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The Effects of Morphine, MK-801, an NMDA Antagonist, and CP-96,345, an NK1 Antagonist, on the Hyperesthesia Evoked by Carageenan Injection in the Rat Paw

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Background: **The spinal mechanisms underlying the hyperesthetic state during inflammation are little understood. To gain a better understanding of these mechanisms, this study evaluated the effects of intrathecal morphine; MK-801, an N-methyl-D aspartic (NMDA) antagonist; and CP-96,345, an NK1 antagonist, on the hyperesthesia observed after carageenan injection of the rat paw.**

Methods: **In rats injected with 2 mg carageenan, the paw withdrawal latency (PWL) for the injected paw was typically 5–6 s less than that for the untreated paw, at 2 h after the carageenan injection. Drugs were administered 2 h after the carageenan injection. The magnitude of hyperesthesia was evaluated with the difference score (DS), which was calculated by subtracting the PWL of the untreated paw from the PWL of the injected paw.**

Results: **Intrathecal morphine increased PWLs of both the injected and the untreated paws equally in a dose-dependent manner, but intrathecal morphine did not affect the level of DS. Intrathecal MK-801 increased PWLs of the injected paw to the level of the untreated paw in a dose-dependent manner and increased the DS levels. Intrathecal CP-96,345 had no effect on PWLs of either the injected or the untreated paw. Coadministration of MK-801 with morphine reduced the DS for each dose of morphine.**

Conclusions: **These data indicate that (1) an NMDA receptor, but not an NK1 receptor, plays an important role in maintaining the hyperesthesia after carageenan injection; and (2) NMDA antagonism has a simple additive interaction with morphine in the carageenan model of inflammatory hyperesthesia. (Key words: Analgesia, opioid: morphine. Antagonists, NK1: CP-96,345. Antagonists, NMDA: MK-801. Pain, inflammatory: carageenan.)**

AS part of the inflammatory response, the inflamed area displays primary hyperesthesia, whereas secondary hyperesthesia occurs in the area surrounding the inflammation.[1] Primary and secondary hyperesthesia are now thought to be mediated by changes in both the peripheral nervous system and the spinal cord. Inflammation induces the release of chemical mediators, such as bradykinin, histamine, prostaglandins, and substance P (sP), and these mediators cause the sensitization of peripheral nerve endings. In addition to the changes in the peripheral nerve endings, peripheral injuries or localized inflammatory lesions cause a state of central facilitation.[2–4]

Activation of chemosensitive afferents with chemical irritants such as mustard oil also is known to generate a state of central facilitation,[5,6] and this facilitation is blocked by the N-methyl-D aspartic (NMDA) antagonist, MK-801.[7] NMDA antagonists are reported to have no analgesic effect, as defined by standard antinociceptive endpoints such as the hot-plate test.[8,9] This observation is consistent with the report that glutamate acting at the NMDA receptor may play a role in the polysynaptic, but not in the monosynaptic events.[10] Repetitive input from C-fibers can evoke a powerful and spinally mediated facilitation (wind-up) of the dorsal horn wide dynamic range neurons.[11] NMDA antagonists block this wind-up phenomenon, though opioids appear to poorly modulate this central facilitation.[12,13] If hyperesthesia depends on wind-up phenomena of the spinal cells, then it may be prevented by an NMDA antagonist.

It has been reported that an NMDA antagonist, but not morphine or an NK1 antagonist, eliminates the hyperesthetic state induced by peripheral nerve injury when administered intrathecally.[14–16] Carageenan injection into the rat hind paw plantar surface induces edema, hyperthermia, and hyperesthesia, and this carageenan-induced hyperesthesia has been used as an animal model of the hyperesthesia during inflammation.[17–19] The mechanisms underlying the hyperesthesia in the inflammation model may differ from that in the

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neurogenic pain model. For example, the sensitization of peripheral nerve endings is important in the inflammation model, and the spontaneous activity in the axons of the injured nerve is important in the neurogenic pain model.[20] In the present study, to clarify the distinction between the neurogenic pain model and the inflammation model, we studied the effects of intrathecally administered morphine; MK801, an NMDA antagonist; and CP-96,345, an NK1 antagonist, on the hyperesthesia evoked by the rat paw carageenan injection.

In addition, the possibility of enhancement of antinociception by the combination of opioids and NMDA antagonists or NMDA antagonists and NK1 antagonists has been proposed.[21,22] We thus sought to clarify the interaction between opioids and NMDA antagonists or between NMDA antagonists and NK1 antagonists, by defining the effects of intrathecal coadministration of morphine and MK-801 or intrathecal coadministration of MK-801 and CP-96,345 on the hyperesthesia observed following the carageenan injection.

Methods

The following investigations were carried out under a protocol approved by the Institutional Animal Care Committee of the authors' institution. Male Sprague-Dawley rats (250–300 g) were prepared with chronic intrathecal catheters and examined for the effects of agents in the carageenan test.

