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# Effect of Capsaicin on Causalgiform Pain in the Rat

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**=ABSTRACT=**

**The purpose of this study is to obtain the effective concentration of capsaicin to relieve pain with no change in the number of C-fibers and its effective duration for pain relief. Capsaicin has been used extremely as a experimental tool and as topical medications for acute or chronic tissue injuries and partial nerve injury is the main cause of causalgiform pain disorders in humans. Here, the left sciatic nerve was ligated unilaterally at the high-level of the thigh to prepare an animal model of this pain condition. The rat developed guarding behavior of the ipsilateral hind paw within a few hours after the operation and this behavior was maintained for several months thereafter, suggesting the possibility of spontaneous pain. These animals were divided into two groups(4-week & 8-week) and each group was subdivided into five groups by different concentration (0.05, 0.1, 0.5, 1.0 & 2.0%). Each capsaicin concentration was treated locally on the spinal cord-side of the ligated nerve and the foot-withdrawal latency was measured. Thereafter, the dorsal roots of L5 were removed from both sides immediately after intracardial perfusion for the counting of C-fibers by the histological procedure. There were no significant differences in the foot-withdrawal latency and the number of C-fibers between the left side treated with 0.05% capsaicin and the right side treated with the vehicle. However, latencies of the left sides treated with 0.1, 0.5, and 1.0 % capsaicin increased significantly throughout 4-6 weeks with almost no change in the number of C-fibers, and the latencies showed the trends to approach slowly to those of the conditions after operation. The latency of subgroup treated with 2.0% increased by approximate 70% more than that of the right side throughout 8 weeks, and the number of C-fibers decreased by about 30% or more These results suggest that the elevated latency with capsaicin(0.1-1.0%) treatment is due to the inhibition of impulse transmission throughout the primary afferent fiber and the data from 2.0% are due to partial destruction of C-fibers. Therefore, capsaicin concentrations from 0.1% to 1.0% are probably very effective for the treatment of causalgiform pain with almost no destruction of C-fibers.**

**Key Words:** Capsaicin, Causalgiform pain, C-fiber, Foot-withdrawal latency

## INTRODUCTION

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the irritant compound of hot pepper in the capsicum plant. This compound was studied at first by the Hungarian Pharmacologist Nicholas-Jancso about an extensive characterization of the pharmacologic effects in the late 1940s. In animal studies it has been used in a large number of ways in neonatal and adult animals including local and systemic injection. Many of reports have revealed a number of effects of capsaicin which include:(i) marked stimulation of peripheral nociceptors followed by a long-lasting insensitivity to chemical pain (Buck & Burks, 1986), (ii) markedly elevated nociceptive chemical and pressure thresholds but no effect or even slightly reduced nociceptive heat thresholds(Hayes & Tyers, 1980, Konietzny & Hensel, 1983,

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McMahon et al, 1991), (iii) alterations in the histochemical content of many C-fibers, reducing the expression of some neuropeptides, such as substance P, calcitonin gene related peptide, cholecystokinin and fluoride resistant acid phosphatase (Ainsworth et al, 1981, Nagy et al, 1981, Helke et al, 1981, Buck et al, 1982, Marley et al, 1982, Lynn & Shakhanbeh, 1988), (iv)increased mechanical nociceptive thresholds but unchanged thermal thresholds (Cervero & McRitchie, 1982), (v) analgesic effect or selective destruction of unmyelinated afferents (Fitzgerald, 1983, Chung et al, 1985, Otsuka & Yanagisawa, 1987), (vi)definitive reduction or block in C-fiber transmission( Williams & Zieglgansberger, 1982, Gamse et al, 1982, Petsche et al, 1983) (vii)excitable properties of sensory nerve: terminals, axon, cell body (Roberts, 1986, Ritter & Dinh, 1988), (viii)selective action in reducing C-nociceptor activity and functional changes by the loss of C-fiber nociceptors (Lynn, 1990), and (ix)reduction in responses of spinothalamic tract(STT) cells to noxious heat stimuli(Chung et al, 1992). Based on these results, it suggests that capsaicin not only acts as a neurotoxic substance but also as a good medicine for relief of severe pain. More recently, the possibility has been raised that some of the long-term physiologic effects of capsaicin on causalia or related severe pain seen in experimental studies might be useful in the clinic (McMahon, 1991).

