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Effects of FK224, a Novel Cyclopeptide NK1 and NK2 Antagonist, and CP-96,345, a Nonpeptide NK1 Antagonist, on Development and Maintenance of Thermal Hyperesthesia Evoked by Carrageenan Injection in the Rat Paw

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Background: The role of tachykinins, such as substance P and neurokinin A, in the development or maintenance of thermal hyperesthesia during inflammation is unclear. In the current study, the authors examined the role of NK1 and NK2 receptors on the thermal hyperesthesia evoked by injection of carrageenan into the rat paw using FK224, a cyclopeptide NK1 and NK2 antagonist, and CP-96,345, a nonpeptide NK1 antagonist.

Methods: In rats injected with 2 mg carrageenan, 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 carrageenan injection. In the pretreatment study, drugs were administered intravenously or intrathecally 10 min before the carrageenan injection. In the posttreatment study, drugs were administered intravenously or intrathecally 2 h after the carrageenan injection.

Results: In the pretreatment study, both intravenous CP-96,345 and intravenous FK224 blocked the development of thermal hyperesthesia and reduced paw edema in a dose-dependent manner 2 h after the carrageenan injection. The effect of CP-96,345 on thermal hyperesthesia was stereospecific, but that on paw edema was not. Posttreatment with intravenous CP-96,345 and intravenous FK224 failed to reduce the level of thermal hyperesthesia or paw edema, and intrathecal injections, either pre- or posttreatment, had no effect on thermal hyperesthesia or paw edema.

Conclusions: These data indicate that: 1) spinal NK1 and NK2 receptors do not play an important role in development and maintenance of thermal hyperesthesia evoked by paw carrageenan, and 2) the peripheral NK1 receptor may play an important role in the development of thermal hyperesthesia, but not of paw edema. (Key words: Antagonists, NK1: CP-

96,345. Antagonists, NK1 and NK2: FK224. Pain, inflammatory: carrageenan.)

THE tachykinins, such as substance P (sP), neurokinin A (NKA), and neurokinin B (NKB), share the common C-terminal sequence Phe-X-Gly-Leu-Met-NH₂, and are found throughout the peripheral and central nervous system. These peptides exert various biologic actions that are mediated by multiple receptors, and these receptors have recently been classified into three subtypes: NK1, NK2, and NK3, of which sP, NKA, and NKB are the most potent natural ligands, respectively.¹ Substance P and NKA are colocalized to a population of capsaicin-sensitive neurons that are believed to be nociceptive afferents.^{2,3}

When the peripheral terminals of polymodal afferent C-fibers are stimulated chemically or electrically, a neuropeptide, such as sP, is released from these terminals.^{4,5} It has been reported that sP induces vasodilatation, plasma extravasation, and hyperalgesia when injected subcutaneously or intraarticularly.^{6,7} In the rat formalin model, intrathecal CP-96,345, a nonpeptide NK1 antagonist,⁸ attenuates the flinching response induced by paw formalin in a dose-dependent manner.⁹ Small afferent neurotoxins, such as capsaicin, will deplete dorsal horn sP and produce a significant thermal antinociception.^{10,11} Thus, NK1 receptors may play an important role in the nociceptive information transmission in both the peripheral nervous system and the spinal cord.

It has been reported that intracutaneous sP, but not NKA, induced cutaneous pain, but NKA potentiated the algic effects of sP when NKA and sP were coadministered intracutaneously in humans.¹² In the spinal cord of rats, a synergistic interaction between sP and NKA has been reported when sP and NKA were coadminis-

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tered intrathecally.¹³ These data indicated that both NK1 and NK2 receptors are involved in the nociceptive information transmission, either at peripheral sites or in the spinal cord.

Recently, a novel cyclopeptide NK1 and NK2 antagonist, FK224, has been reported.¹⁴ FK224 inhibits airway constriction and airway edema induced not only by exogenous sP and NKA, but also by the endogenous neurotransmitters released by neurogenic stimulation.¹⁵ Carrageenan injection into the rat hind paw plantar surface induces edema, hyperthermia, and hyperesthesia, and this carrageenan-induced hyperesthesia has been used as an animal model for the hyperesthesia during inflammation.^{16,17,18} We previously reported that intrathecal CP-96,345 had no effect on the maintenance of thermal hyperesthesia evoked by carrageenan injection, and this data indicates that spinal NK1 receptor does not play an important role in maintaining thermal hyperesthesia. In the current study, to clarify the role of peripheral NK1 and NK2 receptors in the carrageenan model, we studied the effects of intravenous FK224 on the thermal hyperesthesia induced by carrageenan injection in the paw, and compared the effects of FK224 with that of CP-96,345. In addition, we examined the interaction between spinal NK1 and NK2 receptors in the carrageenan model by defining the effects of intrathecal FK224 and intrathecal CP-96,345 on thermal hyperesthesia evoked by carrageenan injection.

