Neurogenic Hyperalgesia: Psychophysical Studies of Underlying Mechanisms

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SUMMARY AND CONCLUSIONS

1. Psychophysical studies were made, in humans, of the sensory characteristics and underlying mechanisms of the hyperalgesia (often termed "secondary hyperalgesia") that occurs in uninjured skin surrounding a local cutaneous injury. The hyperalgesia was characterized by lowered pain thresholds and enhanced magnitude of pain to normally painful stimuli. The "injury" was produced by a single intradermal injection of 10 µl of 100 µg of capsaicin, the algesic substance in hot chili peppers.

2. On injection of capsaicin into the volar forearm, the subjects experienced intense burning pain, accompanied immediately by the formation of three areas of hyperalgesia surrounding the injection site. The largest mean area (55 cm²) was hyperalgesic to a normally painful punctate stimulation of the skin. Nested within this was an area of tenderness to gentle stroking (38 cm²) and a much smaller area of hyperalgesia to heat (7 cm²). An area of analgesia (pinprick, 4 mm in diameter and centered on the injection site, developed within minutes and typically disappeared within 24 h. The hyperalgesia to heat and to stroking disappeared within 1-2 h, whereas the hyperalgesia to punctate stimuli, although gradually decreasing in area, lasted from 13 to 24 h.

3. The radial spread of the mechanical hyperalgesia (to punctate and stroking stimuli) away from the injury was dependent on neural activity and not produced, for example, by algesic substances transported away from the injury. The injection of capsaicin into a small area of anesthetized skin did not produce hyperalgesia in the surrounding, unanesthetized skin. Also, the hyperalgesia in normal skin readily crossed a tight arm band that blocked the circulation of blood and lymph.

4. The spread of mechanical hyperalgesia away from the injury was peripherally mediated via cutaneous nerve fibers because it was blocked by a thin mediolateral strip of cutaneous anesthesia placed 1 cm away from the capsaicin injection site. Hyperalgesia developed normally on the capsaicin side of the strip but not on the other side.

5. Heat stimulation of the skin that produced pain that was equivalent in magnitude and time course to that produced by an injection of capsaicin (10 µg) resulted in much smaller areas of mechanical hyperalgesia. It was postulated that there exist special chemosensitive primary afferent nerve fibers that are more effective in producing mechanical hyperalgesia than are the known thermo- and mechanosensitive nociceptive nerve fibers.

6. Once developed, the mechanical hyperalgesia became only partially dependent on peripheral neural activity originating at the site of injury. Cooling the injection site to 1°C for several minutes reduced but did not eliminate the area of hyperalgesia to stroking, whereas rewarming brought it back. After capsaicin injection, a proximal, mediolateral strip of anesthetic reduced or eliminated the hyperalgesia to stroking on the proximal but not the distal side of the strip. These procedures were generally less effective in reducing the area of hyperalgesia to punctate stimuli.

7. A proximal nerve block before the injection of capsaicin into the distal anesthetic skin prevented the occurrence of any hyperalgesia after recovery from the anesthetic 1-3 h later. A shorter lasting anesthetic greatly reduced or completely eliminated the areas of hyperalgesia to heat and to mechanical stimuli after recovery from the anesthetic. Thus the neurons that are "sensitized" by capsaicin-activated nerve fibers reside in the central and not the peripheral nervous system. This sensitization of one set of neurons by neural activity in another is termed "neurogenic hyperalgesia."

8. A neuronal model of neurogenic hyperalgesia was proposed in which chemospecific peripheral nerve fibers (that either branch widely or are functionally coupled together in the periphery) sensitize low- and high-threshold mechanoreceptive interneurons in the dorsal horn. These, in turn, differentially facilitate the responses of certain projection neurons to innocuous and noxious mechanical stimulation of the skin within the area of mechanical hyperalgesia. Heat-sensitive afferents from the area of heat hyperalgesia are hypothesized to sensitize heat-receptive interneurons, which also converge onto these projection neurons.

INTRODUCTION

Hyperalgesia is characterized by lowered pain thresholds and increased pain to normally painful stimuli. It can occur not only within injured tissue [primary (1°) hyperalgesia] but also in normal, undamaged tissue outside the area of injury [secondary (2°) hyperalgesia] (Lewis 1936; Hardy et al. 1950). It was Lewis (1936) who first published an extensive experimental study of the process by which a large area of undamaged skin surrounding a local cutaneous injury can become hyperalgesic to mechanical stimuli.

Lewis noted that the hyperalgesia surrounding a small cutaneous injury on the forearm could extend beyond the flare to a length of 18-20 cm and corresponding width of 7 cm. Within this area of 2° hyperalgesia, a normally innocuous light stroking evoked soreness or tenderness ("allodynia"), and a normally painful prick from a needle or a von Frey hair produced pain that was abnormally intense and prolonged. The hyperalgesia could be experimentally produced either by locally injuring the skin, for example by crushing a fold of skin with forceps, or by electrically stimulating a cutaneous nerve trunk.

Experimental studies into the mechanisms of 2° hyperalgesia led Lewis to conclude that it was set up and maintained within the peripheral and not the central nervous system. He hypothesized that nerve impulses in certain nerve fibers excited by a local injury, for example in the skin, would travel not only orthodromically but also antidromically along branches to surrounding areas of uninjured skin, thereby triggering from terminal endings the release of pain-enhancing chemical substances (an "axon re-
flex”) that in turn sensitized nociceptors, thereby causing the remote hyperalgesia.

In the attempt to repeat some of Lewis's experiments, Hardy et al. (1950) obtained certain results that were contrary to those of Lewis. They concluded that—although there is, as Lewis hypothesized, a neurogenically mediated spread of hyperalgesia away from a local injury into remote cutaneous areas—the mediating neurons are not peripherally located, for example, in the skin, but instead are located in the CNS.

To determine further the role of peripheral and central processes in 2° hyperalgesia, we conducted a series of psychophysical experiments in humans and parallel neurophysiological studies in anesthetized monkeys. In each experiment, the hyperalgesia was produced by an intradermal injection of capsaicin, the algesic substance in hot chili peppers. Capsaicin has been shown to produce a variety of sensory effects when applied to the skin of humans, including pain; hyperalgesia; and, if given in sufficient amount, hypalgesia (e.g., Buck and Burks 1986). Electrophysiological studies have demonstrated that certain unmyelinated nociceptive afferent fibers, the cutaneous endings of which respond to heat and mechanical stimuli (C-fiber polymodal nociceptors), also respond to capsaicin in low doses injected intra-arterially and may develop lowered thresholds to heat in response to topical capsaicin (Baumann et al. 1991; Konietzny and Hensel 1983; Szolcsányi et al. 1988). However, there is insufficient information on how other types of nociceptors, and low-threshold thermoreceptors and mechanoreceptors, respond and how the responses of each type relate specifically to pain and altered pain states as measured under the same stimulus conditions. Further, there is only limited information concerning how these sensory events produced by capsaicin relate to the responses of higher order neurons to which these primary afferents project. This is particularly important if capsaicin induces prolonged changes in the responsiveness of central neurons.

In the present paper, the results of psychophysical measurements of capsaicin-induced 2° hyperalgesia and its underlying neural mechanisms are described. It is proposed that 2° hyperalgesia is neurogenically produced by injury-induced activity in certain primary afferent fibers that, in turn, sensitize certain neurons in the dorsal horn of the spinal cord. The phrase “neurogenic hyperalgesia” is used to designate the sensitization of nociceptive neurons through activity in other neurons, for example via the release of a sensitizing neuromodulator, as opposed to the sensitization attributed to nonneural tissue, for example because of the release of inflammatory chemical mediators from blood vessels or mast cells (“nonneurogenic hyperalgesia”).

The hypothesized neurogenic sensitization of central neurons receives further support in two companion papers that describe the effects of an intradermal injection of capsaicin in exciting and altering the response properties of nociceptive neurons in the peripheral and central nervous systems. In the next paper it is shown that, although certain primary afferents are excited by the capsaicin injection, none become sensitized. In the third paper it is shown that certain spinothalamic neurons are not only excited by capsaicin but become sensitized in a way that correlates well with the psychophysical measurements of hyperalgesia. Summaries of some of the results described in one or more of the three papers have been published (Baumann et al. 1986; LaMotte et al. 1988; Simone et al. 1988).

METHO[]

Subjects

Forty healthy subjects, 16 women and 24 men of ages ranging from 19 to 65 yr (median 25 yr), participated in one or more of the experiments after giving informed consent to a protocol approved by the university's human investigation committee. Although we never observed an allergic reaction to an injection of capsaicin, candidate subjects with a history of allergies, bronchial asthma, or dermatological disease were excluded from the study, as were subjects who were pregnant, who were taking medication, or who had a history of cardiac irregularities.

Subjects were initially given a training session in which they used numbers of their own choosing that were proportional to the magnitude of pain produced by heat stimuli of different temperatures delivered to the volar forearm (the method of magnitude estimation) (Stevens 1975). The method of heat stimulation is given in LaMotte et al. (1983). In this and all subsequent experiments, subjects were instructed to attend only to the sensation of pain and not to any reactions to this pain, such as how well the pain could be tolerated or to the amount of discomfort produced. Further instructions were given (typically read) to the subject concerning the details of each experiment.

Method of producing cutaneous hyperalgesia

Hyperalgesia was produced by a single intradermal injection of 100 μg of capsaicin (8-methyl N-vanillyl 6-nonalide) in 10 μl of a polyoxyethylene (20) sorbitan monoleate (Tween 80) saline vehicle. Unless otherwise stated, the injections were given to the volar forearm. The capsaicin solution for injection was prepared as follows. Initially, a stock solution was made containing 10% capsaicin (Fluka) in ethanol (weight by volume). A 2-ml aliquot was then withdrawn and diluted to 1% with ethanol. The ethanol in this aliquot was then removed by vacuum, and the capsaicin was dissolved in 0.15 g (0.14 ml) of Tween 80 (7.5% Tween 80 by volume) by heating the mixture to 60°C (the volume of saline was brought into a colloidal mixture with 1.86 ml of 0.9% saline by sonication and then injected through a Millipore filter (0.2-μm pore size) into a sterile injection vial. The vial was stored in a refrigerator when not in use and warmed up to room temperature and shaken by hand for ~30 s before use.

Method for measuring the area and magnitude of hyperalgesia

The border of the area of mechanical hyperalgesia that developed around the capsaicin injection was mapped with handheld devices that delivered either punctate or stroking mechanical stimuli or heat stimuli to the skin. Initially the experimenter delivered the stimuli and a screen prevented the subject from viewing the test area. In some instances the screen was then removed, and the subject was allowed to verify the map by self-stimulation with the same stimuli. Typically the latter resulted in no corrections.

Punctate Stimulation

The punctate stimulus was, for most experiments, a von Frey-type nylon filament, 0.58 mm in diameter, that delivered a force of 275 mN. Hereafter this stimulus will be referred to as the “standard nylon filament.” It typically evoked a sensation of faint pricking pain (a “prickle”) when applied to normal skin. In some instances a 30-gauge needle was also used to...
lightly pricked (not penetrate) the skin. The presence of hyperalgesia was first determined by delivering a series of punctate stimulations (each stimulus 0.5 to 1 s duration and to a different locus approximately every 3 s) within and well outside the anticipated area of hyperalgesia. From the subject's estimates of the magnitude of pain to each stimulation, hyperalgesia was defined as estimates that were greater than the highest of those obtained from estimates in normal skin. The normal estimates were obtained from 10-20 stimulations of homologous areas of skin on the other arm or on the same arm but located well away from the anticipated area of hyperalgesia.