Intrathecal Catheters

Chronic intrathecal catheters were inserted during isoflurane anesthesia by passing a PE-10 catheter through an incision in the atlanto-occipital membrane to a position 9 cm caudal to the cisterna at the level of the lumbar enlargement.[23] The catheter was externalized on the top of the skull and sealed with a steel wire and the wound closed with 3-0 silk sutures. Animals were allowed to recover for a week before being used experimentally. All animals postoperatively displayed normal feeding and drinking behavior. No infection was found a week after the surgery. Rats showing neurologic deficits postoperatively were discarded.

Carageenan Test

Two milligrams lambda carageenan (Sigma Chemical, St. Louis, MO, C-3889) was injected subcutaneously *via* a 24-G needle in the plantar surface of the right hind paw under isoflurane anesthesia. Lambda carageenan was suspended in normal saline by sonication and was administered in a 0.1-ml injection volume. After recovering from isoflurane anesthesia, the animal was then placed in a plexiglass box, which permitted observation. To a separate group, 0.1 ml saline was injected subcutaneously in the plantar surface of the right hind paw to determine whether subcutaneous saline caused the hyperesthetic state.

Paw edema was estimated as an index of inflammation by measuring the dorsal-plantar paw width with a vernier caliper (to 0.1 mm) before carageenan or saline injection and 2 and 3 h after carageenan or saline injection.

Nociceptive Threshold

The thermal nociceptive threshold was measured with a device similar to that previously reported.[18] The rats were placed in a clear plastic cage (10 × 20 × 24 cm) placed on an elevated floor of clear glass (2 mm thick). A radiant heat source (Eye projector halogen lamp JRC-12V-100W, Iwasaki Electric, Tokyo, Japan) with an aperture diameter of 5 mm was contained in a movable holder placed beneath the glass floor. The voltage to the thermal source was controlled by a constant voltage supply. To reduce the variability in plate surface caused by room temperature, the interior of the box under the animal was prepared with a heat source such that the under-plate temperature was regulated to 30°C. The calibration of the thermal test system was such that the average response latency (±SD) in ten normal untreated rats, measured prior to the initiation of an experimental series, was 10 ± 0.5 s.

To initiate a test, a rat was placed in the box and allowed 5–10 min to acclimate. The halogen lamp was then positioned such that it was focused at the plantar surface of one hind paw, where it was in contact with the glass. Care was taken not to focus the lamp on the skin that was off of the glass plate. The light was then activated, which initiated a timing circuit. The time interval between the application of the light beam and the brisk hind paw withdrawal response was manually measured to the nearest 0.1 s. This value was then assigned as its response latency. Cut-off time in the absence of a response was 20 s.

General Behavior

General behavior was evaluated at each test point during the dose-response study by a scoring system of

two specific behaviors: normal or mildly to severely impaired. (1) Placing/stepping reflex: This response was evoked by drawing the dorsum of either hind paw across the edge of the table. This stimulus elicits an upward lifting of the paw from the surface of the table (stepping). (2) Righting reflex: A rat placed horizontally with its back on the table will normally show an immediate coordinated twisting of the body around its longitudinal axis to regain its normal posture.

Experimental Protocol

Consistent with previous reports,[18,19] preliminary studies revealed that maximum hyperesthesia occurred 2 h after the carageenan injection. Thus, drugs were administered intrathecally 2 h after the carageenan injection. Before the carageenan subcutaneous injection, the hind paws were tested alternately three times, with 5-min intervals between the repeated testing of one paw as the base-line data. The left and right test sequence was carried out at 30, 60, and 120 min after carageenan injection. Then drugs were administered intrathecally, and the left and right hind paws were tested at 5, 15, 30, and 60 min after the drug intrathecal injection. After the experiment, the animals were killed with an overdose of barbiturate.

To a separate group of rats, to verify that the analgesic effects of morphine were due to interaction at the opioid receptor, the highest dose of morphine was administered, followed at 30 min by naloxone. To assess the effect of naloxone on morphine analgesia, the left and right paws were tested at 5 min after naloxone injection.

Drugs and Injection

The agents administered intrathecally in this study were morphine hydrochloride (MOR; Takeda, Osaka, Japan), MK-801 ((+)-5 methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine; Research Biochemicals, Natick, MA), and CP-96,345 ([(2S,3S)-cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine]; Pfizer, Groton, CT).[24] These agents were dissolved in normal saline and were administered intrathecally in a volume of 10 μl of vehicle. Ten micrograms normal saline also was injected intrathecally to obtain control data.

For the antagonism study, the following agents were administered: 10 μg morphine followed at 30 min by naloxone HCl (1 mg/kg, intraperitoneally; Sankyo, Tokyo, Japan).

Data Analysis and Statistics

The mean ± SEM values of the paw withdrawal latency (PWL) were plotted. To analyze the magnitude of the hyperesthesia evoked by subcutaneous carageenan, the difference score (DS) was calculated by subtracting the PWL of the control side (left side) from the PWL of the carageenan-injected side (right paw). A negative DS thus indicates a lower threshold on the injected side, *i.e.*, hyperesthesia. To analyze the effects of drugs on the hyperesthesia, the post-drug difference score (post-DS) was calculated by subtracting the maximum PWL (MAX PWL) of the untreated paw (left paw) from the MAX PWL of the carageenan-injected paw (right paw). MAX PWL of each paw was defined as the single longest PWL value during the first 30 min after intrathecal administration of the drug.