Materials and Methods

The following investigations were carried out under a protocol approved by the Institutional Animal Care Committee, Chiba University. Male Sprague-Dawley rats (250–300 g) were prepared with either chronic intravenous catheters or intrathecal catheters, and examined for the effects of agents on the thermal hyperesthesia evoked by carrageenan injection.

Intravenous Catheters

Chronic intravenous catheters were inserted into one jugular vein under pentobarbital anesthesia (50 mg/kg, intraperitoneally). The catheter was externalized on the back of the neck and sealed with a piece of steel wire. Animals were allowed to recover for 3 days before being used experimentally. All animals postoperatively displayed normal feeding and drinking behavior.

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 8 cm caudal to the cisterna at the level of the lumbar enlargement.¹⁹ The catheter was externalized on the top of the skull and sealed with a piece of steel wire. The wound was closed with 3-0 silk sutures. Rats showing neurologic deficits postoperatively were killed and discarded.

Carrageenan Test

Two milligrams of lambda carrageenan (Sigma, C-3889, Sigma, St. Louis, MO) was injected, *via* a 24-G needle, subcutaneously (SC) in the plantar surface of the right hind paw under isoflurane anesthesia. Lambda carrageenan was suspended in normal saline by sonication, and was administered in a 0.1-ml injection volume. After recovering from isoflurane anesthesia, the animals were placed in a plexiglass box that permitted observation.

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 carrageenan injection and 2 and 3 h after carrageenan injection.

Thermal Nociceptive Test

Paw withdrawal latency (PWL) was measured with a device similar to that previously employed.¹⁷ The rats were placed beneath a clear plastic cage (10 × 20 × 24 cm) 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 temperatures resulting from minor changes in room temperature, the interior of the box under the animal was prepared with a heat source such that the glass plate temperature was regulated at 30° C. The calibration of the thermal test system is such that the average response latency (\pm SD) in ten normal untreated rats was maintained at 10 ± 0.5 s before the initiation of an experimental series.

To initiate a test, a rat was placed in the box and allowed 5–10 min to habituate. The halogen lamp beneath the floor was then positioned so that it focused at the plantar surface of one hindpaw that was in contact with the glass. Care was taken not to focus the lamp

on the skin that was off 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 measured to the nearest 0.1 s. The trial was terminated and the lamp removed in the absence of a response within 20 s. This value was then assigned as the response latency.

Behavior Test

Behavior was evaluated at each test point during the dose-response study by a scoring system (normal, or mildly to severely impaired) of two specific behaviors. The first behavior was the placing/stepping reaction, which 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). The second behavior was the 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

Pretreatment Study. Consistent with our previous report,¹⁸ preliminary studies revealed that maximum hyperesthesia occurred 2 h after the carrageenan injection. Therefore, the effects of drugs in this study were evaluated 2 h after the carrageenan SC injection. Before the carrageenan SC injection, the hind paws were tested alternately three times, with 5-min intervals between each testing of one paw, as the baseline data. The average of three measurements was defined as PWL. Then, drugs were administered intravenously or intrathecally. Ten minutes after the drug injection, 2.0 mg carrageenan was injected SC. Two hours after the SC carrageenan, the posttreatment PWLs (post-PWLs) of right and left hind paws were measured alternately.

Posttreatment Study. Before the carrageenan SC injection, the hind paws were tested alternately three times, with 5-min intervals between each testing of one paw, as the baseline data. Two hours after the carrageenan SC injection, the left and right PWLs were measured alternately. Then, the drug was administered intravenously or intrathecally, and the left and right paws were tested at 5, 15, 30, and 60 min after the intravenous or intrathecal drug injection.

The individual who measured PWLs was not blinded to the drug treatment. After the experiment, the animals were killed with an overdose of barbiturate.

Drugs

The agents administered intrathecally or intravenously in this study were CP-96,345 ([*(2S,3S)*-cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine]; Pfizer, Groton, CT), its inactive isomer, CP-96,344 (2R, 3R enantiomer of CP-96,345; Pfizer), and FK 224 ({N-[N²-[N-[N-[N-[2,3-didehydro-N-methyl-N-[N-[3-(2-pentylphenyl)-propionyl]-L-threonyl]tyrosyl]-L-leucynyl]-D-phenylalanyl]-L-allo-threonyl]-L-asparaginy]-L-serine-*v*-lactone}); Fujisawa, Osaka, Japan). Drugs were delivered in 1.5% cyclodextrin (Wako, Osaka, Japan) dissolved in normal saline and administered intravenously, in a volume of 1.0 ml of vehicle, or intrathecally, in a volume of 10 μ l of vehicle. To obtain control data, intravenous 1.0 ml 1.5% cyclodextrin dissolved in normal saline, or intrathecal 10 μ l 1.5% cyclodextrin dissolved in normal saline, was administered.

