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Interaction between intrathecal morphine and glutamate receptor antagonists in formalin test [1]

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Abstract

The analgesic interaction between intrathecally administered morphine and the NMDA receptor antagonist, ((±)-2-amino-5-phosphonopentanoic acid; AP-5), the NMDA receptor glycine site antagonist, (5-nitro-6,7-dichloro-2,3-quinoxaline dion; ACEA 1021), or the AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor antagonist (ACEA 2752) in the formalin test was investigated with a rat model of chronic lumbar intrathecal catheterization. After obtaining dose–response curves for each agent, combinations of morphine and AP-5, ACEA 1021 or ACEA 2752 were tested for their effect on the number of flinches in the formalin test and for associated side-effects, such as motor disturbances, flaccidity, and agitation/allodynia. Using isobolographic analyses, a potent analgesic synergy was observed with decreased side-effects between morphine and ACEA 2752 or AP-5. ACEA 1021 increased the analgesic effect of low-dose morphine. Spinal μ-opioid receptor activation and NMDA or AMPA receptor antagonism showed a synergistic antinociception against tonic pain. These results suggest an important direction in the management of inflammatory pain.

Keywords: Analgesia; NMDA receptor; AMPA receptor; Morphine; Spinal cord; (Rat)

1. Introduction

The excitatory amino acid neurotransmitters have been demonstrated to play a prominent role in spinal nociceptive transmission (Raigorodsky and Urca, 1987; Dickenson and Aydar, 1991). The excitatory effect of glutamate is thought to be mediated by NMDA receptors (Mori and Mishina, 1995) and non-NMDA receptors [mainly AMPA receptors] (Honoré et al., 1988; Long et al., 1990). The NMDA receptor is not postsynaptic to the primary afferent. It is postsynaptic to an interneuron mediating a polysynaptic excitation that is responsible for the spinal nociceptive processing that results in a central facilitatory state induced by ongoing C fiber input (Yaksh, 1993). The AMPA receptor mediates monosynaptic fast excitatory transmission involving both innocuous and acute nociceptive input (Headley and Grillner, 1990). The putative strychnine-insensitive glycine site (glycine-2-type receptor) on the NMDA receptor complex is believed to facilitate excitatory transmission.

The use of selective antagonists has suggested that these receptors have a different role in the processing of nociception. NMDA receptor antagonists have minimal effects upon acute nociception, but appear to block tonic pain processing (Haley et al., 1990; Näsström et al., 1992). In contrast, AMPA receptor antagonists have an analgesic effect on acute nociception (Dougherty et al., 1992; Hunter and Singh, 1994). This is supported by the finding that in the formalin test in rats, NMDA receptor antagonists and non-strychnine-sensitive glycine site antagonists attenuated only the tonic second phase, while AMPA receptor antagonists inhibited the acute phase (Hunter and Singh, 1994).

Morphine, a μ-opioid receptor agonist, can exert an action both pre- and post synaptic to the primary afferent and is well known to have an antinociceptive effect on acute pain (Yaksh and Rudy, 1976) and on tonic pain (Yamamoto and Yaksh, 1992).

Combining the two agents acting on the different receptors, we hypothesized that intrathecal morphine might

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show a potent interaction with spinal glutamate receptor antagonists. In our previous series of experiments, spinal interaction between morphine and AMPA receptor antagonists was synergistic, whereas there was no such interaction between morphine and NMDA receptor antagonists in a rat model of acute thermal nociception (Nishiyama et al., 1998). The purpose of this study was to investigate the hypothesis that intrathecal morphine might have synergistic antinociceptive effects with both NMDA and AMPA receptor antagonists on tonic pain, using a rat model and the formalin test.