The border separating the hyperalgesic and surrounding normal skin was determined by stimulating along a series of at least eight linear paths arranged radially around the injection site. Stimulation along each path started well outside the hyperalgesic area and continued toward the injection site in steps of ~5 mm until the subject reported two consecutive points at which a definitive increase in the magnitude of pain occurred. The first point was then marked on the skin with a felt-tip pen. Later, the dots that enclosed the hyperalgesic area were connected together to form a continuous border and traced onto a clear acetate sheet. This enclosed area was calculated by the use of a digitizing pad connected to an IBM/AT computer.

The threshold for punctate mechanical pain and magnitude estimates of suprathreshold pain were determined within the hyperalgesic area in either of two ways. With the first method, nylon filaments of different diameters were presented once in approximate order of increasing force to the same locus on the skin. A stimulus was presented every 30 s and the subject provided a numerical estimate of the maximum magnitude of pain experienced, if any, according to the method of magnitude estimation. Pain threshold was defined as the lowest of the first two forces each to evoke a sensation of pain. Magnitude estimates of pain were also obtained to the application of the standard nylon filament to a single locus within the hyperalgesic area and compared with estimates obtained for the same spot before capsaicin. The magnitude estimates were normalized in such a way as to eliminate the variability due to individual choice of an internal standard as described elsewhere (e.g., Simone et al. 1989). Each subject was tested both before the injection of capsaicin and again starting ~5 min after.

With the second procedure for threshold determination, carried out with a different group of subjects, the same filament was applied once to 10 different loci circularly arranged around the injection site within a 2- to 3-cm radius. Subjects estimated the magnitude of any pain sensation produced by each stimulus according to the method of magnitude estimation. Before capsaicin injection, four filaments were used to deliver forces that evoked sensations ranging in subjective magnitude from just below to above the threshold for pricking pain. The filament forces in millinewtons (and diameters in millimeters) were 7.95 (0.23), 30.3 (0.33), 90.3 (0.41), and 206.6 (0.55). The magnitude of pain produced by each filament was then recorded again at 10-20 min after the injection of capsaicin. The pain threshold was defined as the interpolated force corresponding to a 50% incidence of pain.

STROKING. The area of hyperalgesia to stroking was measured by the use of a standard 3-in-long cotton-tipped applicator taped to a thin springy strip of metal (a cupping saw blade) held at a designated point between the experimenter's fingers. The cotton swab was calibrated to give an average force (as measured on a microbalance) of 100 ± 3.8 (SE) mN when the metal was just noticeably bent during a stroke. Each stroke was delivered at a velocity of ~5 cm/s over a distance of 1.2 cm. Initially, a series of strokes was delivered to normal skin and then to the anticipated region of hyperalgesia around the injection site. If hyperalgesia was present, the area was mapped by stroking along a series of at least eight linear paths arranged radially around the injection site. For each path, strokes began well outside but progressed toward the hyperalgesic area with slight overlap between successive strokes, until the subject announced the presence of "tenderness" or pain. At that point, a mark was made on the skin with a pen. These points were traced onto a clear acetate sheet and connected together. The enclosed area was then computed as described above. In some experiments, the latency of pain or tenderness to each stroke within the hyperalgesic area was obtained. The experimenter pressed a foot switch while simultaneously beginning a stroke on the skin. The subject released a switch as soon as tenderness was felt. An electrical pulse from each switch was recorded by a computer. Latencies (time between the 2 pulses) were obtained for 10-50 strokes delivered in rotation to four to eight loci arranged obliquely around but ~2 cm away from the injection site.

HEAT STIMULI. The area of hyperalgesia to heat was determined by mapping the borders of the region within which a normally nonpainful heat stimulus was felt as painful. A thermometer of 1-cm diam was maintained at 38°C by circulating water from a constant-temperature bath. The thermometer was applied by hand for 5 s to each of a series of loci in steps of 0.5 cm along each of at least eight linear paths arranged radially around the injection site, starting well outside the hyperalgesic zone. The point at which the subject first reported pain was marked on the skin. The mark was made midway between the center and the edge of the thermode. These marks were copied onto a transparent acetate sheet and connected together, and the enclosed area was measured as described above for mechanical stimuli.

In some experiments, the pain threshold for heat was determined by the use of a specially built Peltier thermode with a 1-cm2 contact area that was centered on the capsaicin injection site. Heat stimuli, each of 5-s duration, were delivered in ascending order of temperature in increments of 2°C above a base of either 28 or 38°C. The interstimulus interval was 25 s. Electrical circuitry maintained the stimulus temperature within ±0.1°C of the desired value via feedback from a thermocouple located at the skin-thermode interface. The rise time was 20 ms, as was the fall time.

**Statistical analyses**

Parametric or nonparametric tests were carried out depending on whether response measures were normally distributed or not, respectively, and are described in RESULTS. The significance level for each test was set at P < 0.05 but corrected when necessary by the Bonferroni procedure (0.05/n, where n is the number of comparisons).

**RESULTS**

We will provide, first, a general description of the pain, local skin reactions, and altered pain states after injection of capsaicin and, second, an investigation into the mechanisms underlying these responses.

**Capsaicin pain**

Severe burning pain was immediate during the injection of capsaicin and typically reached a peak within the first 5-15 s. The pain usually declined rapidly within the next few minutes and then more gradually until it disappeared within 10-30 min after injection (Simone et al. 1989).

**Local skin reactions**

A bleb of ~4 mm diam formed during the injection. The exact size of the bleb depended on the volume of solution
injected and did not differ according to whether the injected substance was capsaicin, normal saline, or the Tween 80-saline vehicle. With few exceptions, no wheal appeared after an injection of capsaicin, and the bleb typically disappeared within 1 h.

A visible area of redness (the “flare”) began to form in the skin surrounding the injection site within the first few minutes; it became most noticeable and reached a maximum size within 3–5 min.

Analgesia

The magnitude estimates of pain produced by lightly pricking the bleb with a needle were less than normal when measured within the first 15 min after injection (Fig. 1). At this time, the hypalgesia was evident during punctate stimulation of 2 s in duration delivered with the standard nylon filament. However, there sometimes occurred, particularly with the first stimulus, a delayed sensation of burning pain that began 3 or 4 s after onset of the poke and lasted several seconds. This delayed pain may have resulted from a minute displacement of capsaicin away from the bleb, thereby activating some adjacent, previously unstimulated nociceptors. We did not pursue this phenomenon further.

The injection site often remained hypalgesic to pinprick after all signs of pain and hyperalgesia had disappeared. In 11 subjects it was determined that initially the entire bleb was analgesic to pinprick and remained so for a median of 22 h. An injection of vehicle also produced a transient analgesia to pinprick at the injection site, but only for a median of 3 h.

Hyperalgesia to heat

The area of skin within which a normally nonpainful stimulus of 38°C (LaMotte et al. 1983) was perceived as painful was mapped, starting ~15 min after injection, in five subjects by the use of the water-heated thermode of 1-cm diam (see METHODS). This area of heat hyperalgesia (pain) was small and confined to within a radius of 1–2 cm around the injection site (Fig. 1) (see also Simone et al. 1991). This result was confirmed in a subsequent experiment in which magnitude estimates of pain produced by heat stimuli of different temperatures were obtained on the injection site and 2 cm away (Simone et al. 1991).

Hyperalgesia to stroking

The sensory quality, time course, and area of hyperalgesia to mechanical stimuli differed according to whether the skin was gently stroked or indented with a punctate stimulus. Subjects reported tenderness or overt pain when the skin was stroked within a relatively wide area around the capsaicin injection site (Fig. 1). The magnitude of tenderness appeared to differ among subjects and sometimes for different experiments with the same subject. In some cases, the movement of a single hair on the arm or a touch without lateral movement was sufficient to evoke pain. It was also noted that the magnitude of tenderness was somewhat greater for strokes closer to the bleb than for strokes near the edge of the hyperalgesic area and that this was not due to traction on the bleb itself.

When asked to attend to the time course of pain produced by a single stroke of the skin, subjects typically reported two separate pain sensations. The first was immediate and temporally contiguous with the sensation of touch during the stroke. There often followed a second pain that began ≥1 s later, lasted for several seconds, and was usually characterized as an increase in “background pain” (or a revival of it, if the background pain had subsided). The cutaneous area for “second pain” was about the same or slightly smaller than the area for “first pain.”

The latency and time course of the “second pain” was measured. Starting 3 min after an injection of capsaicin into the dorsum of the right or left foot, the boundaries of the area of hyperalgesia to the “first” pain were determined. Then, within this area, a single stroke was delivered about every 30 s. The subject depressed a response key before each stroke and released it when first detecting the onset of the second pain. The latency between the start of the stroke and the key release was measured, typically for ~20 strokes. The latency to the first tactile sensation was also measured in normal skin for about the same number of strokes. The median touch latency was subtracted from the median pain latency to obtain an approximate estimate of the latency of pain sensation. On the basis of the results from 14 experiments with eight subjects, this median sensory latency for the second pain ranged from 2.5 to 9.8 s with a grand median of 5.4 s. The median duration of pain was 18 s. The pain was described as growing slowly to a maximum and then subsiding at the same rate, or occasionally faster, and having a “burning” quality, similar to that of the background pain left over from the capsaicin injection. Its magnitude was sometimes less and sometimes greater than that of the first sensation of pain evoked by the stroke.

With the exception of certain experiments in which selective nerve blocks were carried out, the first pain was used in all experiments as the response indicative of hyperalgesia to stroking.

FIG. 1. Areas of analgesia and the flare and areas of hyperalgesia to heating, stroking, and punctate stimulation of the skin surrounding an intradermal injection of 100 µg of capsaicin in the middle volar forearm of a typical human subject. Injection site was located in the center of the bleb.
Hyperalgesia to punctate stimulation

The threshold for punctate mechanical pain was determined within the first 15 min after injection by the use of nylon filaments that delivered a range of forces from below to above threshold either to a single locus (10 subjects) or to multiple loci (a different group of 5 subjects) as described in METHODS. When stimuli were delivered to a single locus 7 mm away from the center of the bleb, the mean pain thresholds obtained after either vehicle or capsaicin were not significantly different from those obtained before (paired t test). Yet when stimuli were delivered to multiple loci 10–20 mm from the injection site, the thresholds were significantly decreased after capsaicin, as evidenced by the increase in the number of stimulations with each filament that were called painful [analysis of variance (ANOVA) with repeated measures; Fig. 2A]. The percentage of trials on which each of the three least-forceful filaments were called painful increased after capsaicin for every subject. By interpolation of the 50% points (defined as threshold) in Fig. 2A, the mean threshold decreased from -65 mN before capsaicin to -8 mN after.

When the same spot was stimulated with the standard filament, the magnitude estimates of pain were significantly increased after capsaicin by 1.8 and 1.6 times for spots located 7 and 20 mm away, respectively, from the injection site. No significant change was found at either distance after an injection of vehicle (ANOVA). When four filaments exerting forces from 7 to 205 mN were delivered to multiple loci, there was a significant increase over normal in the magnitude estimates (Fig. 2B). Every subject experienced an increase in the magnitude of pain evoked by each of the four filaments. The magnitude estimates increased a mean of 3.33 times for the highest force. Thus the measurements of threshold and suprathreshold pain to punctate mechanical stimuli support the conclusion that the magnitude of mechanical hyperalgesia is greater when the stimulus is delivered each time to a new spot and not repeatedly given to the same spot on the skin.

The mean maximum areas of hyperalgesia for stroking and punctate stimulation of the skin and the mean maximum area of the visible flare were determined for 20 subjects (Table 1). The areas of flare and hyperalgesia were compared by use of Friedman's two-way ANOVA by rank (Siegal 1956). The mean maximum area of heat hyperalgesia was also measured in five of these subjects. These data were obtained 3–15 min after the injection. The area of hyperalgesia to punctate stimuli was significantly greater (1.4-fold, mean of ratios for each subject) than that for stroking and the latter significantly greater (1.9-fold) than the area of flare. The flare, in turn, was a median of 16.6 times greater than the area of heat hyperalgesia for the five subjects tested.