Data Analysis and Statistics

To analyze the magnitude of the thermal hyperesthesia evoked by SC carrageenan, the difference score (DS) was calculated by subtracting the PWL of the control side (left side) from the PWL of the carrageenan-injected side (right paw). Thus, a negative DS indicates that the injected paw requires lower thermal energy to evoke paw withdrawal response than the control paw, *i.e.*, thermal hyperesthesia. To analyze the effects of drugs on the hyperesthesia in the pretreatment study, the postdrug difference score (post-DS) was calculated by subtracting the post-PWL of the untreated paw from the post-PWL of the carrageenan injected paw. In the posttreatment study, maximum PWL (MAX PWL) was calculated. MAX PWL of each paw was defined as the maximum PWL value during the first 30 min after the drug administration.

To obtain a dose-response curve, the dose was plotted against the posttreatment PWL, post-DS, or posttreatment paw width. Dose-response curves were established with a least-squares linear regression analysis. Dose dependency was analyzed by one-way ANOVA. ANOVA was carried out with Dunnett's test for multiple comparisons. To verify whether the paw carrageenan injection evoked significant paw edema, and whether drugs affected the amount of edema, we used ANOVA. To compare post-PWLs or MAX PWLs between groups, ANOVA was carried out. To verify the stereospecificity of the CP-96,345 effect on PWL or paw width, we used the *t* test. *P* < 0.05 levels were considered significant.

Results

Behavior Test

Intravenous injection of either 5 mg/kg CP-96,345 or 3.3 mg/kg FK224 had no effect on the placing or stepping reactions or righting reflexes, and intravenous injection of either 15 mg/kg CP-96,345 or 6.6 mg/kg FK224 caused death in one-half of the injected rats. Thus, 5 mg/kg CP-96,345 and 3.3 mg/kg FK224 were the highest intravenous doses employed in this study. Intrathecal injection of CP-96,345 (200 μ g) caused a mild placing or stepping reaction or righting reflex impairment in four of five rats. These animals displayed, to some detectable degree, a lack of hind limb coordination, although they were able to ambulate and were able to lift the hind paw to scratch the head and ears (normal grooming). This impairment lasted no more than 5 min after drug injection, and these animals could be tested 5, 15, 30, and 60 min after the drug administration. Intrathecal CP-96,345, 400 μ g, produced severe behavioral impairment in all injected rats, and this impairment lasted about 30 min. Thus, 200 μ g CP-96,345 was the highest dose employed in this study. Intrathecal injection of 3 μ g FK224 caused death in two of nine rats, and intrathecal injection of 2 μ g FK224 had no effect on the placing or stepping reactions or righting reflexes. Thus, 2 μ g FK224 was the highest intrathecal dose employed in this study.

Nociceptive Test

Pretreatment Study. In the intravenous study, there was no difference between the precarrageenan right and left PWLs in each group. In the intravenous vehicle-treated group, 2 mg of carrageenan resulted in significant thermal hyperesthesia 2 h after the carrageenan injection (post-PWLs of the injected paw [\pm SD] = 5.7 ± 1.6 s, post-PWLs of the untreated paw [\pm SD] = 11.3 ± 0.8 s, $n = 8$). Neither intravenous CP-96,345 nor intravenous FK224 had any effect on the post-PWLs of the untreated paw (left paw), but they increased the post-PWLs of the injected paw (right paw) to the level of the post-PWLs of the untreated paw in a dose-dependent manner (figs. 1 and 2). Thus, the post-PWLs of the two sides became closer after either intravenous CP-96,345 or FK224 in a dose-dependent manner, and the thermal hyperesthesia evoked by carrageenan was reliably and selectively abolished by either intravenous CP-96,345 or FK224. At the highest dose (5 mg/kg), intravenous CP-96,344, the inactive isomer, failed to have any effect on the post-PWLs of the untreated paw