In the current study, we used MAX PWL to estimate the drug effect on the hyperesthesia. The duration of action is different for each drug. If the mean PWL is used for analyzing the drug effect, we underestimate the effects of a drug whose duration of action is short or overestimate the effect of one whose duration of action is long. Thus, we think that MAX PWL is more appropriate for evaluating the effect of a drug than the mean PWL in this study.

To obtain a dose-response curve, the dose was plotted against the MAX PWL or the post-DS. Dose-response curves were established with a least-squares linear regression analysis. Dose dependency was analyzed by one way analysis of variance (ANOVA). To compare the slopes and elevations of the regression lines, we used the *t* test.[25] To verify whether the paw carageenan injection evoked significant paw edema and whether intrathecal drugs affected the amount of edema, we used the paired *t* test. ANOVA was carried out with Dunnett's test for multiple comparisons. $P < .05$ levels were considered significant.

Results

General Behavior

Intrathecal morphine had no effect on placing, stepping, or righting reflexes. Intrathecal injection of 1 μg MK-801 had no effect upon placing, stepping, or righting reflexes. At 10 μg, the placing, stepping or the righting reflex was mildly impaired in two of five rats. Intrathecal injection of 200 μg CP-96,345 also caused a mild placing, stepping, or righting reflex impairment in four of five rats. These animals, though they were

able to ambulate and were able to be tested for thermal nociceptive thresholds, displayed to some detectable degree a lack of hind limb coordination. Thus, 10 μg MK-801 and 200 μg CP-96,345 were the highest doses employed in this study.

Carageenan Effects

Before carageenan injection, the mean PWLs (±SD) for the right and left paws were 10.3 ± 1.1 s and 10.4 ± 1.3 s, respectively. The time course of PWL after paw carageenan injection is illustrated in figure 1. Two hours after the carageenan injection, the mean PWLs (±SD) for the carageenan-injected paw (right paw) and untreated paw (left paw) were 5.7 ± 2.0 s and 11.0 ± 2.0 s, respectively, and the mean DS (±SD) was −5.3 ± 2.2 s. ANOVA showed that 2 mg of carageenan resulted in a significant hyperesthetic state 30 min, 1 h, and 2 h after the carageenan injection (fig. 1).

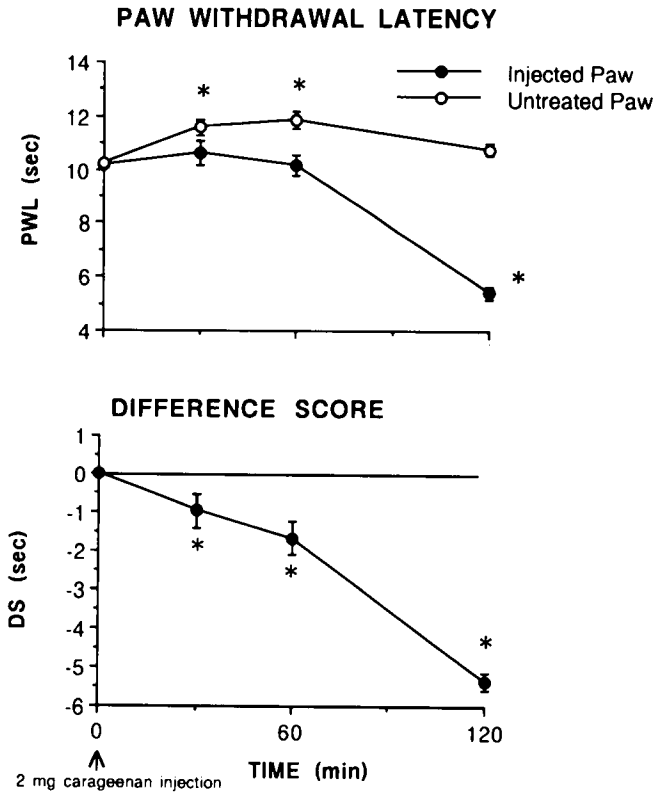

Fig. 1. Effects of 2-mg-carageenan paw injection on the thermal nociceptive threshold. *(Top)* Ordinate: paw withdrawal latency (PWL). Abscissa: time (min) after the carageenan injection. *(Bottom)* Ordinate: difference score (DS), which was calculated by subtracting the PWL of the untreated paw from the PWL of the carageenan-injected paw. Abscissa: time (min) after the carageenan injection. Each line represents the mean ± SEM determination made in 68 rats. *$P < .05$ as compared to time 0. Injected Paw = carageenan-injected paw (right paw); Untreated Paw = left paw.