Causalgia, the extremely painful pain, first described by Mitchel, Morehouse, and Keen in 1964, develops after penetrating wounds in which there is usually a painful injury of the median, ulnar or sciatic nerve (White & Sweet, 1969). In the typical severe forms, the cutaneous dysesthesia may become so intense that the patient cannot bear contacted with clothing or even drafts of air. It has been known that compression or partial damage of nerves causes pain to be resulted from some local ischemia or changes in the transport of chemicals within the unmyleinated C afferents (Zimmermann, 1979). These C-fibers produce pain on electrical stimulation and are responsible for the transport of chemicals that affect spinal cord cells (Wall, 1984). These reports show clearly that nerve injury causes pain by various mechanisms, including causalgiform pain and these pain signals transmit via C-afferent fibers entering the spinal cord.

We modified the method that measures behavioral response to hyperalgesia (Hargreaves et al, 1988). The method used in this study can assess to determine the response time of the foot that withdraw from the heat source, which is radient heat as a thermal stimulus applied to the hind sole of restrained rat.

The purpose of this study is to investigate the effect of capsaicin and its effective duration for relief of causalgiform pain and to attempt solving problem on pain control.

## METHODS

### Subjects

A total of 100 Sprague-Dawley rats (160-200gm, male) was supplied from the Laboratory of Experimental Animals in Korea Research Institute of Chemical Technology at 8 weeks after birth and were kept in the animal room (temperature: 22-24°C) of our school for about 1 month, and their body weight was 250-300 gm at the time of operation

### Behavioral tests before and after operation

The initial study was to obtain the control value of the foot-withdrawal latency from each rat before the operating process. Each rat was placed on the glass floor (50cm × 50cm, 2mm in thickness) covered with the transparent plastic box, and heated locally on the hind paw at 120°C (tip temperature) with the self-designed apparatus under the room temperature of 20-22°C (Fig. 1). The apparatus is composed of the cromel-alumel thermocouple and the temperature controller. The latency that withdraw the hind paw from the heat source was determined alternately six times at the intervals of 5 minutes, and the latencies were obtained from the left (experimental, LT) side and the right (control, RT) side of each rat. The

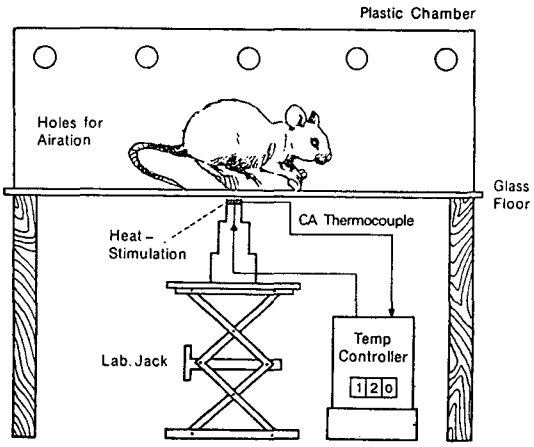


*Fig. 1. The local heating apparatus composed of the CA thermocouple and the temp. controller. Each rat was placed on the glass floor covered with transparent plastic box and the apparatus was moved to and fro under the glass floor for local heating of behind paw.*

latency ratio (LT/RT, %) of both sides were changed into a unit of percent because of individual difference and were compared each other with averaged value before and after operation. The rats used for this study were selected such that the ratios were not significant difference between both sides. Rats were devided into two groups; 4-week and 8-week(50-rat/group), and each group was divided equivalently again into five subgroups in according to five different concentrations of capsaicin solution; 0.05, 0.1. 0.5, 1.0, and 2.0% respectively. Each concentration of capsaicin (Sigma) was suspended in a vehicle of 10% ethanol, 10% Tween 80, and 80 % normal saline. For the controls the vehicle alone was applied on the right sciatic nerve.