Primary versus secondary hyperalgesia

On the basis of the spatially restricted effects of capsaicin in altering the sensitivity of cutaneous nociceptors when injected into or adjacent to their receptive fields (RFs) (Baumann et al. 1991), the spread of capsaicin away from the bleb within the skin is probably limited to 1 or 2 mm. Thus the region within which 1° hyperalgesia to heat or mechanical stimuli could occur (i.e., hyperalgesia within an area directly exposed to the injurious stimulus) is probably very small in the present series of experiments. The hyperalgesia is thus believed to be of the secondary type.

Time course of mechanical and heat hyperalgesia and the flare

The time course of changes in the areas of hyperalgesia to stroking and to punctate stimuli was determined for nine subjects (Fig. 3). The changes in areas of flare and hyperalgesia to heat were also measured for five of the nine subjects.

An area of tenderness to stroking was present immediately after injection and grew in size to reach a maximum

![FIG. 2. Incidence and magnitude of pain produced by punctate stimuli of differing forces delivered to multiple spots on the skin before and after an injection of capsaicin. Each point represents a mean response obtained for 5 subjects. A: percentage of trials on which each stimulus evoked a sensation of pain before and after capsaicin (— — — and — — —, respectively). B: normalized magnitude estimates of pain produced by the same stimuli.](http://jn.physiology.org/)

### Table 1. Maximal areas of flare and hyperalgesia to stroking and punctate mechanical stimuli and to heat after injection of capsaicin

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<th>Flare</th>
<th>Punctate</th>
<th>Stroking</th>
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<td>Area (cm²)</td>
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<td>Mean ± SE</td>
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<td>21.6 ± 1.8</td>
<td>20</td>
<td>54.9 ± 6.2</td>
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<td>37.6 ± 4.5</td>
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<td>(10.8–38.2)</td>
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<td>(13.6–90.8)</td>
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Values are mean areas in cm² ± SE; ranges are in parentheses; n is number of subjects tested.
within the first 15 min. It then gradually decreased over the next 1 h and disappeared altogether within 1.6 h (median 2 h). Similarly, an area of hyperalgesia to punctate stimulation was immediately present after injection and grew to a maximum within 15–30 min. It then decreased gradually, disappearing between 13 and 24 h (median 21 h). The flare reached a maximum size within the first 10 min and then gradually shrank and disappeared within 1–2 h (median 1.5 h). The presence of heat hyperalgesia was tested within 30 min after injection, at which time the area of heat hyperalgesia was maximal. The area gradually shrank and disappeared within 1–2 h (median 1.5 h). Similarly, an area of hyperalgesia to punctate stimulation was immediately present after injection and grew to a maximum within the first 10 min and then gradually shrank and disappeared within 1–2 h (median 1.5 h). The presence of heat hyperalgesia was tested within 30 min after injection, at which time the area of heat hyperalgesia was maximal. The area gradually shrank and disappeared within 1–3 h (median 1.5 h) from the time of injection. Thus there were significant differences in the areas of flare and the areas of hyperalgesia to heat, stroking, and punctate stimuli. However, the durations of the flare and the hyperalgesia to stroking and to heat were similar but significantly shorter than the duration of hyperalgesia to punctate stimuli.

We next investigated the mechanisms underlying the pain and hyperalgesia from capsaicin. Because the area of hyperalgesia to heat was so small, most of the following experiments pertain to the hyperalgesia to mechanical and not to heat stimuli. The results from most of these experiments and the interpretations of these results are summarized in Table 2.

Dissociation between the neural mechanisms mediating the flare and 2° mechanical hyperalgesia

Three separate lines of evidence supported the hypothesis that the flare and mechanical hyperalgesia are mediated by different neural mechanisms. First, the visible flare was typically smaller than the areas of hyperalgesia to either punctate or stroking stimuli. However, it should be mentioned that recent thermographic measurements of the flare produced by histamine iontophoresis yielded significantly larger areas than those based on visual appearance (W. Magerl, personal communication).

Second, in an experiment with two subjects, a solution of 10% capsaicin in 80% ethanol solution was applied topically for 3 days to a 1 × 7-cm strip of skin on the volar forearm. On the fourth day, an injection of capsaicin (usual dose) into this area produced no flare, whereas the area of hyperalgesia to stroking was nearly the same or greater than that surrounding an injection of capsaicin on the other (control) arm. Thus, although the time course and size of the areas of the flare and hyperalgesia to stroking are similar, they are mediated by independent mechanisms.

The third line of evidence supporting the independence of flare and hyperalgesia was obtained when capsaicin was injected near the midline of the body. In seven subjects, capsaicin was injected 1 cm lateral to the midline and 1–3 cm caudal to the spinous process of the seventh cervical vertebra. In another subject, the injection site was located 1 cm lateral to the midline and 2 cm caudal to the sternal notch. Control injections were given well away from the midline but at the same vertebral level.

The borders of the areas of flare and hyperalgesia were traced onto transparent acetate. For an injection close to the midline, a vertical line was drawn through the area at the midline, and the distance between the midline and the injection site was measured.

Table 2. Summary of hypotheses based on results obtained from experimental studies of the mechanisms underlying 2° hyperalgesia to mechanical stimuli (2HM)

2HM is independent of the flare
1. The flare can be abolished while 2HM remains (2/2 experiments).
2. The area of flare may be smaller than the area of 2HM (18/20).
3. The flare but not 2HM crosses the midline (8/8).

Development of 2HM depends on a system of intracutaneous nerve fibers that transmits neural activity from the site of injury to remote surrounding skin
1. No 2HM develops around anesthetized skin injected with capsaicin (CAP) (11/11).
2. 2HM is not produced by transport of CAP away from injection site (i.s.) because 1) 2HM occurs after circulatory block by a pressure cuff (2/2) and 2) 2HM spreads across a tight elastic band (3/3).
3. 2HM does not spread across a narrow "barrier" of anesthetized skin—or, if it does, it is reduced in magnitude (18/20)—but does cross a barrier of injected saline (5/5).

Hypothesized intracutaneous nerve fibers initiating 2HM are chemospecific
Area of 2HM is significantly greater around a CAP injection than around an equally painful heat stimulus that produces little or no visible injury to the skin (6/6).

2HM, once developed, continues to be maintained to varying degrees by neural activity that originates at the site of injury and is transmitted to remote surrounding skin via the hypothesized chemospecific nerve fibers
1. Anesthetizing the skin at the CAP i.s. (by cooling or, less effectively, by injecting anesthetic) reduces the area of the surrounding 2HM to stroking (39/45) and, to a much lesser extent, to punctate stimulation (12/34).
2. A barrier of anesthetic reduces the area of 2HM to stroking (16/20) and to a lesser degree to punctate stimulation (8/20), but only on the side opposite the CAP i.s.

Neurons that are "sensitized" by activity in the hypothesized chemospecificafferents reside in the CNS
A proximal nerve block before the injection of CAP into the distal anesthetic skin prevents occurrence of 2HM after recovery from the anesthetic (19/19).

The value in parentheses after each hypothesis is the number of experiments that had the stated outcome (numerator) out of the total number of experiments (denominator).
Similarly, a vertical line was drawn at this same distance through the areas of flare and hyperalgesia obtained from the control injection of capsaicin away from the midline. Each area was digitized and measured. That portion that crossed the midline (or the corresponding portion that crossed the vertical line drawn on the control area) was divided by the whole area. Comparisons were made of these ratios for control and midline injections for the flare and for each type of hyperalgesia to mechanical stimuli.

The flare developed normally in all cases, extending more or less symmetrically from the injection site (Fig. 4). The areas of hyperalgesia to both stroking and punctate stimuli, however, did not follow the same pattern as the flare. The areas of hyperalgesia developed normally ipsilaterally to the injection site, whereas contralaterally these areas extended <1 cm beyond the midline. (The results of Mann-Whitney U tests demonstrated that for the flare the ratios of control to midline injections were not significantly different for control and midline injections but were significantly different for stroking and punctate stimuli.) Three subjects were tested for hyperalgesia to punctate stimuli 4-5 h after the capsaicin injection, but in no case was hyperalgesia found to have crossed the midline.

EXPERIMENTS TO DETERMINE THE DEGREE TO WHICH THE DEVELOPMENT OF PAIN AND MECHANICAL HYPERALGESIA DEPENDS ON PERIPHERAL NEURAL ACTIVITY AT THE SITE OF INJURY. Effects of injecting capsaicin into a small area of anesthetized skin on the magnitude of evoked pain and the development of mechanical hyperalgesia. In 11 subjects, an approximately circular area of 2-4 cm diam on the volar forearm was anesthetized with an intradermal injection of 0.03-0.50 ml of 2% xylocaine. Nine of these subjects also received an additional injection (0.7-2.0 ml each) subdermally. The area of anesthesia to lightly pricking the skin with a needle or stroking it with a cotton swab averaged (median) 4.1 cm². In all cases, the area of anesthesia was smaller than the area of mechanical hyperalgesia obtained in normal skin by a factor of ≥1.6. Capsaicin was injected into the center of the anesthetized area, and the surrounding skin was examined in the usual way for the presence of any hyperalgesia to mechanical stimulation.

None of the subjects exhibited any hyperalgesia inside or outside the anesthetized area. Although a little pain usually occurred after the injection of capsaicin, the onset was typically delayed (median latency of 30 s), and its peak magnitude was only ~10% (median) of the pain from injection into normal skin. After recovery from the anesthetic, ~1-2 h after capsaicin, when normal cutaneous sensation had returned (e.g., when there was no discernible difference in quality, latency, or magnitude of sensory responses to stimulations of control vs. previously anesthetized skin areas), there was neither spontaneous pain nor hyperalgesia.

The blocking of pain sensation by the anesthetic was not merely due to a "pressure analgesia" or a "dilution" of the injected capsaicin produced by the volume of anesthetic fluid injected. Pain was within normal range in latency and magnitude, and mechanical hyperalgesia developed normally, if capsaicin was injected into an area of skin previously injected with normal saline in volumes approximately the same as those used in the xylocaine experiments (6 subjects tested).

We draw three conclusions from this experiment. First, capsaicin pain is served primarily by sensory nerve endings within and/or in close proximity to the injection site. Capsaicin has a minor and transient effect in evoking pain via activation of nerve endings located deep to or 1-2 cm away from that site (however, this activation is not strong enough to produce hyperalgesia at this remote location). Second, the mechanical hyperalgesia that normally forms outside this radius is the secondary type (i.e., not due to direct exposure to chemical substances traveling from the site of injury). This and further evidence presented below suggest that the 2nd hyperalgesia is neurogenically controlled from the injection site. Third, it is likely that capsaicin is removed from the local tissue and/or inactivated within 1-2 h after injection.

Effects of blocking blood and lymph flow on the development of 2nd mechanical hyperalgesia after a capsaicin injection. The low level of pain evoked when capsaicin was injected into anesthetized skin could be reduced or prevented altogether when the flow of blood and lymph was blocked.

FIG. 4. Results of an experiment demonstrating that the flare crossed the midline on the back, whereas the area of mechanical hyperalgesia did not. A: area of flare (encircled by - - - -) and areas of hyperalgesia to punctate and stroking stimuli (--- and . . . , respectively), shown for 1 subject, developed symmetrically about a capsaicin injection (x) located well away from the midline. B: after a capsaicin injection near the midline, the hyperalgesia developed asymmetrically and only on the side of the injection, whereas the flare crossed the midline and developed fairly symmetrically around the injection site.
by means of a pressure cuff placed on the upper arm (proximal to the injection) and inflated to 200 mmHg just before the capsaicin injection (2 subjects tested). It is possible that capsaicin is taken up by the lymphatic system and while moving proximally can activate to a very minor degree sensory nerve fibers close to but away from the injection site. In fact, a few subjects reported that, during some experiments after a capsaicin injection into normal skin, a local sensation of burning pain was felt to migrate slowly up the arm from the injection site (unaccompanied by any cutaneous hyperalgesia) until it reached the lymph nodes, after which it soon disappeared. This was not a typical finding, however.

