Experimental Neurobiology
Vol. 15, pages 71~79, December 2006

Effects of Capsaicin on the *c-fos* in the Spinal Dorsal Horn and Substance P- and CGRP-like Immunoreactivities in the Dorsal Root Ganglia of the Experimental Arthritic Rat Model

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ABSTRACT

To analyze the effects of capsaicin administered to the arthritic rat model, immunohistochemical stains for *c-fos* protein in the spinal dorsal horn and for substance P and CGRP in the dorsal root ganglia (DRG) were done. Kaolin and carageenan were administered to the knee joint cavity of adult rats to induce arthritis, and capsaicin was administered immediately after kaolin-carageenan injection. A count was conducted of the *c-fos* immunoreactive dorsal horn neurons and substance P and CGRP immunoreactive cells in L5 and L6 DRG to elucidate the effect of capsaicin. The number of *c-fos* immunoreactive neurons in the superficial dorsal horn was increased markedly 2 hours after the kaolin and carageenan injection to the knee joint, and decreased gradually to the control level 1 week after injection. The number of *c-fos* immunoreactive neurons in the deep dorsal horn was increased later than those in the superficial dorsal horn and reached peak level 16 hours after the kaolin and carageenan injection, and decreased gradually thereafter. After capsaicin treatment, the number of *c-fos* immunoreactive neurons in the superficial and deep dorsal horn of capsaicin-treated rats was less than in those rats not treated with capsaicin. The number of substance P and CGRP immunoreactive DRG neurons increased 24 hours after the kaolin and carageenan injection to the knee joint, and also apparently increased 1 week after injection. The number of substance P and CGRP immunoreactive DRG neurons of capsaicin-treated rats was less than in those rats not treated with capsaicin administered rats. Capsaicin reduces the number of *c-fos* immunoreactive neurons in the spinal dorsal horn, and also reduces the number of substance P and CGRP immunoreactive neurons in the DRG of the arthritic rat model, which may be closely related to the analgesic effects of capsaicin.

Key words: capsaicin, *c-fos*, substance P, calcitonin gene-related peptide (CGRP), spinal cord dorsal horn, dorsal root ganglia (DRG), arthritis, pain

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INTRODUCTION

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the

major pungent ingredient of hot peppers (plant genus *Capsicum*). Capsaicin excites peripheral nociceptors on initial administration, but desensitization of pain fibers occur with repeated application in adult animals (Jancs , 1968). Administration of capsaicin on the newborn animals results in a selective and permanent degeneration of small diameter primary sensory neurons including small B type neurons in the dorsal root ganglia, their terminals in the spinal dorsal horn, and their unmyelinated C and small myelinated Ad fibers (Jancs et al., 1977; Hayes et al., 1981, Nagy and Van der Kooy, 1983).

Capsaicin has been used in the field of neurobiology as a powerful tool investigating mechanism of pain transmission, and also used as a therapeutic agent for painful disorders.

The expression of *c-fos* is a morphological and functional marker for identifying the activity of spinal neurons in response to noxious stimulation. It had been used for the assessment for severity of pain and for analgesic efficacy (Hylden et al., 1992; Abbadie and Besson, 1993; Kosai et al., 2001; Bai et al., 2001; Buritova and Besson, 2002).

Capsaicin specifically elicits neurotransmitter releases, especially substance P (SP) and calcitonin gene-related peptide (CGRP) from small sized neurons of dorsal root ganglion and their fibers. SP and CGRP considered to be putative neurotransmitters of the pain transmission (Pernow, 1983; Besson and Chaouch, 1987).

Capsaicin administration has been shown to effective in experimental arthritis in rats (Colpaert et al., 1983; Levine et al., 1986; Ahmed et al., 1995). Antinociceptive effects of capsaicin on the osteoarthritis and rheumatoid arthritis were established clinically with double-blind trial (Deal et al., 1991).