Following the operation schedule to cause the causalgiform pain, rats were anesthetized with sodium pentobarbital(40mg/kg, i.p.) and the skin on the left thigh was opened at the high-level of experimental side. The sciatic nerve was exposed and freed from surrounding tissues. Thereafter, the nerve was ligated loosely three times with silk thread (No.1) so as to inhibit the blood flow at two points aparted from 5mm each other. Almost of all rats showed discomfortable behaviors and the signs of allodynia, suggesting causalgifrom pain. The rats that showed significant differences(over 30%) in the foot- withdrawal latency between both sides were selected to compare the capsaicin effect for 4 or 8 weeks. Capsaicin was treated locally on the spinal cord-side of the ligated sciatic nerve at 1 week after the latency measurement. A small piece of parafilm was placed under the nerve to minimize the spread of capsaicin solution and similar size of a small piece of gelform soaked in each concentration was placed around a 5mm-length of the nerve. After wounds were sutured, rats were returned to recover in their cage prepared with the soft bedding. The foot-withdrawal latency determine on 7th day in according to previously described procedures.

## Histological study

After the determination of the latency is over, rats were anesthetized with sodium pentobarbital(40mg/kg,i.p.). Each rat was perfused intracardially with 300ml(room temperature) of saline containing 400 units of heparin and 0.4 ml of 1% sodium nitrite. When the auricular effluent was free of blood, the perfusion fluid was changed to a fixative solution (500ml) of 3% paraformaldehyde, 3% glutaraldehyde, and 0.1 % picric acid in 0.1M cacodylate buffer (pH 7.4). The dorsal root of L5 was exposed by laminectomy, and removed together with comparable segment of the right sciatic nerve as control material. The tissue was placed in a petri dish containing cold fixative solution. Under a dissecting microscope, 1cm long blocks of the roots were dissected at about 1 cm from the dorsal root ganglion. The specimens were stored in cold fixative solution for 8-12 hours, rinsed with cacodylate buffer, and postfixed in 1% osmium tetroxide in 0.1M cacodylate buffer for 2 hours. Following en bloc staining with 0.5% uranyl acetate, the pieces were dehydrated by using a graded series of ethanol and embedded in an Epon-Araldite mixture. Thin sections (1$\mu$m in thickness) were cut with an ultramicrotome (Sorvall, type MT-5000), and after stain-

ing with 1% of toluidine blue, the sections were examined with a light microscope (Olympus BH-2), and photographed at a magnification of 400.

Photomicrographs were printed with an additional magnification of 2.5. Montages representing the whole cross section of the dorsal root were reconstructed from photomicrographs. Unmyelinated fibers were counted with the aid of 4x magnifying glass, and each axon was counted to avoid potential error due to an uneven distribution of fibers. The counting error, in terms of repeated counts for the same tissue was ±8%.

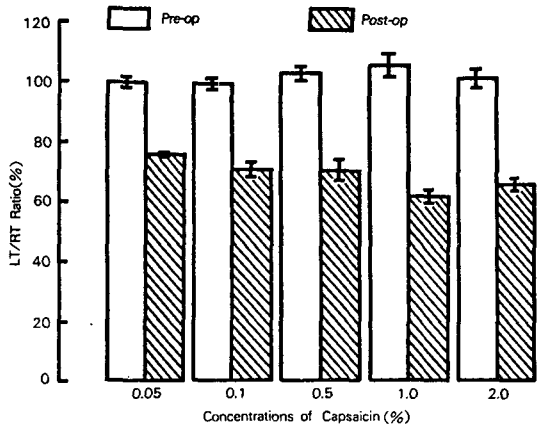


*Fig. 2. The ratio changes in pre-and post-operation group. In the pre-operation group, the ratios between both sides were about 100% in all subgroups with no significant differences. In the postoperation, however, the ratios showed significant differences. A ratio of = 100% represents that the latencies between experimental and control side have no difference and <100% represents that experimental side is more sensitive to heat stimulation than control side.*

