Kor J Physiol (1994) 28(2), 123-132

Conduction Block of the Primary Afferent Fibers by Topically Applied Allyl Isotheocyanate*

Hong Kee Shin and Kee Soon Kim

Department of Physiology, College of Medicine, Hanyang University, Seoul 133-791, Korea

=ABSTRACT=

The present study was undertaken to elucidate the desensitization of cutaneous receptors and the conduction block of the afferent nerves induced by direct application of allyl isotheocyanate (mustard oil) to the receptive field (RF) or onto the afferent nerve, respectively. Dorsal horn cell responses to mechanical stimulations of RF were completely suppressed when mustard oil was applied to either the afferent nerve or the whole area of RF. C-fiber responses of dorsal horn cells were more susceptive to mustard oil than A-fiber activities. This was confirmed by the experiment in which the compound action potentials recorded from rat tibial nerve before and after topical application of mustard oil were compared. The higher the concentration of mustard oil and the longer the application time, the more powerful desensitization or conduction block was induced.

From the results of the present study, it is suggested that the desensitization of the afferent fiber and sensory receptors induced by mustard oil results mainly from the conduction block of C-fiber in the primary afferent nerve.

Key Words: Mustard oil, Neurogenic inflammation, Desensitization, Dorsal horn cell responses, Compound action potential

INTRODUCTION

Antidromic stimulation of the sensory nerves results in cutaneous vasodilation and plasma extravasation associated with an increased vascular permeability(Lembeck & Holzer, 1979; Rosell et al, 1981; Couture & Cuello, 1984). For the development of this neurogenic inflammation, unmyelinated nociceptive fibers must be intact. Neurogenic inflammatory response such as antidromic vasodilation occurs only when unmyelinated C-fibers are stimulated (Hinsey & Gasser, 1930; Celander & Folkow, 1953) and fails to occur after chronic denervation of skin with neonatal capsaicin treatment(Jancsó et al, 1967;Gamse et al, 1980), Many of the peptides that can be localized to unmyelinated afferents have been implicated in neurogenic inflammation. One of the most probable candidates responsible for the production of neurogenic inflammation is believed to be substance P(SP) (Lembeck & Holzer, 1979; Rosell et al, 1981; Couture & Cuello, 1984) and calcitonin gene-related peptide (CGRP) is also known to have nociceptive and vasodilatory actions(Brain et al, 1985; Oku et al, 1987).

The neurogenic inflammatory responses induced by antidromic nerve stimulation are known to have much in common with those evoked by topical application of mustard oil

* This study was supported by 1993 Research Grant from Hanyang University

(allyl isotheocyanate). Mustard oil topically applied onto a peripheral sensory nerve also produces plasma extravasation and vasodilation (Jancsó et al, 1967; Kocher et al, 1985). However, mustard oil does not evoke inflammatory responses after neonatal capsaicin treatment or surgical denervation of sensory nerves(Jancsó et al, 1967; Jancsó et al, 1968; Gamse et al, 1980). Reeh et al(1986) reported that following topical application of mustard oil onto the rat saphenous nerve, thermal sensitivity and ongoing discharges of C-fibers were generally increased but in several units, the initial excitation of C-fibers was followed by delayed desensitization. Using single unit recording, Woolf and Wall (1986) confirmed that mustard oil activates the majority of cutaneous C-fibers with the minimal effect on A delta afferents, indicating that the inflammatory responses evoked by mustard oil is likely to be of neurogenic type.

In our previous study on the responsiveness of cat dorsal horn cells during mustard oil-induced inflammation, we demonstated that subcutaneous injection of mustard oil into the receptive field could elicit desensitization of the dorsal horn neurons(Yun et al, 1990). Repeated administration of capsaicin is also known to cause desensitization of the nociceptors to chemical stimuli (Jancsó et al, 1967). The present study was undertaken to elucidate the desensitization induced by direct application of mustard oil to receptive fields or onto the afferent nerves.

