Comparison of Responses of Primate Spinothalamic Tract Neurons to Pruritic and Algogenic Stimuli

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STT neurons contributed to the pain and hyperalgesia produced by noxious stimuli can cause itch (Bickford 1938). In addition, itch and pain appear to be transmitted via the spinothalamic tract (STT) since both sensory modalities as well as temperature sensation are abolished following cordotomies and their possible role in mediating histamine-induced hyperalgesia. Furthermore, nearly all STT neurons exhibited vigorous and persistent responses to histamine consistent with their unlikely role in mediating alloknesis. Neither type of neuron exhibited significant changes in response to mechanical stimuli as wide dynamic range (WDR) or high-threshold (HT). Approximately half of the WDRs and one of the HTs responded weakly to histamine, some with a duration >5 min, the maximal time allotted. WDRs but not HTs exhibited a significant increase in response to punctate stimulation after histamine consistent with their possible role in mediating histamine-induced hyperalgesia. Neither type of neuron exhibited significant changes in response to stroking, consistent with their unlikely role in mediating alloknesis. Furthermore, nearly all STT neurons exhibited vigorous and persistent responses to capsaicin, after which they became sensitized to stroking consistent with the enhanced sense of itch (<i>praevia</i>) the itch evoked by intradermal injection of histamine, 2) the enhanced sense of itch evoked by innocuous stroking (alloknesis), and 3) the enhanced pain evoked by punctate stimulation (hyperalgesia) of the skin surrounding the injection site. Responses to intradermal injections of histamine and capsaicin were compared in STT neurons recorded in either the superficial or the deep dorsal horn of the anesthetized monkey. Each neuron was identified by antidromic activation from the ventral posterior lateral nucleus of thalamus and classified by its initial responses to mechanical stimuli as wide dynamic range (WDR) or high-threshold (HT). Approximately half of the WDRs and one of the HTs responded weakly to histamine, some with a duration >5 min, the maximal time allotted. WDRs but not HTs exhibited a significant increase in response to punctate stimulation after histamine consistent with their possible role in mediating histamine-induced hyperalgesia. Neither type of neuron exhibited significant changes in response to stroking, consistent with their unlikely role in mediating alloknesis. Furthermore, nearly all STT neurons exhibited vigorous and persistent responses to capsaicin, after which they became sensitized to stroking and to punctate stimulation. We conclude that the STT neurons in our sample are more likely to contribute to pain, allodynia, and hyperalgesia than to itch and alloknesis.

INTRODUCTION

Chronic pruritis is a characteristic feature of many cutaneous disorders, such as atopic, allergic, and irritant contact dermatitis, psoriasis, AIDS, and other infectious and neoplastic diseases (Bernhard 1994). Given its clinical significance, it is surprising that the neural basis underlying this sensory modality remains poorly understood. However, itch shares some common mechanisms with pain, even though they are distinct sensations. For example, most cutaneous nociceptors are also responsive to pruritic chemical stimuli (Handwerker et al. 1991; Schmelz et al. 1997; Tucket and Wei 1987). Pruritic stimuli can sometimes elicit mild pain and hyperalgesia (Atanassoff et al. 1999). Conversely, mild cutaneous injuries produced by noxious stimuli can cause itch (Bickford 1938). In addition, both itch and pain appear to be transmitted via the spinothalamic tract (STT) since both sensory modalities as well as temperature sensation are abolished following cordonotomies that sever this pathway (Hyndman and Wolkin 1943; Nathan 1990; White et al. 1950).

Application of histamine and capsaicin to the skin of humans has been used to study psychophysical attributes of itch and pain and the characteristics of the dysesthesiae associated with each. Iontophoresis (Heyer et al. 1989; Magerl et al. 1990) or intradermal injection (Bickford 1938; LaMotte 1996; Simone et al. 1989, 1991a) of histamine into the skin produces a flare and a wheal and the sensation of itch with little or no pain. In the surrounding skin there develops local alloknesis to lightly stroking the skin with a cotton swab and hyperknesis and hyperalgesia (enhanced itch and pain, respectively, evoked by different punctate stimuli) (Atanassoff et al. 1999; Simone et al. 1991a). Intradermal injection of capsaicin produces a flare, burning pain, hyperalgesia to heat in a small area of skin surrounding the injection, and a larger surrounding area of mechanically evoked allodynia and hyperalgesia (LaMotte et al. 1991; LaMotte 1992; Simone et al. 1989). Experimental itch and accompanying dysesthesiae were enhanced by a local anesthetic (Atanassoff et al. 1999) and attenuated in hyperalgesic skin surrounding a capsaicin injection (Brull et al. 1999).