Three observations indicated that capsaicin does not result in second mechanical hyperalgesia by coming in contact with nerve endings within the second area via transport by the circulatory or lymphatic systems. First, the hyperalgesia developed fairly symmetrically on either side of an injection in the middle forearm, which would not be expected if lymphatic uptake were involved (cf. Lewis 1936; Hardy et al. 1950). Second, when the flow of blood and lymph was blocked in the upper forearms of two subjects by a pressure cuff (200 mmHg) proximal to an injection of capsaicin, the area of hyperalgesia to either stroking or punctate stimulation was within the normal (Table 1) range. Third, in experiments with three subjects, a tight elastic band, 2 cm wide, was wrapped around the arm to block lymph flow and venous return. After capsaicin was injected through a small hole in the center of the band, the hyperalgesia developed symmetrically on both sides of the band and occupied the same area as it had after a control injection of capsaicin without the band.

EXPERIMENT TO DETERMINE WHETHER THE AREA OF SECOND HYPERALGESIA IS DIRECTLY RELATED TO THE MAGNITUDE OF PAIN EVOKED BY A NOXIOUS STIMULUS AND WHETHER EQUALLY PAINFUL CHEMICAL AND NONCHEMICAL NOXIOUS STIMULI PRODUCE DIFFERENT AREAS OF HYPERALGESIA. The purpose of the next experiments was to see whether capsaicin, a putative potent activator of chemosensitive nociceptive afferent fibers, was more effective in producing second mechanical hyperalgesia than a physical stimulus, in this case heat, when each stimulus produced about the same magnitude and duration of pain. However, we anticipated that very injurious levels of heat might be required to match the pain from the usual capsaicin dose. This was undesirable because an injury would be accompanied by a release of endogenous inflammatory chemicals that might activate the same chemosensitive afferents. In an attempt to minimize this possibility, we reduced the pain from capsaicin by injecting 10 µg instead of the usual 100 µg. Before injection into one forearm, we placed the Peltier thermode on the other forearm. The subject was instructed to change the temperature of the thermode by turning a dial in such a way as to continually match (in magnitude and time course) the pain produced by capsaicin with the pain produced by the thermode. In addition, every 15 s the subject called out a number proportional to the maximum magnitude of pain experienced during the previous 15-s interval.

In all six subjects tested, the maximum magnitude of pain was 40–50% less after 10 µg of capsaicin than after 100 µg. Also, the duration of pain was shorter for the 10 µg dose as well, lasting 9–12 min. The maximum temperature used by any subject to match the pain was 51°C and the mean maximum temperature was 49 ± 0.3°C (SE). The skin in contact with the thermode remained erythematous but not edematous for several hours to 1 or 2 days. Thus the heat stimulus produced relatively little or no injury of the skin. A typical time course of temperature adjustments required to produce equivalent magnitude estimates of pain is shown for one subject in Fig. 5A. Also shown are the areas of hyperalgesia to stroking and to punctate stimuli mapped at two times, the first between 3 and 5 min after injection and the second between 10 and 12 min (Fig. 5B). In addition, this subject and one other were tested again for hyperalgesia after removing the thermode from the skin. This and every subject tested had a considerably larger area of second hyperalgesia produced by capsaicin in comparison with that produced by heat. This was true at all times of testing. The mean areas of hyperalgesia to stroking and punctate stimuli were 20.6 ± 1.4 (SE) and 43.0 ± 5.8 cm², respectively, after capsaicin and 1.6 ± 1.7 and 8.2 ± 3.1 cm² during heat stimulation. (The values for heat include the 1-cm² area occupied by the thermode.) Two subjects had no hyperalgesia to stroking after a capsaicin injection (2 subjects tested). It is possible that the heat stimulus produced relatively little or no injury of the skin.
peralgesia around the thermode and for these a value of zero was used in the average. For the two subjects tested after removing the thermode, there were no overall changes in the hyperalgesic areas after heat or capsaicin as tested during the next 10 min.

Thus, in spite of the fact that the pain in each arm was comparable in magnitude and time course, 2° hyperalgesia developed to a much greater degree on the arm injected with capsaicin. That is, the peripheral and central consequences of activity in heat-sensitive nociceptive afferent fibers (e.g., C- and Aδ-polymodal nociceptive afferents), which presumably evoked pain comparable in magnitude with that produced by capsaicin, did not produce a comparable degree of hyperalgesia. We therefore conclude that certain types of nociceptive afferents, which we hypothesize are selectively responsive to algic substances, are more effective—directly or indirectly—in producing 2° mechanical hyperalgesia than are others.

**EXPERIMENT TO DETERMINE WHETHER THE DEVELOPMENT OF 2° MECHANICAL HYPERALGESIA DEPENDS ON A SYSTEM OF INTRACUTANEOUS NERVE FIBERS THAT TRANSMIT NEURAL ACTIVITY FROM THE SITE OF INJURY (CAPSAICIN INJECTION SITE) TO REMOTE SURROUNDING SKIN.** The following experiment was carried out in 12 subjects, 8 tested 1 time and 4 tested 3 different times, for a total of 20 experiments.

A thin mediolateral strip of skin across the middle volar forearm was anesthetized with 1% xylocaine (a string of ~8-12 injections, i.e., of ~0.05 ml each). A spatially contiguous strip of anesthesia to light pinpricks was mapped and found to be ~1 cm wide (proximal-distal) by 8-10 cm long (medial-lateral). The skin of each side of the anesthetic strip was stimulated with nylon filaments or lightly stroked with the cotton swab to ensure that there was normal sensitivity near the strip vs. stimulations of the skin on the control arm). Capsaicin was then injected 1 cm from either the distal or proximal border of the strip (18 experiments were distal and 2 were proximal). During a period of 3-10 min after the injection of capsaicin, the area of 2° hyperalgesia was mapped with the usual mechanical stimuli. The strip was periodically tested to be certain that it remained anesthetic.

To determine whether the anesthetic strip acted as a barrier to significantly reduce or prevent the development of hyperalgesia on the side opposite the capsaicin injection, it was necessary to calculate the "predicted area" of hyperalgesia that would have extended beyond the strip had the strip not been there.

In these anesthetic-strip experiments, injections were given into the distal half of the volar forearm. In 22 control experiments with 14 subjects in which capsaicin was injected into the distal half of the volar forearm, it was found that the fully developed area of hyperalgesia was slightly asymmetrical with respect to the injection site. When the map of each hyperalgesic area was divided into two sections (Fig. 6) by a line drawn mediolaterally through the capsaicin injection site (X), it was found that the distal (HD) section was slightly smaller in area than the proximal (HP). For each experiment, the ratio, ZD of HD to HP was obtained and averaged for each subject. The mean ratio was 0.83 for stroking and 0.72 for punctate stimuli. In the anesthetic strip experiments, the predicted area (HD or HS) of the section containing the strip was determined from HD = HD/ZD if the strip was placed in the proximal section, or HD = HD/ZD if the strip was placed in the distal section. The area, K, that was predicted to have extended beyond the strip had the strip not been there was obtained by subtracting the subsections J (the area of hyperalgesia between the injection site and the strip) and J (the area occupied by the strip) from HP or HD. The median value of K for subjects participating in the strip experiments was 8.5 cm² for stroking (range 5.4-38.4), and 21.5 cm² for punctate stimuli (range 4.4-75.3). Such analyses could not be carried out for the area of flare or area of hyperalgesia to heat because these areas were too small.

For each of the 12 subjects, in at least one experiment, the anesthetic strip partially or completely blocked the spread of hyperalgesia (to both stroking and punctate stimuli) across the strip to the side opposite the injection of capsaicin. The development of hyperalgesia was always unaffected on the capsaicin side. In 10 of the 20 experiments, there was no hyperalgesia to either stroking or to punctate stimuli on the side opposite the capsaicin injection (see Fig. 7 for typical result), whereas in 2 experiments hyperalgesia developed normally (was 100% or more of the predicted area) on both sides of the strip. In the remaining eight experiments, the strip had a partial effect in blocking the spread of hyperalgesia, reducing the area to a median of 30% of the value predicted (K') for stroking and 37% of the area predicted for punctate stimuli. The two failures to
achieve any reduction in area were probably due to an unevenness in the concentration of anesthetic delivered or to the strip being so short that the collaterals of axons that supplied the skin on each side of the strip were not all blocked.

In one of the experiments in which the block was partial and in another one in which there was no reduction in area, magnitude estimates of pain were obtained on each side of the strip in response to a series of stimulations with the standard nylon filament. The median magnitude estimates were 30 and 50% lower on the side opposite the capsaicin injection.

There was a suggestion of individual differences among those subjects given three repetitions of the experiment. For three of the subjects, the hyperalgesia was blocked only partially or not at all during each test, whereas for another subject it was always completely blocked.

In several instances, the anesthetic blocked the development of hyperalgesia as described until the anesthetic began to wear off at a spot along the strip. At this time a small area of hyperalgesia began to develop in the area of skin lying beyond the barrier. In two other subjects, although small spotty areas of hyperalgesia to punctate stimuli developed beyond the barrier after the anesthetic began to wear off, there was no hyperalgesia to stroking.

The remote possibility that the mere volume of anesthetic injected acted as a kind of mechanical barrier that blocked, for example, diffusion or transport of capsaicin away from the injection site (even though such mechanisms as a basis for 2° hyperalgesia were previously discredited) was disproved. In tests with five subjects, the spread of hyperalgesia was not blocked by a mediolateral strip of normal saline injected intracutaneously in amounts equivalent to those used with xylocaine.

We conclude that the spread of 2° mechanical hyperalgesia depends on a peripheral rather than a central transmission of neural activity—probably via nerve fibers within the skin—for considerable distances away from the injection site.

EXPERIMENTS TO DETERMINE WHETHER 2° MECHANICAL HYPERALGESIA, ONCE FULLY DEVELOPED, IS MAINTAINED BY NEURAL ACTIVITY ORIGINATING AT THE SITE OF INJURY. Two sets of experiments were carried out to determine whether 2° mechanical hyperalgesia is continuously maintained by peripheral neural activity within or close to the injection site. Once hyperalgesia was fully developed and its boundaries determined by stroking and punctate stimuli (5–10 min after capsaicin injection), either of two procedures was carried out in an attempt to block or at least reduce neural activity at the injection site. In the first set of experiments, the skin in and adjacent to the injection site was cooled, whereas in the second set it was injected with local anesthetic.

Effects of cooling the injection site on the maintenance of 2° mechanical hyperalgesia. A 1-cm² area of skin centered on the injection site was maintained at 1°C for 2–5 min by the Peltier thermode, starting ~15 min after the capsaicin injection. This procedure rendered the cooled skin analgesic to light pinpricks. The experiment was given once to 21 subjects. The area of hyperalgesia to stroking was mapped for each subject before the onset of cooling, again during cooling, just before the skin was rewarmed, and once more after the skin was rewarmed. In 10 of the subjects, the area of hyperalgesia to punctate stimuli was measured as well.

1) Hyperalgesia to stroking. During cooling, the area of hyperalgesia to stroking decreased for all but one subject (Fig. 8A and Table 3). The area during cooling was a median of 26.7% of the original size (range 5.5–79.5%). The reduced size typically amounted to a circular area around the thermode. After a rewarming period of 2–5 min, which brought the cooled area up to ~34°C, the reduced hyperalgesic area for each subject was substantially increased to a size that was equal to or greater than the area before cooling (10 subjects) or slightly less than the area before cooling (11 subjects). The decrease in area from the original size, after rewarming, may represent merely the usual decrease due to the passage of time.

