2085 (2.8 ng) with pretreatment of naloxone (90 μ g, intraperitoneally). Each point represents the mean \pm SEM of 10 animals. * P < 0.05 versus morphine + ACEA 2085, ** P < 0.01 versus morphine + ACEA 2085, † P < 0.05 versus morphine + ACEA 2085 + naloxone

nists have been reported to have few or no selective antinociceptive effects on acute nociception.^{17,18} As in the present work, NMDA receptor antagonists do not produce a behaviorally defined analgesia until doses are used that result in evident motor dysfunction,^{18,19} typically flaccidity. Occupancy of the glycine site by glycine is believed necessary to generate the allosteric interaction, which permits efficient activation of the NMDA channel by glutamate. Accordingly its functional properties will resemble those of an NMDA antagonist. Thus, not surprisingly, it serves to block facilitated processing^{12,20} but has little effect on acute nociception.

The AMPA receptor may be involved in mediating the acute excitation evoked by high-intensity stimuli in the dorsal horn. Thus acute stimuli serve to release glutamate, which may then act on the AMPA receptor.¹¹ Intrathecal injection of AMPA/kainate receptor antagonists produced dose-dependent antinociception on the thermal tail withdrawal reflex test in rats.²¹ Accordingly in the present study, ACEA 2085 (an AMPA antagonist) had a significant effect on acute thermal nociception.

The spinal antinociceptive mechanism of opioids is believed to be a presynaptic inhibition of unmyelinated primary afferent terminal excitability and a hyperpolar-

ization of higher-order spinal neurons.⁹ Intrathecal morphine is thought to act presynaptically on μ -opioid receptors, whereas higher concentrations have been argued to have an additional postsynaptic action.²² Spinally administered opioids are thought to decrease the release of neurotransmitters, such as glutamate or peptides, from small primary afferent fibers.²³⁻²⁵

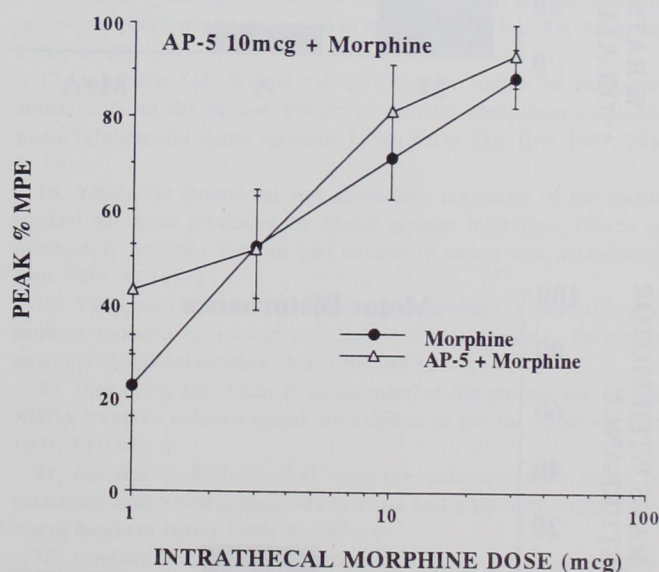
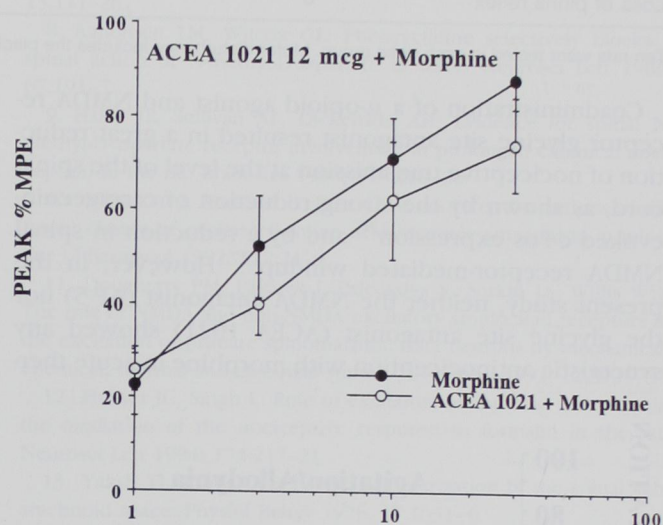


Fig. 5. Interaction (as measured by the hot-plate response latency (expressed as the % of the maximum possible effect) between morphine and ACEA 1021 (upper) or AP-5 (lower). Both ACEA 1021 and AP-5 showed no synergistic effects. Each point represents the mean \pm SEM of 10 animals.

Table 1. Number of Rats That Showed Each Side Effect with the Usable Maximum Dose

| | Saline | Morphine 30 μ g | ACEA 2085 750 ng | AP-5 30 μ g | Vehicle | ACEA 1021 24 μ g |
|------------------------|--------|------------------------|------------------------|--------------------|---------|----------------------------|
| Agitation or allodynia | 2 | 4 | 3 | 1 | 5 | 7 |
| Motor disturbance | 0 | 2 | 8 | 8 | 0 | 4 |
| Flaccidity | 0 | 0 | 8 | 3 | 0 | 0 |
| Loss of pinna reflex | 0 | 2 | 0 | 0 | 0 | 1 |

Ten rats were tested in each dose group. Motor disturbance includes the placing/stepping reflex and the righting reflex. No rats showed loss of corneal reflex.

Coadministration of a μ -opioid agonist and NMDA receptor glycine site antagonist resulted in a great reduction of nociceptive transmission at the level of the spinal cord, as shown by the strong reduction of carrageenin-evoked c-Fos expression²⁶ and by a reduction in spinal NMDA receptor-mediated windup.²⁷ However, in the present study, neither the NMDA antagonist (AP-5) nor the glycine site antagonist (ACEA 1021) showed any synergistic antinociception with morphine in acute ther-

mal nociception. This lack of interaction is consistent with binding studies, which show no significant direct interaction between opiates and the NMDA receptor subtype²⁸ nor a role for this receptor in acute spinal nociceptive processing.