These studies demonstrated functional interactions between pruritic and nociceptive systems and lend support to the hypothesis that the mechanisms subserving itching are inhibited centrally by mechanisms that underlie pain and hyperalgesia (Atanassoff et al. 1999; Brull et al. 1999; Graham et al. 1951; Nilsson et al. 1997; Ward et al. 1996).

In an earlier study (Simone et al. 1991b), we investigated the role of the STT in capsaicin-evoked pain and hyperalgesia. It was found that STT neurons were excited vigorously by an intradermal injection of capsaicin and that their response profile correlated well with psychophysical measures of pain magnitude and duration. In addition, STT neurons developed enhanced responses to heat stimuli at the injection site, and enhanced responses throughout their receptive fields (RFs) to stroking the skin and to indentations with punctate stimuli. The results demonstrated that the activation and sensitization of STT neurons contributed to the pain and hyperalgesia produced by capsaicin.

In the present study a similar approach was used to investigate the role of STT neurons in encoding histamine-evoked itch and dysesthesiae and to compare the responses of individual cells to histamine and capsaicin.

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METHODS

Subjects

A total of 11 monkeys (Macaca fascicularis) weighing between 1.8 and 3.5 kg were used. Each animal was initially sedated with ketamine (10 mg/kg im) followed by a mixture of nitrous oxide, halothane, and oxygen. The right cephalic vein was catheterized and an initial dose of α-chloralose (60 mg/kg iv) was given. One carotid artery was catheterized for continuous monitoring of blood pressure. The experiment was terminated if blood pressure dropped below 60 mmHg. Following a tracheotomy, animals were paralyzed with gallamine triethiodide and artificially ventilated. Bilateral pneumothoraces were done when necessary to reduce movements of the spinal cord. End-tidal CO₂ was continuously monitored and maintained at 3.5–4.5%. A feed-back-controlled heating blanket was used to maintain body temperature near 37.5°C. A stable level of anesthesia, as indicated by small, areflexic pupils, was maintained by a constant infusion of pentobarbital sodium (2–7 mg/kg/h).

All protocols were approved by the Animal Care Committee of the University of Minnesota.

The lumbar sacral enlargement was exposed by a laminectomy, and a bilateral craniotomy was performed to allow a stimulating electrode access to both sides of the thalamus. The animal was placed in a stereotaxic frame and the spinal cord was covered with a pool of warm mineral oil. The exposed cortex was covered with a mixture of warm petroleum jelly and mineral oil.

Stimulation and recording procedures

STT neurons were identified by antidromic activation produced by electrical stimulation of the thalamus. A stainless steel monopolar electrode was inserted into the ventral posterior lateral (VPL) nucleus of the thalamus. The position of this electrode was adjusted until the maximal response evoked by tactile stimulation of the contralateral leg and foot was found. Once the thalamic electrode was in its final position, it was used to activate STT neurons antidromically in the lumbar dorsal horn. Electrical search stimulation of the thalamus consisted of 750-μA pulses delivered for a duration of 0.2 ms at 10 Hz. Once an antidromically activated action potential was found, the stimulating electrode was moved systematically in 0.5-mm steps through a number of tracks in the same anterior–posterior plane until a point was located at which the antidromic threshold was ≤30 μA (Dado et al. 1994; Zhang et al. 1999).

Antidromically activated cells were recorded using stainless steel microelectrodes (2–6 MΩ). Electrodes were slowly lowered into the spinal cord in 5- to 10-μm steps using an electronic microdrive. A cell was identified as belonging to the STT by a constant latency to thalamic stimulation, its ability to follow high-frequency stimulation of the thalamus (3 or 4 impulses at >333 Hz), and collision of the antidromic and orthodromic impulses. STT cells were selected that had RFs on the leg and foot.

Functional classification of STT neurons

STT neurons were classified functionally as high-threshold (HT), wide dynamic range (WDR), or low threshold (LT) according to their responses to graded intensities of mechanical stimuli applied to the skin. Mechanical stimuli were used that were comparable with those employed previously (brushing with a camel-hair brush, application of arterial clips with different closing forces, and squeezing the skin with forceps), allowing us to verify that our classification of cells was consistent with that described in previous studies (Willis and Coggeshall 1991). Cells that responded at highest frequencies to pinching with little (<1.5 impulses/s) or no response to innocuous stimulation were classified as HT. WDR neurons were those that responded in a graded fashion to stimuli of increasing intensity. Cells that responded maximally to brushing without increased response to noxious stimulation were classed as LT cells and were not studied further.