2) Hyperalgesia to punctate stimulation. Cooling reduced the area of hyperalgesia to punctate stimuli in only 4 of the 10 subjects tested (median, 74% of the original size; range, 52.3–88.5%). In three experiments for which cooling failed to reduce the area of hyperalgesia to punctate stimuli, magnitude estimates of pain evoked by the standard nylon filament were obtained. Median magnitude estimates ranged from 66 to 80% of values obtained before cooling. That is, the hyperalgesia was still partially dependent on

FIG. 7. Effect of a strip of local anesthetic in blocking the spread of 2° mechanical hyperalgesia away from the capsaicin injection site when the anesthetic was given before the injection. A: normal areas of hyperalgesia to stroking and to punctate stimuli (— and —), respectively, surrounding a capsaicin injection (x) on a subject's forearm. B: hyperalgesia did not cross a strip of skin that was anesthetized (■) in the same subject before a capsaicin injection.
neural activity from the capsaicin injection site, even though cooling did not produce a reduction in area. Nevertheless, it is clear that the hyperalgesia to punctate stimuli, once developed, is less dependent on peripheral neural activity originating at the capsaicin injection site than is the hyperalgesia to stroking.

Two control experiments for cooling were carried out. In the first, the thermode cooled the injection area to 20°C for 2-5 min to determine whether mild cooling (which might be expected to silence any heat-sensitized nociceptors made spontaneously active by the warmth of normal skin temperature) was as effective as the anesthetizing effect of the 1°C stimulus. For each of the six subjects tested, cooling to 20°C failed to reduce the hyperalgesic area, or, if there was a slight shrinkage, there was no expansion on rewarming, indicating that the reduction was due to the passage of time and not to cooling (Table 3).

The second control experiment tested whether the effect of cold on reducing the area of hyperalgesia was specific to the injection site or just a result of the cold stimulus per se regardless of where it was applied. When the thermode was centered on a spot located 1.5 cm proximal to the injection site, the results were the same as those obtained with the first control experiment. Cooling this control spot to 1°C did not alter the area of 2°C hyperalgesia (Table 3).

Effects of anesthetizing the injection site with local anesthetic on the maintenance of 2°C mechanical hyperalgesia. The following results indicated that locally anesthetizing the capsaicin injection site had approximately the same overall effect as cooling, i.e., hyperalgesia to stroking was reduced more than hyperalgesia to punctate stimuli. A total of 24 experiments in 13 subjects was carried out (3 subjects tested 3 times, 5 tested 2 times, and 5 tested 1 time). After hyperalgesia produced by a capsaicin injection was fully developed, an area centered on the injection site was anesthetized with 2% xylocaine administered in amounts of 0.04-0.5 ml ic followed in some subjects by 1-2 ml sc. This resulted in a median area of cutaneous anesthesia of 5.2 cm² that was approximately circular. The areas of hyperalgesia to punctate and to stroking stimuli were measured for each subject before and after the administration of the anesthetic.

1) Hyperalgesia to stroking. In all but one subject (who was given only 1 experiment), the xylocaine completely or partially reduced the area of hyperalgesia to stroking in at least one of the experiments. The anesthetic failed to reduce the area in 5 experiments, eliminated it completely in 7, and partially reduced it in 12 (median, 40% of the original size; range, 2-78%). In the cases for which hyperalgesia was not reduced, we think it likely that the injections of anesthetic displaced the capsaicin and brought it in contact with adjacent unanesthetized nociceptive afferents. Indeed, on occasion, a surge of pain was felt as the anesthetic was injected, and this was sometimes accompanied by an expansion of the flare and hyperalgesic area.

2) Hyperalgesia to punctate stimuli. A complete reduc-

### Table 3. Effects of cooling and rewarming the skin on and off the capsaicin injection site on the areas of hyperalgesia to stroking and punctate mechanical stimuli

<table>
<thead>
<tr>
<th>Type of Stimulus</th>
<th>Cooling Site</th>
<th>Cooling Temp., °C</th>
<th>Before Cooling</th>
<th>n</th>
<th>During Cooling</th>
<th>n</th>
<th>After Rewarming</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punctate Inj.</td>
<td>1</td>
<td>70.0 ± 8.3</td>
<td>10</td>
<td>64.9 ± 9.5</td>
<td>10</td>
<td>75.6 ± 9.1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29.4-110)</td>
<td></td>
<td>(23.4-115)</td>
<td></td>
<td>(31.2-97.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroking Inj.</td>
<td>1</td>
<td>49.3 ± 7.1</td>
<td>21</td>
<td>16.2 ± 4.3</td>
<td>21</td>
<td>42.5 ± 6.8</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16.0-134.0)</td>
<td></td>
<td>(1.3-70.0)</td>
<td></td>
<td>(8.0-132.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroking Inj.</td>
<td>20</td>
<td>38.7 ± 8.1</td>
<td>5</td>
<td>35.8 ± 9.9</td>
<td>5</td>
<td>32.8 ± 13.1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20.1-61.4)</td>
<td></td>
<td>(14.6-63.2)</td>
<td></td>
<td>(7.9-76.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroking 1.5 cm from inj.</td>
<td>1</td>
<td>35.1 ± 5.3</td>
<td>6</td>
<td>34.1 ± 7.5</td>
<td>6</td>
<td>31.1 ± 8.1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20.6-61.8)</td>
<td></td>
<td>(17.3-61.5)</td>
<td></td>
<td>(14.1-31.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean areas in cm² ± SE; ranges are in parentheses; n, number of subjects tested. Inj., capsaicin injection site.
tion was achieved in only one experiment and a partial reduction in seven. The partial reductions were characterized by reduced regions in which isolated spots were hyperalgesic (2 experiments) or contiguous hyperalgesic areas were 50–86% the original sizes. In three of the experiments in which the anesthetic failed to reduce the area of hyperalgesia, magnitude estimates of pain were obtained. The median magnitude estimates for these subjects were 40, 44, and 50% less than those obtained before the anesthetic. Thus the hyperalgesia was maintained to a small degree by neural activity from the capsaicin injection site, even though the area of hyperalgesia was not diminished.

When the effects of cooling and injecting xylocaine are considered together, the area of hyperalgesia was reduced significantly more for stroking (39 of 45 experiments) than for punctate stimuli (12 of 34 experiments) ($\chi^2$ test).

On the basis of results obtained in both the cooling and xylocaine experiments, we conclude that once the hyperalgesia to mechanical stimuli is developed, it is maintained to varying degrees by peripheral neural activity originating at the site of injury. This dependence is more so for stroking than for punctate stimuli and, for reasons not yet apparent, can vary in degree from one experiment to another even for the same subject. Possibly some of this variability is due to regional differences in the depths of the nerve fibers that must be reached by the anesthetic and/or to variations in the density of innervation.

**EXPERIMENT TO DETERMINE WHETHER 2" MECHANICAL HYPERALGESIA, ONCE FULLY DEVELOPED, IS CONTINUOUSLY MAINTAINED BY A SYSTEM OF CUTANEOUS NERVE FIBERS THAT TRANSMIT NEURAL ACTIVITY AWAY FROM THE SITE OF INJURY TO REMOTE SURROUNDING SKIN.** In 20 experiments with 12 subjects (1 tested in 4 experiments, 2 tested in 3, and 1 tested in 2), once the area of 2" hyperalgesia was mapped on the skin, a mediolateral strip of skin ~1 cm wide by 10 cm long was anesthetized. A series of intradermal injections of 2% xylocaine was given, ~8–12 injections of 0.05 ml each, requiring ~10 min to complete. Then, during the next 5 min, the skin on both sides of the strip was tested periodically to map any change in the area of hyperalgesia.

The area of hyperalgesia on the side of the strip farthest away from the capsaicin injection site was designated according to whether it was measured before ($K_0$) or after ($K_t$) the strip. If $K_0$ was not sufficiently large, for example because of an overly large area of anesthesia, then the experiment was terminated (and the data not included in our analyses). Otherwise, if the strip acted as a barrier to the transmission of neural activity away from the injection site, then it should reduce or completely eliminate $K_t$. However, one might expect $K_t$ to decrease anyway with the passage of time. To correct for this, we multiplied $K_t$ by the proportion ($H_t/H_0$) by which the area of hyperalgesia (distal or proximal to the capsaicin injection site) not containing the strip changed during the same period of time to produce the predicted value of $K'_t$. ($H_0$ and $H_t$ designate hyperalgesic areas after and before the strip, respectively.) If the anesthetic strip eliminated or reduced the hyperalgesia on the side of the strip away from the injection site, then $K'_t$ should be less than $K'_0$ (the area corrected for shrinkage with time).

The areas of hyperalgesia to punctate and stroking stimuli are illustrated for one subject before and after the injection of anesthetic in Fig. 9, A and B, respectively.

![Fig. 9](http://jn.physiology.org/)

**1) Hyperalgesia to stroking.** This was partially or completely eliminated on the side of the strip opposite the capsaicin injection in at least one experiment for every subject. The hyperalgesia on the same side of the strip as the capsaicin injection was unaffected by the anesthetic. A partial reduction was arbitrarily designated as a shrinkage to a size ≤90% of the area, $K'_t$, predicted to occur with the passage of time. The areas that were partially reduced, occurring in 10 of the 20 experiments, were reduced to a median of 47% the original size (range, 8–78%). The hyperalgesia to stroking was completely eliminated ($K_0/K_t = 0$) in six experiments but not reduced at all in four experiments.

**2) Hyperalgesia to punctate stimuli.** This was completely eliminated in only 1 of the 20 experiments and partially reduced in 7 (reduced to a median of 76% of the original size; range, 49–88%). Thus the anesthetic was significantly less effective in eliminating or reducing the hyperalgesia to punctate stimuli (8 of 20 experiments) than the hyperalgesia to stroking (16 of 20) ($\chi^2$ test). In two experiments for which there was no reduction in the area of hyperalgesia (e.g., Fig. 9B) and in three experiments in which there was a partial reduction, magnitude estimates evoked by stimulations with the standard nylon filament were obtained for each side of the anesthetic strip within the hyperalgesic zone. For each subject, these magnitude estimates were less on the side of the strip opposite the capsaicin injection (averaging a median of 66% of the estimates on the other side). Thus, in these experiments, the hyperalgesia remote to the capsaicin injection site was partially dependent on neural activity transmitted away from this site, presumably through intracutaneous branches of peripheral nerve fibers, even when the anesthetic strip failed to reduce the area of hyperalgesia.

In two experiments in which the strip partially reduced
the areas of hyperalgesia to stroking but not punctate stimuli, the capsaicin injection site was infiltrated with xylocaine. This failed to reduce the total area of hyperalgesia to either type of mechanical stimulus on either side of the strip. The effects of the anesthetic on magnitude of pain, however, were not tested.

We conclude that, once hyperalgesia to mechanical stimuli develops around a site of injury, it continues to be maintained to some degree by neural activity transmitted away from the site of injury via capsaicin-activated neurons that either arborize over an unusually large area of skin (larger than RFs of known peripheral neurons) or are functionally coupled together. This maintenance by peripheral neural activity is greater for the hyperalgesia to stroking than it is for the hyperalgesia to punctate stimuli.

**EXPERIMENT TO DETERMINE WHETHER THE NEURONS THAT BECOME SENSITIZED AS A RESULT OF ACTIVITY IN CAPSAICIN-ACTIVATED AFFERENT FIBERS RESIDE IN THE PERIPHERAL OR THE CENTRAL NERVOUS SYSTEM.** Effects of a prolonged, complete nerve block on the hyperalgesia to punctate stimulation. The question considered next is whether the hyperalgesia to punctate stimuli, which lasts 13–24 h after capsaicin injection, results from the “sensitization” (e.g., lowered response thresholds and enhanced responses to suprathreshold stimuli) of neurons in the peripheral or the central nervous system. Because the direct effects of capsaicin are not long lasting, the postanesthesia testing would not be complicated by the presence of the original cause of the hyperalgesia, as it is after the kind of physical injuries produced in the experiments by Lewis (1936) and Hardy et al. (1950).