A biphasic nociceptive response to formalin has been widely accepted as a model of prolonged noxious stimulation and is characterized by both behavioral (Wheeler-Aceto and Cowan, 1991) and electrophysiological measures (Haley et al., 1990). It consists of an acute phase (0–5 min) involving direct activation of monosynaptic primary afferent input into the superficial laminae of the spinal cord which, following a quiescent period of 5–10 min, appears to trigger a subsequent polysynaptic response involving sensitization of the deeper laminae dorsal horn neurons and manifested in the behavioral model as a more prolonged, tonic phase of activity, lasting up to approximately 60 min. The tonic phase is also accompanied by the onset of a persistent inflammatory response that lasts beyond the cessation of the nociceptive response (Tjolsen et al., 1992).

2. Materials and methods

2.1. Animal preparations

Experiments were carried out according to a protocol approved by the Institutional Animal Care Committee of the University of California, San Diego. Male Sprague–Dawley rats (250–300 g; Harlan Industries, Indianapolis, IN) were implanted with chronic lumbar intrathecal catheters under halothane (2%) anesthesia according to a modification of the method described by Yaksh and Rudy (1976). Briefly, 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 to 7 days after surgery. They were used three times, with an interval of at least 5 days, for behavioral study but only once for the formalin test. One hundred rats were used for behavioral study and 240 rats were used for the formalin test.

2.2. Drugs and injection

Drugs for intrathecal injection were dissolved in a solvent such that 10 μl contained the desired quantity of the agent. Morphine (morphine sulfate, opioid receptor agonist; Merck, Sharpe and Dohme, West Point, PA), AP-5 ((±)-2-amino-5-phosphonopentanoic acid, NMDA receptor antagonist; Research Biochemical International, Natick, MA), and ACEA 2752 (AMPA receptor antagonist; CoCensys, Irvine, CA) were dissolved in normal saline. ACEA 1021 (5-nitro-6,7-dichloro-2,3-quinoxaline dion, non-strychnine-sensitive NMDA receptor 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 μl of normal saline or vehicle. A micro injector syringe was used for all injections. In each dose group, 8 rats for the formalin test and 10 rats for behavioral study randomly received one of these doses of morphine (1, 3, 10, 30 μg), AP-5 (1, 3, 10, 30 μg), ACEA 1021(2.4, 8, 12, 24 μg), ACEA 2752 (0.1, 1, 50, 100 μg), saline or vehicle.

2.3. Formalin test

The formalin test was carried out as described by Malmberg and Yaksh (1992). Rats were anesthetized with 3% halothane until a transient loss of spontaneous movement was observed. Rats were then quickly removed from the anesthesia box and 50 μl of 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. Immediately after injection, the rat was placed in an open Plexiglas chamber and observed for 60 min. Pain behavior was quantified from the number of spontaneous flinches/shaking of the injected paw at 1–2 min, 5–6 min and at 5 min intervals during 10–60 min after formalin injection. The animals were then killed with an overdose of barbiturate (intraperitoneal injection of 100 mg/kg Beuthanasia, Schering-Plough Animal Health, Kenilworth, NJ). As previously described (Malmberg and Yaksh, 1992), two distinct phases were observed: phase 1, during the 0–6 min interval after injection, and phase 2, beginning approximately 10 min after injection.

2.4. Behavioral and motor function test

General behavior (including agitation and allodynia), motor function, flaccidity, pinna reflex, and corneal reflex were examined. These were judged as present or absent. Agitation was judged as spontaneous irritable movement and/or vocalization. The presence of allodynia was determined 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 watching 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 muscle weakness. Pinna and corneal reflexes were examined by application of a small flexible probe.

2.5. Experimental design

The first series of experiments were performed to determine the dose-dependence and time course of the analgesic actions of each intrathecally administered agent in the formalin test. The formalin-evoked flinching responses were assessed over 60 min after formalin injection. Behavioral and motor function tests were performed before and at intervals of 15, 30, 60, 90, 120, and 180 min after intrathecal drug injection in a separate group of animals.