Localization of recording and stimulation sites

After each cell was studied the recording site was marked by a lesion produced by passing current (25 μA for 20 s) through the tip of the stainless steel microelectrode. At the end of most experiments, the site of thalamic stimulation was also marked with a lesion. Animals were perfused with physiological saline followed by 10% Formalin. Potassium ferrocyanide (1%) was added to the perfusate to stain iron deposits from the electrode (Prussian blue reaction). The thalamus and lumbar spinal cord were removed, blocked, and stored in 10% Formalin and 1% potassium ferrocyanide for 48 h. The tissue was then transferred and maintained for several days in 10% Formalin containing 30% sucrose. Frozen serial sections (50 μm) were stained with neutral red. Stimulation and recording sites were identified by Prussian blue marks.

Chemical stimuli

Histamine hydrochloride was dissolved in normal saline at a concentration of 2 mg/ml (0.2%). Capsaicin (8-methyl-N-vanillyl-6-nonamide) was dissolved at a concentration of 10 mg/ml (1%) in a vehicle containing 7% polyethylene(20)sorbite monooleate (Tween 80) in normal saline. All solutions were passed through a Millipore filter (0.22 μm) and stored in sterile glass injection vials. Intradermal injections of 20 μg histamine, 100 μg capsaicin, and vehicle (saline and Tween 80) were given in a volume of 10 μl using a 0.5-ml insulin syringe with a 28-gauge needle.

Experimental design

Once an STT cell was classified, its RF was mapped. Six test sites, spaced approximately 1 cm apart, were marked on the skin within the most sensitive part of the RF. The orientation of these test sites with respect to each other was dependent on the size and location of the RF, but they were usually spaced linearly in one or two rows. First, the vehicle was injected into one of the test sites. This was followed by an injection of 20 μg of histamine into another test site, and, subsequently, 100 μg capsaicin into a third site. These doses were chosen because they evoked strong and reliable sensations of itch and allodynia, and pain and hyperalgesia, respectively, in psychophysical studies in humans (LaMotte et al. 1991; Simone et al. 1987, 1989, 1991a). The test sites designated for injection were selected randomly. Injections were a minimum of 1 cm apart and were separated by approximately 30 min. Care was taken to inject superficially in the skin. The time taken to insert the needle, inject, and withdraw the needle was approximately 5–7 s. The response to each injection was recorded for 5 min beginning when the needle was withdrawn from the skin. Before and at 5 min after each injection, responses evoked by mechanical and heat stimuli were obtained. These were 1) stroking the skin lightly with a cotton swab both in an area adjacent to the injection site and within the RF. The skin was stroked 6–10 times within an area of approximately 4 cm surrounding the injection site, with each stroke lasting approximately 1 s and covering a distance of 1 cm; 2) indentation of the skin with a punctate stimulus (a von Frey monofilament with a diameter of 500 μm and bending force of 149 mN) applied for 2 s to each test site in sequence three times; and 3) heat stimuli of 45 and 50°C, each applied for 5 s at each injection site. The punctate stimulus elicits a faint pinching pain but not itch when applied to the volar forearm of humans (unpublished observations). The heat stimuli were applied with a Peltier device with a contact area of 0.8 cm², starting approximately 5 min after the mechanical stimuli. The thermode was centered over the injection site and applied with enough pressure to just dimple the skin. The mechanical and heat stimuli were similar or identical to those used in human psychophys-
ical studies to characterize and quantify alloknesis, allodynia, and hyperalgesia following injection of histamine or capsaicin (LaMotte et al. 1991; Simone et al. 1989; 1991a). In those studies, stroking the skin with a cotton swab evoked a sensation of itch after histamine and tenderness or pain after capsaicin. The von Frey monofilament used in those studies produced a sensation of enhanced pricking pain after histamine and capsaicin. In the present study, an experiment was performed only once on each hindlimb. In most experiments, one STT cell was studied from each side of the spinal cord.

Data analyses

A response evoked by an injection was defined as an increase in discharge rate if it 1) was clearly above the level of spontaneous activity exhibited just prior to injection, 2) was greater than that produced by injection of vehicle (when given), and 3) persisted for ≥1 min after injection. Responses of individual neurons evoked after the injection of vehicle, histamine, and capsaicin were divided into 15-s bins over a 5-min recording period. The mean rate of spontaneous activity, obtained for a minimum of 30 s just prior to each injection, was subtracted from the rate obtained during each 15-s bin after injection. Changes in mean responses of all HT and WDR neurons evoked by each injection were determined using one-way repeated measures ANOVAs, and posthoc comparisons of discharge rates before and at various times after injection were made using Newman–Keuls procedure. A $P$ value $\leq 0.05$ was considered significant.