METHODS

Responsiveness of dorsal horn cells

Twenty adult cats weighing 2.5 to 3.5 kg were used in this experiment. After pretreatment with ketamine HCl (10 mg/kg), the animals were anesthetized by the intravenous injection of α-chloralose (60 mg/kg). The animals were artificially ventilated with a respirator and paralyzed by continuous infusion of pancuronium bromide (0.3 mg/kg/hr). Throughout the entire experiment, end-tidal CO_2 level was kept between 3.5 and 4.5% and the rectal temperature was maintained near 37 ℃ with a homeothermic blanket system. The arterial blood pressure was also monitored. Laminectomy was performed to expose lumbosacral spinal cord at the L4-S1 levels. The common peroneal and tibial nerves were dissected free from the surrounding tissues at popliteal fossa and placed on two sets of tripolar platinum electrodes for electrical stimulation. The distance between two electrodes was about 3 to 4 cm. Liquid paraffin pools were made over the exposed spinal cord and peripheral nerves to prevent drying.

Single unit activity of the spinal neuron elicited by the electrical stimulation of the afferent nerves was recorded with the carbon filament microelectrode. Once single activity of a dorsal horn cell was recorded, the type of neuron was determined according to the response pattern to mechanical stimulation of the receptive field (RF). Both high threshold (HT) and wide dynamic range (WDR) cells with both A- and C-fiber inputs were used in this experiment. The evoked activities were amplified (WPI, DAM 80), displayed on oscilloscope and fed into a window discriminator, output of which was used by a computer to compile the poststimulus time histograms. The A- and C-fiber responses of the dorsal horn cells were compiled from 20-30 consecutive stimulations of the afferent nerve with single pulse (0.1 msec, 10 T) or a train of three pulses (0.5 msec, 150-250 T), respectively. The stimulus strength was expressed as times the threshold (T) of the largest A-fiber and A-fiber threshold was determined by recording the cord dorsum potential. Mechanical stimulation was applied to RF for 10 sec:brushing the skin with a hair brush (BR), placing a large arterial clip on a skin fold (PR), and pinching a fold of skin with forceps(PI).

The control responses of the dorsal horn cell

to mechanical stimulations were compared with those obtained after topical application of 20 % mustard oil to the whole or the part of RF. Changes in A- and C-fiber responses of spinal neurons to electrical stimulation at graded intensities were also recorded before and after topical application of mustard oil (5 or 20 %)-soaked cotton strip (3×3 mm) to afferent nerves between two sets of stimulating electrodes (proximal and distal electrodes) for 5 min. The location of dorsal horn cells was established by the depth of microelectrode tip below the surface of the spinal cord. Because the size of evoked responses was different from one unit to another, data were expressed as a percentage of discharges in the control state.

Compound action potential recordings

In this experiment the compound action potentials were recorded from peripheral nerve in rats. Most experimental procedures were the same as in the cat experiments except that laminectomy was not performed. The afferent nerve dissected at popliteal fossa was placed on a tripolar stimulating electrode and the recording electrode was installed about 2 cm proximal to the stimulating electrode. The most proximal lead of the tripolar stimulating electrode toward the recording electrode was grounded to prevent direct current spread from the stimulating to the recording electrode. The compound action potentials evoked by 20 consecutive stimuli were averaged. The control compound action potentials of the A- and C-fiber volleys were compared with those obtained after placing a small cotton strip soaked in 5 % mustard oil between the recording and stimulating electrode for 1 minute.

RESULTS

After topical application of mustard oil to the part of large RF as shown in Fig. 1A3, WDR cells were still activated by the mechanical stimuli applied to the part of RF distant from the mustard oil-applied area(Fig. 1A2). On the other hand, the responses of WDR cells to mechanical stimulations of RF were completely depressed(Fig. 1B2) when mustard oil was applied to the whole area of RF (Fig. 1B3, n= 9). But in both cases, spontaneous activities greatly increased. Single sweep oscilloscopic recordings showed that dorsal horn cells were still activated by the electrical stimulation of afferent nerve with suprathreshold intensity even after topical application of mustard oil to the whole area of RF (Fig. 1C1 & C2)

The responses of WDR (Fig.2 A) and HT (Fig. 2B) cells to mechanical stimulations of RF were not evoked following direct topical application of mustard oil to afferent nerve (Fig. 2A2 & B2). This implies that mustard oil induced conduction block of afferent nerves as in the receptive field.

