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Involvement of spinal NK_2 and NMDA receptors in aversive behavior induced by intra-arterial injection of capsaicin

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Abstract

The spinal processing by which intra-arterial injection of capsaicin (CAP) induces vocalization response (VOR) was investigated in guinea pigs. Intrathecal pre-treatment with CP-96,345 (a selective NK_1 receptor antagonist, 50 nmol) did not affect the CAP-induced VOR. However, significant attenuation of the VOR was observed by intrathecal pre-treatment with a selective NK_2 receptor antagonist MEN-10,376 (40 nmol) accompanied with a significant change in the response modality. MK-801 [an *N*-methyl-D-aspartate (NMDA) receptor antagonist, 20 and 40 nmol] inhibited the CAP-induced VOR dose-dependently without affecting the response modalities. Furthermore, intrathecal co-treatment with 40-nmol MEN-10,376 and 40-nmol MK-801 resulted in a marked inhibitory effect on the VOR followed by a significant alteration of response modalities. Intrathecal pre-treatment with neurokinin A (NKA; a tachykinin NK_2 receptor agonist, 1 nmol) enhanced the CAP-induced VOR. These behavioral results suggested that spinal NK_2 and NMDA receptors might have priority over NK_1 receptors in the spinal processing of nociceptive information from the CAP-sensitive nociceptor.

Keywords: Vocalization; Capsaicin; Tachykinin receptors; NMDA receptor; Guinea pig

1. Introduction

A small amount of capsaicin (CAP), a pungent principal of the *Capsicum* genus, has been shown to have powerful algogenic activity in a previous study of blister base (Szolcsanyi, 1977), and there are several lines of evidence for nociceptive effects of CAP injection into the arteries (Andoh et al., 1980, 1982) in animals. It is well known that arterial chemogenic nociceptors excite the nociceptive neurons in somatic nerves (Beck and Handwerker, 1974) and spinothalamic tracts (Andoh et al., 1982; Besson et al., 1972). At peripheral sites, the algesic actions of CAP are interpreted as due to the selective activation of unmyelinated C-fibers through the polymodal nociceptor (Szolcsanyi et al., 1988). In the central nervous system, CAP evokes the release of neurokinins such as substance P (SP) and neurokinin A (NKA) but not neurokinin B (Hua et al., 1986) and excitatory amino acids (EAAs) (Sorkin and McAdoo, 1993) from the spinal cord. These observations indicated that the nociceptive phenomena evoked by CAP are involved in the C-fiber-mediated and/or direct co-release of neurokinins and EAAs at the spinal cord.

SP and NKA have been suggested to play major roles in sensory processing in the mammalian spinal cord, and their specific binding sites on neurons include NK_1 and NK_2 receptors, respectively (Helke et al., 1990). Immunohistochemical evidence indicated that SP and NKA coexist in dorsal ganglion cells, dorsal roots and synaptic endings in the superficial laminae of the spinal dorsal horn (Dalsgaard et al., 1985), many of which also contain the EAA glutamate (Merighi et al., 1991) display selectivity for *N*-methyl-D-aspartate (NMDA) receptor (Mclennan and Lodge, 1979). In vitro, both neurokinins (Hope et al., 1990) and EAAs

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(Kangrga and Randic, 1991) have been shown to be released from the spinal cord following electrical stimulation of C-fibers. Iontophoretically applied SP (Henry, 1977), NKA (Fleetwood-Walker et al., 1993) and EAAs (Aanonsen et al., 1990) augment the responses of spinal neurons to peripheral noxious stimuli. Further, several behavioral investigations have indicated that intrathecal (it) SP (Hylden and Wilcox, 1981) and NMDA (Aanonsen and Wilcox, 1987) cause pain-related responses such as biting or licking of the hind paws and scratching with the hind paws, and intrathecal NKA (Fleetwood-Walker et al., 1990) showed hyperalgesic effects on thermal nociception. Thus, these neurochemicals have been suggested to act as transmitters of primary afferents involved in processing of nociceptive information in the spinal cord. However, it has not been clarified which of these neurochemicals are predominant in the processing of nociceptive information from peripheral nociceptors. In the present study, to elucidate the priority of the involvement of NK_1, NK_2 and NMDA receptors in spinal processing of nociceptive information from the CAP-sensitive nociceptor, we examined the spinal effects of their antagonists on the vocalization responses (VORs) induced by injection of CAP into the femoral artery, chemogenic nociceptors of which are innervated by somatic nerves in the hindlimbs (Guzman et al., 1962) and their responses are opiate sensitive (Andoh et al., 1982). The VOR is a natural reaction to noxious sensation in conscious animals (Levine et al., 1984) and has been suggested as a useful model for the study of pain (Adachi and Ishii, 1979). Therefore, this model can be used as a parameter to assess its nociceptive sensitivity and characteristics in the present experiment.