To investigate the interaction, a fixed dose of ACEA 1021 was coadministered with various doses of morphine because ACEA 1021 did not itself produce a response to give an ED_{50}. Isobolographic analysis (Tallarida et al., 1989) was used for the interaction between morphine and AP-5 or ACEA 2752. The method is based on comparisons of dose ratios that are determined to be equieffective. The dose–response curves of the agents alone are used to determine the respective ED_{50} values. Subsequently, a dose–response curve is obtained by coadministration of the two drugs in a constant dose-ratio based on the ED_{50} values of the single agents (1/2, 1/4, 1/8, and 1/16 ED_{50}). The ED_{50} value of the total dose of the mixture is calculated from this dose–response curve. Behavioral and motor function tests were also performed.

2.6. Data analysis and statistics

The number of flinches was calculated as a percentage of the control (saline or vehicle). Dose response curves were obtained using the maximum values for each dose. The first and second phase data were examined separately. ED_{50} and confidence intervals were calculated using a linear expression program.

Data were expressed as means ± standard error (S.E.M.). The differences between groups were analyzed with a two-way repeated measures analysis of variance (ANOVA) followed by the Contrasts as a post hoc test.

To obtain a value for describing the magnitude of the interaction between morphine and AP-5 or ACEA 2752, a total dose fraction value was calculated as follows (Roerig and Fujimoto, 1988): [(ED_{50} dose of drug 1 in combination)/(ED_{50} value for drug 1 given alone)] + [(ED_{50} dose of drug 2 in combination)/(ED_{50} value for drug 2 given alone)]. Fractional values indicate what portion of the single ED_{50} value was accounted for by the corresponding ED_{50} value for the combination. Values near 1 indicate an additive interaction, values greater than 1 imply an antagonistic interaction, while values less than 1 indicate a synergistic interaction. To compare the theoretical additive point with experimentally derived ED_{50}, isobolographic analysis was used. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of morphine and glutamate antagonists

Intrathecal administration of morphine, AP-5 (NMDA receptor antagonist), and ACEA 2752 (AMPA receptor antagonist) resulted in a dose-dependent decrease in the number of flinches in both phases 1 and 2 of the formalin test, although ACEA 1021 (NMDA receptor glycine site antagonist) did not decrease the flinches (Figs. 1 and 2). The ED_{50}s were: 7.1 μg [95% confidence interval (CI): 4.6–13.5 μg] in phase 1, 3.6 μg (95% CI: 1.3–9.5 μg) in

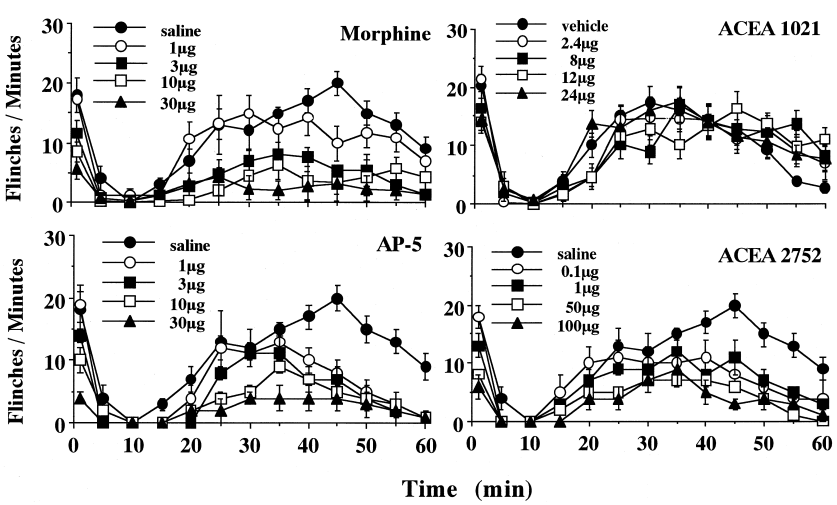

Fig. 1. Time course of the effect (mean flinches per min) for intrathecal morphine (opioid receptor agonist), AP-5 (NMDA receptor antagonist), ACEA 1021 (NMDA receptor glycine site antagonist), and ACEA 2752 (AMPA receptor antagonist). Each point presents the mean ± S.E.M. for 8 animals. Morphine, AP-5, and ACEA 2752, but not ACEA 1021 showed dose-dependent decreases in flinches in both phase 1 (1–5 min) and phase 2 (10–60 min).