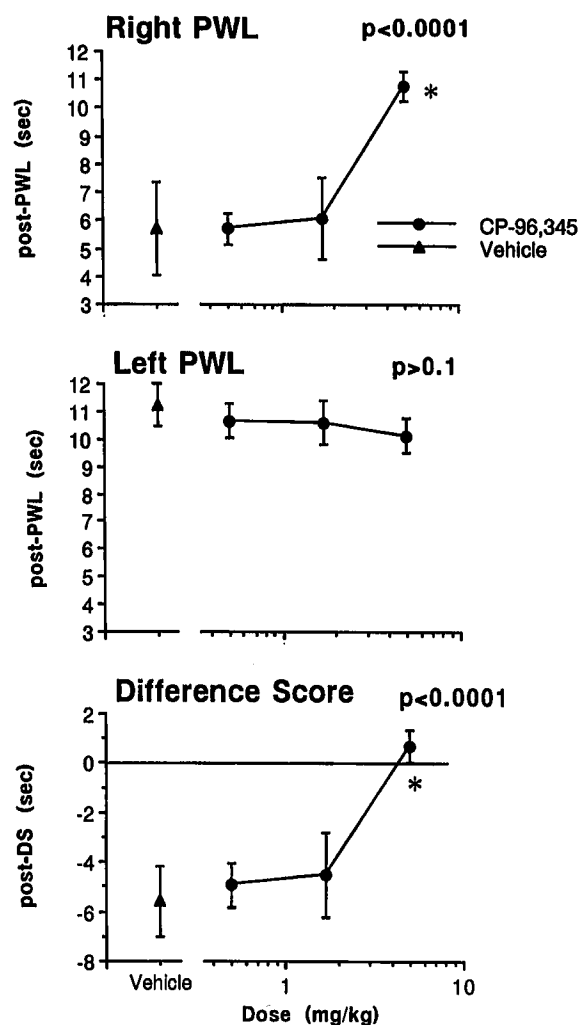


Fig. 1. Log dose-response curve for the effects of intravenous CP-96,345 on the thermal nociceptive threshold in the pretreatment study. (*Top*) Ordinate: postpaw withdrawal latency (post-PWL) of right paw. Abscissa: log dose (μ g) of CP-96,345. (*Middle*) Ordinate: post-paw withdrawal latency (post-PWL) of left paw. Abscissa: log dose (μ g) of CP-96,345. (*Bottom*) Ordinate: postdifference score (post-DS). Abscissa: log dose (μ g) of CP-96,345. Each point represents the mean \pm SD of determinations made in five to eight rats. *P* value in the significance level when analyzed by ANOVA. **P* < 0.05, as compared with vehicle level.

(post-PWL of injected paw [\pm SD] = 7.0 ± 1.5 s, post-PWL of untreated paw [\pm SD] = 10.8 ± 0.8 s, $n = 5$). The post-DS level (\pm SD) in the 5 mg/kg CP-96,344-treated rats was -4.0 ± 2.0 s, and this level is significantly lower than that in the 5 mg/kg CP-96,345-treated rats (0.68 ± 0.7 s, $n = 5$) ($P < 0.005$, *t* test). In the intrathecal study, there was no difference be-

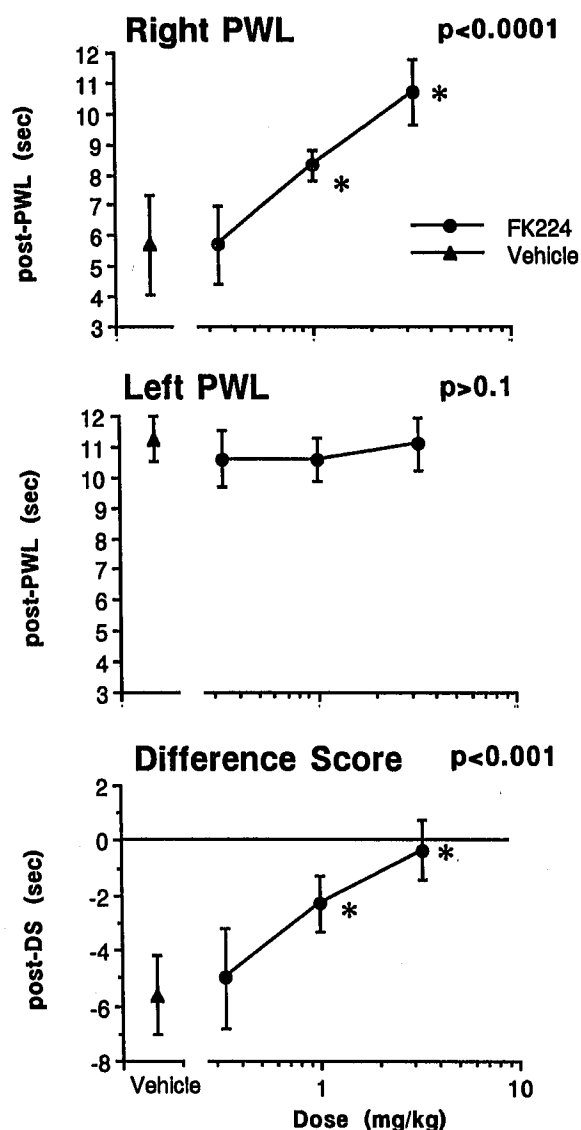


Fig. 2. Log dose-response curve for the effects of intravenous FK224 on the thermal nociceptive threshold in the pretreatment study. (Top) Ordinate: post-paw withdrawal latency (post-PWL) of right paw. Abscissa: log dose (μ g) of FK224. (Middle) Ordinate: post-paw withdrawal latency (post-PWL) of left paw. Abscissa: log dose (μ g) of FK224. (Bottom) Ordinate: postdifference score (post-DS). Abscissa: log dose (μ g) of FK224. Each point represents the mean \pm SD of determinations made in five to eight rats. *P* value in the significance level when analyzed by ANOVA. **P* < 0.05 as compared with vehicle level.

tween the precarrageenan right and left PWLs in each group. In the intrathecal vehicle group, significant thermal hyperesthesia developed 2 h after the carrageenan injection. Neither 200 μ g intrathecal CP-96,345

nor 2 μ g intrathecal FK 224 had any effect on the PWLs (\pm SD) of either the carrageenan-injected (right) or untreated (left) paw (CP-96,345: right paw = 7.6 ± 1.9 s, left paw = 11.3 ± 1.1 s, *n* = 5; FK224: right paw = 7.9 ± 1.3 s, left paw = 10.6 ± 1.4 s, *n* = 5) compared with vehicle-treated rats (right paw = 7.9 ± 1.3 s, left paw = 11.5 ± 1.0 s, *n* = 5).