2. Methods

2.1. Animal preparation

Experimental procedures were conducted according to protocols approved by the Committee of Animal Experiments of the Tohoku Pharmaceutical University (Approval No. 96022) and in accordance with the ethical guidelines for investigation of experimental pain in conscious animals (Zimmerman, 1983). All experiments were performed on male Hartley guinea pigs (Charles River Japan, Yokohama) weighing 500–550 g. Animals were housed in groups of two in standard stainless-steel cages (W 35.0 × D 42.0 × H 20.0 cm) at a constant temperature (23 ± 1°C) and relative humidity (55 ± 1%) under a 12-h light/dark cycle, and were given food (CG-7, CLEA Japan, Tokyo) and water ad libitum. Arterial and thecal cannulae were made of silicon-coated polyethylene tubing (PE-10) tapered to an appropriate size by heating. Intra-arterial and intrathecal cannulations were performed simultaneously under ether anesthesia using a facemask containing ether-soaked cotton. Prior to the surgery, animals were treated with atropine sulfate (0.05 mg/kg ip). The arterial cannula for CAP injection filled with glycerin solution containing heparin (100 U/ml, approximately 50 μl) was inserted retrogradely 4–5 cm into the right or left femoral artery via the saphenous artery. The end tip of the cannula reached the bifurcation where the abdominal artery branches off to the common iliac artery. The CAP was then infused into the contralateral common iliac and femoral arteries, which were confirmed by angiography. Following laminectomy between L3 and L4, an intrathecal cannula filled with sterilized artificial cerebrospinal fluid (CSF; 126.7-mM NaCl, 2.5-mM KCl, 2.0-mM $MgCl_2$, 1.3 mM-$CaCl_2$, 15 μl) was caudally inserted into an opening in the dura. Its tip was carefully placed in the subarachnoid space of L5 or L6. With the cannula in this position, all parts of the lumbar cord and the caudal part of the thoracic cord were stained by injection of trypan blue solution (10 μl plus wash out with artificial CSF, 17 μl). The opposite end of each cannula tip was passed through and fixed to the skin of the nape of the neck, and cannulated animals were medicated with an anti-inflammatory analgesic (ketoprofen, 5 mg/kg im) and laid on a warming chamber for recovery for a few hours. The animals were allowed to recover for over 14 days in individual cages (W 35.0 × D 42.0 × H 20.0 cm).

2.2. Recording and analysis of VOR

Prior to the onset of the experiment, each animal was placed in a testing cage (W 8.0 × D 21.0 × H 8.0 cm) in a normal posture for 1 h for adaptation. The VOR was recorded on a FM data recorder (VR-500WB, TEAC, Japan) through an electric condenser microphone (MZ-110, JVC, Japan) located on the side of the testing cage approximately 8 cm from the animal's head. All recorded VORs were analyzed with a signal processor (DP1100, NEC, Japan). The signal processor could summate with amplitudes (mV) of collected vocal sound waves for every 50 μs in a wide dynamic range (DC∽10 000 Hz) and displayed the rectified form (Fig. 1). For quantitative analysis, the value of integration (mV s), which was obtained by integrating the area of the rectified form within VOR, was used as the vocalization count (VC). CAP at a dose of 1 μg afforded VC values of 98.6–331.4 mV s (mean ± S.D. = 190.7 ± 65.4 mV s, $n = 118$) in the pre-treated (control) animals. The VC induced by CAP has been confirmed to be dose dependent (Andoh et al., 1982). As illustrated in Fig. 1, the latency was the period measured from the beginning of injection to the appearance of VOR (1.4–4.6 s, mean ± S.D. = 3.0 ± 1.0 s, $n = 118$), and the duration was the period of VOR (4.4–13.1 s, mean ± S.D. = 7.7 ± 2.3 s, $n = 118$). We interpreted these data including the latency and the duration of the VOR as the sensitivity to the arterial CAP in various experimental settings. In general, no tachyphylaxis was observed following repeated injection of CAP (1 μg) when at least 20 min elapsed between injections. Therefore, each CAP injection was performed at intervals greater than 20 min in this study.