Although capsaicin has been reported to reduce pain by neural mechanism, its effects on sensory dorsal root ganglion neurons and spinal dorsal horn neurons has not been clarified. The purpose of this study is to analyze the effects of capsaicin administered to the arthritic rat model by immunohistochemical stains for *c-fos* protein in the spinal dorsal horn and for substance P and CGRP in the dorsal root ganglia (DRG).

MATERIALS AND METHODS

Sixty-five adults Sprague-Dawley rats (200～250 gm) were used in this study. The rats were divided into (a) normal control group (NC), (b) arthritis induced group (EC) which were given kaolin and carageenan, (c) capsaicin treated group (CAP) that were given capsaicin immediately after inducing arthritis. All animal procedures were carried out according to a protocol approved by the Yonsei University Animal Care Committee.

Arthritis was induced experimentally by intrasynovial injection of a 4% kaolin dissolved in distilled water into the knee joint and the knee was flexed and extended for 15 min. Then, 0.15～0.3 ml of 2% carageenan solution was injected into the joint cavity and the knee was flexed and extended for 5 min.

Capsaicin (50 mg/kg, Sigma Chemical Co., St. Louis, MO, USA) was prepared with 10% ethanol, 10% Tween80 and 80% saline solution and was injected to the adult rat subcutaneously. Only the vehicle was administered into the control rats.

The arthritis induced group and capsaicin treated group were sacrificed 2 h, 4 h, 8 h, 16 h, 24 h, and 1 week after inducing arthritis, 5 rats in each respectively.

The animals were anesthetized by sodium pentobarbital and then perfused through the left ventricle with saline followed by 4% paraformaldehyde (0.1 M phosphate buffered saline, PBS, pH 7.4). L5 and L6 spinal segments were obtained to perform immunohistochemical staining for *c-fos* protein. L5 and L6 spinal ganglia were obtained to perform immunohistochemical staining for SP and CGRP. The extracted spinal cords was fixed in additional fixing solution for 2～24 hours and sectioned on a vibratome at 50 μm-thickness. The spinal ganglia were embedded in paraffin after being dehydrated and cleared. Tissues were sectioned into 10 μm-thick slices using a microtome.

For immunohistochemical staining, the sliced tissues were washed several times using PBS and were immunohistochemically stained by the peroxidase-antiperoxidase (PAP) method of Sternberger (1986). Sections were placed in 10% normal goat serum (NGS; Gibco Lab., New York, NY, USA) for 1 hour followed by incubation with anti-*c-fos* anti-

body (Oncogene, Boston, MA, USA) diluted at 1 : 500 ratio, antirat rabbit SP antibody (Oncogene, Boston, MA, USA) diluted at 1 : 2,000, and antirat rabbit CGRP antibody (Oncogene, Boston, MA, USA) diluted at 1 : 2,000 for 72 hours at 4°C.

Following several rinses in phosphate buffer (pH 7.4, containing 0.1% Triton X-100), the tissue was incubated with goat anti-rabbit gamma globulin (GAR; Chemicon International Inc., Temecula, CA, USA) at a dilution of 1 : 50 for 1 hour at room temperature. The sections were rinsed several times with the same phosphate buffer and then placed in peroxidase-antiperoxidase (PAP) conjugates (Chemicon International Inc., Temecula, CA, USA) at a dilution of 1 : 100 for 1 hour at room temperature. Following several rinses in phosphate buffer, the sections were placed in 0.05% diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO, USA) solution containing 0.01% hydrogen peroxide for 10 minutes. Controls were prepared using incubating solution without primary antibodies. All procedures were performed in a moisture chamber.

Stained samples were used to measure the degree of positive immunoreactivity using an image analyzer. The number of *c-fos* stained neurons in the posterior horn of the spinal cord of L5 and L6 segments was counted in the superficial layer and deep layer. The numbers of SP immunoreactive cell and CGRP immunoreactive cell were calculated in L5 and L6 spinal ganglia. These numbers counted were analyzed in each group. Results were statistically analyzed by the Mann-Whitney U test.