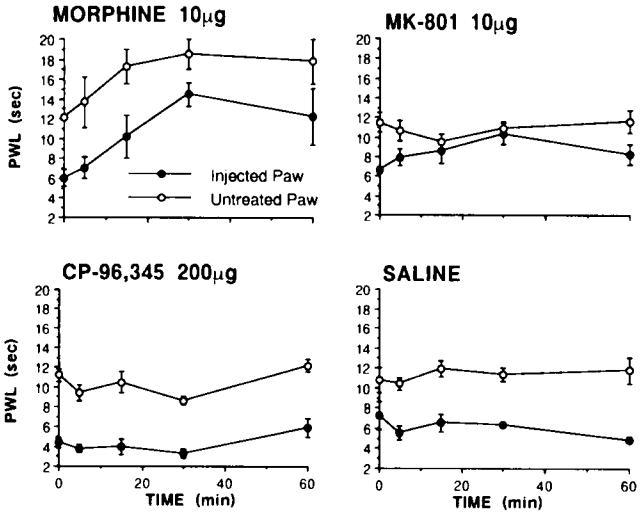

Fig. 2. Effects of intrathecal morphine (10 μg), MK-801 (10 μg), CP-96,345 (200 μg), and saline on the thermal nociceptive threshold. Ordinate: paw withdrawal latency (PWL). Abscissa: time (min) after drug injection. Each line represents the mean ± SEM determination made in four or five rats. Injected Paw = carageenan-injected paw (right paw); Untreated Paw = left paw.

Subcutaneous saline did not cause the hyperesthetic state 2 h after the injection [before the injection (mean ± SD), right PWL = 10.9 ± 0.6 s, left PWL = 10.9 ± 0.7 s; 2 h after the injection (mean ± SD), right PWL = 11.0 ± 1.2 s, left PWL = 10.9 ± 0.5 s; $P > .8$, paired t test].

Intrathecal Saline, Morphine, MK801, and CP-96,345

Saline. Intrathecal saline had no effect on the PWLs of either the carageenan-injected or the untreated paw [injected paw (mean ± SD): after carageenan but pre-drug PWL = 7.2 ± 3.0 s, MAX PWL = 7.0 ± 1.6 s; untreated paw (mean ± SD): after carageenan but pre-drug PWL = 10.8 ± 2.8 s, MAX PWL = 12.5 ± 1.1 s; n = 5] (fig. 2).

Morphine. Intrathecal morphine produced approximately equal increments in PWLs of the injected and untreated paws, *i.e.*, the morphine dose-response curve for the injected paw was shifted to the right from the morphine dose-response curve for the untreated paw in a parallel fashion (figs. 2 and 3). Thus, the difference between the PWLs of the injected and untreated paws

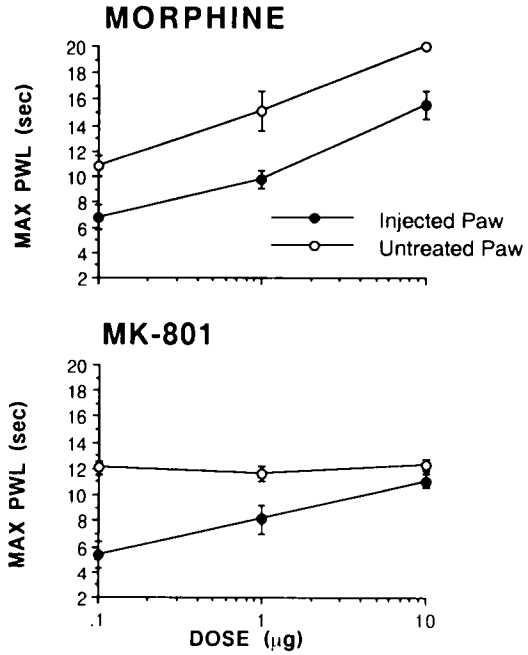

Fig. 3. Log dose-response curve for the effects of morphine and MK-801 on the thermal nociceptive threshold. Ordinate: maximum paw withdrawal latency (MAX PWL). Abscissa: log dose (μg). Each point represents the mean ± SEM of four or five rats. Injected Paw = carageenan-injected paw (right paw); Untreated Paw = left paw.

was preserved even as the PWLs on each side increased with intrathecal morphine (figs. 3 and 4).

MK-801. MK-801 had no effect on the PWLs of the untreated paw. In every rat tested, the PWLs on the two sides became closer following intrathecal MK-801 in a dose-dependent manner, and the hyperesthetic state evoked by carageenan was reliably and selectively abolished by intrathecal MK-801 (figs. 2 and 3). Thus, intrathecal MK-801 increased the post-DS levels in a dose-dependent manner (fig. 4).

CP-96,345. When the highest dose of CP-96,345, 200 μg, was administered intrathecally, mean MAX PWLs (±SD) of the carageenan-injected paw and the untreated paw were 4.6 ± 1.6 s and 10.7 ± 2.3 s, respectively, and the post-DS (±SD) was −6.1 ± 3.3 s. These data are not different from the after carageenan but pre-drug PWL (±SD) and DS (±SD) [PWL of the injected paw = 4.4 ± 1.1 s, PWL of the untreated paw = 11.2 ± 1.5 s, DS = −6.8 ± 1.9 s]. Thus, 200 μg intrathecal CP-96,345 had no effect on the PWLs of either the carageenan-injected or the untreated paw (fig. 2).