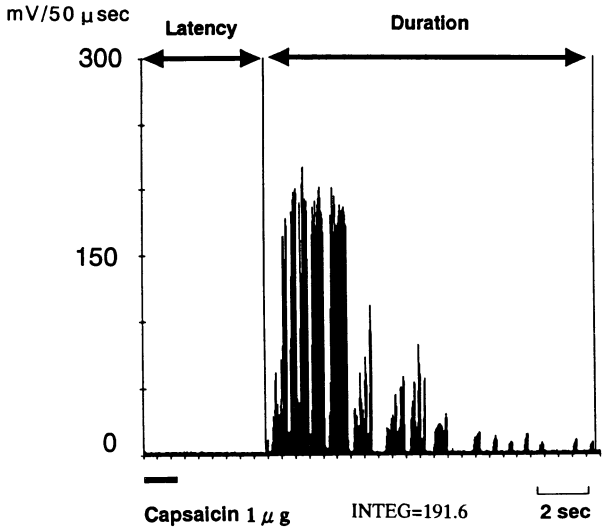

Fig. 1. A typical tracing of rectified form with CAP (1 μg)-induced vocalization collected and summated for every 50 μs by the signal processor. The thick bar indicates the injection time (approximately 1.2 s) of CAP into the femoral artery. Each double-arrowed section indicates the latency and the duration of VOR, respectively. Ordinate: the amplitude of summated vocal sounds/50 μs (50 mV/division). Abscissa: the passage of time in s (0.5 s/division). Abbreviation: INTEG, the value of integration for the rectified form within the duration (VOR, mV s).

At the end of each experiment, we verified that intrathecal injection of lidocaine HCl (1000 μg/10 μl; Fujisawa Pharmaceutical, Japan) elicited transient paralysis of both hindlimbs. All animals were used only once.

2.3. Drugs

CP-96,345 (Pfizer, USA) and MK-801 (Research Biochemicals, USA) were dissolved in artificial CSF, and MEN-10,376 (Research Biochemicals) was dissolved in 50% dimethyl sulfoxide (DMSO; Nacalai Tesque, Japan). SP, NKA (Peptide Institute, Japan) and NMDA (Research Biochemicals) dissolved in artificial CSF were used as agonists for NK_1, NK_2 and NMDA receptors, respectively. Each drug or vehicle alone was injected intrathecally in a volume of 10 μl followed by 17 μl of artificial CSF to flush the cannula at a constant speed (10 μl/30 s). In a few animals, we confirmed that intrathecal injection of each vehicle alone and maximum dose of drug did not cause any motor dysfunction. CAP (Merck, Germany) was dissolved in 0.05 ml of ethanol and diluted (50 μg/ml) in the mixed vehicle (ethanol, 1%; Tween 80, 1%; Ringer's solution, 98%). The CAP with a dose of 1 μg was injected with Ringer's solution at a constant speed (0.5 ml/30 s). Ringer's