Paired $t$-tests (with Bonferroni correction) were used to determine whether mean responses of individual cells evoked by stroking and by von Frey stimulation obtained before injection were altered following injection of vehicle, histamine, or capsaicin. Each electrophysiologically recorded response evoked by a mechanical stimulus was adjusted by subtracting the mean background activity occurring just before the stimulus during a period of time equal to or greater that the duration of the stimulus. For punctate stimuli, responses were averaged across the test sites. Responses to each heat stimulus (occurring during 5 s of heating and 5 s after onset of cooling) were adjusted by subtracting 10 s of background activity occurring just before stimulus onset. One-way ANOVAs with repeated measures were used to determine differences in mean responses of WDR and HT neurons to stroking and to punctate stimuli following injections of vehicle, histamine, and capsaicin. Paired $t$-tests were used to determine the effects of vehicle, histamine, and capsaicin on mean responses of HT and WDR neurons to heat stimuli. $P$ values $\leq 0.05$ were considered significant. All data are expressed as the mean ± SE.

RESULTS

General characteristics of STT neurons

Recordings were made from a total of 20 STT neurons identified by antidromic activation from the ventral lateral aspect of the thalamus. Stimulation sites were recovered histologically for 11 neurons. The stimulation site for 1 neuron was located in the ventral posterior inferior nucleus and all others were located in the VPL nucleus, primarily in the ventral portion of the nucleus (Fig. 1A).

All mechanosensitive RFs were located on the posterior surface of the lower leg and/or the glabrous skin of the foot. Seven neurons were classed functionally as HT and 13 as WDR. Five of 7 HT neurons and all WDR neurons had spontaneous activity. HT neurons exhibited lower rates of spontaneous activity (0.1 to 3.6 Hz) than WDR neurons (0.26 to 36.3 Hz). The mean spontaneous discharge rates for HT and WDR neurons were 0.86 ± 0.50 and 8.9 ± 2.83 Hz, respectively, and differed significantly ($t$-test, $P < 0.05$). Recording sites identified and reconstructed for 11 neurons (6 HT and 5 WDR) were distributed throughout the dorsal horn (Fig. 1B). All the recording sites for HT neurons were located in the superficial dorsal horn while those for WDR neurons were located in the superficial ($n = 2$) and deep ($n = 3$) dorsal horn.

Responses evoked by intradermal injections of histamine and capsaicin

Figures 2 and 3 illustrate the discharges of individual WDR and HT neurons before and after injections of vehicle, histamine, and capsaicin. Intradermal injection of vehicle produced a weak response in 2 of 10 WDR neurons and in 1 of 5 HT neurons tested. Injection of histamine excited 7 of 11 WDR and 1 of 6 HT neurons. The responses of WDR neurons always began within the first few seconds after injection and persisted.
for more than 5 min in 3 of these (Fig. 2). For the remaining WDR neuron, histamine-evoked responses lasted for 1–2 min. The peak discharge rates usually occurred during the first 15–30 s after injection.

For the one HT neuron that was excited by histamine, a response began within a few seconds after injection and persisted more than 5 min (Fig. 3). Histamine-evoked discharge rates were generally lower for the single HT than for the WDR neurons, but did not differ significantly. The peak discharge rates that occurred during the 5-min recording period (per 15-s bin) of individual HT and WDR cells evoked following histamine were 10.3 ± 3.4 and 22.7 ± 4.5 Hz, respectively.

The majority of STT neurons (4 of 4 HT and 9 of 10 WDR) responded immediately and vigorously to the injection of capsaicin. The greatest discharge rate occurred within seconds after injection, and responses persisted for more than 5 min in all but one WDR neuron in which the response lasted approximately 2 min. The mean peak discharge rates evoked by capsaicin did not differ for WDR and HT neurons (51.4 ± 5.4 and 49.1 ± 10.5 Hz, respectively), and a greater discharge was evoked by capsaicin in each WDR and HT neuron than by histamine (Figs. 2 and 3). Peak discharge rates of WDR and HT neurons evoked by capsaicin were significantly (P < 0.003, t-test) higher than those evoked by vehicle (13.6 ± 4.8 and 3.8 ± 1.8 Hz, respectively) or histamine (22.7 ± 4.5 and 10.3 ± 3.4 Hz, respectively). All STT neurons that were sensitive to histamine (n = 8) were also excited by capsaicin. There was little overall difference in responses to capsaicin for histamine responders and nonresponders. We did not encounter any STT neurons that were excited by histamine but not by capsaicin. In contrast, 3 HT and 2 WDR neurons were activated by capsaicin but not by histamine.