The lateral antebrachial cutaneous nerve of the forearm was infiltrated with 5–10 ml of 1% xylocaine at the antecubital space in 16 experiments with 10 subjects. In three additional tests with two subjects, the superficial radial nerve was anesthetized instead. The area of cutaneous analgesia to light pinpricks was mapped, as well as any region of diminished touch to lightly stroking with a cotton swab. Capsaicin was then injected within the analgesic area either near the center (17 experiments) or near the edge (2 experiments). Some subjects received an injection of capsaicin at the same time in the other (control) arm. The other subjects received the control injection on another day.

When capsaicin was injected into the center of the analgesic area, a sensation of faint pain typically occurred within the first minute but was only 11.5% (median for 11 experiments) of the peak magnitude of pain produced in control skin for each subject. Furthermore, the pain was transient, typically lasting <1–2 min. A flare always developed normally (i.e., within range shown for flare in Table 1), but none of the subjects experienced any sensation to mechanical stimulation within the analgesic area (Fig. 10A).

Within a mean of 2.6 h (range, 1.2–3.7 h) after the capsaicin injection, the formerly analgesic skin recovered normal sensation (i.e., no discernible differences in quality, latency, or magnitude of mechanically evoked touch or pain sensations on control skin vs. previously blocked skin) without any evidence of hyperalgesia to stroking or punctate stimuli (Fig. 10A). In contrast, hyperalgesia to mechanical stimuli on the control arm was still present for many hours beyond this period of time (Fig. 10B).

When capsaicin was injected inside but near the edge of the analgesic zone, the pain was again reduced to a median of 9% of the control peak magnitude. However, within the first few minutes, a small area of hyperalgesia to both punctate and stroking stimuli developed nearby, but only outside the analgesic zone (Fig. 11A). A similar occurrence was observed for two of the subjects given an injection at a more central site within the analgesic zone. In each case the duration of hyperalgesia was short (2–9 min) (Fig. 11, A and B), and no hyperalgesia was observed within the formerly analgesic area once normal sensation returned. However, for three subjects, the area of hyperalgesia lying outside the analgesic zone remained hyperalgesic even after recovery of normal sensation within the formerly analgesic area.

In two experiments, the injection of capsaicin was delayed until sensations evoked by mechanical and thermal stimulation of the skin began to recover (i.e., the sensations were not discriminably different from stimulations of control skin). Normal areas of hyperalgesia (see Table 1) to heat, to stroking, and to punctate stimuli ensued. This suggests that it is unlikely that there was a novel type of primary afferent that became sensitized to mechanical or heat stimuli but was somehow more susceptible to the anesthetic...
and therefore remained blocked long after the return of normal sensation.

We draw three conclusions. First, capsaicin remains active (capable of activating chemosensitive afferents) for only a short period of time, e.g., ≤1–2 h; otherwise, pain and hyperalgesia would have developed after the anesthetic wore off. Second, the neurons that become sensitized are located within the CNS; otherwise, the proximal nerve block would not have eliminated the hyperalgesia. Finally, the small region of hyperalgesia that sometimes developed outside the anesthetic zone is hypothesized to result from the activation, by capsaicin, of branches of chemospecific nociceptive afferent fibers in an unanesthetized nerve; activity in these fibers then sensitizes certain neurons in the spinal cord.

Effects of a complete but shorter lasting nerve block on mechanical and heat hyperalgesia. Testing for the presence of the shorter lasting hyperalgesia to stroking or heating the skin presented a methodological problem: if the duration of the anesthesia were too short, capsaicin would still be active and the hyperalgesia would be produced as the anesthetic wore off. Conversely, if the anesthesia lasted too long, e.g., 2 h, hyperalgesia to heat or to stroking might have disappeared anyway. Thus it was particularly important to compare hyperalgesia in the nerve-blocked skin with hyperalgesia in normal (control) skin tested in the same subject at comparable times.

In nine experiments with five subjects, the superficial branch of the radial nerve was anesthetized with 2% chloroprocaine HCl, a short-acting anesthetic. In one experiment, 1% xylocaine was injected instead. The areas of analgesia to pinprick and anesthesia to lightly stroking the skin were mapped and marked on the skin. The skin was also tested for loss of sensation to warm and cool. After determining that the skin was analgesic and anesthetic, we injected 1% capsaicin near the middle of the analgesic area. Within 20–30 min after the injection, the opposite arm was given a control injection of capsaicin. On recovery from the nerve block, the areas of hyperalgesia to mechanical and to heat stimuli were mapped in the usual manner. In addition, pain threshold and magnitude estimates of pain were obtained in response to heat stimuli of 29–51°C (see METHODS).

In all cases in which the skin was anesthetic to pinprick and to tactile stimuli, the maximum magnitude of pain to capsaicin was reduced to a median of 30% of control magnitude estimates of pain. Furthermore, the pain duration was decreased, lasting only 1–3 min.

In four of the experiments, at 3–10 min after the capsaicin injection, the subject reported that stimulation of the skin, which was anesthetic just before the injection, became hyperalgesic to mechanical stimulation within a small area around the injection site (Fig. 12A). The hyperalgesic areas to stroking and to punctate stimuli were of comparable size but only 10% as large as those present at comparable times on control skin (medians of 2 vs. 20 cm²) (Fig. 12B). The area was also hyperalgesic to heat, as evidenced by the pain to contact with a test tube filled with water at 38–40°C, even though warm and cool could not be distinguished. The pain was not a result of pressure because testing with a test tube filled with cool water evoked no sensation. During these tests the surrounding skin that was previously anesthetic remained so. The hyperalgesia disappeared and was replaced by anesthesia before complete recovery from the anesthetic.

In two experiments with two subjects, hyperalgesia to stroking and to punctate stimuli developed after a delay of
smaller (median of 1.5 cm² for 4 of the 8 subjects and absent for the other 4) (Figs. 12A and 13). 2) The area of hyperalgesia to punctate stimuli was a median of 19 cm² on the control skin but no larger than 1.5 cm² on the previously blocked skin (6 subjects tested). 3) A normal area of heat hyperalgesia (within range of values in Table 1) was present on the control skin for five subjects (2 others had none) as tested with the heat stimuli of ascending temperature. For each of the five subjects, the pain threshold was lower than normal (preexperiment control values) on the skin receiving the control injection of capsaicin (median of 35°C) but normal (within range of preexperiment values) on the previously blocked skin (median of 43°C).

Presumably a proximal nerve block that prevented primary afferent neuronal activity from reaching the CNS would not affect a sensitization of peripheral nerve endings. But because hyperalgesia was reduced or eliminated by such a block a peripheral sensitization appears unlikely. We therefore conclude that the neurons that presumably become sensitized to stroking or punctate mechanical stimulation and the neurons that become sensitized to heat are in the CNS and are not primary afferent fibers.

Effects of selective nerve blocks on the development of 2nd hyperalgesia. 1) Conduction block in myelinated peripheral nerve fibers. We attempted to block conduction in myelinated nerve fibers selectively by compressing the superficial radial nerve either by means of a band attached to weights, totaling 5 kg (11 subjects), or by applying a pressure cuff to the upper arm and inflating it to 200 mmHg (3 subjects). The sense of touch was monitored on the skin distal to the block by the use of light pressure and stroking, and the sensations of cool (26°C) and warm (39°C) were monitored with handheld thermodes. Several experiments were performed that differed as to the time of capsaicin injection or in the types of sensory measurements obtained. The results of each experiment were compared with those obtained after control injections of capsaicin without nerve block (typically not administered at the same time as the nerve-block injection).

In one series of experiments with eight subjects, we mapped the borders of an area within which the sense of touch to light stroking was lost and the sense of cool in response to abrupt cooling was present but delayed by 1–3 s. When the area of anesthesia was larger than the area of mechanical hyperalgesia normally obtained after a control injection, capsaicin was injected into the center of this area of diminished sensation and produced pain, the magnitude and duration of which was normal compared with published values (Simone et al. 1989). Starting 3–5 min after the injection, an area of hyperalgesia to stroking was mapped. The subjects, having no sense of touch and not allowed to view the skin, were notified when each stroke was to begin and then reported whether any pain or an increase in background pain occurred. Each area was found to be within the normal range of sizes for that subject. Four of these subjects experienced the usual dual sensation of a short latency "first" pain followed several seconds later by a "second" pain. However, both pains had slightly longer-than-normal latencies. One subject experienced only one sensation of pain that occurred at a delay of a few seconds. This soon happened with the subjects that earlier had experienced two sensations of pain. These results suggest that the
hyperalgesia to stroking is mediated by thinly myelinated nerve fibers and not by thickly myelinated fibers serving the sense of touch.

In a second series of experiments with five subjects, capsaicin was injected only after the sense of cool was lost as well as the sense of touch (i.e., the block progressed further than it did in the experiments just described because the sense of cool was lost as opposed to merely being delayed). The magnitude estimates of pain evoked by capsaicin were within range of control values. Testing for hyperalgesia was begun 3-5 min after the injection of capsaicin. Two subjects had no hyperalgesia to either stroking or to punctate stimuli and felt little or no pain at all in response to the standard von Frey filament. The other subjects had only one pain sensation in response to stroking, which occurred at about the same latency as second pain to stroking in control experiments.

In all of the above nerve-block experiments, the sense of warmth was intact within the area anesthetic to cool and/or touch. In addition, heat hyperalgesia was present around the injection site, as evidenced by the pain produced by the normally nonpainful stimulus of 38°C. Also, when the block was released, the hyperalgesia to mechanical stimuli was present and occupied the usual area of skin surrounding the injection site.

Thus the results of the studies of remaining sensation after conduction block in myelinated peripheral nerve fibers support the conclusion that the enhanced "first" pain to mechanical stimulation in the hyperalgesic skin is served by activity in thinly myelinated afferent fibers. Also, activity in unmyelinated fibers contributes more to capsaicin pain than does activity in myelinated fibers.

2) Conduction block in unmyelinated peripheral nerve fibers. For three subjects the superficial radial nerve at the wrist was infiltrated with 1% xylocaine. This produced an area of analgesia on the dorsum of the hand that was only slightly larger than the area of mechanical hyperalgesia that would normally be produced by an injection of capsaicin in this region. The median nerve was also blocked at the same time by a separate injection of anesthetic in two of the subjects.

The sense of touch recovered first in the area innervated by the superficial radial nerve followed by a delayed sense of cool. At this moment, when the sense of warmth was still absent, capsaicin was injected into the center of the area sensitive to touch. All subjects experienced pain that was characterized as having a stinging (turning to aching in one subject) rather than the usual burning quality. The magnitude estimates of pain were within range of those evoked by capsaicin on control skin for one subject but for two other subjects were reduced and shorter lasting in comparison with control values. The subject experiencing the more intense pain developed hyperalgesia to heat around the injection site, whereas the other subjects did not. All subjects had only a very small area of hyperalgesia to stroking (~20% of control areas). An area of hyperalgesia to punctate stimuli was measured in only one subject and found to be about one-half that of the control. Thus the pain from capsaicin was reduced in two of the three subjects tested, and the development of hyperalgesia was greatly reduced in all three subjects even though the sense of touch was normal (i.e., no discernible differences from touch sensations on control skin).

The presence of a delayed sense of cool suggests that thinly myelinated fibers were not completely blocked. Consequently, activity in these fibers might have mediated the stinging pain to capsaicin and, for the one subject, hyperalgesia to heat. However, the absent or reduced areas of hyper-
peralgesia to mechanical stimuli suggest the importance of activity in unmyelinated fibers for the initiation and lateral cutaneous spread of this type of hyperalgesia.