To elucidate the susceptive components of afferent nerves to mustard oil, changes in dorsal horn cell responses to graded electrical stimulation of afferent nerves were recorded prior to and after placing 5 % mustard oil-soaked cotton strip between the proximal and distal stimulating electrode for 5 minutes (Fig. 3 & 4, n=12). After the application of mustard oil, A-fiber responses of WDR cells to the electrical stimulation delivered through the proximal electrode did not change significantly (Fig. 3B1 & B2) but those through the distal electrode were weakly depressed (Fig. 3A1 & A2). A-delta fiber responses (67.8 %) were a little more strongly inhibited than the responses to the electrical stimulation of A$\alpha\beta$ fibers (97.0 %).

In sharp contrast, C-fiber responses of WDR cells elicited by the electrical stimulation delivered through the distal electrode were almost completely disappeared after topical application of 5 % mustard oil (Fig. 4A1 & A2) while those through the proximal electrode were not affected as was the case with A-fiber responses. On the other hand, A- as well as C-

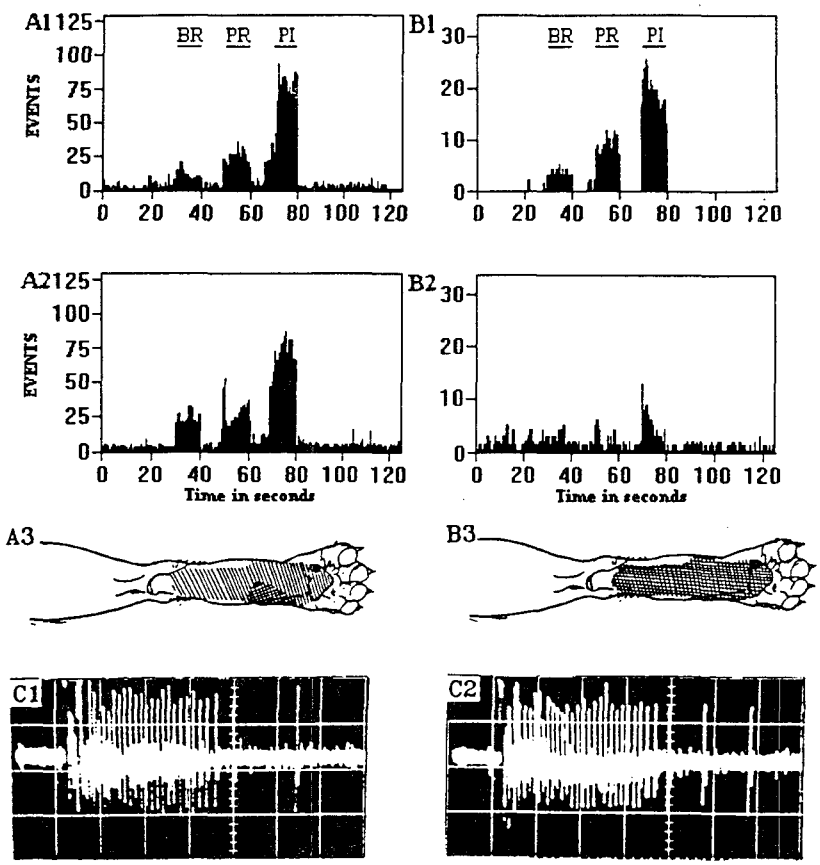

Fig. 1. Desensitizing action of allyl isotheocyanate on the responses of WDR cell to mechanical stimulation. BR: brush, PR:pressure, PI:pinch. A1 & B1:the control responses of WDR cell to mechanical stimulation. A2:after applying allyl isotheocyanate (20 %) to the restricted small area of receptive field, WDR cells still responded to mechanical stimulation and generally spontaneous activities were increased. B2:following application of allyl isotheocyanate (20 %) to the whole receptive field, responses of WDR cell to mechanical stimulation completely disappeared. A3 & B3:drawings showing the location of receptive field and the area to which allyl isotheocyanate was applied. Black dot:the area to which mechanical stimuli were applied. Cross-hatched area:the area to which allyl isotheocyanate was applied. C1 & C2:single sweep oscilloscopic recordings of dorsal horn cell activities during electrical stimulation of afferent nerves with suprathreshold intensity (0.5 msec, 7 mA) before (C1) and after (C2) application of allyl isotheocyanate to the whole receptive field.

fiber responses of WDR cells were almost completely disappeared after the topical application of higher concentration (20 %) of mustard oil to afferent nerves for a longer time (10 min.) (Fig. 5A & B, n=5).