One-way ANOVAs with repeated measures were used to determine whether mean discharge rates of WDR and HT neurons increased after injection of vehicle, histamine, or capsaicin relative to the spontaneous discharge rate before injection. Regarding WDR neurons, injection of vehicle had no effect on their mean discharge rates (Fig. 4A). In contrast, mean discharge rates of WDR neurons increased significantly after histamine (one-way ANOVA, P < 0.0001). Posthoc comparisons using the Newman–Keuls method revealed that the mean discharge rates increased above preinjection values at 15 s after injection and remained elevated at nearly all time points until 4 min and 45 s after injection, at which time mean discharge rates did not differ from preinjection values. Mean discharge rates of WDR neurons also increased following injection of capsaicin (one-way ANOVA, P < 0.001). Although mean discharge rates were elevated throughout the 5-min recording period, the increased discharge rates were elevated significantly only during the first 2 min after injection. Discharge rates (per 15-s bin) were higher following capsaicin than after histamine. Following histamine, the mean peak discharge rate of all WDR neurons occurred at 30 s after injection and was 19.9 ± 4.4 impulses/s whereas the mean peak discharge rate evoked by capsaicin was 50.8 ± 5.9 impulses/s.

Injection of the vehicle did not increase mean discharge rates of HT neurons for more than a few seconds (Fig. 4B). A one-way ANOVA with repeated measures indicated that mean discharge rates of HT neurons increased following injection of histamine (P < 0.05). Small increases in discharge rates were observed only at 15 s and 1.5 min after injection. HT neurons were excited vigorously by capsaicin (one-way ANOVA, P < 0.001) and the mean discharge rates were similar to those of WDR neurons. The peak mean response occurred at 15 s after injection, and discharge rates were significantly elevated relative to preinjection values for over 3 min (Newman–Keuls method).

Responses evoked by mechanical stimuli

RESPONSES TO STROKING. Mean responses evoked by stroking were obtained from responses across all test sites. A one-way ANOVA with repeated measures indicated significant differences in mean responses of WDR neurons as a function of the type of injection given (P < 0.03). Stroking the skin with the cotton swab excited all WDR neurons. Responses evoked by stroking were not altered after injection of vehicle (n = 11). The mean number of impulses evoked by each stroke was 21.2 ± 2.8 before and 21.9 ± 3.2 after the injection of vehicle (Fig. 5A). After injection of histamine, the mean number of impulses evoked by each stroke (27.3 ± 4.2) did not differ significantly from the preinjection mean responses or from the mean response obtained after the injection of vehicle (Bonferroni t-test). Responses to stroking increased significantly (>25%) in only 1 of 10 neurons. For 3 additional neurons, responses to stroking increased approximately 20% after histamine, but this did not differ significantly from baseline values for these cells. In contrast, more than half (6/10) of the WDR neurons tested exhibited an increased response to stroking after capsaicin. Mean responses of WDR neurons to stroking increased to 29.8 ± 3.5 and differed significantly from the preinjection mean response of 21.2 ± 2.8 (Bonferroni t-test, P < 0.046).

Figure 5B illustrates a similar trend for HT neurons, and a one-way ANOVA with repeated measures indicated a significant difference in responses to stroking as a function of the type of injection (P < 0.03). Prior to any injection, stroking the skin with a cotton swab weakly excited 3 of 7 HT neurons. The
FIG. 3. Responses of a single HT neuron to intradermal injections of vehicle, histamine, and capsaicin. Reconstruction of the stimulation site in VPL for antidromic activation (A) and the recording site in lamina I of the dorsal horn (B). C: localization of the RF, injection sites, and test sites for application of punctate stimuli. D: peristimulus time histograms showing responses evoked by brush, pressure, pinch, and squeeze that were used to class this neuron functionally as HT. Discharge rates evoked by each stimulus are indicated. Binwidth of histograms is 100 ms. Discriminated output pulses are provided below the histogram. E: line graph showing mean discharges rates (15-s binwidth) for this neuron following injection of vehicle, histamine, and capsaicin. F: peristimulus time histograms showing responses evoked by injection of vehicle, histamine, and capsaicin.
mean number of impulses evoked by a single stroke for all neurons was 4.7 ± 2.9, and this did not differ following injection of vehicle (5.8 ± 3.4, Bonferroni t-test). Similarly, mean responses to stroking were not altered significantly after histamine (2.7 ± 1.8), although responses to stroking increased by about 100% in 1 of 6 HT neurons from an average of 1.7 impulses before histamine to 3.6 impulses after the injection. In contrast, responses to stroking increased in 3 of 6 HT neurons after capsaicin, and the responses of two neurons each increased by 100% or more. The mean number of impulses for all HT neurons evoked by each stroke increased significantly from 4.7 ± 2.9 to 11.1 ± 5.7 after capsaicin (Bonferroni t-test, P < 0.024).