## RESULTS

### Sign of causalgiform pain

The rats were awake within approximately 10 minutes or more early after the termination of the surgical anesthesia. An hour later they were fully alert and responsive. For this time the rats placed on the glass floor showed the discomfortable behavior that flexed the operated hind limb and bore less weight on the operated side than the contralateral side. This behavior lasted several weeks, suggesting that the contact with the floor appeared to be an aversive stimulus. Furthermore, beginning about 1 hour post-operation and for a period of several weeks thereafter, the rats at rest clearly preferred the contralateral intact hind limb. The ipsilateral hind limb was usually placed on the cushioned scrotum, with the paw hanging in mid-air. While resting, the rats paid considerable attention to the operated side in a manner unlike usual grooming. This behavior was not observed in the sham operated rats.

### Foot-withdrawal latencies

*Difference between pre- and post-operation in each subgroup*

All the animals were tested for foot-withdrawal latency before and after operation throughout 4 weeks or 8 weeks. The average ratios of all rats before operation were not significantly different between both sides, however, rats used for this study showed the significant difference in the ratio after operation. In Fig. 2 the difference of the ratios in pre- and post-operation of each subgroup are plotted. A ratio of <100 % represents that the experimental side was more sensitive to heat stimulation than the control side, and >100%, the opposite effect. For the ratios in all subgroups before operation, the difference shows no significant variations with about 100%. However, the ratios of all subgroups after operation were reduced markedly with no significant difference among them ($p<0.005$).

*Different concentrations of capsaicin and their effective durations*

The change of the reduced ratio after capsaicin treatment and its effective duration showed in Fig. 3 and 4. In the Fig. 3 the ratio from the 0.05% capsaicin-treated animals was cons-