By the comparison of the compound action potentials recorded from rat tibial nerve (n=9) before and after topical application of mustard oil, it was confirmed that C-fiber volleys are more susceptive to mustard oil than A-fiber volleys (Fig. 6). As shown in Fig. 6A2 and B2, C-fiber volley was completely disappeared whereas A-fiber volley was strongly depressed after topical application of mustard oil to afferent nerve.

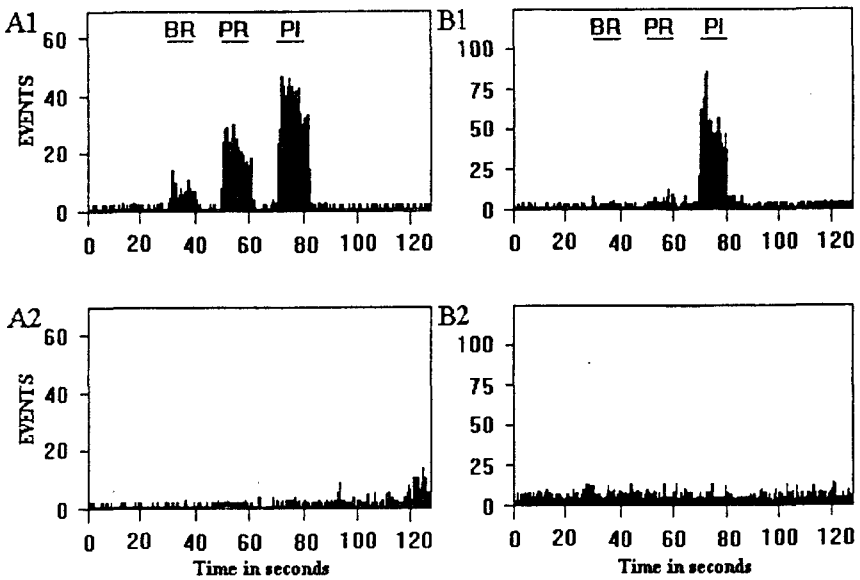

Fig. 2. The conduction block induced by the topical application of allyl isotheocyanate (5 %) to afferent fibers. BR:brush, PR:pressure Pl:pinch. A1 & B1:the control responses of WDR (A1) and HT (B1) cells to mechanical stimulation. A2 & B2:the responses of WDR (A2) and HT (B2) cells to mechanical stimuli were completely blocked following direct application of allyl isotheocyanate to afferent fibers.

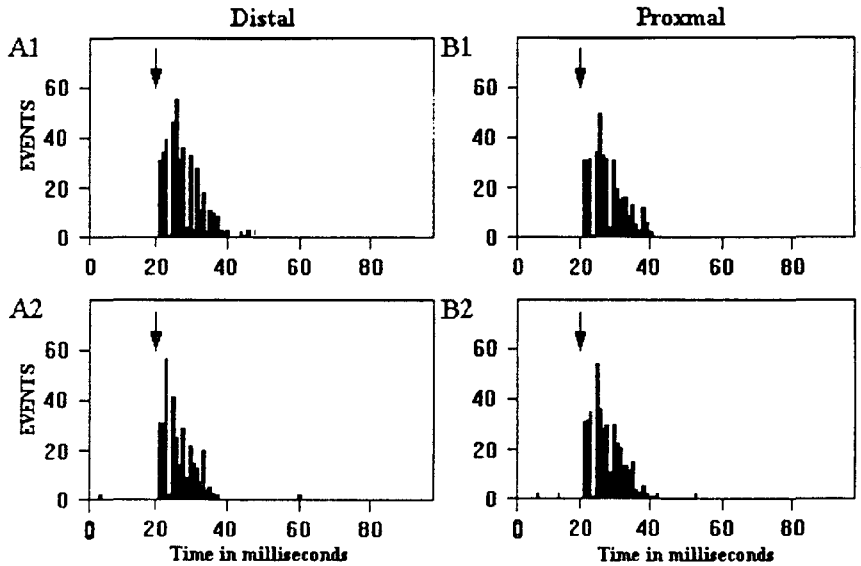

Fig. 3. Effects of allyl isotheocyanate (5 %) on the A-fiber responses of dorsal horn cells. Arrows indicate the time at which single stimulus (0.1 msec, 10 T) was applied. A1 & B1:the control A-fiber responses evoked by the stimulation of afferent nerves with the distal (A1) and proximal (B1) stimulating electrodes. A2 & B2:after application of allyl isotheocyanate onto nerve between the distal and proximal stimulating electrodes, A-fiber responses to the electrical stimulation with distal electrode (A2) were weakly suppressed whereas those to the electrical stimulation with proximal electrode (B2) were not changed.