Posttreatment Study. Two hours after injection of 2 mg carrageenan, the PWLs of the injected paw (right paw) decreased significantly (precarrageenan PWL [\pm SD]: right paw = 10.4 ± 0.6 s, left paw = 10.4 ± 0.6 s; PWL [\pm SD] 2 h after carrageenan injection: right paw = 5.1 ± 1.5 s, left paw = 10.7 ± 0.8 s, *n* = 34), and resulted in a significant hyperesthetic state (*P* < 0.0001, *t* test). Neither 5 mg/kg intravenous CP-96,345 nor 3.3 mg/kg intravenous FK224 had any effect on the PWLs of either the injected paw or the untreated paw when compared with the vehicle-treated rats. There was no difference between the MAX PWLs (\pm SD) of intravenous CP-96,345-treated rats (right paw = 5.1 ± 1.5 s, left paw = 12.1 ± 1.6 s, *n* = 5), intravenous FK224-treated rats (right paw = 6.2 ± 2.4 s, left paw = 12.9 ± 1.3 s, *n* = 5), and intravenous vehicle-treated rats (right paw = 4.3 ± 2.4 s, left paw = 12.4 ± 1.4 s, *n* = 8). Neither 200 μ g intrathecal CP-96,345, nor 2 μ g FK 224, nor the vehicle had any effect on the PWLs of either the injected paw or the untreated paw. There was no difference between MAX PWLs (\pm SD) of intrathecal CP-96,345-treated rats (right paw = 5.1 ± 1.1 s, left paw = 10.6 ± 1.2 s, *n* = 5), intrathecal FK 224-treated rats (right paw = 5.4 ± 1.5 s, left paw = 11.6 ± 6.4 s, *n* = 6), and intrathecal vehicle-treated rats (right paw = 6.5 ± 1.7 s, left paw = 11.5 ± 1.0 s, *n* = 5).

Paw Edema

Pretreatment Study. Either intravenous CP-96,345 or intravenous FK224 partly inhibited carrageenan-induced paw edema 2 h after the carrageenan injection in a dose-dependent manner (fig. 3), but had no effect on the paw width of the untreated paw 2 h after carrageenan injection (CP-96,345 [\pm SD]: 5 mg/kg = 3.2 ± 0.1 mm, 1.7 mg/kg = 3.4 ± 0.09 mm, 0.5 mg/kg = 3.4 ± 0.1 mm; FK224 (\pm SD): 3.3 mg/kg = 3.5 ± 0.1 mm, 1.0 mg/kg = 3.3 ± 0.09 mm, 0.33 mg/kg = 3.4 ± 0.2 mm). The paw width (\pm SD) of the carrageenan-injected paws in the 5 mg/kg intravenous CP-96,345-treated rats was 5.0 ± 0.4 mm (*n* = 5), the same as that in the 5 mg/kg intravenous CP-96,344-treated rats (5.6 ± 0.8 mm, *n* = 5) (*P* > 0.1, *t* test). Neither intrathecal 200 μ g CP-96,345 nor 2 μ g intrathecal FK224 had any effect on the paw

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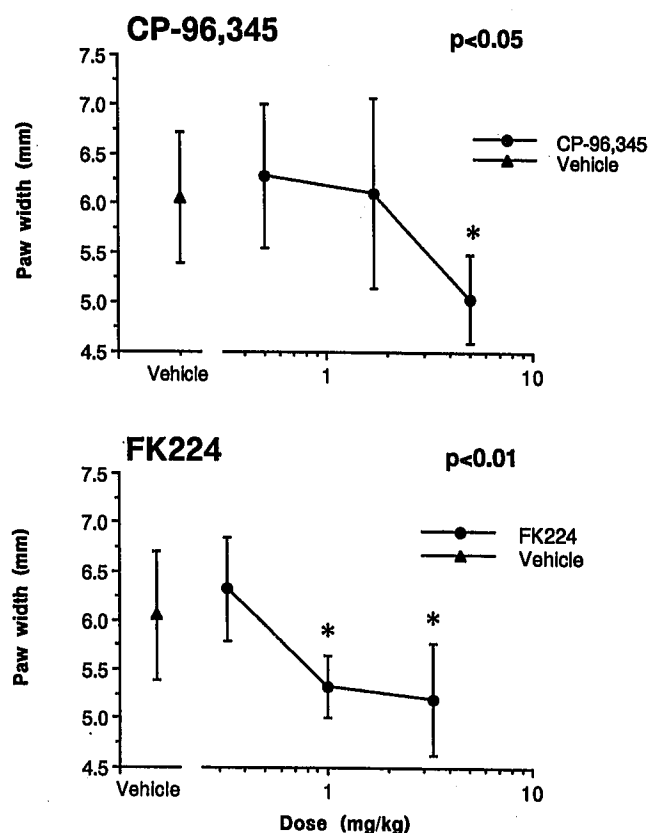


Fig. 3. Log dose-response curve for the effects of intravenous CP-96,345 and intravenous FK224 on the paw width of the carrageenan-injected paw 2 h after the injection in the pretreatment study. Each line represents the mean \pm SD of determinations made in five to eight rats. P value in the significance level when analyzed by ANOVA. * $P < 0.05$ as compared with vehicle level.

width in either the carrageenan-injected paws or untreated paws 2 h after the carrageenan injection when compared with the vehicle-treated group (table 1).