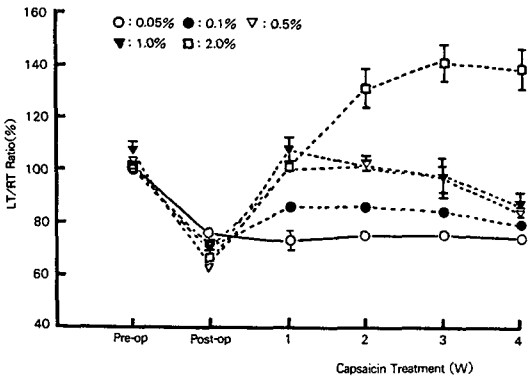


*Fig. 3. The ratio changes for 4 weeks after capsaicin treatment*

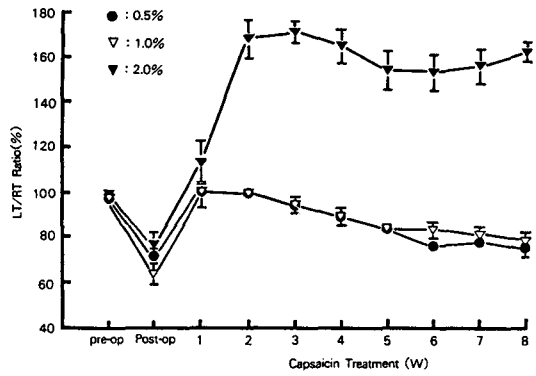


*Fig. 4. The ratio changes for 8 weeks after capsaicin treatment*

tant throughout 4 weeks without any significant change(indicative of hyperreflexia). Those from the 0.1%, 0.5 %, and 1% were the singificant differences in the ratio throughout 4 weeks showing the trend to approach gradually the ratios before capsaicin treatment. However, the ratio from 2.0% showed great changes which the ratio of capsaicin-treatment side is increased by approximate 70% (indicative of hyporeflexia) on the contrary compared to the control side throughout 4 weeks. In the Fig. 4 there are compared with the ratio changes that continued capsaicin effect over 4 weeks. The data from 2.0% show continously stronger effect throughout 8 weeks and did not approach to the ratio of the control side. These results suggest that the lower concentration less than 0.1% has no effect to relieve the causalgiform pain and the higher concentrations more than 0.1% have considerable sustained effect on the control of the causalgiform pain disorders for at least 4 weeks and, however, the data from 2.0% show the long-lasting stronger effect over 8 weeks.

## Histological study

The ratio changes resulted from capsaicin treatment suggest that impulse transmission was inhibited for several weeks, and it may be due to the destruction of C-fibers themselves. In Fig. 5 the data from all subgroups were not

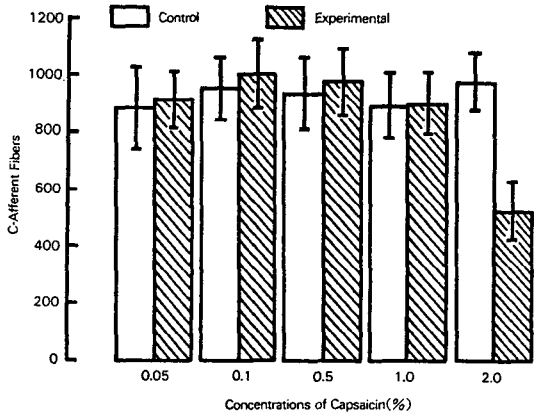


*Fig. 5. The Changes in the number of C-fiber afferents. the lower concentrations than 1.0% capsaicin show almost no reduction in the number, however the higher concentration than 1.0% shows marked reduction.*

significant difference in the number of C-fibers between both sides except the subgroup of 2.0%. These results suggest that the lower concentrations less than 1.0% can cause the inhibition with almost no change in the number of C-fibers and, however, 2.0% capsaicin shows significant reduction in the number of C-fibers.

## DISCUSSION

In our previous study(Chung et al. 1992), we showed that application of capsaicin to the sciatic nerve in monkeys causes a reduction in the responsiveness of STT cells to noxious heat stimuli that lasts at least several weeks. We believed that the reduction in responses of the STT cells was due to the partial destruction of C-fibers in the peripheral nerve. In addition, other mechanisms that capsaicin application can reduce responses to C-fiber volleys and to noxious heat stimuli are inhibition of axonal transport of peptides (Gamse et al. 1982), chemical changes in the spinal cord terminals (Palermo et al. 1981, Nagy et al. 1980, Theriault et al. 1979, Jessel et al. 1978) and long-lasting conduction block of C-fibers(Petsche et al. 1983, Williams & Zieglgansberger, 1982, Wall & Fitzerald, 1981).

In this study the results suggest that the sciatic nerve ligated loosely can cause causalgiform pain to last constantly for several weeks, and that local treatment of capsaicin from 0.1% to 1.0% to the nerve can cause the elevation of foot-withdrawal latency to noxious heat stimulation. And the elevated effect lasts at least 4-6 weeks with almost no change of C-fibers in the number. The treatment of 2.0% capsaicin, however, can cause the marked elevation of the latency until 8 weeks or more, leading to the dramatic loss of C-fiber afferents.