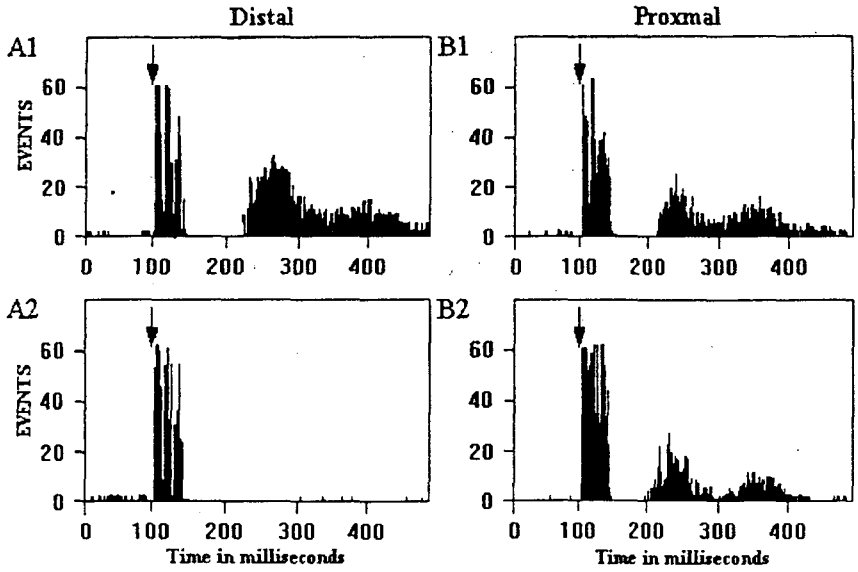

Fig. 4. Changes in C-fiber responses of WDR cells induced by the application of allyl isotheocyanate (5 %) onto the afferent nerve. Arrows indicate the time at which 3 train stimuli (0.5 msec, 200 T) were applied. A1 & B1: the control C-fiber responses of WDR cell to electrical stimulation of afferent fibers with the distal (A1) and proximal (B1) electrodes. A2 & B2:C-fiber responses eroked by the electrical stimulation of afferent fibers with the distal electrode (A2) were completely inhibited but those induced by the electrical stimulation with the proximal electrode were not affected.

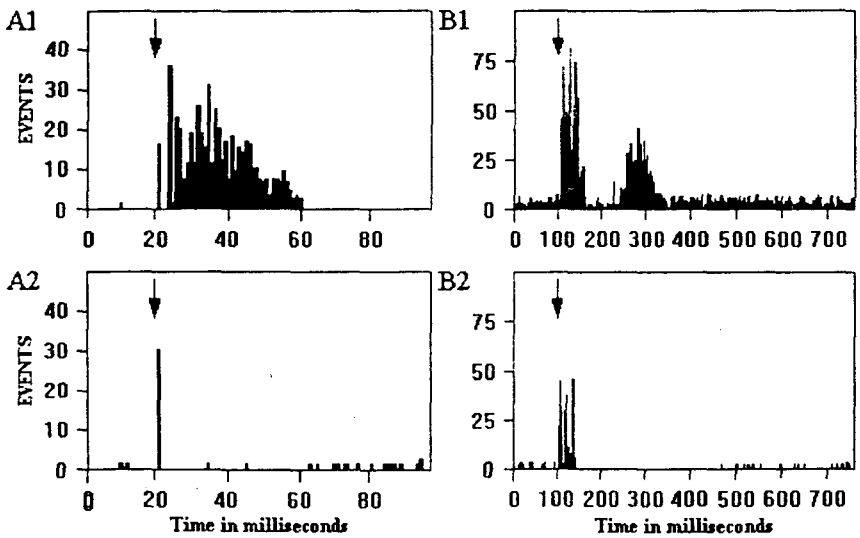

Fig. 5. Inhibition of A- and C-fiber responses of the dorsal horn cell by the application of higher concentration (20 %) of allyl isotheocyanate for a longer time (10 min). Arrows indicate the time at which single or train stimuli were applied to afferent nerve. A1 & B1:the control A-(A1) and C-(B1) fiber responses of WDR cell to graded electrical stimulations. A2 & B2:A-(A2) and C-(B2) fiber responses were very strongly depressed after topical application of higher concentration of mustard oil (20 %) for a longer time (10 min) to afferent nerves.