Interaction Study

Morphine and MK-801. Coadministration of 10 μg MK-801 with morphine did not alter the effects of morphine in the untreated paw ($P > .5$), but shifted the log dose-response curve of MAX PWL for the untreated paw to the left, and the log dose-response curve for the untreated paw overlapped that of the injected paw ($P > .2$; fig. 5). Thus, coadministration of 10 μg MK-801 with morphine reduced the post-DS for each dose of morphine (fig. 6).

CP-96,345 and MK-801. Coadministration of 200 μg CP-96,345 with MK-801 did not alter the effects of MK-801 in either the injected or the untreated paw ($P > .2$; fig. 5). The log dose-response curve of the post-DS for the effects of MK-801 had the same slope and elevation as that for the effects of coadministration of 200 μg CP-96,345 with MK-801 ($P > .5$; fig. 6).

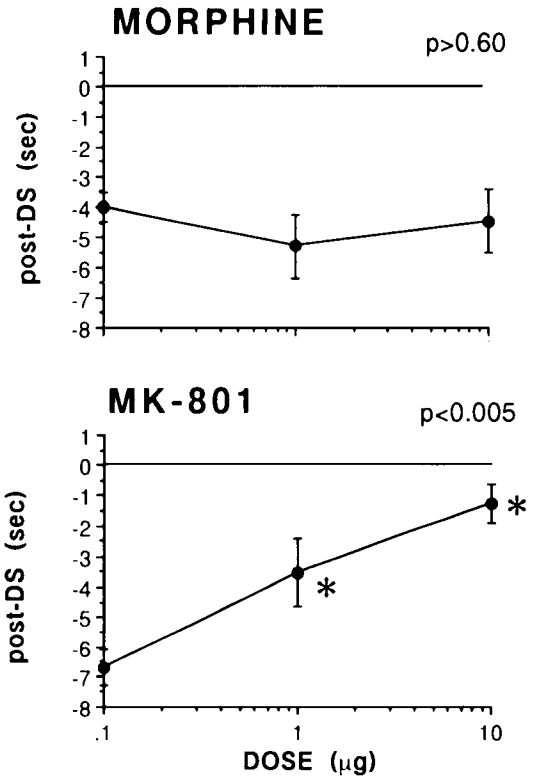

Fig. 4. Log dose-response curve for the effects of morphine and MK-801 on the post-drug difference score (post-DS), where post-DS = maximum paw withdrawal latency (MAX PWL) of the carageenan untreated paw − MAX PWL of the carageenan-injected paw. *P* value in the significance level when analyzed by ANOVA. *$P < .05$, Dunnett test, compared to 0.1 μg MK-801. Ordinate: post-DS. Abscissa: log dose (μg). Each point represents the mean ± SEM of four or five rats.

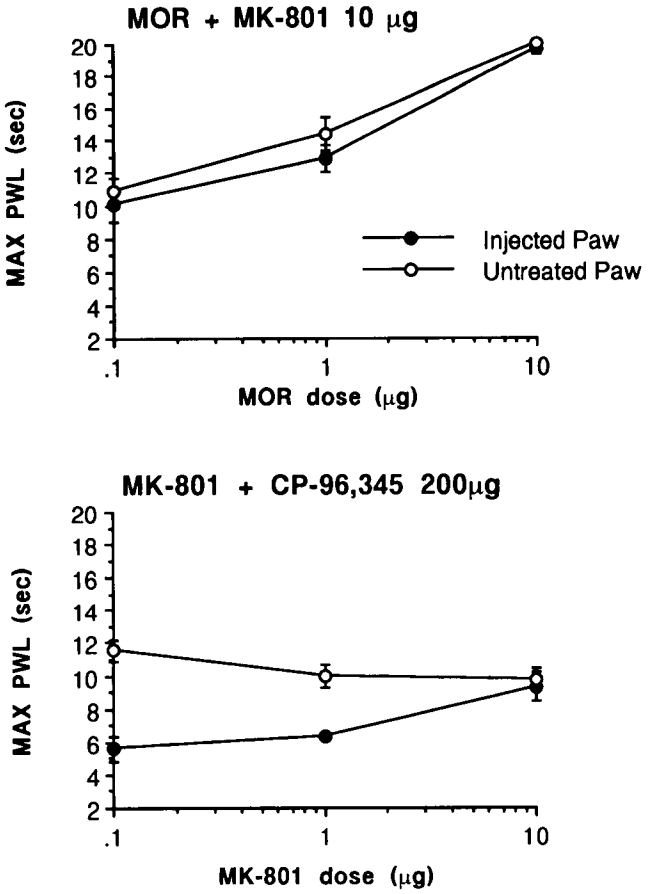

Fig. 5. Log dose-response curve for the effects of coadministration of 10 μg MK-801 with morphine (MOR) and coadministration of 200 μg CP-96,345 with MK-801 on the paw withdrawal latency (PWL). Ordinate: maximum paw withdrawal latency (MAX PWL). Abscissa: log dose (μg). Each point represents the mean ± SEM of four or five rats. Injected Paw = carageenan-injected paw (right paw); Untreated Paw = left paw.