DISCUSSION

Skin reactions and altered pain states resulting from intradermal injection of capsaicin

Typically, the only visible skin reaction to the injection of capsaicin was a flare and the slightly raised area of skin at the injection site caused by the volume of fluid injected. Although there was considerable variability between subjects, the areas of flare after 100 μg of capsaicin were comparable with published values in experiments in which 100 μg of histamine were injected intradermally (Simone et al. 1987b, 1989) or local injuries were sustained (e.g., Hardy et al. 1950). When capsaicin was injected close to the midline (cervical spine) the flare crossed to the opposite side, i.e., developed symmetrically about the injection site. This is in contrast to the observation by Helme and McKernan (1985) that the flare produced by capsaicin on the forehead was an area typically considerably larger than the visible skin at the injection site caused by the volume of fluid injected. This difference between our result and theirs may reflect slight differences in the neuronal innervation pattern for these two areas of skin or possibly differences in experimental methodology.

Four concentric areas of altered pain sensibility and sensitivity developed, centered on the capsaicin injection site. The bleb at the injection site was analgesic to pinprick. Around this area were three hyperalgesic areas. The smallest was a thin ring of hyperalgesia to heat and to both types of mechanical stimuli (punctate and stroking). Around this was an area typically considerably larger than the visible flare that was hyperalgesic to both stroking and punctate stimuli but not to heat. Around this area, in turn, was a slightly larger area that was hyperalgesic only to punctate stimuli.

ANALGESIC BLEB. Topical applications of capsaicin, while initially lowering thresholds to heat, can subsequently elevate thresholds (Carpenter and Lynn 1981; Szolcsanyi 1977). Elevations of thresholds for heat pain, pain to capsaicin, and itch from histamine were also obtained in preliminary experiments carried out in our laboratory after intradermal injections of capsaicin (Shain, Simone, and LaMotte, unpublished observations). A correlative measure was obtained in the elevated response thresholds or in loss of responsiveness of heat- and mechanosensitive nociceptive afferents with A- or C-fibers (AMHs and CMHs, respectively) to heat and mechanical stimuli after a capsaicin injection into their RFs in the monkey hairy skin (Bau- mann et al. 1991). The loss of sensitivity to pain in an area directly exposed to capsaicin may result, in part, from the loss of sensitivity in these nociceptive afferents.

HEAT HYPERALGESIA. This was characterized by a lowering of pain thresholds to as low as 32°C (Simone et al. 1987a) and by an enhanced sensitivity to normally painful heat. The area of hyperalgesia occupied a small area confined to within 1–2 cm of the edge of the analgesic bleb. A single topical application of 1% capsaicin can lower thresholds for heat pain (Carpenter and Lynn 1981; Szolcsanyi 1977; Szolcsanyi et al. 1973) and lower the response thresholds of CMII nociceptors (Baumann et al. 1991; Konietzny and Hensel 1983). A sufficiently large dose of capsaicin can block the responses of CMH nociceptors (Baumann et al. 1991; Szolcsanyi et al. 1988). It is possible that less capsaicin reaches the nociceptor after a topical delivery than after an intradermal injection and that the heat hyperalgesia around a capsaicin injection site is due partially to a lesser dose reaching (by passive diffusion) and then sensitizing heat-sensitive nociceptive afferents (e.g., CMHs) surrounding the desensitized fibers within the bleb. Arguing against this idea is the absence of any evidence for the sensitization of any nociceptive afferent to heat after a capsaicin injection within, adjacent to, or outside its RF (Baumann et al. 1991). We hypothesize that capsaicin, when applied topically, produces 1° hyperalgesia by sensitizing heat-sensitive nociceptive afferent fibers. However, when injected, capsaicin produces primarily 2° and not 1° hyperalgesia via sensitization of certain neurons in the spinal cord. Of course, in the case in which nociceptors are sensitized within an area of injury, it is possible that a central neurogenic hyperalgesia may contribute as well to the 1° hyperalgesia within the injury.

MECHANICAL HYPERALGESIA. In the present study, the areas of mechanical hyperalgesia surrounding a capsaicin injection appear to be within the range of sizes obtained after an electrical, mechanical, or thermal injury, as illustrated in figures published by Lewis (1936) and Hardy et al. (1950). The distinction was not made in these earlier studies between the areas of hyperalgesia mapped by stroking as opposed to punctate stimuli. In the present experiments it was determined that the area of hyperalgesia was typically larger and lasted longer for punctate than for stroking stimuli.

The time course of hyperalgesia after a capsaicin injection differed slightly from that of hyperalgesia after certain physical injuries to the skin. The hyperalgesia to stroking and to punctate stimuli was present immediately with the onset of capsaicin pain and reached a maximum size typically within 15 min after the injection. It disappeared to stroking within 1–2 h but lasted 13–24 h for punctate stimulation. In contrast, the area of 2° hyperalgesia resulting from a local burn, mechanical or electrical injury, or electrical stimulation of a cutaneous nerve trunk developed more gradually and reached maximum within 15–60 min. The hyperalgesia lasted 2–48 h (Lewis 1936; Hardy et al. 1950), possibly depending on the extent of the injury. Unlike the effects of a physical injury, which might produce 1° hyperalgesia for days during the onset of the healing process, the capacity of a capsaicin injection to produce hyperalgesia is lost after ~1 h, as determined by injecting capsaicin into an anesthetic area of skin and noting the absence of hyperalgesia after the anesthetic has worn off.

As a measure of pain sensibility to punctate mechanical stimuli, Hardy et al. (1950) determined the force exerted by a von Frey filament required to produce the same amount of pain on normal skin as on an area of 2° hyperalgesia around a burn injury. A force 2–10 times greater (depending on the severity of injury) on the control skin was required to match the pain produced by a force of 1 g applied to hyperalgesic skin. Yet it was determined that the thresli-
Peripheral and central neural mechanisms contributing to 2° hyperalgesia after capsaicin injection

INITIAL DEVELOPMENT AND SPREAD OF 2° MECHANICAL HYPERALGESIA. Our results indicate that the extension of hyperalgesia away from the injection site is mediated by a neurogenic mechanism. The hyperalgesia does not result from the direct action of capsaicin itself, for example, via local diffusion or uptake in lymphatic or blood vessels. The same was held to be true for 2° mechanical hyperalgesia from local physical injuries of the skin (Lewis 1936; Hardy et al. 1950).

Our results support the proposition that the neurogenic mechanism responsible for the initial spread of mechanical hyperalgesia away from the injection site is in the periphery and involves nerve fibers that either arborize much more widely than the widths of the well-known nociceptors or arborize within a lesser area but are functionally coupled to one another so that activity in one elicits activity in its neighbor. There is a discrete anatomic boundary to these nerve fibers, as evidenced by the failure of the hyperalgesia to cross the midline (when capsaicin was injected close to the cervical spine).

A thin strip of anesthetic injected into the skin acted as a barrier to the development of hyperalgesia on the side away from the injection site. For the few experiments in which the anesthetic failed to block the spread of hyperalgesia, we hypothesize that the nerve fibers in question branched beneath or even more proximal to the anesthetized skin or, in any case, were not reached by a sufficient concentration of anesthetic.

A small region of anesthetized skin can also block the spread of hyperalgesia from a local physical injury of the skin (Lewis 1936) and can block the spread of "itchy skin" ("alloknesis") away from the site of an intradermal injection of histamine (Bickford 1938; LaMotte et al. 1988; M. Alreja, D. A. Simone, and R. H. LaMotte, unpublished observations). Curiously, Lewis' experiments with the anesthetic barrier were not discussed by Hardy et al. (1950), who favored the hypothesis that the neural mechanism responsible for the spread of 2° hyperalgesia was located in the dorsal horn of the spinal cord.

Could the anesthetic barrier block the radial spread of neuronal activity in the central as opposed to the peripheral nervous system? For example, could the anesthetic eliminate ongoing activity in certain primary afferents that otherwise would facilitate the central "spread" of neural activity from capsaicin-activated spinal neurons to neighboring neurons that are then sensitized to input from mechanoreceptive afferents innervating the area of 2° mechanical hyperalgesia? Candidate fibers that arc spontaneously active in normal skin are the low-threshold thermoreceptors and slowly adapting type II mechanoreceptors. But, as shown in the present study, an ischemic block of the larger-diameter afferents that mediate touch sensation does not necessarily block mechanical hyperalgesia to capsaicin. Similarly, we did not observe significantly different areas of hyperalgesia when the skin was cool versus warm, nor did a cold metal tube laid across the skin block the spread of hyperalgesia from one side to the other (unpublished observations).

Thus the idea of central facilitation via spontaneously active low-threshold afferents seems to us less likely to be a mechanism responsible for the spread of 2° hyperalgesia than does the lateral spread of neural activity via special intracutaneous fibers.

It is unlikely that the peripheral nerve fibers responsible for mediating the spread of hyperalgesia belong to any of the well-documented types of cutaneous sensory afferents. Mechanically and thermally sensitive afferents in the monkey, including CMH and AMH nociceptive fibers, do not typically respond to injections of capsaicin remote to their cutaneous RFs (Baumann et al. 1991), nor do CMHs in humans (LaMotte et al. 1987). Similarly, CMHs in monkey are not remotely sensitized by a small physical injury of the skin outside their RFs (Campbell et al. 1988). More evidence against a major role for sensory afferents such as those mediating heat pain is that the presence and magnitude of 2° hyperalgesia are not necessarily linked to the presence and magnitude of the pain produced by the initial injurious stimulus. In the present study, when the pain from a capsaicin injection into one arm was matched in magnitude and time course to pain produced by locally heating the other arm, the resulting area of 2° hyperalgesia was far greater on the capsaicin-injected arm. Also, the find-
ing that the area of 2° hyperalgesia reaches a maximal size more gradually after a physical injury such as blistering heat (which is immediately severely painful) (Lewis 1936) than it does after a capsaicin injection suggests to us that the afferent fibers responsible for the spread of hyperalgesia are selective for algesic chemical substances, whether externally applied, as in the case of an irritant, or endogenously released, for example during the acute inflammatory response to injury. It is therefore postulated that chemonociceptive afferents, which may contribute only slightly to capsaicin pain, release a chemical substance that increases the responsiveness of certain neurons in the CNS to neural activity in certain mechanosensitive peripheral nerve fibers.

MAINTENANCE OF 2° MECHANICAL HYPERALGESIA ONCE FULLY DEVELOPED VIA PERIPHERAL NEURAL ACTIVITY. The area of hyperalgesia to stroking could be abolished or significantly reduced by anesthetizing an area of skin centered on the capsaicin injection site by either cooling it to 1°C or injecting it with a local anesthetic. In contrast, the anesthetization was less effective or ineffective in reducing the area of hyperalgesia to punctate stimuli, although there was evidence that the magnitude of pain evoked by these stimuli could be reduced. Thus Lewis’ (1936) statement that the hyperalgesia surrounding a local cutaneous injury, once developed, is no longer dependent on peripheral neural activity at the site of injury, and the statement to the contrary by Hardy et al. (1950), must be qualified. The qualification must take into account the difference between stroking and punctate stimuli, whether the response measure is the area of hyperalgesia or the magnitude of pain evoked by stimulating the hyperalgesic area, the possibility of individual differences between subjects, and the variability that occurs between tests with the same subject.

A related issue is whether the hypothesized radial transmission of neural activity through intracutaneous nerve fibers away from the injury to remote skin is needed to maintain 2° hyperalgesia once it is fully developed and once capsaicin is no longer active in the skin. We found that a medial lateral strip of anesthetic 1 cm proximal to the capsaicin injection site was less effective in reducing the hyperalgesia to punctate stimuli proximal to the strip after hyperalgesia was fully developed than it was in preventing its occurrence if delivered before the injection of capsaicin. In contrast, the strip of anesthetic was typically equally effective in reducing the hyperalgesia to stroking after as opposed to before the hyperalgesia had developed. Anesthetizing the capsaicin injection site had analogous effects: anesthetizing before capsaicin was injected prevented the development of any hyperalgesia; whereas anesthetizing after hyperalgesia was fully developed, although typically reducing or eliminating the hyperalgesia to stroking, had a lesser effect in reducing or eliminating the hyperalgesia to punctate stimuli. Thus, once the hyperalgesia to stroking developed, it continued to be maintained by peripheral neural activity both at the site of injury and through radial transmission of neural activity to areas remote to the injury. In contrast, once the hyperalgesia to punctate stimuli was fully developed, it became less dependent on or independent of such peripheral neural activity.