RESULTS

Change of c-fos protein-like immunoreactivity in the dorsal horn of the spinal cord

The immunoreactivity of *c-fos* protein was determined with dark brown granules in the nuclei of neurons in the dorsal horn of the spinal cord (Fig. 1). The positive immunoreactivities appeared as dark spots in the superficial (Rexed lamina I and II) and deep layer (Rexed lamina III, IV, V, VI) of spinal posterior horn.

Changes in the superficial layer of the dorsal horn of the spinal cord

The number of *c-fos* positive neurons in the superficial layer of the dorsal horn was quantified. In normal control rats, the number was 0.83 ± 0.08 (n=5) in L5 and 0.81 ± 0.21 (n=5) in L6 in average (not shown in table). Compared with normal control rats, this number was increased significantly at all time periods after inducing arthritis (Table 1). In the experimental group, *c-fos* positive neurons were observed in the contralateral side from 2 hr to 8 hr after inflammation ($p < 0.01$). The number of positive cells increased significantly by 2 hr and decreased gradually afterwards (Table 1). Comparing to the contralateral side, the number of the *c-fos* positive neurons in the inflammed side was increased statistically significant at all time periods ($p < 0.01$). When capsaicin was injected after inflammation induction, the number of *c-fos* protein positive neurons was not significantly changed in the

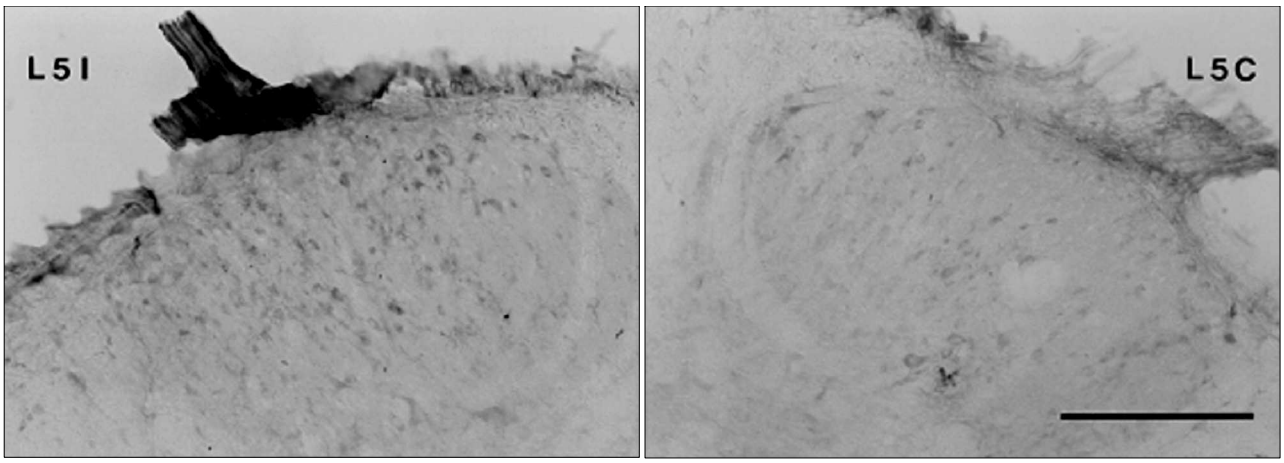

Fig. 1. Photomicrograph of *c-fos* protein immunostained L5 dorsal horn. L5I: inflammed side, L5C: control side. bar=0.1 mm.

contralateral side. However, the number of *c-fos* protein positive neurons was decreased and it was statistically significant. The overall pattern of changes was similar in L6 as in L5 (Table 2).