Paw Edema Evoked by Carageenan Injection

Table 1 shows the level of edema evoked by the carageenan injection and the effects of intrathecal morphine, MK-801, and CP-96,345 on the edema. These data show that carageenan injected into the rat paw induced paw edema significantly. Intrathecal morphine, MK-801 and CP,96-345 had no effect on paw edema. Subcutaneous saline did not induce paw edema 2 h after the injection (before injection (±SD) right paw width = 3.5 ± 0.3 mm, left paw width = 3.5 ± 0.1 mm, 2 h after injection (±SD) right paw width = 3.6 ± 0.1 mm, left paw width = 3.6 ± 0.1 mm; $P > .2$).

Antagonist Study

Naloxone antagonized the morphine antinociceptive effect significantly in this model (fig. 7), reversing the increased PWLs of the injected and untreated paws by approximately the same degree.

Discussion

It has been shown that subcutaneous carageenan will yield a pronounced time-dependent thermal hyperesthesia.[18] In the present study, subcutaneous carageenan decreased the PWLs of the carageenan-injected paw 2 h after the injection, though subcutaneous carageenan

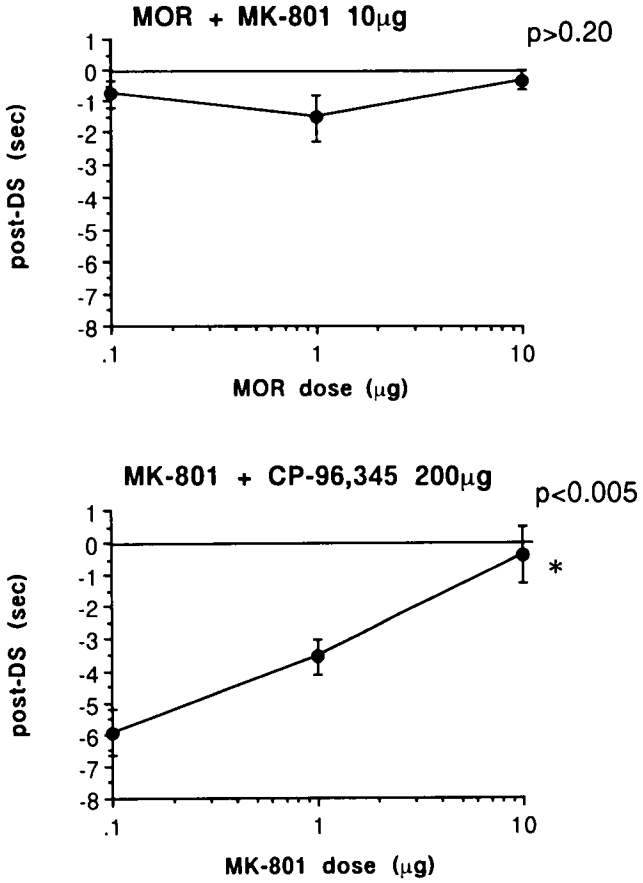

Fig. 6. Log dose-response curve for the effects of coadministration of 10 μg MK-801 with morphine (MOR) and coadministration of 200 μg CP-96,345 with MK-801 on the post-drug difference score (post-DS), where post-DS = maximum paw withdrawal latency (MAX PWL) of the untreated paw − MAX PWL of the carageenan-injected paw. *P* value in the significance level when analyzed by ANOVA. *$P < .05$, Dunnett test, compared to 0.1 μg MK-801. Ordinate: post-DS. Abscissa: log dose (μg). Each point represents the mean ± SEM of four or five rats.

Table 1. The Dorsal–Plantar Paw Width after Carageenan Injection

	Baseline (mm)	2 h after Carageenan sc (mm)	1 h after Drug it (mm)
Saline			
R	4.2 ± 0.2	6.0 ± 0.7*	6.8 ± 0.8
L	3.7 ± 0.3	3.8 ± 0.3	3.8 ± 0.4
Morphine 0.1 μg			
R	3.9 ± 0.3	7.3 ± 1.0*	7.3 ± 0.7
L	3.7 ± 0.3	3.8 ± 0.3	3.8 ± 0.4
Morphine 1.0 μg			
R	3.6 ± 0.2	7.0 ± 0.3*	7.1 ± 0.1
L	3.6 ± 0.1	3.8 ± 0.4	3.7 ± 0.3
Morphine 10 μg			
R	3.9 ± 0.3	7.0 ± 0.5*	7.2 ± 0.9
L	3.7 ± 0.2	3.8 ± 0.1	3.8 ± 0.2
MK-801 0.1 μg			
R	3.7 ± 0.5	6.3 ± 0.2*	6.5 ± 0.2
L	3.6 ± 0.3	3.9 ± 0.3	3.8 ± 0.2
MK-801 1.0 μg			
R	3.7 ± 0.3	5.9 ± 0.5*	6.7 ± 0.7
L	3.8 ± 0.3	4.0 ± 0.3	4.0 ± 0.4
MK-801 10 μg			
R	3.6 ± 0.4	6.1 ± 0.4*	6.3 ± 0.3
L	3.8 ± 0.5	4.1 ± 0.1	4.2 ± 0.3
CP-96,345 200 μg			
R	3.7 ± 0.3	7.2 ± 0.7*	7.0 ± 0.2
L	3.6 ± 0.3	4.0 ± 0.5	3.8 ± 0.3