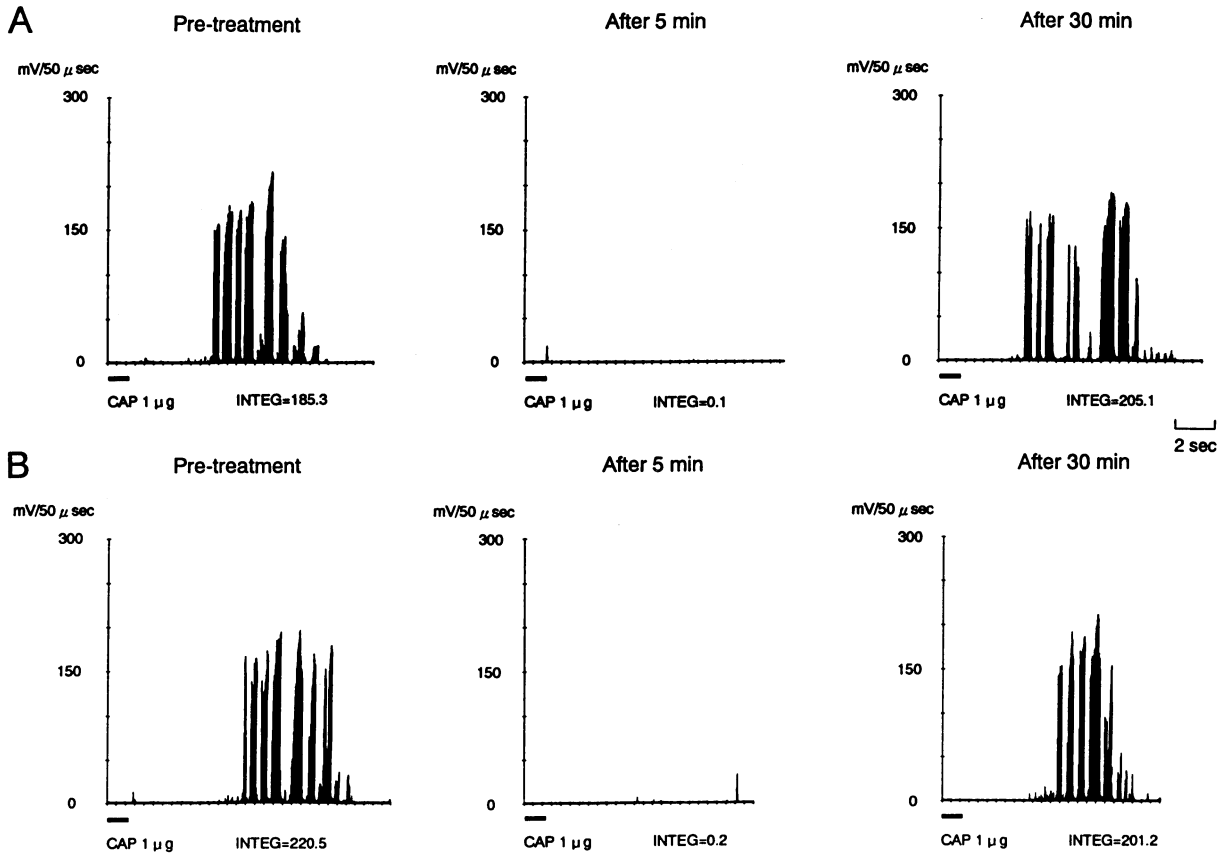

Fig. 2. Vocalization tracings for temporary blocking effects of intra-arterial (0.8 mg/min for 3 min, Panel A) and intrathecal (1000 μg/10 μl, Panel B) pretreatment with lidocaine on intra-arterial CAP (1 μg). Ordinate: the amplitude of summated vocal sounds/50 μs (50 mV/division). Abscissa: the passage of time in s. Abbreviation: INTEG, the value of integration for rectified form within the duration (VOR, mV s).

solution and the mixed vehicle did not cause any behavioral responses when intra-arterially injected alone, respectively. The effect of each antagonist on the CAP-induced VOR was measured at 10, 30, 60, 90 and 120 min after intrathecal treatment. In the agonists, VOR was measured at 5 and 30 min after intrathecal treatment.

2.4. Statistics

The results of intrathecal treatment with drugs were calculated as percentages of the mean pre-drug VOR taken as 100%. The data were shown as mean ± S.E.M. and were analyzed with repeated-measures ANOVA followed by

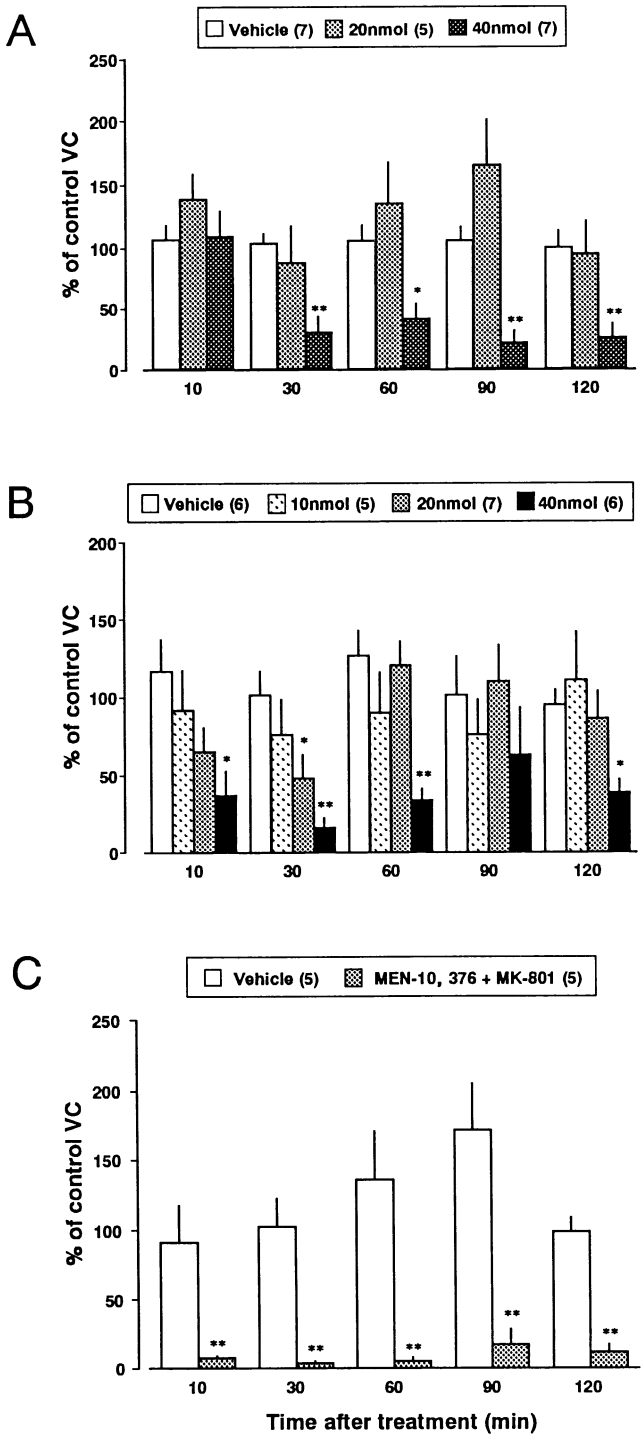

Fig. 3. The effects of intrathecal pre-treatment with MEN-10,376 (Panel A), MK-801 (Panel B) and MEN-10,376 + MK-801 (Panel C) on VC of VORs by intra-arterial CAP (1 μg). Results are expressed as means ± S.E.M. Values in parentheses are the number of animals used in each group. Significant differences between each antagonist- and vehicle-treated groups are shown as $*P<.05$, $**P<.01$ (Dunnett's post-hoc procedure).