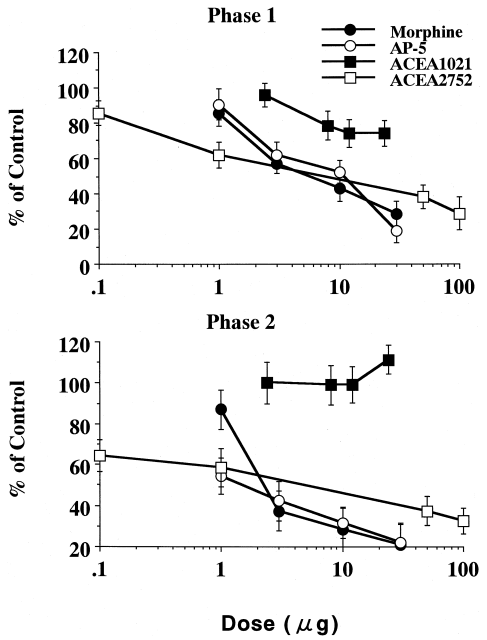

Fig. 2. Dose–response curves for intrathecal morphine (opioid receptor agonist), AP-5 (NMDA receptor antagonist), ACEA 1021 (NMDA receptor glycine site antagonist), and ACEA 2752 (AMPA receptor antagonist). Each point represents the mean ± S.E.M. for 8 animals. ACEA 1021 could not decrease the flinches to less than 50% of the control (saline).

phase 2 for morphine, 7.6 μg (95% CI: 5.3–12.5 μg) in phase 1, 1.4 μg (95% CI: 0.1–12.5 μg) in phase 2 for AP-5, 7.2 μg (95% CI: 4.3–16.4 μg) in phase 1 and 3.1 μg (95% CI: 1.6–17.5 μg) in phase 2 for ACEA 2752. The ED_{50} of ACEA 1021 could not be obtained at the maximum usable dose. The rank order of potency was morphine ≥ ACEA 2752 ≥ AP-5 > ACEA 1021 in phase 1 and AP-5 > ACEA 2752 ≥ morphine > ACEA 1021 in phase 2.

3.2. Interaction between morphine and NMDA antagonists

Isobolographic analysis could not be used for the interaction study between morphine and ACEA 1021 (NMDA receptor glycine site antagonist), because ACEA 1021 did not induce analgesic effects of more than 50%. ACEA 1021 increased the efficacy of lower doses of morphine (1 and 3 μg in phase 1, and 1 μg in phase 2) (Fig. 3).

Coadministration of morphine and AP-5 (NMDA receptor antagonist) intrathecally showed a significant decrease in flinches compared to morphine or AP-5 alone (Fig. 4). The experimentally obtained ED_{50} for the combination of morphine and AP-5 were morphine 0.082 μg (95% CI: 0.036–0.185 μg) with AP-5 0.032 μg (95% CI: 0.014–0.072 μg) in phase 1 and morphine 0.022 μg (95% CI: 0.007–0.067 μg) with AP-5 0.009 μg (95% CI: 0.002–0.026 μg) in phase 2. These doses were significantly lower than the theoretical additive doses (morphine 3.55 μg with AP-5 3.8 μg in phase 1 and morphine 1.80 μg with AP-5 0.7 μg in phase 2). The total fractional dose values were

