Facial Injections of Pruritogens and Algogens Excite Partly Overlapping Populations of Primary and Second-Order Trigeminal Neurons in Mice

T. Akiyama, M. Iodi Carstens, and E. Carstens
Department of Neurobiology, Physiology and Behavior, University of California, Davis, California

Submitted 23 June 2010; accepted in final form 20 August 2010


Overlapping Populations of Primary and Second-Order Trigeminal Neurons

Intradermal injection of histamine in the mouse cheek elicited bouts of hindlimb scratching directed to that site, whereas capsaicin elicited an ipsilateral forelimb wiping response but little or no scratching (Shimada and LaMotte 2008). We recently confirmed and extended this (Akiyama et al. 2010a) by showing that other pruritogens, including serotonin (5-HT) and agonists of the protease-activated receptor subtypes PAR-2 and PAR-4, elicit scratching and little wiping, whereas algogens including mustard oil [allyl isothiocyanate (AITC)] and bradykinin elicit mainly forelimb wipes but little hindlimb scratching. These findings are summarized in Fig. 1, which plots the number of hindlimb scratch bouts and ipsilateral forelimb wipes elicited by each agent. Interestingly, intradermal insertion of a spicule of cowhage (Mucuna pruriens) elicited approximately equal numbers of scratch bouts and wipes, consistent with recent reports that cowhage evokes concomitant sensations of itch and burning or stinging (LaMotte et al. 2009; Sikand et al. 2009). The main aim of this study was to begin to investigate possible neural circuits mediating these distinct behavioral responses.

Recent evidence supports the existence of largely separate pathways for histaminergic and nonhistaminergic itch. Whereas intradermal injection of histamine elicits itch and associated flare that is prevented by antihistamines, intradermal insertion of cowhage spicules elicits itch that is not accompanied by flare and is not reduced by antihistamines (Johane et al. 2007). Cowhage contains a substance called mucunain that elicits itch via activation of protease-activated receptor subtypes PAR-2 and PAR-4 (Reddy et al. 2008). PAR-2 has been implicated in chronic itch of atopic dermatitis (Steinhoff et al. 2003), and agonists of PAR-2 and PAR-4 elicit scratching behavior (Akiyama et al. 2009c; Shimada et al. 2006; Tsujii et al. 2009). Another receptor potentially involved in nonhistaminergic itch is mrgprA3 (Liu et al. 2010). This receptor is expressed in superficial cutaneous sensory nerve endings and selectively binds chloroquine, an antimalarial drug that frequently induces itching. Chloroquine and histamine elicited scratching in wildtype mice, whereas histamine, but not chloroquine, elicited scratching in mice lacking mrgprA3 (Liu et al. 2010).

Histamine and cowhage activate largely separate populations of human primary afferent C-fibers (Namer et al. 2008) and monkey spinothalamic tract neurons (Davidson et al. 2007). However, most of these pruripensive neurons additionally respond to the algogen capsaicin, raising the issue of how the CNS discriminates between itch and pain. This study used calcium imaging and single-unit electrophysiological recording methods to investigate activation by pruritogens and algo-

Address for reprint requests and other correspondence: E. Carstens, Dept. of Neurobiology, Physiology and Behavior, Univ. of California, 1 Shields Ave., Davis, CA 95616 (E-mail: ec.carstens@ucdavis.edu).
FIG. 1. Behavioral scratching and wiping elicited by cheek injection of pruritogens and algogens. Scatter plot shows mean numbers of scratch bouts and ipsilateral forelimb wipes (error bars: SE) elicited by each pruritich and/or algicch agent shown. Each chemical was injected intradermally in a volume of 10 μL. Doses (in nmol) were as follows: histamine, 272; protease-activated receptor (PAR)-2 agonist, 76; PAR-4 agonist, 73.4; chloroquine, 194; 5-HT, 47; capsaicin, 98.2; allyl isothiocyanate (AITC), 100.9; bradykinin, 1.0. The formalin dose was 16.7 μmol. For coughage, a single spicule was inserted intradermally, left in for 5 s, and removed. Dashed line: equal numbers of wipes and scratch bouts. Data for histamine, PAR-2, and -4 agonists, 5-HT, capsaicin, AITC, bradykinin, and coughage modified from Akiyama et al. 2010a.

gens of primary sensory trigeminal ganglion (TG) cells and second- or higher-order neurons in trigeminal subnucleus caudalis (Vc), which relays orofacial sensory information to the brain. We wished to determine whether cheek microinjections of pruritogens or algogens, which elicit distinct behavioral responses, activate separate or overlapping populations of TG and Vc neurons.

METHoDS

Behavior

Details of the cheek microinjections and video recording of scratching and wiping behavior are provided in our recent study (Akiyama et al. 2010a), from which much of the data in Fig. 1 was adapted. In this study, we additionally tested chloroquine. Briefly, following habituation to the test environment, ICR mice (n = 6) received an intradermal microinjection of chloroquine (100 μg in saline, 10 μL) in the cheek and were videotaped from below. Two reviewers blinded to treatment reviewed the videotapes to count the numbers of ipsilateral forelimb wipes and hindlimb scratch bouts directed to the injection site at 5-min intervals over a 40-min period. Total numbers of wipes or scratch bouts per 40 min were compared with saline controls using an unpaired t-test with P < 0.05 considered to be statistically significant.