Changes in the deep layer of the posterior horn of the spinal cord

The degree of *c-fos* protein expression in the deep layer of the posterior horn of the spinal cord, i.e., Rexed lamina II, IV, V and VI was more prominent compared to the superficial layer in control rats. In normal control group, the number of *c-fos* protein positive immunoreactive neurons in the deep layer of the posterior horn of the L5 was 4.12 ±0.35 (n=5) in the average. In L6, this number was 3.11±0.54 (n=5). In the L5 of inflammation group, the number of *c-fos* protein positive cells in the deep layer was increased gradually from 2 hours after inflammation induction and reached maximum by 16 hours. They were recovered to almost normal level by 24 hours later (Table 3). Comparing the number of *c-fos* protein positive cells between the inflammed side and contralateral side, 8 hours group and 16 hours group showed significant difference ($p<0.01$), but after that there was no difference (Table 3). When capsaicin was

Table 1. Number[1] of *c-fos* protein-immunoreactive neurons in the L5 superficial dorsal horn

		2 hrs[2]	4 hrs[2]	8 hrs[2]	16 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Inflamed side[2]	49.71±5.81	20.53±4.09	14.21±3.84	11.67±2.72	9.56±1.97	7.49±1.39
	Control side[2]	4.26±0.52	3.34±0.50	3.27±1.02	1.47±0.21	1.83±0.26	0.84±0.08
Capsaicin treated	Inflamed side[2]	31.55±6.90*	15.87±3.72*	9.17±1.58*	7.44±1.42*	6.38±1.08†	3.23±0.74*
	Control side[2]	3.89±0.69	3.51±0.79	3.13±0.59	1.66±0.33	1.39±0.29	1.01±0.16

[1]average±standard error, [2]n=5 each. *$p<0.01$, †$p<0.05$ to control side of each group.

Table 2. Number[1] of *c-fos* protein-immunoreactive neurons in the L6 superficial dorsal horn

		2 hrs[2]	4 hrs[2]	8 hrs[2]	16 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Inflamed side[2]	36.43±4.33	16.59±2.38	11.83±1.83	8.41±1.64	5.32±1.08	4.13±0.40
	Control side[2]	3.27±0.90	2.27±0.12	2.82±1.01	1.30±0.16	1.33±0.26	0.82±0.14
Capsaicin treated	Inflamed side[2]	30.77±6.00*	13.17±2.74*	9.37±1.55*	7.98±1.33*	3.94±1.65*	3.55±0.74†
	Control side[2]	2.91±0.59	2.44±0.51	2.71±0.54	1.83±0.88	1.41±0.39	0.90±0.24

[1]average±standard error, [2]n=5 each group. *$p<0.01$, †$p<0.05$ to control side of each group

Table 3. Number[1] of *c-fos* protein-immunoreactive neurons in the L5 deep dorsal horn

		2 hrs[2]	4 hrs[2]	8 hrs[2]	16 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Inflamed side[2]	5.49±0.78*	6.59±0.50*	6.89±0.75*	7.11±1.04*	4.12±0.54	4.81±0.79
	Control side[2]	3.43±0.17	3.05±0.70	5.57±0.62	4.92±0.33	4.19±0.29	3.08±0.11
Capsaicin treated	Inflamed side[2]	4.71±0.66†	4.61±0.55*†	5.22±0.82*†	5.01±0.81*†	4.41±0.69	4.07±0.60
	Control side[2]	3.32±0.55	3.65±0.49	4.37±0.55†	4.17±0.70	4.54±0.91	3.98±0.77

[1]average±standard error, [2]n=5 each group. *$p<0.01$ to control side of each group, †$p<0.01$ to inflamed side of capsaicin untreated group.

Table 4. Number[1] of *c-fos* protein-immunoreactive neurons in the L6 deep dorsal horn

		2 hrs[2]	4 hrs[2]	8 hrs[2]	16 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Inflamed side[2]	4.04±0.81	5.11±0.77*	5.93±0.91	7.88±1.24*	5.12±0.80	3.82±0.90
	Control side[2]	3.27±0.91	2.48±0.70	5.75±0.82	5.95±0.16	4.88±0.43	2.90±0.18
Capsaicin treated	Inflamed side[2]	3.41±0.53	4.05±0.74*†	4.40±1.08*†	4.61±0.94*†	3.31±0.90†	3.28±0.48
	Control side[2]	2.96±0.54	3.02±0.57	3.55±0.79†	3.18±0.50†	2.93±0.34†	3.17±0.38

[1]average±standard error, [2]n=5 each group, *$p<0.01$ to control side of each group, †$p<0.01$ to inflamed side of capsaicin untreated group.

injected after inflammation induction, the number of *c-fos* protein positive neurons was decreased statistically by 4 hours, 8 hours and 16 hours ($p<0.01$).