A number of recent studies have involved the induction of peripheral neuropathies in several animal models that have quite different characteristics from those produced by nerve damage in this study. For example, Benett and Xie (1988) produced such a mononeuropathy by tying 4 loose ligatures around the sciatic nerve in rats. The animals develop a hyperresponsiveness to thermal stimuli that lasts a month or more. Different model was described by Seltzer et al.(1990) in which about 1/3 to 1/2 of the sciatic nerve of rats was interruped by a tight ligature. These animals also show the signs of hyperalgesia that last a month or more. More recently, Kim and Chung(1991) described new model, in which one or two lumbar spinal nerves are ligated tightly in rats. These animals develop a hyperalgesia that includes a prominent reduction in threshold for responses to stimulation with Von Frei hairs, suggesting a mechanical allodynia. However, in this study the nerve tied by 2 loose ligature so as to inhibit the blood flow and the hyperresponsiveness to thernal stimuli was similar to those of early reports. Especially, the two preparations described by Seltzer et al. and this study produce some similar and some distinctly different findings in the postoperative follow-up of the withdrawal latencies to heat stimulation. The behavioral disorders and the withdrawal latencies of experimental side are similar to each other but the latencies of control side are not consistent with each other.

Trauma in some peripheral tissue first activates unmyelinated (C) nociceptors. This activity excites WDR (and other) neurons in the spinal cord and also cause these neurons to become more responsive to all subsequent afferent inputs. If this sensitization of WDR neurons is present over time, then the WDR neurons will continue to give a vigorous response to mechanical stimulation of A-fiber mechanoreceptors even after healing is complete, leading to touch-evoked pain or allodynia(Roberts, 1986). Based on this theory, we used the method that capsaicin is treated locally on the sciatic nerve in experimental side for the purpose of the inhibition of noxious signal transmission throughout C-fibers and we also thought that the sensitization of WDR neurons becomes to be reduced as a result of capsaicin treatment.

We believe that the marked reduction of the withdrawal latency to local heat stimulation on the hind paw was due to the depression of signal transmission throughout the C-fiber afferents in the peripheral nerve, especially from 0.1% to 1.0%. It is clear that those concentrations were so effective for the reduction of the latency at least 4-6 weeks. However, though we do not know whether additional mechanisms decribed previously was involved in the results of this study, we also believe the possibility that

capsaicin exerts a number of roles for the inhibition of signal transmission with the interpretation of many results in early reports.

## REFERENCES


Ainsworth A, Hall P, Wall PD, Allt G, MacKenzie ML, Gibson S & Polak JM (1981) Effects of capsaicin applied locally to adult peripheral nerve. II. Anatomy and enzyme and peptide chemistry of peripheral nerve and spinal cord. *Pain* **11**, 379-388

Bennett GJ & Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33**, 87-107

Buck SH, Miller MS & Burks TF(1982) Depletion of primary afferent substance P by capsaicin and dihydrocapsaicin without altered thermal sensitivity in rats. *Brain Research* **233**, 216-220

Buck SH & Burks TF(1986) The pharmacology of capsaicin: review of some recent observations. *Pharmacological Reviews* **38**(3), 179-226

Cervero F & McRitchie HA(1982) Neonatal capsaicin does not affect unmylinated efferent fibers of the autonomic nervous system: functional evidences. *Brain Research* **239**, 283-288

Chung JM, Paik KS, Kim JS, Nam SC, Kim KJ, Oh UT, Hasegawa T, Chung K & Willis WD(1992) Chronic effect of topical application of capsaicin to the sciatic nerve on responses of spinothalamic neurons. *J Neurophysiol* (submitted)

Chung K, Schwen RJ & Coggeshall RE(1985) Ureteral axon damage following subcutaneous administration of capsaicin in adult rats. *Neuroscience Letters* **53**, 221-226

Fitzgerald M(1983) Capsaicin and sensory neurons - a reviews. *Pain* **15**, 109-130

Gamse R, Petsche U, Lembeck F & Jancso G(1982) Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. *Brain Research* **239**, 447-462

Hargreaves K, Dubner R, Brown F, Flores C & Joris J(1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* **32**, 77-88

Hayes AG & Tyers MB(1980) Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. Pain Research **189**, 561-564

Helke CJ, DiMicco JA, Jacobowitz DM & Kopin IJ(1981) Effect of capsaicin administration to neonatal rats on the substance P content of discrete CNS regions. *Brain Research* **222**, 428-431