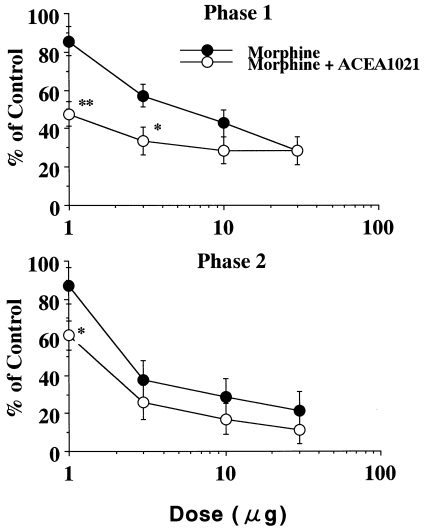

Fig. 3. Interaction [as measured by the number of flinches (expressed as the % of the control)] between morphine and ACEA 1021. Dose–response curves were shifted down in both phase 1 and 2 by addition of ACEA 1021, which means that ACEA 1021 increased the effects of morphine. Each point represents the mean ± S.E.M. for 8 animals. $^{*}P < 0.05$ vs. Morphine, $^{**}P < 0.01$ vs. Morphine.

calculated to be 0.016 in phase 1 and 0.012 in phase 2, which indicate synergistic interactions.

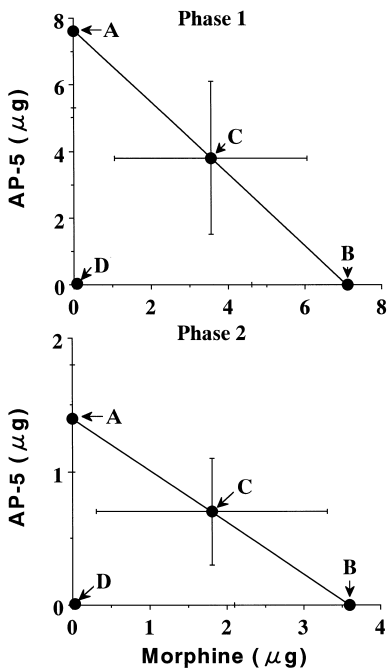

Fig. 4. Isobolograms for the interaction of intrathecal morphine and AP-5 in phase 1 (upper) and phase 2 (lower). The ED_{50} values for the single agents are plotted on the *X* and *Y* axes (A: ED_{50} for AP-5, B: ED_{50} for morphine). Horizontal and vertical bars indicate 95% confidence interval. The oblique line between the *X* axis and *Y* axis is the theoretical additive line. The point in the middle of this line (C) is the theoretical additive point calculated from the ED_{50} values and their variance. The experimental ED_{50} point (D) lies interior to the line of additivity in both phases 1 and 2, indicating synergy. Calculation of the fractional dose values of the combination gave the values 0.016 in phase 1 and 0.012 in phase 2, indicating synergy (see text for details).

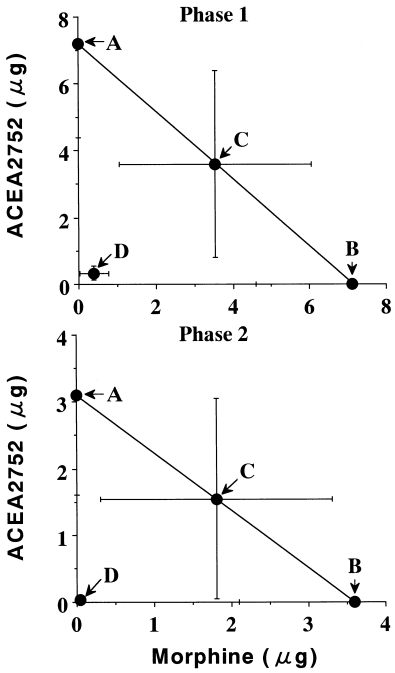

Fig. 5. Isobolograms for the interaction of intrathecal morphine and ACEA 2752 in phase 1 (upper) and phase 2 (lower). The ED_{50} values for the single agents are plotted on the *X* and *Y* axes ((A) ED_{50} for ACEA 2752, (B) ED_{50} for morphine). Horizontal and vertical bars indicate 95% confidence interval. The oblique line between the *X* axis and *Y* axis is the theoretical additive line. The point in the middle of this line (C) is the theoretical additive point calculated from the ED_{50} values and their variance. The experimental ED_{50} point (D) lies interior to the line of additivity in both phase 1 and 2, indicating synergy. Calculation of the fractional dose values of the combination gave the values 0.102 in phase 1 and 0.019 in phase 2, indicating synergy (see text for details).