In L6, the changes of the *c-fos* positive neurons were generally similar to those of L5 dorsal root ganglia (Table 4). In the deep layer of the dorsal horn of kaolin-carageenan injection side, the number of *c-fos* positive neurons were increased slowly than those of the control animals to peak at 16 hours and then decreased to normal level by 1 week. In the contralateral side, the number of *c-fos* positive neurons were elevated statistically ($p<0.01$) at 8, 16 and 24 hours than those of control animals. Between inflammed side and contralateral side, there were differences in the number of *c-fos* positive neurons at 4 and 6 hours after injection. The number of *c-fos* positive neurons of inflammed side is more elevated than contralateral side ($p<0.01$).

In L6, the number of *c-fos* positive neurons of the capsaicin injected side were decreased statistically ($p<0.01$) 4 hours, 8 hours, and 16 hours after kaolin-carageenan injection than those of the contralateral side. The number of *c-fos* positive neurons of the capsaicin injected side of the deep layer of spinal dorsal horn were decreased statistically ($p<0.01$) 4 to 24 hours after kaolin-carageenan injection than those of the arthritis induced animals without capsaicin treatment. In the contralateral side of the capsaicin injection, the number of *c-fos* positive neurons were decreased statistically ($p<0.01$) 8 to 24 hours after injection than those of the only arthritis induced animals (Table 4).

Changes in the Substance P and calcitonin gene-related peptide like immunoreactivities in the dorsal root ganglia

In the affected side of inflammation induced rats, the numbers of SP and CGRP positive immunore-

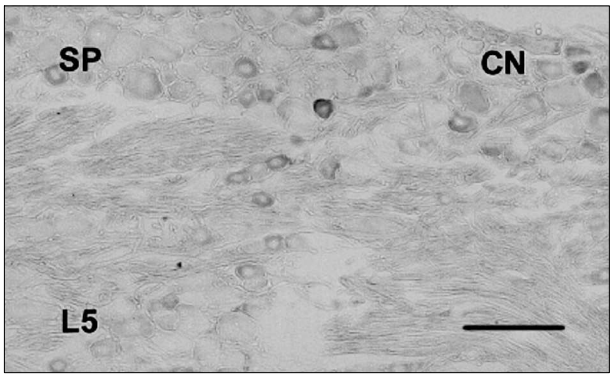

Fig. 2. Substance P (SP) immunoreactive neurons of L5 dorsal root ganglion of control side. bar=30㎛.

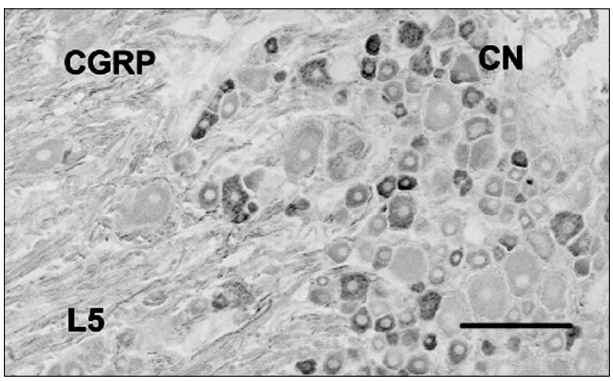

Fig. 3. CGRP immunoreactive neurons of L5 dorsal root ganglion of control side. bar=30㎛.