Calcium imaging

A total of 12 adult male ICR mice (32–50 g) were used under a protocol approved by the UC Davis Institutional Animal Care and Use Committee. The animal was killed under sodium pentobartal anaesthesia, and trigeminal ganglia were acutely dissected and enzymatically digested at 37°C for 10 min in Hanks’s balanced salt solution (HBSS; Invitrogen, Carlsbad, CA) containing 20 units/ml papain (Worthington Biochemical, Lakewood, NJ) and 6.7 mg/ml l-cysteine (Sigma), followed by 10 min at 37°C in HBSS containing 3 mg/ml collagenase (Worthington Biochemical). The ganglia were mechanically triturated using fire-polished glass pipettes. TG cells were pelleted, suspended in MEM Eagle’s with Earle’s BSS (Gibco) containing 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), 1× vitamin (Gibco), and 10% horse serum (Quad Five, Ryegate, MT), plated on poly-D-lysine-coated glass coverslips, and cultured for 16–24 h.

TG cells were incubated in Ringer solution (pH 7.4; 140 mM NaCl, 4 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM HEPES and 4.5 mM NaOH) with 10 μM of Fura-2 AM and 0.05% of Pluronic F-127 (Invitrogen). Coverslips were mounted on a custom made aluminum perfusion block and viewed through an inverted microscope (Nikon TS100, Technical Instruments, San Francisco, CA). Fluorescence was excited by UV light at 340 and 380 nm alternately, and emitted light was collected via a CoolSnap camera attached to a Lambda LS lamp and a Lambda optical filter changer (Sutter Instrument, Novato, CA). Ratiometric measurements were made using Simple PCI software (Compix, Cranberry Township, PA) every 3 s.

Solutions were delivered by a solenoid-controlled eight-channel perfusion system (ValveLink, AutoMS). Histamine (100 μM), the PAR-2 agonist SLIGRL-NH2 (100 μM), and 5-HT (100 μM) were delivered in randomized order. The 100 μM concentration selected for each pruritogen was the same as used in prior studies (histamine: Han et al. 2006; Nicholson et al. 2007; Shim et al. 2007; PAR-2 agonist: Amadesi et al. 2004; Steinhoff et al. 2000; 5-HT: Nicolson et al. 2002). This was followed by AITC (100 μM), capsaicin (1 μM), and 144 mM potassium in the same order for all experiments. Stimulus duration was 30 s (10 s for capsaicin). Ratios were normalized to baseline. Cells were judged to be sensitive if the ratio value increased by >10% of the resting level following chemical application. Only cells responsive to high-K+ were included for analysis. Between-group differences were compared by t-test and Fisher’s exact probability test.

SINGLE-UNIT RECORDINGS FROM VC. Experiments were performed using 55 adult male ICR mice (Harlan, Oxnard, CA) (32–50 g) under a protocol approved by the UC Davis Animal Care and Use Committee. The methods were similar to those reported in our recent study (Akiyama et al. 2009a) and are briefly summarized as follows. Anesthesia was induced with sodium pentobartal (60 mg/kg, ip) and maintained by intermittent supplemental injections to achieve a level of ~10–20 mg/kg/h. The upper cervical (C1-C2) spinal cord and caudal medulla were exposed by laminectomy, and a tungsten microelectrode (FHC, Bowdoin, ME) recorded extracellular single-unit activity. A chemical search strategy was used to isolate units in superficial laminae of Vc. Using a 30.5-gauge needle connected to a Hamilton microsyringe, a small volume (~0.25 μL, ~12 μg) of either histamine, SLIGRL-NH2, or AITC was microinjected in the cheek intradermally, and the recording electrode was driven into the ipsilateral medulla to isolate an action potential in the superficial medullary dorsal horn (<300 μm from surface) that exhibited ongoing activity. If no unit was isolated, the procedure was repeated on the opposite cheek. We waited ≥10 min until firing decreased to a steady low level. Then, the same chemical used to isolate the unit was reinjected in a volume of 1 μL. Responsive units (i.e., >30% increase above baseline) were studied further. The chemically evoked response was recorded for ≥20 min, after which a second 30.5-gauge needle containing a different chemical was inserted in the cheek intradermally. The order of chemical injections was counterbalanced, with histamine (50 μg) first followed by SLIGRL-NH2 (50 μg), 5-HT (10 μg), vehicle, AITC (10 μg), and capsaicin (30 μg) (n = 25 units), SLIGRL-NH2, histamine, 5-HT, vehicle, AITC, and capsaicin (15 units), or AITC, histamine, SLIGRL-NH2, 5-HT, vehicle, and capsaicin (18 units). The second chemical was injected, and unit activity was recorded for another 20–30 min. After the first three chemicals, we recorded unit responses to light brushing with a cotton wisp and pinching using forceps. Units were classified as wide dynamic range (WDR) if they responded at higher firing rate to pinch than light touch, nociceptive specific (NS) if they responded to pinch but not
light touch, or mechanically insensitive if the responded to neither. Only one unit was studied per cheek in a given animal.