Posttreatment Study. Carrageenan injection induced a significant increase in the dorsal-plantar paw width (table 2). Neither CP-96,345 (5 mg/kg intravenously or 200 μ g intrathecally), nor FK224 (3.3 mg/kg intravenously or 2 μ g intrathecally), nor the vehicle (intravenously or intrathecally) had any effect on the carrageenan-induced paw edema 1 h after the drug injection when compared with after-carrageenan but predrug levels (table 2).

Discussion

It has been shown that SC carrageenan will yield a pronounced time-dependent thermal hyperes-

thesia.^{17,18} In the posttreatment study, SC carrageenan significantly decreased the PWs of the carrageenan-injected paw 2 h after the injection, but did not affect the PWs of the untreated paw. We found that SC carrageenan induced significant paw edema in the injected paw 2 h after the injection in the posttreatment study. These data indicated that SC carrageenan induced the local inflammation in the injected paw and produced a reliable thermal hyperesthetic state 2 h after the SC carrageenan injection in the current study. Thus, we believe that the appropriate time to verify the effects of drugs on the inflammation and thermal hyperesthesia evoked by SC carrageenan in a pretreatment study is 2 h after the carrageenan injection.

Pretreatment Study

In the current study, we found that CP-96,345, a nonpeptidic NK1 antagonist, reduced the level of paw edema and thermal hyperesthesia evoked by SC carrageenan in a dose-dependent manner only when drugs were administered intravenously. These findings were consistent with the report that SC (\pm)-CP-96,345 abolished carrageenan-induced mechanical hyperalgesia and significantly reduced paw edema.²⁰ The level of paw edema in the 5 mg/kg intravenous CP-96,344-treated rats was the same as that in the 5 mg/kg intravenous CP-96,345-treated rats, although the post-DS level in 5 mg/kg intravenous CP-96,344-treated rats was significantly lower than that in the 5

Table 1. Effect of Intrathecal CP-96,345, FK224, and Vehicle on Dorsal-Plantar Paw Width in the Pretreatment Study

	Paw Width	
	Precarrageenan (mm)	2 h after Carrageenan (mm)
CP-96,345 (200 μ g) (n = 5)		
R	3.3 \pm 0.1	6.0 \pm 0.4*
L	3.3 \pm 0.1	3.3 \pm 0.1
FK224 (2 μ g) (n = 5)		
R	3.4 \pm 0.1	5.9 \pm 0.3*
L	3.4 \pm 0.2	3.4 \pm 0.2
Vehicle (n = 5)		
R	3.3 \pm 0.1	6.0 \pm 0.9*
L	3.3 \pm 0.1	3.2 \pm 0.2

Data are mean \pm SD.

R = right hind paw; L = left hind paw.

* $P < 0.05$ as compared to the precarrageenan paw width.

Table 2. The Effects of Intravenous CP-96,345, FK224, and Vehicle and Intrathecal CP-96,345, FK 244, and Vehicle on Dorsal-Plantar Paw Width in the Posttreatment Study

	Paw Width		
	Precarrageenan (mm)	After Carrageenan but Predrug (mm)	1 h after Drug Injection (mm)
Intravenous study			
CP-96,345 (5 mg/kg) (n = 5)			
R	3.3 ± 0.05	6.7 ± 0.6*	7.2 ± 0.9*
L	3.4 ± 0.1	3.3 ± 0.04	3.3 ± 0.1
FK 224 (3.3 mg/kg) (n = 5)			
R	3.3 ± 0.2	6.5 ± 0.3*	6.7 ± 0.9*
L	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.2
Vehicle (n = 8)			
R	3.4 ± 0.1	6.4 ± 0.3*	6.6 ± 0.5*
L	3.4 ± 0.2	3.4 ± 0.1	3.4 ± 0.2
Intrathecal study			
CP-96,345 (200 µg) (n = 5)			
R	3.4 ± 0.1	6.4 ± 0.5*	6.9 ± 0.5*
L	3.4 ± 0.08	3.4 ± 0.07	3.4 ± 0.2
FK224 (2 µg) (n = 6)			
R	3.3 ± 0.2	6.5 ± 0.5*	7.0 ± 0.3*
L	3.4 ± 0.2	3.3 ± 0.1	3.3 ± 0.1
Vehicle (n = 5)			
R	3.4 ± 0.2	6.4 ± 0.4*	6.8 ± 0.4*
L	3.4 ± 0.3	3.3 ± 0.05	3.3 ± 0.1

Data are mean ± SD.