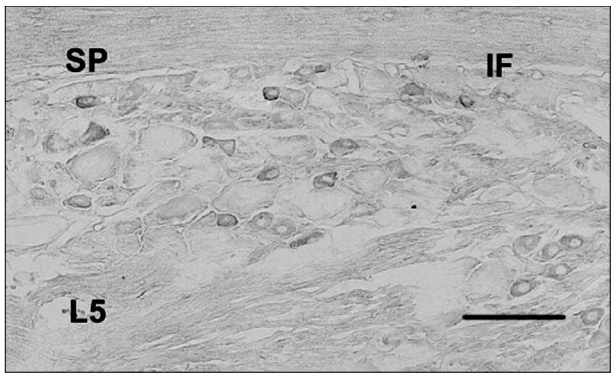

Fig. 4. Substance P (SP) immunoreactive neurons of L5 dorsal root ganglion of inflamed side 24 hours after kaolin-carageenan injection, bar=30㎛.

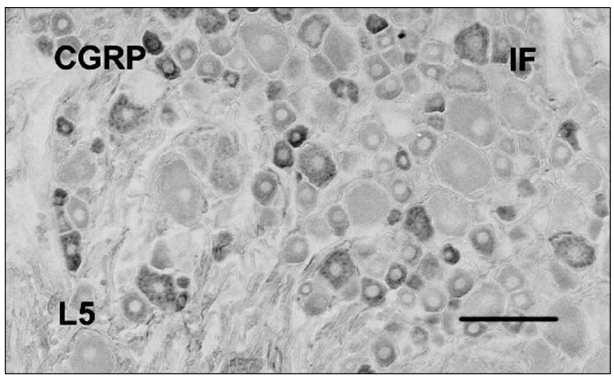

Fig. 5. CGRP immunoreactive neurons of L5 dorsal root ganglion of inflamed side 24 hrs after kaolin-carageenan injection, bar=50㎛.

active neurons did not differ significantly by 2 hours and 24 hours after arthritis induction but were increased by 1 week compared with the control side (Fig. 2～5).

The number of SP immunoreactive neurons was calculated in L5 spinal ganglia and there was no change up to 2 hours after inflammation induction. The number was increased by 24 hours later ($p<0.05$) and was increased by 1 week later ($p<0.01$) (Table 5). The similar pattern was seen in L6 as in L5. After the administration of capsaicin, the number of SP immunoreactive neurons in L5 and L6 in the control side did not increase. Significant decrease was seen in the number of SP immunoreactive neurons by 24 hours and 1 week after capsaicin administration in the inflammation induced side (Tables 5, 6).

On the other hand, no change was seen in the control side. The number of CGRP immunoreactive neurons in the spinal ganglia in L5 was quantified and no change was seen until 2 hours but increased significantly by 24 hours and 1 week later

Table 5. Number[1] of substance P-immunoreactive neurons in the L5 dorsal root ganglion

L5		2 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Control side[2]	965±97.8	1,034±169.5	987±126.4
	Inflamed side[2]	978±139.0	1,267±88.5†	1,327±66.5*
Capsaicin treated	Control side[2]	1,077±157.7	961±106.9	1,038±94.8
	Inflamed side[2]	994±141.3	1,030±88.3‡	1,051±88.6†

[1]average±standard error, [2]n=5 each group. *$p<0.01$, †$p<0.05$ to control side of each group, ‡$p<0.01$ to inflamed side of capsaicin untreated group.

Table 6. Number[1] of substance P-immunoreactive neurons in the L6 dorsal root ganglion

L6		2 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Control side[2]	1,065±144.0	871±43.3	956±131.9
	Inflamed side[2]	957±174.6	1,113±39.7	1,257±130.8*
Capsaicin treated	Control side[2]	956±66.2	967±96.6	1,116±140.8
	Inflamed side[2]	1,009±80.0	914±102.7†	1,040±133.3†

[1]average±standard error, [2]n=5 each group. *$p<0.01$ to control side of each group, †$p<0.01$ to inflamed side of capsaicin untreated group.