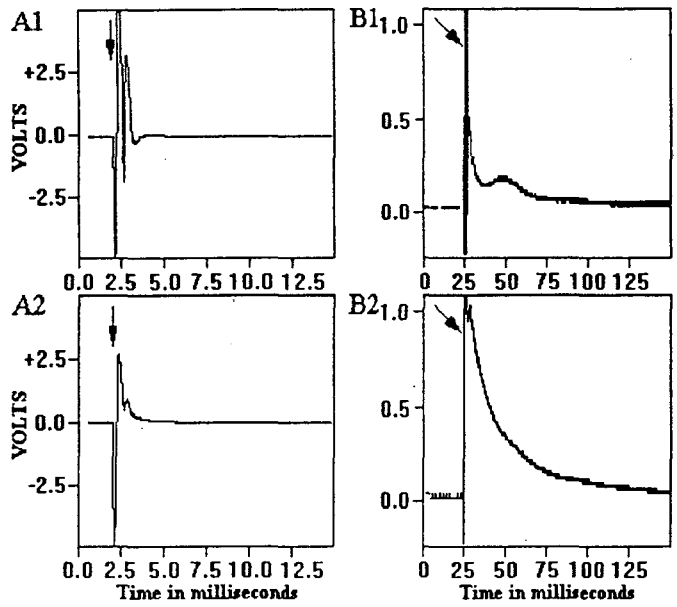

Fig. 6. Effects of allyl isotheocyanate on the A- and C-fiber volleys in the rat. Arrows indicate the time at which electrical stimulus was applied to a nerve. A1 & B1:the control A-(A1) and C-(B1) fiber volleys induced by electrical stimulation of tibial nerve. A2 & B2:A- and C-fiber volleys were greatly depressed after the topical application of mustard oil (5 %) to the afferent nerve in the rat.

DISCUSSION

It is known that spinal neurons as well as nociceptive neurons are generally sensitized after induction of local inflammations including neurogenic ones (Yun et al, 1990). In the present study, the responses of dorsal horn cells to mechanical stimulations of RF completely disappeared while spontaneous activities generally increased following topical application of mustard oil to the whole area of RF. However, dorsal horn cells invariably responded to the mechanical stimulations when mustard oil had been topically applied to the restricted area of RF distant from the stimulated area. On the other hand, responses of dorsal horn cells to electrical stimulations of afferent fibers were blocked if mustard oil had been topically applied to afferent nerves proximal to the distal electrode. In general the C-fiber responses of cat dorsal horn cells were strongly suppressed than A-fiber ones and higher concentration of mustard oil appeared to have stronger blocking effect. These experimental findings strongly suggest that mustard oil can induce strong desensitization of RF and conduction block of primary afferent nerve. These results agree well with the reporst of Yun et al (1990) and Reeh et al (1986) who observed delayed desensitizations of single afferent nerve fiber and dorsal horn cells preceded by initial sensitization.

The excitatory action of mustard oil is believed to result from the activation of small diameter fibers, especially C-fibers. Topical application of mustard oil to RF or afferent nerves has been known to strongly activate small unmyelinated fibers with the minimal effect on myelinated fibers (Woolf & Wall, 1986; Russell et al, 1987). The mustard oil-induced inflammatory responses are not

observed in animals without an intact C-fiber innervation (Jancsó et al., 1967; Jancsó et al, 1968; Gamse et al, 1980). This strong activation of high threshold nociceptive afferent by mustard oil may cause the release of some substances from the nerve endings, which facilitate the transmission of nociceptive sensory signals in the spinal cord and these same substances also seem to produce plasma extravasation and vasodilation in the peripheral tissue. SP and CGRP are likely to be the candidate substances to meet the aforementioned requirements. These two substances have strong vasodilatory action (Moochhala & Sawynok, 1984; Brain et al, 1985) and are released only by the stimulation of unmyelinated afferent fibers and noxious stimuli applied to RF (Yaksh et al., 1980;Duggan & Hendry, 1986; Duggan et al, 1988). Antagonists against SP and CGRP effectively attenuate plasma extravasation and vasodilation induced by mustard oil or by antidromic nerve stimulation (Rosell et al, 1981; Couture & Cuello, 1984; Louis et al, 1989). SP has been reported to induce the release of histamine, which is one of the well known inflammatory products (Erjavec et al, 1981; Fewtrell et al, 1982) and excitatory amino acids such as aspartate and glutamate (Skilling et al, 1988; Smullin et al, 1990).