Values are mean ± SD. R = right hind paw; L = left hind paw.
* $P < .05$ *versus* baseline data with paired *t* test.

did not result in shorter PWLs of the injected paw than the control PWL until after 60 min. The average DS observed in this study was approximately −5 s 2 h after subcutaneous carageenan. We found that subcutaneous carageenan induced significant paw edema. On the other hand, subcutaneous saline had no effects on the PWLs of either the injected or the untreated paw and did not induce paw edema 2 h after the injection. Thus, the carageenan injection, not the injection itself, caused the hyperesthetic state, which was defined by the level of DS, 2 h after the injection. Intrathecal saline had no effect on the PWLs of the carageenan-injected or untreated paw during this experiment.

Morphine

Intrathecal morphine resulted in a dose-dependent increase in the PWLs of both the carageenan-injected and the untreated paws, and the dose-response curve of the carageenan-injected paw was shifted to the right from the dose-response curve of the untreated paw in a parallel fashion. These data indicate that the injected and untreated paws were equally sensitive to intrathecal morphine. During inflammation evoked by subcutaneous carageenan, sensitization of peripheral nerve endings occurs, and the input to the spinal cord generated by a given thermal stimulus at the site of the inflammation is increased.[26] Thus, the dose-response curve of the carageenan-injected paw starts at a lower baseline than that of the untreated paw. It would be anticipated that the carageenan-injected paw would indeed require an increased dose to reach any given response criteria, compared to the untreated paw. We think that this is why the post-DS was not affected by intrathecal morphine. No alteration of μ, κ, and δ receptor binding was observed at 4 h or 4 days after carageenan-induced inflammation.[27] This also supports our data that the morphine sensitivity of the PWLs in the carageenan-injected paw is the same as that in the untreated paw.

Hylden *et al.*[28] reported that when [D-Ala^2, N-Me-Phe^4, Gly^5-ol] enkephalin (DAMGO, a μ-selective agonist) was administered intrathecally, the dose-response curve of DAMGO for the carageenan-injected paw overlapped that of the contralateral untreated paw. DAMGO has been reported to have a higher intrinsic efficacy than morphine.[29] When the intensity of a stimulus is increased, the dose-response curve for an agent with a lower intrinsic activity shows a greater shift to the right than that for an agent with a higher intrinsic

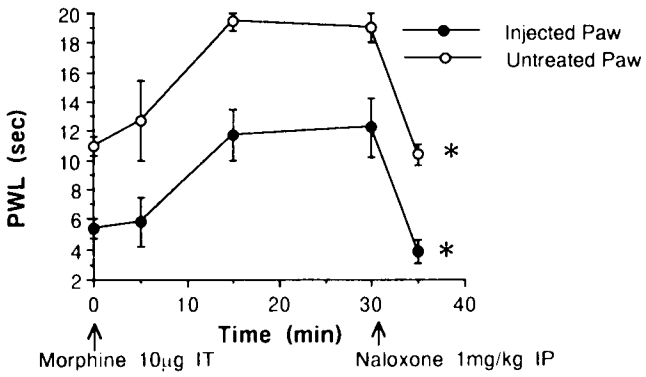

Fig. 7. Time course of paw withdrawal latency (PWL) of the morphine (10 μg intrathecally) plus naloxone (1 mg/kg intraperitoneally) study. *$P < .05$ as compared to the time 30. Ordinate: PWL. Abscissa: time (min). Each line represents the mean ± SEM of determinations made in five rats. Injected Paw = carageenan-injected paw (right paw); Untreated Paw = left paw; IT = intrathecal injection; IP = intraperitoneal injection.

activity.‡ In this model, as described above, a given thermal stimulus to the carageenan-injected paw evokes higher intensity input to the spinal cord than one to the untreated paw. Thus, the dose-response curve of intrathecal morphine for the carageenan-injected paw shows a great shift to the right from that of the untreated paw, while the dose-response curve of intrathecal DAMGO for the carageenan-injected paw shows a slight shift to the right from that of the untreated paw.