Table 7. Number[1] of CGRP-immunoreactive neurons in the L5 dorsal root ganglion

L5		2 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Control side[2]	2,539±158.1	2,643±359.4	2,719±397.4
	Inflammed side[2]	2,611±241.8	3,171±298.8*	3,304±162.0*
Capsaicin treated	Control side[2]	2,669±202.5	2,804±277.8	2,688±230.8
	Inflamed side[2]	2,519±230.4	2,880±232.9§	2,966±176.6††

[1]average±standard error, [2]n=5 each group. *$p<0.01$, †$p<0.05$ to control side of each group, ‡$p<0.01$, §$p<0.05$ to inflamed side of capsaicin untreated group.

Table 8. Number[1] of CGRP-immunoreactive neurons in the L6 dorsal root ganglion

L6		2 hrs[2]	24 hrs[2]	1 wk[2]
Capsaicin untreated	Control side[2]	1,645±185.4	1,568±176.6	1,689±242.4
	Inflamed side[2]	1,588±145.3	1,932±132.1*	2,123± 87.6*
Capsaicin treated	Control side[2]	1,701±198.1	1,639±166.3	1,715±123.9
	Inflamed side[2]	1,603±125.6	1,597±180.4†	1,789±160.7†

[1]average±standard error, [2]n=5 each group. *$p<0.01$ to control side of each group, †$p<0.01$ to inflamed side of capsaicin untreated group

($p<0.01$). No other change was seen in L6 until 2 h, and increase was slight by 24 hours ($p<0.05$). Statistically significant increase was observed by 1 week ($p<0.01$) (Tables 7, 8).

After capsaicin treatment in L5, the number of CGRP immunoreactive neurons in the control side was slightly increased by 1 week in the inflammation induced side ($p<0.05$). Increase was not significant by 2 h, 24 hours and 1 week after arthritis induction in L6. In the inflammation induced side in rats with capsaicin treatment, the number of CGRP immunoreactive neurons was decreased significantly by 24 hours and 1 week in inflammation induced group with no capsaicin treatment.

In short, the numbers of SP and CGRP immunoreactive neurons in the spinal ganglia (DRG) in the arthritis induced side were increased slightly by 24 hours after arthritis induction and increased definitely by 1 week later. Furthermore, these numbers were decreased significantly by 24 hours and 1 week after capsaicin treatment.

DISCUSSION

Since Hunt et al (1987) first developed the immunohistochemical method using the *c-fos* mRNA byproduct, *c-fos* protein, as the antigen in order to assess the degree of pain transmitting neuronal activation in the dorsal horn of the spinal cord, this method had been used extensively for the assessment for severity of pain and for analgesic efficacy.

In this study, *c-fos* immunoreactive neurons of spinal dorsal horn were increased significantly in the same side of the inflamed knee joint induced by injecting kaolin and carageenan. Two hours after injection, *c-fos* protein was highly expressed and gradually decreased afterwards. However, it was significantly increased even by 7 days after its expression compared with control rats. According to the study by Bullitt et al. (1992) maximal immunoreactivity appears approximately 2 hours after intense electrical stimulation has ceased, and disappears within hours. Castro et al. (1999) reported that, in Freund's adjuvant induced monoarthritic animals, the basal expression of *c-fos* (without any additional stimulation), 14 days after the induction of the arthritis, was similar to that of control normal animals without any stimulation.

Spinal neurons that express *c-fos* after noxious stimulation, are located in laminae I and IIo, and laminae V and VI of the dorsal horn, corresponding to the terminal fields of primary nociceptive afferent fibers and to the distribution of nociresponsive neurons identified by electrophysiological recordings (Harris, 1998). The expression of *c-fos* mRNA in the nerve system would first increased significantly but would decrease gradually with repeated stimuli. Furthermore, the expression of *c-fos* protein was definite in the superficial layer of the posterior horn of the spinal cord, i.e., Rexed layers I and II according to the findings of the present study. These results shows same pattern of *c-fos* activation as in other studies (Hunt et al., 1987; Williams et al., 1990; Abbadie and Besson, 1993).