The mechanism by which mustard oil induces desensitization is far from clear and needs to be explored by further studies. One possible hypothesis is that degradative product of SP is responsible for the mustard oil-induced desensitization. Larson (1988) reported that repeated intrathecal injection of SP(1-11) resulted in the desensitization of SP(1-11)-induced pain behaviors. Repeated injection of C-terminal fragments of SP(5-11) did not evoke desensitization but elicited SP-like pain behaviors in animals (Cridland & Henry, 1988). On the other hand, N-terminal fragments of SP(1-7) did not have SP-like action and inhibited the pain behaviors induced by the injection of SP(1-11) or SP(5-11) (Hall & Stewart, 1983; Igwe et al, 1988). Prevention of N-terminal production with the inhibition of SP(1-11) degradative enzymes was reported to block the development of the SP-induced desensitization and enhanced the SP-induced behavioral episodes (Igwe et al, 1988; Larson, 1988). These experimental findings suggest that excessive amount of SP released by strong stimulation of mustard oil applied to RF may be degraded enzymatically and these N-terminal fragments produced by SP degradation may cause the development of desensitization, This hypothesis may explain the desensitization phenomena induced in RF but not the conduction block of the afferent fibers.

At present, there is no direct evidence that mustard oil has depolarizing action, but judging from its irritative property it is possible that mustard oil has a strong depolarizing action. If this is the case, prolonged excessive depolarization induced by mustard oil may cause conduction block of the nerve.

The facts that mustard oil induces more selective activation of C-fibers and also causes the development of delayed desensitization are comparable to the actions of capsaicin, It has been reported that after topical application of capsaicin to afferent nerves, the C-fiber responses of spinothalamic tract cells to graded electrical stimulation of afferent nerves were strongly depressed with little effect on the responses to innocuous stimuli (Chung et al, 1985) and that repeated application of capsaicin also resulted in delayed desensitization of RF (Jancsó et al, 1967; Petsnhe et al, 1983). This conduction block induced by capsaicin can be caused by strong depolarizing action resulting from an increase in Na^+ and Ca^{++} conductances (Hayes et al, 1984;Marsh et al, 1987).

From the results obtained from the present work it is not possible to intelligently discuss the mechanism underlying the desensitization induced by mustard oil. Our findings suggest that the desensitization of the afferent fibers and sensory receptors induced by mustard oil results mainly from the conduction block of C-fibers.

REFERENCES

Brain SD, Williams TJ, Tippins JR, Morris HR & Macintyre I (1985) Calcitonin gene-related peptide is a potent vasodilator. *Nature* **313**, 54-56

Celander O & Folkow B (1953) The nature and the distribution of afferent fibers provided with the axon reflex arrangement. *Acta Physiol Scand* **29**, 359-370

Chung JM, Lee KH, Hori Y & Willis WD (1985) Effects of capsaicin applied to peripheral nerve on the responses of primate spinothalamic tract cells. *Brain Res* **329**, 27-38

Couture R & Cuello AC (1984) Studies on the trigeminal antidromic vasodilation and plasma extravasation. *J Physiol(London)* **346.** 273-285

Cridland RA & Henry JL (1988) N- and C-terminal fragments of substance P:spinal effects in the rat tail flick test. *Brain Res Bull* **20**, 429-432

Duggan AW & Hendry IA (1986) Laminar localization of the sites of release of immunoreactive substance P in the dorsal horn with antibody-coated microelectrodes. *Neurosci Lett* **68**, 134-140

Duggan AW, Hendry IA, Morton CR, Hutchison WD & Zhao ZQ(1988) Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Res* **451,** 261-273

Erjavec F, Lembeck F, Florjanc-Irman T, Skofitsch G, Donnerer J, Saria A & Holzer P (1981) Release of histamine by substance P. *Naunyn-Schmiedeberg's Arch Pharmacol* **317**, 67-70