Dunnett's post-hoc procedure (for the antagonists) or Fisher's protected least significant difference (for the agonists). Differences were considered statistically significant at $P<.05$.

3. Results

3.1. Effects of local anesthetics on CAP-induced VOR

The injection of 1 μg CAP into the femoral artery of conscious guinea pigs usually induced VOR accompanied with shaking or stamping of the infused hindlimb. These excitatory responses were temporarily blocked by previous intra-arterial infusion of lidocaine (0.8 mg/min for 3 min; Fig. 2A) through the same cannula accompanied with muscle tonus and flexion reflex of the infused hindlimb in response to pinching of the skin with forceps ($n=3$). Blockade of VORs was almost restored at 30 min after infusion of lidocaine. Similar results were observed in guinea pigs pre-treated intrathecally with lidocaine (1000 μg/10 μl) in addition to transient paralysis of both hindlimbs ($n=3$; Fig. 2B).

3.2. Effects of CP-96,345, MEN-10,376 and MK-801 on CAP-induced VOR

The effects of intrathecal pre-treatment with the tachykinin NK_1 receptor antagonist CP-96,345 (Snider et al., 1991) on the CAP-evoked VOR were studied to assess the role of NK_1 receptors at the level of the spinal cord in the response to CAP. Pre-treatment with CP-96,345 (25 and 50 nmol) had no significant effect on the VC, latency and duration of the CAP-evoked VOR as compared with vehicle-treated controls [data not shown; overall repeated-measures ANOVA: $F(2,10)=0.48$, 0.85, 0.84; $P>.05$ for the VC, latency and duration, respectively].

Fig. 3A illustrates the effects of MEN-10,376, a tachykinin NK_2 receptor antagonist (Maggi et al., 1991), on the CAP-induced VOR. Pre-treatment with MEN-10,376 (40 nmol) caused a marked and significant decrease in the VC values at 30 (30.6±12.6%, $P<.01$), 60 (41.5±12.9%, $P<.05$), 90 (21.8±9.1%, $P<.01$) and 120 min (25.5±11.4%, $P<.01$) as compared with vehicle-treated group. The latencies of VOR were significantly prolonged at 30 (175.9±20.5%, $P<.05$) and 90 min (144.6±8.4%, $P<.05$) after treatment as compared with the vehicle-treated control group (Table 1). The duration was slightly but not significantly reduced (Table 1). Intrathecal pre-treatment with 20-nmol MEN-10,376 had no apparent effects on VC value or latency of CAP-evoked VOR as compared with vehicle-treated group by Dunnett's post-hoc procedure [Fig. 3A and Table 1; repeated-measures ANOVA: $F(2,10)=5.61$, 1.97; $P<.01$, $P<.05$ for the VC and latency, respectively]. Repeated-measures ANOVA indicated a nonsignificant effect of 20-nmol MEN-10,376 for the duration [$F(2,10)=1.12$, $P>.05$].

MK-801, a selective NMDA receptor antagonist acting as a cation-channel blocker (Huettner and Bean, 1988), showed a dose-dependent decrease in VC of the VOR at 10 and 30 min following intrathecal administration (Fig. 3B). MK-801 at a dose of 20 nmol significantly decreased VC only at 30 min (48.0±15.5%, $P<.05$) after administration. A significant decrease in the VC was observed 10 (37.4±15.5%, $P<.05$), 30 (15.7±6.4%, $P<.01$), 60 (34.0±7.1%, $P<.01$) and 120 min (38.4±8.8%, $P<.05$) after administration of 40 nmol of MK-801 (Fig. 3B). MK-801 did not cause significant changes in the latency or duration of the CAP-induced VOR at any dose examined [data not shown; overall repeated-measures ANOVA: $F(3,15)=1.79$, 1.23, 1.16; $P<.05$ for the VC, latency and duration, respectively].