3.3. Interaction between morphine and AMPA receptor antagonist

Coadministration of morphine and ACEA 2752 intrathecally also showed significant decreases in flinches compared to morphine or ACEA 2752 alone (Fig. 5). The experimentally obtained ED_{50} for the combination of morphine and ACEA 2752 were morphine 0.397 μg (95% CI: 0.016–0.85 μg) with ACEA 2752 0.332 μg (95% CI: 0.016–0.61 μg) in phase 1 and morphine 0.035 μg (95% CI: 0.005–0.097 μg) with ACEA 2752 0.03 μg (95% CI: 0.001–0.028 μg) in phase 2. These doses were significantly lower than the theoretical additive doses (morphine 3.55 μg with ACEA 2752 3.6 μg in phase 1 and morphine 1.80 μg with ACEA 2752 1.55 μg in phase 2). The total fractional dose values were calculated to be 0.102 in phase 1 and 0.019 in phase 2, which indicate synergistic interactions.

3.4. Behavior and motor function

Agitation and/or allodynia was observed at 1 μg morphine (20% of rats), 2.4 μg ACEA 1021 (50% of rats), 3 μg AP-5 (10% of rats), and 1 ng ACEA 2752 (20% of rats) and increased with higher doses. Motor disturbance (tested by the placing/stepping reflex and the righting reflex) was observed at 30 μg morphine (20% of rats), 12 μg ACEA 1021 (20% of rats), 3 μg AP-5 (10% of rats), and 100 μg ACEA 2752 (20% of rats) and increased with higher doses. Flaccidity occurred in rats that received AP-5 30 μg. Intrathecal morphine 30 μg and ACEA 1021 12 μg and 24 μg induced loss of the pinna reflex. No rat showed a loss of corneal reflex in the present study.

In the combination drug studies, the combinations of morphine with ACEA 1021, AP-5 or ACEA 2752 caused fewer side-effects than when equianalgesic or less analgesic doses of the agents were administered alone (Table 1).

4. Discussion

In the present study, the opioid receptor agonist, (morphine), NMDA receptor antagonist, (AP-5) and AMPA receptor antagonist, (ACEA 2752), but not the NMDA receptor glycine site antagonist, (ACEA 1021), produced a

Table 1
Behavioral and motor effects of intrathecal morphine and glutamate antagonists
The number of rats which showed agitation and/or allodynia, loss of pinna reflex, motor disturbance, or flaccidity is shown on the table as a percentage (%) of total rats ($n = 10$).

	Agitation and/or Allodynia	Loss of pinna reflex	Motor disturbance	Flaccidity	Analgesic effects[a] (% of control)	
					Phase 1	Phase 2
Morphine (30 μg)	40	20	20	0	28.6	21.1
ACEA 1021 (24 μg)	70	10	40	0	73.9	111.3
AP-5 (30 μg)	10	0	80	30	19.0	22.0
ACEA 2752 (100 μg)	20	0	20	0	28.6	32.5
Morphine (30 μg) + ACEA 1021 (12 μg)	40	20	0	0	28.6	11.4
Morphine (3.6 μg) + AP-5 (1.4 μg)	10	0	0	0	4.8	0
Morphine (1.8 μg) + ACEA 2752 (1.5 μg)	10	0	0	0	28.6	7.3

[a]Analgesic effect on phase 1 and phase 2 of the formalin test is shown as % of the number of flinches against the control ($n = 8$), 100: no analgesic effect, 0: complete analgesia.

dose dependent decrease in the number of flinches in both phases in the formalin test. The NMDA receptor antagonist and AMPA receptor antagonist showed a potent synergistic antinociception with morphine in both phases of the formalin test. This enhancement occurred without an augmented side-effect. The NMDA glycine site antagonist increased the analgesic effect of low dose morphine with decreased side-effects.