MK-801

Intrathecal MK-801 had no effect on the PWLs of the untreated paw at the dose used in this study. These data are consistent with the previous report that NMDA antagonists have little or no selective antinociceptive effects.[8,9]

Intrathecal MK-801 increased the PWLs of the carageenan-injected paw in a dose-dependent manner and selectively abolished the hyperesthetic state evoked by subcutaneous carageenan at a dose that did not alter the PWLs of the untreated paw. The effects of intrathecal MK-801 on the hyperesthetic state were in contrast to that of intrathecal morphine. Intrathecal MK-801 did not affect the level of edema at the dose used in this study. One possible mechanism underlying the hyperesthetic state evoked by subcutaneous carageenan is the property whereby repetitive C-, but not A-fiber stimulation yields a central facilitation (wind-up).[11] Some polymodal nociceptors inside the inflamed area evoked by carageenan injection exhibited spontaneous activities,[26] and these activities may induce a wind-up like central facilitation in the spinal cord. Opioids appear to modulate the wind-up phenomenon poorly, and NMDA antagonists markedly attenuate this central facilitation.[12,13] Thus, the hyperesthetic state evoked by subcutaneous carageenan is abolished by intrathecal MK-801, not by intrathecal morphine. Hargreaves *et al.*[18] reported that intraperitoneal indomethacin, whose analgesic effect is thought to be a peripheral one that acts primarily on the synthesis of prostaglandins at the site of inflammation,[30] prevented the development of hyperesthesia following paw carageenan injection. We think that both peripheral and spinal mechanisms are required to maintain the hyperesthetic state after carageenan injection.

CP-96,345

CP-96,345, a novel nonpeptide NK1 tachykinin receptor antagonist, showed no effect on the PWLs of either the carageenan-injected paw or the untreated paw at a dose that did not affect motor function. The affinity of CP-96,345 for functional NK1 receptors is species-dependent, and CP-96,345 was approximately 30–120-fold less potent at inhibiting [3H]-sP binding in the rat or mouse cerebral cortex than in other mammalian species.[24,31,32] The lack of a suppressive effect of CP-96,345 in this study may reflect an inadequate dose, although 200 μg intrathecal CP-96,345 has been reported to produce depression of the agitation behavior induced by the injection of formalin into a rat's hind paw.[33] Thus, we think that in this model, 200 μg CP-96,345 is an adequate dose to study its antinociceptive effect. Our data suggested that the spinal NK1 receptor does not play an important role in maintaining the hyperesthetic state after subcutaneous carageenan injection in the rat. Although both subcutaneous formalin and subcutaneous carageenan induce localized inflammation, there are several differences between the carageenan test and the formalin test. Paw formalin injection induces biphasic spontaneous nociceptive behavior, such as flinching and licking, and the duration of the formalin response is about 1 h.[17] On the other hand, paw carageenan induces no flinching response in the rat,[17] and 2 mg carageenan induces much more severe paw edema than does formalin. The different sensitivity of CP-96,345 in the formalin test and the carageenan test may reflect the different characteristics of these two tests.

Interaction Study

In the present study, we demonstrated that coadministration of 10 μg MK-801 with morphine did not alter the morphine effect on the untreated paw but reduced the post-DS for each morphine dose, a finding similar to that in the hyperesthesia model induced by peripheral nerve injury.[34] NMDA antagonists act to block polysynaptic but not monosynaptic excitation of dorsal horn neurons in the spinal cord,[10] and spinal opioids appear to exert the action by inhibitory C-fiber input and by hyperpolarizing the dorsal horn projection neurons.§ As described above, an NMDA antagonist blocked

‡ Yaksh TL: Intrathecal dose-response curve for morphine (MOR) and sufentanil (SUF): Role of drug efficacy in the right shifts produced by increasing stimulus intensity. Society of Neuroscience Abstract 16:409, 1990.

§ Sabbe MB, Yaksh TL: Pharmacology of spinal opioids. Journal of Pain and Symptom Management 5:191–203, 1990.

the wind-up phenomena and an opiate poorly modulated this central facilitation. We believe that the NMDA antagonist simply blocks that spinal facilitation and renders the spinal system equivalent to the system that processes pain information in the absence of conditions that lead to or augment the facilitatory processes. Thus, the effects of spinal morphine under normal conditions and under conditions of facilitation in the presence of spinal NMDA antagonism will be equivalent.

Coadministration of 200 μg CP-96,345 with MK-801 did not alter the effects of MK-801 in either the injected or the untreated paw. This data suggested that the NK1 receptor does not play an important role in maintaining the hyperesthetic state in this carageenan model.

Common Role of NMDA Receptor in the Hyperesthetic State

In the current study, we demonstrated that MK-801, an NMDA receptor antagonist, selectively abolished the hyperesthetic state evoked by carageenan injection in the rat. In the formalin test, Haley *et al.* reported that an intrathecal selective NMDA receptor antagonist, 5-amino-phosphonovaleric acid, caused a marked dose-related reduction in the second prolonged phase evoked by formalin injection.[35] With regard to the neurogenic pain model, thermal hyperesthesia induced by constriction injury of the rat sciatic nerve is selectively abolished by MK-801, and morphine has no effect on this thermal hyperesthesia.[15,16] These observations suggested that the NMDA receptor plays an important role in the processing of afferent input in both the neurogenic hyperesthetic state and inflammation induced in the hyperesthetic state.

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