RESPONSES TO PUNCTATE STIMULI. Responses of WDR and HT neurons evoked by the von Frey monofilament before and after injections of vehicle, histamine, and capsaicin are shown in Fig. 6. Mean responses evoked by a single application of the von Frey monofilament were obtained from responses across all test sites. A one-way ANOVA with repeated measures indicated differences in mean responses of WDR neurons as a function of the type of substance injected (P < 0.001). Injection of vehicle did not alter the mean number of impulses evoked by the von Frey monofilament (46.2 ± 6.2 impulses before and 51.0 ± 8.0 impulses after injection (Bonferroni t-test; Fig. 6A). Only 1 of 11 neurons exhibited an increased response after vehicle. Responses of WDR neurons increased significantly after injection of histamine. Responses increased 31–38% in 4 of 12 neurons after histamine and were elevated 4.8–25% in an additional 4 neurons. The mean number of impulses evoked after histamine was 56.6 ± 9.4, which differed from responses obtained prior to any injection (46.2 ± 6.2; Bonferroni t-test, P < 0.012). Responses of WDR neurons also increased significantly after capsaicin relative to the base-
line condition ($P < 0.001$). The mean number of impulses evoked by the von Frey monofilament after capsaicin was 65.4 ± 12.0. Five of 7 WDR neurons became sensitized after capsaicin and the response of each neuron increased 50\% or more compared with baseline values. For those neurons that exhibited increased firing after histamine, evoked responses of all but 1 neuron increased further after capsaicin.

A one-way ANOVA with repeated measures also revealed a significant difference in responses of HT neurons as a function of the chemical substance injected ($P < 0.001$). Injection of the vehicle did not increase responses of any neuron and had no effect on the mean number of impulses evoked by the monofilament (7.9 ± 2.5 before and 12.8 ± 3.7 after injection). Unlike WDR neurons, HT neurons did not exhibit a significant increase in mean responses after histamine. The mean number of impulses evoked by the punctate stimulus increased in only 1 of 7 HT neurons after histamine (the same neuron that responded to histamine). The mean number of impulses evoked for all neurons, 12.4 ± 4.4, did not differ from the means obtained before the injection of histamine or after the injection of vehicle. However, like WDR neurons, most HT neurons (4 of 6 tested) increased their responses to the punctate stimulus after capsaicin. The mean number of impulses evoked by the punctate stimulus increased from 16.2 ± 4.9 before capsaicin to 26.2 ± 4.6 after ($P < 0.03$).

Responses of STT neurons to heat

We also determined whether intradermal injection of histamine or capsaicin altered responses evoked by heat stimuli of 45 or 50°C. The response of each neuron to heat was defined as the number of impulses occurring during the period starting at the onset of the 5-s stimulus and for 5 s after its termination. This number was divided by 10 to obtain the discharge rate (impulses/s). Figure 7 illustrates the change in responses to heat for individual STT neurons following injections of vehicle, histamine, and capsaicin. Shown for each neuron is the change in discharge rate evoked after injections of vehicle histamine and capsaicin compared with the discharge rate evoked before each injection. Five of six WDR neurons tested responded to heat stimuli of 45°C and all were excited by 50°C. Prior to any injection, the mean discharge rates for all WDRs evoked by 45 and 50°C were 4.7 ± 1.4 and 26.9 ± 9.0 impulses/s, respectively, and were not changed after an injection of the vehicle (6.4 ± 2.1 and 21.7 ± 7.5 impulses/s, respectively) (paired $t$-test). Although the responses of three WDRs to each heat stimulus increased dramatically after the injection of histamine (1 of which responded to histamine for more than 5 min), the responses of others exhibited little change. Consequently, there were no significant differences in the mean rates evoked by 45 and 50°C before (7.4 ± 3.5 and 12.5 ± 5.7) and after (14.4 ± 5.7 and 30.6 ± 16.4 impulses/s), respectively. Only one WDR neuron was excited to an intradermal injection of capsaicin and its response to heat stimuli did not change.