In this study, changes of the number of *c-fos* positive neurons show different pattern in superficial (lamina I and IIo) and in deep layers (lamina V, VI). The number of *c-fos* positive neurons in the superficial layer peaked at 2 hours after the carageenan-kaolin injection, but in deep layer, there was no change in the number of *c-fos* positive neurons at 2 hours after the injection, but increased at 4 hours and peaked at 12 hours, gradually decreased thereafter.

Changes in the deep layer of the posterior horn of the spinal cord were not examined thoroughly. Hunt et al (1987) reported that the expression of *c-fos* was increased only in the superficial layer of the posterior horn of the spinal cord after pain was induced but did not change in the deep layer, in which the expression of *c-fos* protein was increased only slightly in the deep layer by non-painful stimuli. However, differences in the laminar distribution of spinal *c-fos* expression induced by different types of noxious stimulation (chemical, thermal, or mechanical) have been reported (Lima et al., 1993). Lee et al. (2001) reported that *c-fos* protein was expressed later and lasted further in the deep layer compared with the superficial layer in a neuropathic pain model. In this study, the number of *c-fos* positive neurons in the deep layer was increased by 4~8 hours after the induction of inflammatory arthritis and increased further by 12 hours. Because both neuropathic pain and arthritic pain are chronic, continuous type of noxious stimulation, continuous noxious stimulation in the region of carageenan-

kaolin induced arthritis could be a cause of appearance of *c-fos* positive neurons in the deeper layer in later stages.

In the present study, the number of *c-fos* immunoreactive neurons in the both superficial and deep dorsal horn of capsaicin-treated rats was less than in those rats not injected with capsaicin. Although no definite result was reported on the effect of capsaicin on arthritic animal models, it was reported that the inflammation-induced increases in *c-fos* immunoreactive neurons were greatly attenuated by neonatal capsaicin treatment (Hylden et al., 1992). Clinically, capsaicin has been successful in the treatment of rheumatoid arthritis and osteoarthritis (Cordell and Araujo, 1993; Fraenkel et al., 2004). As for the effect of capsaicin in L5 is more pronounced than those of L6, it was presumed that this difference was due to the distribution of more pain transmitting nerve fibers to the knee joint in L5 compared with L6.

Another frequently used method of assessing pain is immunohistochemical staining of SP and CGRP which believed to be the neurotransmitters of pain transmission in primary sensory neurons. SP and CGRP synthesized in DRG have been implicated in nociception in experimental and clinical inflammatory arthritis (Bulling et al., 2001). SP and CGRP immunoreactive terminals distributed in spinal dorsal horn were the axon terminals of the DRG neurons. For the quantitation, DRG were processed for immunohistochemistry. In the present study, the number of SP and CGRP immunoreactive DRG neurons increased 24 hours after the kaolin and carageenan injection to the knee joint, and also apparently increased 1 week after injection. Arthritis-induced increase of *c-fos* was faster than increase of CGRP and SP because it takes sometime to synthesize neurotransmitters.

In this study, the number of SP and CGRP immunoreactive DRG neurons of capsaicin-treated adult rats was less than in those rats not treated with capsaicin. SP and CGRP were decreased by neonatal and adult capsaicin administration (Jessell et al., 1978; Gamse et al., 1980; Holzer et al., 1982; Skofitsch and Jacobowitz, 1985; Franco-Cereceda et al., 1987; Carr et al., 1990; Lee et al., 1996). Intra-articular injection of capsaicin solution (1～5 weeks previously) virtually abolishes arthritis induced by substance P injection (Lam and Ferrell, 1989). In adjuvant arthritis-induced rats those pretreated with capsaicin had significantly lower concentrations of SP and CGRP by radioimmunoassay in dorsal root ganglia compared to the arthritic controls (Ahmed et al., 1995).

There are no direct relationship between *c-fos* activation and SP and CGRP increase because *c-fos* activation is not occurred in DRG, and both neuropeptides were not found in *c-fos* activated neuronal cell bodies in dorsal horn.

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