It is not clear whether the stroking and punctate stimuli are activating the same or different types of primary afferent fibers. Because the punctate stimulus is more intense than stroking, the different sensory effects of this stimulus versus stroking may be due, in part, to different levels of activity in the same class of afferents. It would be useful in future experiments to see whether a less intense punctate stimulus, one closer to or just below normal pain threshold, would yield results closer to those obtained with the stroking stimulus. It is also possible that these two types of stimuli activate different types of primary afferent fibers that terminate centrally on different types of second-order neurons. The sensitization of those central neurons receiving input from afferents activated by punctate stimuli might be less dependent on a continual input from chemoceptive afferents from the site of injury than the sensitization of neurons activated by stroking.

POSSIBLE LOCATION AND TYPE OF NEURON THAT IS SENSITIZED TO MECHANICAL STIMULATION WITHIN THE AREA OF 2° HYPERALGESIA. Lewis (1936) and later Hardy et al. (1950) looked for the presence of hyperalgesia after electrically stimulating a cutaneous nerve above versus below a local anesthetic block of the nerve. (In the absence of the anesthetic, the electrical stimulation produced an area of hyperalgesia in the cutaneous distribution of the nerve comparable in size and sensory characteristics to that produced by a physical injury of the skin.) Opposing results were obtained: Lewis concluded that the “sensitized neurons” (our phrase) were peripheral and Hardy’s group concluded they were in the dorsal horn of the spinal cord. The controversy centered, in part, on 1) whether the nerve was completely blocked, 2) whether the hyperalgesia was immediately present or took time to develop after the anesthetic block wore off, and 3) whether the electrical stimulus produced a tissue injury that could be the cause of the hyperalgesia after the block wore off. A problem with these studies is that it is difficult to assess the effective amount and duration of local anesthetic delivered and the extent and magnitude of any injury produced by the electrical stimulus.

The advantage of using capsaicin to produce the hyperalgesia as shown in the present study is that, when injected intradermally, its capacity to initiate the development of hyperalgesia is transient in relation to the time it takes for a physical injury to heal. Thus the original cause of the hyperalgesia would presumably be gone by the time a local anesthetic wore off. With this in mind, we conducted experiments in which capsaicin was injected into skin rendered anesthetic by a proximal anesthetic block of a peripheral nerve. Despite the weak but transient pain produced by the injection, hyperalgesia was either absent or occupied a very small area after the anesthetic wore off, even in experiments in which the anesthetic lasted <1 h. It was concluded that 2° hyperalgesia to mechanical stimuli and to heat stimuli as well results from the sensitization of second- or higher-order neurons within the CNS.

As shown in a companion paper, a comprehensive search for changes in the response properties of several classes of nociceptive and low-threshold afferent fibers in the monkey failed to obtain a single instance of clear-cut sensitization to mechanical or heat stimuli in any fiber after injection of capsaicin outside, adjacent to, or within its cutaneous re-
ceptive field. Similar negative findings were obtained in recordings of the responses of single CMH primary afferents to intradermal injections of capsaicin in human (LaMotte et al. 1987).

In a second companion paper, spinothalamic neurons recorded in the dorsal horn in anesthetized monkeys were found that responded vigorously to capsaicin injected into their cutaneous RFs. For most of these neurons, response thresholds were lowered to heat, but usually only for heat stimuli delivered within the immediate vicinity of the injection site. Also, responses increased to punctate stimuli delivered to various loci within their RFs. In addition, there were enhanced or newly developed responses to light stroking of the skin with a cotton swab. These results were in accordance with the lowered pain thresholds and increased magnitude of pain to heat and to mechanical stimuli obtained in our psychophysical studies in humans. Thus the enhanced responses to heat and to mechanical stimuli in these spinothalamic neurons in the monkey, if representative of altered responses in such neurons in human, might account for the 2° hyperalgesia after an intradermal injection of capsaicin.

Torebjork et al. (1990) studied the quality of cutaneous sensation referred to an area of skin produced by intraneural electrical stimulation of myelinated mechanoreceptive peripheral nerve fibers in humans before and after an injection of capsaicin 7–20 mm outside the area. When this area developed 2° hyperalgesia, the electrically evoked activity in these afferents that produced only a nonpainful tactile sensation before capsaicin then evoked an additional component of pain (soreness) after capsaicin. This pain had the same quality and the same time course as the pain evoked by stroking the skin. This result supported the conclusion that certain neurons in the CNS had become sensitized to the normally innocuous neural activity evoked by stroking the skin.

Taken together, the results of the present experiments with nerve blocks, the absence of peripheral sensitization of at least the known types of nociceptive primary afferent fibers, the altered sensations evoked by intraneur al electrical stimulation of A-fibers after capsaicin, and the presence of sensitization in spinothalamic neurons support the conclusion that the sensitized neurons contributing to neurogenic hyperalgesia are located in the dorsal horn of the spinal cord.

In certain experiments in the present study, when a distal-proximal strip of skin on the forearm was anesthetized by a proximal block of a cutaneous nerve and capsaicin was then injected into the center of the strip, some hyperalgesia appeared outside the anesthetized region but was typically of short duration. Even when this border hyperalgesia persisted after recovery from the anesthetic block, no hyperalgesia was present or developed subsequently within the formerly anesthetized skin. We suggest that the hyperalgesia bordering the anesthetized zone was the result of remote activation of unblocked chemonociceptive fibers innervating the bordering unanesthetized skin and extending through wide arborizations into the anesthetized region. These neighboring fibers then evoked the relatively low level of pain experienced (even though the skin was anesthetic to thermal or mechanical noxious stimuli) and also central mechanisms of sensitization that were weakly activated because of the absence of facilitory input from the anesthetized region.

Model of peripheral and central neural mechanisms contributing to capsaicin pain and hyperalgesia

The following neural events are hypothesized to occur after an intradermal injection of capsaicin (refer also to Fig. 14). The same or similar mechanisms are assumed to occur after a local physical injury of the skin. With the injection of the 100-µg dose of capsaicin there is immediate, intense, burning pain due to the vigorous responses of heat nociceptors and, to a lesser extent, of mechanosheat nociceptors (CMHs and type II AMHs). In addition, chemonociceptors respond directly to capsaicin with prolonged discharges of nerve impulses that travel along widely arborizing branches located entirely or partially within the skin. [Either individual fibers branch very widely in the skin (as illustrated in Fig. 14) or they are functionally (chemically, ephaptically) linked together (cf. Meyer et al. 1985) such that chemically evoked activity in one fiber can evoke activity in neighboring chemonociceptive fibers that are remote from the site of injury.] Presumably, in the case of a local physical injury, certain algesic substances released from injured tissue as part of the acute inflammatory response would also be capable of exciting these same fibers.

The proposed chemonociceptive afferents are hypothesized to terminate in the dorsal horn on interneurons that receive input from cutaneous mechanoreceptors with myelinated axons that are either low threshold (and have larger-diameter axons) or nociceptive (with smaller-diameter axons). In response to a neuromodulator released by activated chemonociceptive afferents, these interneurons become sensitized, i.e., they develop lowered thresholds and enhanced suprathreshold responses to normal evoked activity in both the low-threshold and nociceptive afferents. The sensitization is initially dependent on continual input from the chemonociceptive afferents for both types of interneurons but subsequently may persist to varying degrees without input, particularly in the nociceptive interneurons.

The heat-selective nociceptive afferent fibers project onto a set of dorsal horn interneurons that receive a convergent input from these afferents and from fibers with low-threshold warm receptors. These interneurons become sensitized by the intense discharge in the heat-sensitive nociceptive afferents activated by capsaicin. Activity elicited in low-threshold warm afferents, which would normally evoke only a weak response, then evokes enhanced responses in the sensitized interneurons, thereby contributing to the lowered thresholds for heat pain at or close to the site of injury. In addition, a small proportion of heat-specific afferents may become peripherally sensitized as well. Presumably these heat-sensitive neurons also respond to endogenous algesic chemical substances as well as to externally applied irritants such as capsaicin. Those heat-selective nociceptive fibers that have peripheral terminals in direct contact with capsaicin would supply a cutaneous area of 1° hyperalgesia. Other such fibers with endings that terminate adjacent to the skin containing capsaicin but that project to heat-sensitized interneurons would supply an area of 2° hyperalgesia to heat.
FIG. 14. A model of neurogenic hyperalgesia. A local cutaneous injury, in this example produced by an intradermal injection of capsaicin, activates chemonociceptive afferent fibers (Chem), which branch very widely. Proximal terminals of these afferents release a neuromodulator (\(\theta\)) that sensitizes certain dorsal horn neurons that receive input from myelinated low- or high-threshold mechanoreceptive afferents (LT and HT mech, respectively). These sensitized low- and high-threshold mechanoreceptive 2nd-order neurons (LT, HT mech) then facilitate the responses of wide-dynamic-range (WDR) and certain high-threshold (HT) spinothalamic neurons to innocuous as well as painful mechanical stimulation of the skin well outside the injury, thereby accounting for the existence of remote hyperalgesia to mechanical but not to heat stimuli. Spread of hyperalgesia away from the injury is due to neural activity in lateral branches of the hypothesized chemosensitive afferents and can be blocked by a cutaneous strip of local anesthetic. High-threshold afferents responsive only to noxious heat (HT heat) and also to certain algesic chemicals release a neuromodulator that sensitizes dorsal horn neurons receiving a convergent input from these heat-sensitive afferents and from low-threshold afferents with warm receptors (LT heat). Once sensitized, these 2nd-order neurons facilitate the responses of certain WDR and HT neurons to warming and painful heat stimulation, but only at the site of injury. Capsaicin locally desensitizes CMH and AMH (Mechanoheat) nociceptive afferents innervating the injection site.

Both the heat- and mechanoreceptive interneurons project onto wide-dynamic-range (WDR) spinothalamic neurons and some high-threshold (HT) spinothalamic neurons as well. In response to capsaicin injected into their cutaneous receptive fields, these WDR and HT neurons behave as follows (Simone et al., 1991). 1) They respond vigorously to capsaicin via input from interneurons and from those CMH and AMH (type II) fibers directly activated by capsaicin. 2) They develop lowered thresholds and enhanced suprathreshold responses to innocuous stroking and noxious punctate stimuli throughout their RFs in response to the increased discharges of sensitized mechanoreceptive interneurons. 3) They develop lowered thresholds and increased suprathreshold responses to heat delivered in the immediate vicinity of the injury, but not elsewhere in their RFs, via facilitory input from sensitized heat-specific interneurons.

The model in Fig. 14 provides an explanation for why a mediolateral strip of skin can block the development of 2\(^{o}\) hyperalgesia on the side opposite the side of injury and why, when capsaicin is injected into nerve-blocked skin, hyperalgesia does not develop after recovery from the block. The existence of widely branching (or functionally coupled) chemonociceptive fibers would account for the fact that an injection of capsaicin into skin that is analgesic to mechanical or thermal pain can still evoke a low level of pain and sometimes hyperalgesia within adjacent unanesthetized skin. The model also accounts for the finding that there is hyperalgesia even though none of the known types of nociceptive afferent fibers became sensitized to mechanical or thermal stimuli after a capsaicin injection within, adjacent to, or remote from their cutaneous RFs (Baumann et al. 1991). These and other results are discussed in the following two articles.

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REFERENCES

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