R = right hind paw; L = left hind paw.

* $P < 0.05$ as compared to the precarrageenan paw width with Dunnett's test.

mg/kg intravenous CP-96,345-treated rats. This indicates that the effect of CP-96,345 on thermal hyperesthesia evoked by SC carrageenan is stereospecific, but the effect on the level of paw edema was not stereospecific. Thus, the effect of CP-96,345 on thermal hyperesthesia is probably caused by the interaction between the NK1 receptor and CP-96,345, and the effect on the level of paw edema is not caused by the interaction between the NK1 receptor and CP-96,345. It has been reported that SC CP-96,345 inhibited the mechanical hyperalgesia induced by SC carrageenan injection, and this effect was not stereospecific.²¹ This indicates that the NK1 receptor does not play an important role in developing mechanical

hyperesthesia evoked by SC carrageenan injection. Both CP-96,345 and CP-96,344 have been reported to interact with Ca^{2+} channel-binding sites.²² Such nonspecific effects of CP-96,345 may be important in blocking mechanical hyperesthesia or reducing the level of paw edema evoked by SC carrageenan.²¹ The inhibition of sP-induced vasodilatation or neurogenic plasma extravasation by CP-96,345 has been reported to be stereospecific, as CP-96,344 had no effect.²³ Thus, the pharmacologic characteristics of carrageenan-induced paw edema are different from those of neurogenic plasma extravasation, and, in the carrageenan model, the NK1 receptor plays an important role in development of thermal hyperesthesia, but not in development of paw edema.

In the intrathecal CP-96,345 study, pretreatment with 200 µg CP-96,345 had no effect on the development of thermal hyperesthesia when thermal hyperesthesia was tested 2 h after carrageenan injection. It has been reported that CP-96,345 has an effective half life after intrathecal administration of approximately 1 h,⁹ and this indicates that a reasonable amount of CP-96,345 exists in the spinal cord during the first 2 h after the drug injection. Thus, we think that the NK1 receptor does not play an important role in the development of thermal hyperesthesia evoked by carrageenan injection. It has been reported that 200 µg intrathecal CP-96,345 produced depression of the agitation behavior induced by the injection of formalin into a rat's hind paw when CP-96,345 was administered 1 min before the formalin injection.⁹ Although both subcutaneous formalin and subcutaneous carrageenan induce localized inflammation, there are several differences between the carrageenan test and the formalin test. Paw formalin injection induces biphasic spontaneous nociceptive behavior, such as flinching.¹⁶ However, carrageenan injection induces no flinching response in the rat, and 2 mg carrageenan induces a much more severe paw edema than does formalin.¹⁶ The difference in sensitivity produced by CP-96,345 in the formalin test and the carrageenan test may reflect the different characteristics of these two tests.

FK224, a cyclopeptide NK1 and NK2 antagonist, significantly blocked the development of carrageenan-induced thermal hyperesthesia, and significantly reduced carrageenan-induced paw edema when administered intravenously. It is proposed that the NK1 receptor interacts with the NK2 receptor in the nociceptive information transmission at both peripheral sites and in the spinal cord.^{12,13} Either intravenous FK224 or CP-

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96,345 completely blocked the development of thermal hyperesthesia at the doses we employed. In the current study, the effect of an NK2-selective antagonist on the development of thermal hyperesthesia was not examined. We do not know whether the effect of FK224 on the development of thermal hyperesthesia was caused by the interaction between the NK1 receptor and FK224, or by the interaction between both the NK1 and NK2 receptor and FK224. The CP-96,345 study showed that the NK1 receptor does not play an important role in the development of paw edema after carrageenan injection. We believe that the FK224 effect on carrageenan-induced paw edema is mediated by the NK2 receptor. We recognize, however, the possibility that other receptor systems may also be involved.

Tissue distribution experiments demonstrated that systemic FK224 administration does not enable FK224 to enter the central nervous system.[#] Thus, the site of action of intravenous FK224 is peripheral. Intrathecal injection of CP-96,345 had no effect on either carrageenan-induced paw edema or thermal hyperesthesia at a dose that had no effect on the general behavior. This indicates that the site of action of intravenous CP-96,345 on carrageenan-induced thermal hyperesthesia is not the spinal cord, but peripheral sites.

Posttreatment Study

Neither intravenous CP-96,345 nor intravenous FK224 had any effect on carrageenan-induced thermal hyperesthesia or carrageenan-induced paw edema at doses that blocked thermal hyperesthesia in the pretreatment study. Thus, we believe that, once inflammation has developed and thermal hyperesthesia has become established after a carrageenan injection, neither the NK1 nor the NK2 receptor play an important role in maintaining thermal hyperesthesia.

In the intrathecal study, neither CP-96,345 nor FK224 had any effect on carrageenan-induced thermal hyperesthesia or carrageenan-induced paw edema, and this CP-96,345 data is consistent with our previous report.¹⁸ Thus, we believe that the spinal NK1 and NK2 receptors do not play an important role in the development and maintenance of thermal hyperesthesia or paw edema evoked by SC carrageenan.