Fewtrell CMS, Foreman JC, Jordan CC, Ochme P, Renner H & Stewart JM (1982) The effects of substance P on histamine and 5-hydroxy-tryptamine release in the rat. *J Physiol* **330**, 393-411

Gamse R, Holzer P & Lembeck F (1980) Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br J Pharmacol* **68**, 207-213

Hayes AG, Hawcock AB & Hill RG (1984) The depolarising action of capsaicin on rat isolated sciatic nerve. *Life Sci* **35**, 1561-1568

Hall ME & Stewart JM (1983) Substance P and behavior:Opposite effects of N-terminal and C-terminal fragments. *Peptides* **4**, 763-768

Hinsey JC & Gasser HS (1930) The component of the dorsal root in mediating vasodilation and the Sherrington contracture. *Am J Physiol* **92**, 679-689

Igwe OJ, Sun X & Larson AA (1988) Role of substance P (SP) metabolism in SP-induced desensitization in mice. *Soc Neurosci Abstr* **14**, 709

Jancsó N, Jancsó-Gabor A & Szolcsanyi J (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br J Pharmacol Chemother* **31**, 138-151

Jancsó N, Jancsó-Gabor A & Szolcsanyi J (1968) The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. *Br J Pharmacol Chemother* **32**, 32-41

Kocher L, Jung S, Reeh PW & Handwerker HO (1985) Mustard oil but not antidromic nerve stimulation affects sensitivity of cutaneous polymodal C-fibers in the rat. *Pflüger Arch* **403**, R59

Larson AA (1988) Desensitization to intrathecal substance P in mice:possible involvement of opioids. *Pain* **32**, 367-374

Lembeck F & Holzer P (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. *Naunyn-Schmiedeberg's Arch Pharmacol* **310**, 175-183

Louis SM, Johnstone D, Russell NJW, Jamieson A & Dockray GJ (1989) Antibodies to calcitonin gene-related peptide reduce inflammation induced by topical mustard oil but not that due to carrageenin in the rat. *Neurosci Lett* **102**, 257-260

Marsh SJ, Stansfeld CE, Brown DA, Davey R & McCarthy D (1987) The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro. *Neuroscience* **23**, 275-289

Moochhala SM & Sawynok J (1984) Hyperalgesia produced by intrathecal substance P and related peptide:desensitization and cross de-

sensitization. *Br J Pharmacol* **82**, 381-388

Oku R, Satoh M, Fujii N, Otaka A, Yajima H & Takagi H (1987) Calcitonin gene-related peptide promotes mechanical nociception by potentiating release of substance P from the spinal dorsal horn in rats. *Brain Res* **403**, 350-354

Petsche U, Fleischer E, Lembeck F & Handwerker HO (1983) The effect of capsaicin application to a peripheral nerve on impulse conduction in functionally identified afferent nerve fibers. *Brain Res* **265**, 233-240

Reeh PW, Kocher L & Jung S (1986) Does neurogenic inflammation alter the sensitivity of unmyelinated nociceptors in the rat? *Brain Res* **384**, 42-50

Rosell S, Olgart L, Gazelius B, Panopoulos P, Folkers K & Horig J (1981) Inhibition of antidromic and substance P-induced vasodilation by a substance P antagonist. *Acta Physiol Scand* **111**, 381-382

Russell NJW, Heapy CG & Jamieson A (1987) Afferent activity in models of inflammation. *Pain Suppl* **4**, S255

Skilling SR, Smullin DH & Larson AA (1988) Extracellular amino acid concentration in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. *J Neurochem* **51**, 127-132

Smullin DH, Skilling SR & Larson AA (1990) Interactions between substance P, calcitonin gene-related peptide, taurine and excitatory amino acids in the spinal cord. *Pain* **42**, 93-102

Woolf CJ & Wall PD (1986) Relative effectiveness of C primary afferent fibers of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. *J. Neurosci* **6**, 1433-1442

Yaksh TL, Jessell TM, Gamse R, Mudge AW & Leeman SE (1980) Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. *Nature* **286**, 155-157

Yun YB, Kim JH, Shin HK & Kim KS (1990) Modification in the responsiveness of dorsal horn cells during allyl isotheocyanate-induced inflammation in the cat. *Kor J Physiol* **24**, 305-317