The mean discharge rates of HT neurons evoked by heat stimuli did not change significantly after injection of vehicle, histamine, or capsaicin. Prior to any injection, three of four HT neurons were excited by heat stimuli and responses were not altered after injection of vehicle. Mean discharge rates evoked by 45°C were 0.6 ± 0.3 Hz before injection and 0.2 ± 0.1 impulses/s after injection. Similarly, the mean discharge rates evoked by 50°C before and after injection of vehicle were 3.9 ± 2.9 and 2.9 ± 4.0 impulses/s, respectively. The mean discharge rates evoked by 45 and 50°C before and after histamine were 0.6 ± 0.5 and 3.1 ± 2.1 and 3.1 ± 1.9 and 9.7 ± 4.4 impulses/s respectively.

The effect of intradermal injection of capsaicin on responses to heat was examined in four HT neurons. The mean discharge rate evoked by the stimulus of 45°C was significantly increased from 3.5 ± 1.8 impulses/s before to 11.3 ± 4.3 impulses/s after the injection of capsaicin ($P < 0.05$, paired $t$-test). There was no significant difference in mean discharge rates evoked by 50°C before and after capsaicin (17.0 ± 5.5 and 16.1 ± 7.9 impulses/s, respectively).

Discussion

The STT neurons that were recorded in this study in monkeys were polymodal with respect to their excitation by pruritic and painful stimuli. Similar results were obtained in rats in which the majority of nociceptive dorsal horn neurons responsive to histamine were also responsive to noxious stimuli (Carstens 1997; Jinks and Carstens 2000, 2002). It was concluded in those studies that histamine-responsive neurons had a predominant role in nociception rather than pruritis.

Neuronal response properties that might contribute to itch and itchy skin in humans

It is useful to consider what properties of a hypothetical itch-mediating (“pruriceptive”) neuron might be required to account for experimentally produced itch in humans. When
such properties are measured in animals, it is assumed that the animal in question (e.g., cat or monkey) and humans experience similar sensations in response to the same pruritic stimulus.

One property of a candidate pruriceptive neuron is that its responses to pruritic stimuli have a profile that is similar in shape and time course to that of the itch response evoked by the same stimuli in humans. A corollary response property often postulated is that the responses of the neuron are specific or at least “selective” (preferential) for pruritogenic as opposed to other types of stimuli, such as those eliciting pain. In the case of primary afferent neurons, for example, a subpopulation of C mechanoheat (CMH) nociceptors has been found responsive to intracutaneous application of histamine (Handwerker et al. 1991; Tuckett and Wei 1987). A small proportion of these recorded in humans exhibited responses that were comparable in time course to the sensation of itch and of greater magnitude than those elicited by mustard oil, a stimulus that elicits a sensation of burning (Handwerker et al. 1991). The responses of most other CMHs to histamine were usually weak and of insufficient duration to account for itch sensation. The variety of responses of these CMHs is similar to that exhibited by histamine-responsive STT neurons in the present study.

Mechanically insensitive afferent (MIA) C fibers with particularly slow conduction velocities in humans responded to histamine with a time course of excitation that resembled the sensation of itch and were thus said to be itch specific (Schmelz et al. 1997). Subsequently, STT neurons with similar properties were identified in the superficial dorsal horn of the cat (Andrew and Craig 2001). A subpopulation of STT neurons, identified antidromically in cat, were not responsive to mechanical stimuli and more than half of these were excited by histamine in a manner appropriate for the duration of itch in humans. Two of the latter neurons were not responsive to mustard oil and thus were suggested to be itch specific. In addition, the axonal projections to thalamus exhibited slower conduction velocities than those of mechanically sensitive nociceptive STT neurons, suggesting that they are part of an itch coding system separate from that coding for pain. Future studies are needed to search in the primate for mechanically insensitive STT neurons with slow axonal conduction velocities. Such neurons would have likely been overlooked in the present experiments.