Jessel TM, Iversen LL & Cuello AC(1978) Capsaicin-induced depletion of substance P from primary sensory neurons. *Brain Research*, **152**, 183-188

Kim SH & Chung JM(1991) An alternative experimental model for peripheral neuropathy. *Pain* (submitted)

Konietzny F & Hensel H(1983) The effect of capsaicin on the response characteristic of human C-polymodal nociceptors. *J Therm Biol* **8**, 213-215

Lynn B & Shakhanbeh J(1988) Substance P content of the skin, neurogenic inflammation and numbers of C-fibers following capsaicin application to a cutaneous nerve in the rabbit. *Neuroscience* **24**(3), 769-775

Lynn B(1990) Capsaicin: actions on nociceptive C-fibers and therapeutic potential. *Pain* **41**, 61-69

Marley PD, Nagy JI, Emson PC & Rehfeld JF(1982) Cholecystokinin in the rat spinal cord: distribution and lack of effect of neonatal capsaicin treatment and rhizotomy. *Brain Research* **238**, 494-498

McMahon SB, Lewin G & Bloom SR(1991) The Consequences of longterm topical application in the rat. *Pain* **44**, 301-310

Miller MS, Buck SH, Sipes IG, Yamamura HI & Burks TF(1982) Regulation of substance P by nerve growth factor: disruption by capsaicin. *Brain Research* **250**, 193-196

Miller MS, Buck SH, Sipes IG & Burks TF(1982) Capsaicinoid-induced local and systemic antinociception without substance P depletion. *Brain Research* **244**, 193-197

Nagy JI, Vincent SR, Staines WMA, Fibiger HC, Reisine TD & Yamamura HI(1980) Neurotoxic action of capsaicin on spinal substance P neurons. *Brain Research* **186**, 435-444

Nagy JI, Emson PC & Iversen LL(1981) A re-evaluation of the neurochemical and antinociceptive effects of intrathecal capsaicin in the rat. *Brain Research* **211**, 497-502

Otsuka M & Yanagisawa M(1987) Effect of a substance P antagonist on capsaicin-induced nociceptive reflex in the isolated spinal cord-tail preparation of the rat. *Acta Physiologica Hungarica* **69(3-4)**, 363-366

Palermo NN, Brown HK & Smith DL(1981) Selective neurotoxic action of capsaicin on glomerular C-type terminals in rat substantia gelatinosa. *Brain Research* **208**, 506-510

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 Research* **265**, 233-240

Ritter S & Dinh TT(1988) Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. *J Comp Neurol* **271**, 79-90

Roberts WJ(1986) A hypothesis on the physiological basis for causalgia and related pains. *Pain* **24**, 297-311

Seltzer ze'ev, Dubner R & Shir Y(1990) A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain* **43**, 205-218

Theriault E, Otsuka M & Jessell T(1979) Capsaicin-evoked release of substance P from primary sensory neurons. *Brain Research* **170**, 209-213

Wall PD & Fitzerald M(1981) Effects of capsaicin applied locally to adult peripheral nerve. I. Physiology of peripheral nerve and spinal cord. *Pain* **11**, 363-377

Wall PD(1984) Mechanisms of acute and chronic pain. *Advances in pain Research and Therapy* **6**, 95-104

White JC & Sweet WH(1969) I. Causalgia, Chapter IV. Other Varieties of Peripheral Neuralgia. In: *Pain and Neurosurgeon* (A Forty-Year Experience). Charles C Thomas Publisher, Springfield, USA, pp 87-122

Williams JT & Zieglgansberger W(1982) The acute effects of capsaicin on rat primary afferents and spinal neurons. *Brain Research* **253**, 125-131

Zimmermann M(1979) Peripheral and central nervous mechanisms of nociception, pain, and pain therapy: facts and hypothesis. *Advances in Pain Research and Therapy* **3**, 3-32