In the current study, we demonstrated that peripheral NK1 and NK2 receptors play an important role in the development of the inflammation after the chemical

stimuli. This indicates the possibility of the clinical use of NK1 and NK2 receptor antagonists as antiinflammatory analgesic agents.

References

1. Guard S, Watson SP: Tachykinin receptor types: Classification and membrane signalling mechanisms. *Neurochem Int* 18:149-165, 1991
2. Dalsgaard C-J, Haegerstrand A, Theodorsson-Norheim E, Brodin E, Hökfelt T: Neurokinin A-like immunoreactivity in rat primary sensory neurons: Coexistence with substance P. *Histochemistry* 83:37-39, 1985
3. Foreman JC: Peptides and neurogenic inflammation. *Br Med Bull* 43:386-400, 1987
4. Yaksh TL: Substance P release from knee joint afferent terminals: Modulation by opioids. *Brain Res* 458:319-324, 1988
5. White DM, Helme RD: Release of substance P from peripheral nerve terminals following electrical stimulation of the sciatic nerve. *Brain Res* 253:125-131, 1985
6. Nakamura-Craig M, Smith TW: Substance P and peripheral inflammatory hyperalgesia. *Pain* 38:91-98, 1989
7. Lam F, Ferrell WR: Mediators of substance P-induced inflammation in the rat knee joint. *Agents Actions* 31:298-307, 1990
8. Snider RM, Constantine JW, Lowe JA III, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW, Hess H-J: A potent nonpeptidic antagonist of the substance P (NK1) receptor. *Science* 252:435-437, 1991
9. Yamamoto T, Yaksh TL: Stereospecific effects of a nonpeptidic NK1 selective antagonist, CP-96,345: Antinociception in the absence of motor dysfunction. *Life Sci* 49:1955-1963, 1991
10. Yaksh TL, Farb DH, Leeman SE, Jessell TM: Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. *Science* 206:481-483, 1979
11. Jhamandas K, Yaksh TL, Harty G, Szolcsanyi J, Go VLW: Action of intrathecal capsaicin and its structural analogues in the content and release of spinal substance P: Selectivity of action and relationship to analgesia. *Brain Res* 306:215-225, 1984
12. Pedersen-Bjergaard U, Nielsen LB, Jensen K, Edvinsson L, Jansen I, Olesen J: Algesia and local responses induced by neurokinin A and substance P in human skin and temporal muscle. *Peptides* 10:1147-1152, 1989
13. Xu X-J, Wiesenfeld-Hallin Z: Intrathecal neurokinin A facilitates the spinal nociceptive flexor reflex evoked by thermal and mechanical stimuli and synergistically interacts with substance P. *Acta Physiol Scand* 144:163-168, 1992
14. Morimoto H, Murai M, Maeda Y, Yamaoka M, Nishikawa M, Kiyotoh S, Fujii T: FK244, a novel cyclopeptide substance P antagonist with NK1 and NK2 receptor selectivity. *J Pharmacol Exp Ther* 262:398-402, 1992
15. Murai M, Morimoto H, Maeda Y, Kiyotoh S, Nishikawa M, Fujii T: Effects of FK244, a novel compound NK1 and NK2 receptor antagonist, on airway constriction and airway edema induced by neurokinins and sensory nerve stimulation in guinea pigs. *J Pharmacol Exp Ther* 262:403-408, 1992
16. Wheeler-Aceto H, Porreca F, Cowan A: The rat paw formalin test: Comparison of noxious agents. *Pain* 40:229-238, 1990

[#] Fujii T: Personal communication. 1993.

17. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32:77-88, 1988
18. Yamamoto T, Shimoyama N, Mizuguchi T: The effects of morphine, MK-801, an NMDA antagonist, and CP-96,345, an NK1 antagonist, on the hyperesthesia evoked by carrageenan injection in the rat paw. *ANESTHESIOLOGY* 78:124-133, 1993
19. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 17:1031-1036, 1976
20. Birch PJ, Harrison SM, Hayes AG, Rogers H, Tyers MB: The non-peptide NK1 receptor antagonist, (\pm)-CP-96-345, produces antinociceptive and anti-oedema effects in the rat. *Br J Pharmacol* 105:508-510, 1992
21. Nagahisa A, Asai R, Kanai Y, Murase A, Tsuchiya M, Nakagaki T, Shieh T-C, Taniguchi K: Non-specific activity of (\pm) CP-96,345 in models of pain and inflammation. *Br J Pharmacol* 107:273-275, 1992
22. Schmidt AW, McLean S, Heym J: The substance P receptor antagonist CP-96,345 interacts with Ca^{2+} channels. *Eur J Pharmacol* 215:351-352, 1992
23. Lembeck F, Donnerer J, Tsuchiya M, Nagahisa A: The non-peptide tachykinin antagonist, CP-96,345, is a potent inhibitor of neurogenic inflammation. *Br J Pharmacol* 105:527-530, 1992