4.1. NMDA receptor antagonists

NMDA receptor antagonists produce antinociceptive effects when noxious inputs are tonically active and produce hyperexcitability in the dorsal horn (Dickenson and Aydar, 1991). Spinal glutamate receptors do not appear to be involved in the mediation of responses to innocuous and noxious mechanical stimuli applied under normal conditions. They appear to be important in the generation of inflammation-evoked hyperexcitability of spinal cord neurons (Neugebauer et al, 1994) or pain behaviors in a rat postoperative pain model (Zahn and Brennan, 1998). Intrathecal administration of NMDA receptor antagonists will attenuate nociceptive responses in both phases of the formalin test (phase 2 is more suppressed than phase 1) in rats (Yamamoto and Yaksh, 1992), which is consistent with the results of the present study, whereas the NMDA receptor antagonist had no marked antinociceptive effects on acute thermal stimulus in our previous study (Nishiyama et al., 1998).

4.2. NMDA receptor glycine site antagonists

Block of NMDA receptor function by inhibition of the coupled glycine site preferentially elicits antinociception against prolonged (chemical) noxious stimulation in the absence of a marked influence on motor coordination (Millan and Seguin, 1994). Thus, we expected an antinociceptive effect of an NMDA receptor glycine site antagonist in the formalin test (Dickenson and Aydar, 1991; Hunter and Singh, 1994). However, the present results showed no antinociceptive effect of ACEA 1021, an NMDA receptor glycine site antagonist in the dose range used. Chapman and Dickenson (1992) also reported that an NMDA receptor glycine site antagonist did not affect facilitated state of pain processing shown as wind-up. Regarding ACEA 1021, it is a selective antagonist at the strychnine-insensitive glycine site of the NMDA receptor at low concentrations. However, high doses of ACEA 1021, such as 10 mg/kg in vivo, may have actions through non-NMDA receptors (Lingenhohl and Pozza, 1998). The doses used in the present study (maximum 24 μg/300 g rat) might have been too small to act on non-NMDA receptors. Therefore, the results of the present study were considered to be effects of NMDA receptor glycine site antagonism. The differences in effects between ACEA 1021 in the present study and other NMDA receptor glycine site antagonists (Dickenson and Aydar, 1991; Hunter and Singh, 1994) might have been due to the different efficacy of the agents used. Twenty-four micrograms of ACEA 1021, which was the maximum dose soluble in the vehicle, might not be enough to induce sufficient effects. However, higher doses were not usable because 24 μg induced motor disturbance in 40% of rats.

4.3. AMPA receptor antagonists

The AMPA receptor may be involved in mediating the acute excitation evoked by high-intensity stimuli in the dorsal horn. Intrathecal injection of AMPA receptor antagonists produced dose-dependent antinociception in the tail flick test (Advokat and Rutherford, 1995) and hot plate test (Nishiyama et al., 1998) in rats, although apparently not against formalin-induced pain (Coderre and Melzack, 1992). The AMPA receptor antagonist did not inhibit the hyperalgesia induced by intraplantar injections of prostaglandin E_2 or carrageenin (Ferreira and Lorenzetti, 1994). Hyperalgesia evoked by an inflammatory stimulus is considered to cause a continuous release of spinal glutamate, which via an NMDA-type receptor, promotes retrograde sensitization of the primary sensory neurons. However, in the present study, an AMPA receptor antagonist, ACEA 2752, could produce dose-dependent analgesia in phase 2 of the formalin test. Our results are consistent with the finding that AMPA/kainate receptor antagonists produce a marked decrease in pain behaviors in a rat model of postoperative pain (Zahn et al., 1998). AMPA/kainate receptors in the spinal cord are thought to be involved in the processing of intensified mechanical stimuli of animals sensitized by injury. The phase 2 response to formalin is initiated by an ongoing barrage, which is produced in phase 1. Therefore, when the phase 1 component of the formalin stimulus is decreased by the AMPA receptor antagonist, the phase 2 response might also be reduced. Thus, both phase 1 and phase 2 responses were dose dependently inhibited by ACEA 2752, an AMPA receptor antagonist in the present study.