3.3. Effects of co-administration of MEN-10,376 and MK-801 on CAP-induced VOR

Co-administration of MEN-10,376 (40 nmol) and MK-801 (40 nmol) markedly inhibited the VOR of CAP. There was a significant decrease in the VC value 10 (7.2±2.4%, $P<.01$), 30 (4.2±1.3%, $P<.01$), 60 (5.6±2.4%, $P<.01$), 90 (16.9±11.8%, $P<.01$) and 120 min (11.6±5.4%, $P<.01$) after intrathecal co-administration of both antago-

Table 1
The effects of intrathecal pre-treatment with MEN-10,376 on the latency (L) and duration (D) of VOR evoked by CAP

Drugs		% of control (mean±S.E.M.) After 10 min	After 30 min	After 60 min	After 90 min	After 120 min
Vehicle						
	L	99.9±28.2	104.9±22.0	91.7±13.6	96.0±17.7	106.6±18.9
	D	109.2±10.3	101.8±9.6	100.0±10.7	90.7±7.4	88.1±8.5
MEN-10,376						
20 nmol	L	87.0±12.1	109.0±10.6	89.7±6.5	79.1±17.1	93.0±11.9
	D	138.7±14.6	115.1±27.0	148.1±23.1	135.7±27.0	110.7±30.9
40 nmol	L	99.3±18.9	175.9±20.5 *	119.7±10.6	144.6±8.4 *	117.3±5.4
	D	110.8±19.9	85.8±17.2	74.7±11.6	80.0±7.8	51.0±19.5

Values were obtained in the intra-arterial CAP (1 μg) in vehicle- and MEN-10,376-treated groups ($n=5-7$).
* $P<.05$, significantly different from vehicle-treated group (Dunnett's post-hoc procedure).

Table 2
The combined effects of intrathecal pre-treatment with MEN-10,376 and MK-801 on the latency (L) and duration (D) of VOR evoked by CAP

	% of control (mean ± S.E.M.)				
Drugs	After 10 min	30 min	60 min	90 min	120 min
Vehicle					
L	128.7 ± 15.2	117.5 ± 19.8	110.3 ± 12.5	109.5 ± 13.7	99.3 ± 9.3
D	110.2 ± 16.3	88.7 ± 8.3	94.8 ± 9.7	121.2 ± 17.2	97.9 ± 13.2
MEN-10,376 (40 nmol) + MK801 (40 nmol)					
L	188.1 ± 30.9	195.1 ± 27.0 *	206.26 ± 32.9 *	193.0 ± 31.6 *	197.2 ± 27.7 * *
D	55.6 ± 10.6 *	52.1 ± 16.3	54.1 ± 14.5 *	50.8 ± 13.4 * *	65.8 ± 21.8

Values were obtained in the intra-arterial CAP (1 μg) in vehicle- ($n = 5$) and MEN-10,376 + MK-801-treated groups ($n = 5$).
* $P < .05$, significantly different from vehicle-treated group (Dunnett's post-hoc procedure).
** $P < .01$, significantly different from vehicle-treated group (Dunnett's post-hoc procedure).

nists [Fig. 3C; repeated-measures ANOVA: $F(1,5) = 6.35$; $P < .05$]. Repeated-measures ANOVA showed a statistically significant difference in the main effect for VC in the co-treated group as compared with the MK-801 (40 nmol)-treated [$F(1,5) = 7.23$, $P < .05$] but not MEN-10,376 (40 nmol)-treated group [$F(1,5) = 3.65$, $P > .05$]. Significant prolongation of the latency was also observed from 30 to 120 min after co-administration (Table 2). In the duration, significant variation was also observed at 10, 60 and 90 min after injection [Table 2; overall repeated-measures ANOVA:

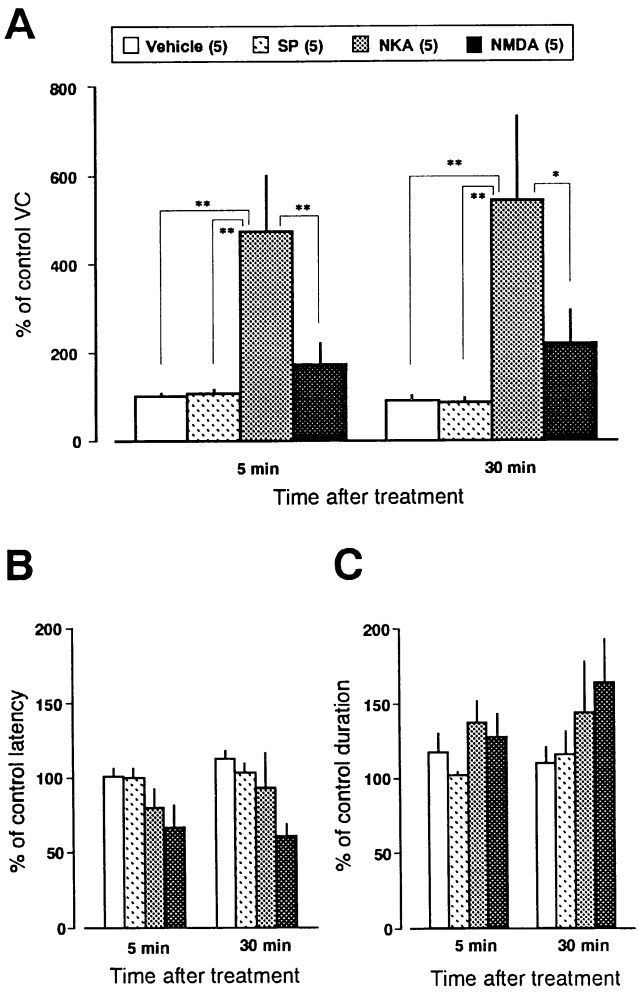

Fig. 4. The effects of intrathecal pre-treatment with SP, NKA and NMDA on VC (Panel A), latency (Panel B) and duration (Panel C) of CAP (1 μg)-evoked VORs. Results are expressed as means ± S.E.M. 5 and 30 min after intrathecal administration of SP, NKA and NMDA. Values in parentheses are the number of animals used in each group. Significant differences between NKA- and vehicle-treated (ACSF) and SP- and NMDA-treated groups are shown as * $P < .05$, * * $P < .01$ (Fisher's protected least significant difference).

$F(1,5) = 3.26$ and 2.55; $P < .05$ for the latency and duration, respectively]. The latency was significantly different between the co-treated and the MK-801 (40 nmol)-treated groups [repeated-measures ANOVA: $F(1,5) = 4.06$; $P < .01$] but not MEN-10,376 (40 nmol)-treated group [repeated-measures ANOVA: $F(1,5) = 1.49$; $P > .05$].

3.4. Effects of SP, NKA and NMDA on CAP-induced VOR

Pre-treatment with NKA (1.0 nmol) produced a marked increase in the CAP-evoked VOR, whereas pre-treatment with SP (1.0 nmol) or NMDA (1.0 nmol) showed no effect. As illustrated in Fig. 4A, the NKA-treated group showed $474.4 \pm 124.8\%$ (after 5 min) and $543.8 \pm 72.9\%$ (after 30 min) of the control VC values, and these values were significantly different from the vehicle, SP and NMDA-treated groups [$P < .01$; repeated-measures ANOVA: $F(3,6) = 2.97$; $P < .05$]. However, there was no significant effect in the latency and duration of the CAP-induced VOR analyzed by repeated-measures ANOVA [$F(3,6) = 1.21$ and 0.98; $P > .05$ for the latency and duration, respectively; Fig. 4B and C).

4. Discussion

We observed that intra-arterial pre-treatment with lidocaine transiently blocked arterial CAP-induced VOR excluding the response to skin pinching. On the other hand, intrathecal pre-treatment with lidocaine also provoked temporary disappearance of VOR accompanied with paralysis of the hindlimbs. A previous study (Tallarida et al., 1979) in generally anesthetized rabbits showed that injection of bradykinin (BK), an endogenous algogenic, into the femoral artery evoked nociceptive cardiovascular and respiratory reflexes that were inhibited by arterial pre-infusion of local anesthetic or sectioning of femoral and sciatic nerves. Another group (Guzman et al., 1962) demonstrated that nociceptive VORs to femoral injection of BK or potassium chloride were blocked by ganglionectomy of the ipsilateral dorsal root ganglion from L2 to S2 but not by excision of the ipsilateral sympathetic chain from L2 to L5 in mammals. These findings and our results suggested that injection of CAP into the femoral artery might stimulate chemosensitive nociceptors in the arterial and/or deep peri-arterial tissues, e.g. the skeletal muscles of the hindlimb that are mainly innervated by the somatic nerves and their nociceptive information is transmitted to the caudal spinal cord through the dorsal root ganglion.

Previous behavioral studies have indicated that a selective NK_1 antagonist, CP-96,345, produces antinociceptive effects to several noxious stimuli (Besson et al., 1972), and that its antagonistic effects on NK_1 agonists evoked nociceptive behavior (Picard et al., 1993). Another study (Radhakrishnan and Henry, 1991) demonstrated that systemic treatment with CP-96,345 (0.5 mg/kg ip) depresses the noxious radiant heat-evoked responses of the SP-sensitive but not the SP-insensitive neurons in cats. On the other hand, Dougherty et al. (1994) reported that CP-96,345 prevented the CAP-induced sensitizing effects on the responses of primate spinothalamic tract neurons to noxious mechanical stimulation of the skin but not the responses to noxious input by itself. In the rat nociceptive reflex model (Xu et al., 1992), CP-96,345 administered intrathecally did not inhibit the skin C-fiber-mediated flexion reflex induced by single electrical stimulation. In addition, CAP-induced evoked potentials in rat spinal neurons in vitro were also unaffected by CP-96,345 (Urban et al., 1993). The present results also indicated that neither the tachykinin NK_1 receptor agonist (SP) nor the antagonist (CP-96,345) changed the CAP-induced VOR in guinea pigs, although they possessed much higher affinity for brain sites in the guinea pig than in the rat (Gitter et al., 1991). These findings suggested that spinal NK_2 receptors might play a major role in the processing of nociceptive information from CAP-sensitive nociceptors.