There are no published studies regarding the interaction between μ -agonist and AMPA antagonism on acute nociception. The present study confirmed our hypothesis that μ -agonist and AMPA antagonism show synergistic analgesia on acute thermal nociception. These synergistic effects were suppressed by naloxone, which suggests that the synergism is mediated by the opioid receptor. There are no published data on whether the AMPA antagonist alters the interaction of morphine with its receptor, although there is no *a priori* reason to suspect such an interaction. Why naloxone completely blocked the combined effect of morphine and ACEA 2085 could not be drawn in this study. Further study is necessary to elucidate the reason using the combination of naloxone and ACEA 2085 and that of AMPA and ACEA 2085 or morphine.

Several classes of antinociceptive agents, such as the δ -agonist,²⁹ midazolam,³⁰ cholinesterase inhibitors,³¹ α_2 -agonist (ST-91),³² have been shown to possess a synergistic interaction with morphine. A variety of mechanisms have been proposed to underlie synergistic antinociceptive interactions between drugs in the spinal cord.³²⁻³⁴ Synergistic interactions between μ -opioid and δ -opioid receptor agonists may be explained by allosteric interactions between opioid receptor subtypes that result in increased agonist affinities.³⁵ Opioid and α_2 -adrenoceptors may also coexist on individual neurons and share second messenger mechanisms.^{36,37} Functional interactions may result from distinct drug effects at separate anatomic sites that may act independently as well as multiplicatively to inhibit spinal nociceptive processing.³⁸ In the present study, we believe a likely explanation of the potent synergy reflects the joint effects on release produced by morphine from small afferents and the reduction in the postsynaptic excitation by the

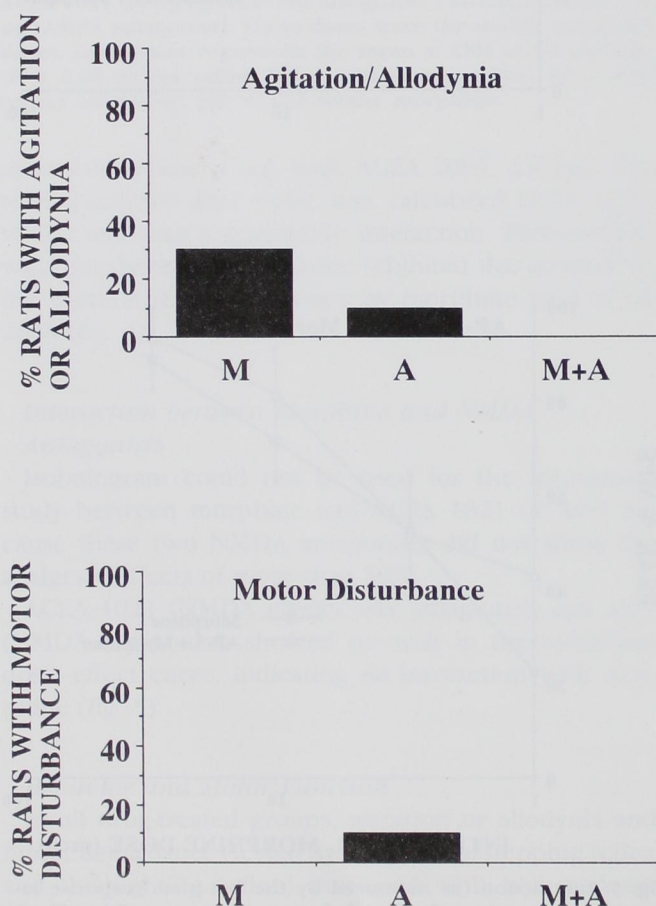


Fig. 6. Side effects of intrathecal morphine, 1 μ g (M); ACEA 2085, 2.8 ng (A); and morphine, 1 μ g, + ACEA 2085, 2.8 ng (M + A). Total number of rats administered each drug was 10.

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blockade of the principal route of monosynaptic excitation.

An important attribute of the present synergy was the lack of an augmentation in the observed side effect profile. Such a reduction of motor actions, although sustaining the antinociception, suggests that the combined drug delivery will serve in principle to enhance the therapeutic ratio of the treatment. Clinically, epidural analgesia with combinations of opioids and local anesthetics has been found to be useful in the obstetric and postoperative settings.^{39,40} Intrathecal ketamine (NMDA antagonist) enhances the analgesic effect of morphine in patients with terminal cancer, thus reducing the dose of intrathecal morphine.⁴¹ This coadministration adds the benefit to decrease side effects such as respiratory and circulatory depression, itching, and so on. In the present study also, a high dose of intrathecal ACEA 2085 (AMPA antagonist) also showed respiratory depression, flaccidity, and motor disturbance. These supraspinal side effects might affect the measurement of analgesic effect. However, rats were not completely paralyzed. Therefore we could test an analgesic effect in those rats. Coadministration of morphine and the AMPA antagonist decreased side effects (agitation or allodynia, motor disturbance, flaccidity) with a sustained analgesia. In the present study, we could not see respiratory depression by intrathecal morphine. Regarding itching, pruritus, and nausea, we could not test these side effects in this study.

In conclusion, intrathecal administration of morphine and an AMPA antagonist induced a significant analgesia on acute thermal nociception, and their codelivery revealed a potent synergy without an increase in the side effect profile. These results suggest an important direction for development of acute pain strategies may focus on the AMPA receptor in the spinal cord.

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