4.4. Opioid receptor agonist

Intrathecally administered morphine inhibits presynaptic primary afferent excitability and higher doses have an additional postsynaptic action (Lombard and Besson, 1989). As shown in the present study, intrathecal morphine is effective on both phase 1 and 2 of the formalin test.

4.5. Opioids and NMDA receptor antagonists

Spinally administered opioids are thought to diminish the release of neurotransmitters such as glutamate or peptides from small primary afferent fibers (Dickenson and Sullivan, 1986; Kangra and Randic, 1991; Malmberg and Yaksh, 1995). The ability of opioids to act presynaptically

on C fiber terminals to reduce transmitter release produces synergistic inhibitions with postsynaptically acting NMDA receptor antagonists (Dickenson, 1997). NMDA receptor antagonists significantly increase morphine's antinociceptive effect on thermal stimuli (Plesan et al., 1998). We now showed marked synergistic antinociceptive effects in both phases of the formalin test between the NMDA receptor antagonist and morphine.

4.6. Opioids and NMDA receptor glycine site antagonists

Coadministration of a μ-opioid receptor 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 expression (Honoré et al., 1996) and by inhibition of wind up (Chapman and Dickenson, 1992), while no synergistic interaction was found on acute thermal nociception (Nishiyama et al., 1998). In the present study, although a NMDA receptor glycine site antagonist alone could not inhibit the response to formalin, co-administration of the NMDA receptor glycine site antagonist with low-dose morphine intensified the analgesic effect of morphine. Even an insufficient dose of NMDA receptor glycine site antagonist to block tonic pain processing alone could decrease the activation of NMDA receptor thus adding some analgesic effects to a weak antinociception by low-dose morphine.

4.7. Opioids and AMPA receptor antagonists

There are no other studies of the interaction between μ-opioid receptor agonists and AMPA receptor antagonism in the formalin test nor is there any evidence of the coupling of μ-opioid receptors and AMPA receptors. However, our results suggest a strong functional correlation between the two receptors and the excitation of monosynaptic primary afferents and a polysynaptic response during facilitated processing.

4.8. Clinical application

From a clinical point of view, the drug combinations in the present study appear to enhance the therapeutic ratio of the treatment because they could decrease side-effects at dose combinations which induced a greater effect than did a large dose of either drug alone. Clinically, intrathecal ketamine, (NMDA receptor antagonist), enhances the analgesic effect of morphine in terminal cancer patients, thus reducing the dose of intrathecal morphine (Yang et al., 1996), and also decreasing the side-effects such as respiratory depression, itching, pruritus or nausea. However, ketamine should not be administered intrathecally because of its neurotoxicity (Malinovsky et al., 1991). The neurotoxicity of the agents used in the present study should be investigated to facilitate clinical application of this combination analgesic therapy.

In conclusion, in the formalin test, intrathecal administration of morphine, a NMDA receptor antagonist, and an AMPA receptor antagonist, but not a NMDA receptor glycine site antagonist, induces dose-dependent analgesia. Coadministration of morphine with a NMDA receptor antagonist or an AMPA receptor antagonist induced a potent synergy without an increase in side-effects. The NMDA glycine site antagonist increased the analgesic effect of low-dose morphine. These drug combinations could play a major role in the management of continuous pain.

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