Because itch is a fundamental sensory modality that differs from pain, it is reasonable to hypothesize the existence of pruriceptive neurons that respond specifically to pruritogenic as opposed to allogenic or other types of nonpruritic stimuli. But to prove specificity for itch, it would be desirable to demonstrate a lack of response to stimuli that elicit intense pain (not pain that is merely weak or moderate). This was our rationale for choosing to inject 100 μg/10 μl of capsaicin that is known to elicit an exceedingly intense sensation of pain in humans (LaMotte et al. 1991; Simone et al. 1989). Another rationale was to inject doses of capsaicin (100 μg) and histamine (20 μg) that were roughly matched for the overall durations of evoked pain and itch, respectively, if injected intracutaneously into humans (Atanassoff et al. 1999; Simone et al. 1987, 1989). Thus we were able to show that the STT neurons recorded in the present study were neither specifically nor selectively responsive to histamine. The mechanically insensitive neurons recorded by Andrew and Craig (2001) from the dorsal horn of the cat were not tested for their responses to capsaicin or other algesic stimuli aside from mustard oil. It will be important to do so in the future because the histamine-sensitive MIA s in humans were recently found not to be specific for itch as previously supposed in the study by Schmelz et al. (1997). Instead, these neurons responded not only to one or more painful chemical agents such as capsaicin but also to prostaglandin E2, a stimulus that elicited little or no sensation (Schmelz et al. 2003). Further experiments are required to establish the selectivity of these neurons and of STT neurons for pruritic stimuli.

It would be useful to know whether there are peripheral and/or central neurons that are “nociceptive specific,” i.e., responsive to allogenic but not to pruritogenic stimuli. This would be necessary in support of an “occlusion” theory of itch in which a population of pruriceptive neurons that responds also to allogenic stimuli are interpreted by the brain as signaling itch only in the absence of concomitant activity in a population of nociceptive specific neurons (Handwerker 1992).

The present experiment revealed the existence of several candidate nociceptive-specific neurons that responded well to capsaicin but did not respond to histamine in confirmation of results previously obtained in recordings from the dorsal horn of the cat (Hirata et al. 1990). However, the same caveat must be applied regarding the necessity of future testing as to whether such neurons fail to respond to a higher dose of histamine or to other types of pruritic stimuli that are equally intense or more so.

Another property that might be expected of a pruriceptive neuron is that its activity is increased by stimuli that increase the sensation of itch, for example, warming the skin (Fruhstorfer et al. 1986) or spinal administration of opiates (Ballantyne et al. 1988; Ko and Naughton 2000). In addition, central pruriceptive neurons should become sensitized to the mechanical stimuli that evoke enhanced itch in areas of alloknesis and hyperknesis after application of a pruritic chemical (Atanassoff et al. 1999; Bickford 1938; Simone et al. 1991, 1991a). The STT neurons in the present study generally did not become sensitized to innocuous stroking of the skin after histamine and thus appear to be incapable of contributing to alloknesis. The punctate stimulus to which they did sensitize elicits enhanced pain (hyperalgesia) and not itch after intradermal injection of histamine in humans.

Another property expected of a candidate pruriceptive neuron in the CNS is that its activity is inhibited by algesic stimuli applied to normal skin and by the development of allogenic dysesthetic states such as allodynia and hyperalgesia (Bickford 1938; Brull et al. 1999; Graham et al. 1951; Koppert et al. 1993; Nilsson et al. 1997; Ward et al. 1996). Rather than being inhibited by capsaicin, most of the STT neurons in the present study were vigorously excited, after which they became sensitized to stroking and to punctate stimuli. In addition, approximately half of the neurons examined exhibited an increased response to heat after histamine and capsaicin. It is well known that capsaicin causes hyperalgesia to heat (LaMotte et al. 1991; Simone et al. 1987), and preliminary data suggest that intradermal injection of histamine also produces heat hyperalgesia at the injection site (RH LaMotte, unpublished observations). Thus it seems more likely that the STT neurons in this study contribute to pain, hyperalgesia, and allodynia rather than to itch and alloknesis.

There is psychophysical evidence that histamine activates an
antipruritic system that acts to reduce the magnitude of itch and the associated areas of cutaneous dysesthesiae. The magnitude and duration of itch is enhanced and the dysesthetic areas of allodynia, hyperknesis, and hyperalgesia are significantly greater when histamine is injected into a bleb of anesthetized skin as opposed to a bleb of saline (Atanassoff et al. 1999). It was hypothesized that, in the absence of an anesthetic, histamine-evoked activity in a subpopulation of nociceptors and nociceptive dorsal horn neurons that lasted for the duration of the itch and acted to reduce activity in central neurons mediating itch and the associated dysesthesiae. Those STT neurons exhibiting enduring responses to histamine in our experiments may contribute to the antipruritic rather than the pruritic effect of the chemical. Whether activity in these neurons acts to inhibit pruriceptive neurons remains to be tested.

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