There is accumulating evidence for the involvement of NK_2 receptor activity in the mediation of high-threshold afferent-evoked responses in the spinal cord. NK_2 antagonists have been shown to inhibit the nociceptive transmission in the mammalian spinal cord. Spinal treatment with selective NK_2 antagonists inhibits the NK_2 agonist-facilitated neural and behavioral responses to noxious heat stimuli (Fleetwood-Walker et al., 1990). The exogenous NKA and electrical conditioning (tonic) stimulation of the gastrocnemius nerve (at C-fiber intensity) causes the facilitation, which was inhibited by a NK_2 antagonist in the flexor reflex of the rat (Xu et al., 1991). A selective NK_2 antagonist, MEN-10,376, reduces responses of neurons in the in vitro spinal cord dorsal root preparations to brief application of CAP at the dorsal root ganglia (Urban et al., 1993). Nagy et al. (1994) reported that small diameter primary afferents, which produce slow synaptic potentials in neonatal rat spinal neurons, are significantly attenuated by MEN-10,376. Furthermore, the responses of primate dorsal horn neurons to intradermal injection of CAP are attenuated by MEN-10,376 (Dougherty and Willis, 1991). In the present study, MEN-10,376 (40 nmol) significantly attenuated the CAP-induced VOR, and intrathecal NKA (1 nmol) markedly facilitated the CAP-induced VOR (cf. Figs. 3A and 4A and Table 2). Therefore, our results indicated that spinal NK_2 receptors might be principally responsible for activation of CAP-sensitive nociceptors in this experimental model.

There is substantial evidence that NMDA-type EAA receptors are involved in nociception. Dougherty and Willis (1991) reported that iontophoretically applied NMDA can induce excitatory effects on dorsal horn nociceptive neurons. Competitive antagonists APV (D-2-amino-5-phosphonopentanoic acid) and CPP [D-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid] and the non-competitive NMDA antagonist MK801 have been shown to attenuate the increases in

excitability of dorsal horn nociceptive neurons induced by formalin (Haley et al., 1990), mustard oil (Woolf and Thompson, 1991), Freund's complete adjuvant and carrageenan (Ren et al., 1992). These NMDA antagonists can also inhibit the behavioral responses to the nociceptive mechanical and thermal stimuli (Cahusac et al., 1984) and hyperalgesia induced by constriction injury of the sciatic nerve (Yamamoto and Yaksh, 1992). Furthermore, the NMDA antagonist APV co-injected intrathecally with CAP resulted in a marked inhibition of the aversive behavior to CAP (Okano et al., 1994). These observations and the present results of the inhibitory effects of MK-801 on the CAP-evoked VOR (cf. Fig. 3B) indicate that NMDA receptors take part in nociceptive processing in the spinal cord.

Rusin et al. (1992) reported that the neurokinins (SP and NKA) potentiate NMDA-induced currents modulated by the cyclic-nucleotide transduction cascade in acutely isolated neurons from the rat dorsal horn. Exogenous administration of neurokinins increased glutamate and aspartic acid release, and this was inhibited by neonatal CAP pre-treatment in spinal cord slices in vitro (Kangrga and Randic, 1990). Recently, intrathecal NMDA was reported to increase NK_1 receptor expression and release of SP in the rat spinal cord (Liu et al., 1997). These observations suggested that the NMDA and neurokinin (SP and NKA) receptors are closely linked in their functions concerning nociceptive transmission in the spinal cord. These observations suggest that the CAP-induced VOR is delivered through the release of neurokinins and EAAs in primary afferent terminals of the spinal cord. The marked inhibition of VOR by the co-treatment with MEN-10,376 and MK801 (cf. Fig. 3C and Table 2) supported the suggestion that there is a specific interaction between NK_2 and NMDA receptors involved in sensory development from the CAP-sensitive nerves in the arteries and/or muscles. These observations are in agreement with previous electrophysiological results in rat spinal preparations (Urban et al., 1993). The mechanism of this interaction is unclear, but it is possible that selective cooperation between NK_2 and NMDA receptors may occur through activation of protein kinase C (Rusin et al., 1992).

In conclusion, the present study demonstrated that intrathecal treatment with either agonists or antagonists of NK_2 and NMDA but not NK_1 receptors showed significant effects on the arterial CAP-induced aversive behavior. These observations suggested that NK_2 and NMDA receptors might have priority over NK_1 receptors in spinal processing of nociceptive information from the CAP-sensitive nociceptors.

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