Action potentials were recorded to a computer and counted using Chart software (AD Instruments, Colorado Springs, CO). Unit activity was usually quantified as number of action potentials per minute and displayed in peristimulus time histogram (PSTH) format with 1-s bins. Group responses at 1-min intervals following a given stimulus were compared with activity 1 min before the stimulus by paired t-test or repeated-measures ANOVA (SPSS 9.0, SPSS, Chicago, IL), with $P < 0.05$ set as significant.

At the end of the experiment, an electrolytic lesion was made through the microelectrode. The medulla was fixed in 10% buffered formalin, and 50-μm sections were cut and mounted on slides for microscopic verification of the lesion site.

**RESULTS**

**Behavior**

Behavioral data are summarized in Fig. 1, which plots mean numbers of scratch bouts versus forelimb wipes/40-min observation period. Chloroquine elicited significantly more scratch bouts ($P < 0.05$) but not ipsilateral forelimb wipes compared with vehicle (saline) controls, comparable with the effects of histamine and the PAR-2 and -4 agonists. In contrast, algogens bradykinin, capsaicin, and AITC elicited significantly more wipes ($P < 0.05$ for all), but not scratch bouts, compared with vehicles as previously reported (Akiyama et al. 2010a). 5-HT and formalin elicited more scratch bouts than wipes, whereas insertion of a cowhage spicule elicited equivalent numbers of scratch bouts and wipes.

**Calcium imaging of TG cells**

A total of 853 TG cells was imaged for responsiveness to histamine, SLIGRL-NH2, AITC, and capsaicin. Examples of TG cell responses are shown in Fig. 2. The cell indicated by the red trace responded to SLIGRL-NH2, AITC, and capsaicin but not histamine, whereas the cell indicated by the blue trace responded to both histamine and SLIGRL-NH2 but not to AITC or capsaicin.

Overall, 15.4% of TG cells responded to histamine, 5.8% to the PAR-2 agonist, 11% to 5-HT, 13.4% to AITC, and 36.7% to capsaicin when tested in that order. The percentages of TG cells activated by these chemicals were similar, regardless of whether histamine, the PAR-2 agonist, or 5-HT was tested first (Table 1), arguing against order effects.

The mean diameter of all 853 TG cells tested was $20.0 \pm 0.22$ (SE) μm. There were no marked differences in mean diameter among cells responsive to histamine ($18.3 \pm 0.4$ μm; $n = 133$), the PAR-2 agonist ($15.7 \pm 0.5$ μm, $n = 46$), 5-HT ($15.3 \pm 0.35$ μm; $n = 81$), capsaicin ($18 \pm 0.25$ μm; $n = 315$), and AITC ($17.3 \pm 0.37$ μm; $n = 113$). The mean diameter of TG cells unresponsive to any of the tested chemicals was $24.03 \pm 0.34$ μm ($n = 384$).

Figure 3A shows that 9.6% of TG cells responded only to histamine, 1.9% only to the PAR-2 agonist, and 6.9% only AITC, whereas the remainder responded to two or all three agents (proportions indicated within overlap regions in Fig. 3A). Capsaicin activated 36.7% of all TG cells (Fig. 3B), and 40% of cells were excited by capsaicin and/or AITC (Fig. 3C). Of the capsaicin-sensitive TG cells, 29% also responded to histamine, 10.2% also responded to the PAR-2 agonist, and some responded to both, whereas 63% did not respond to either pruritogen (Fig. 3B). In addition, 20.5% of capsaicin-sensitive TG cells were also activated by 5-HT. The percentages of coincident responses were similar when capsaicin- and AITC-sensitive TG cell populations were combined (Fig. 3C). Overall, 3.7% of the TG cells only responded to histamine and 1% only responded to the PAR-2 agonist, with the remainder also responding to AITC and/or capsaicin (Fig. 3C).

Eleven percent of TG cells responded to 5-HT. 5-HT activated 23% of histamine-responsive TG cells and 36% of PAR-2 agonist-responsive TG cells (Table 2). Histamine, the PAR-2 agonist, AITC, and capsaicin activated 32, 19, 32, and 72%, respectively, of 5-HT–sensitive TG cells.

**Vc electrophysiology**

Data were obtained from a total of 58 Vc units identified by histamine ($n = 25$), PAR-2 agonist ($n = 15$), or AITC ($n = 18$) search stimuli. Most units (88%) had a mechanosensitive receptive field on the cheek including the intradermal injection site, with 62% classified as WDR and 26% as nociceptive-

![Graph](image-url)
specific (NS), whereas 12% were classified as mechanically insensitive. It should be noted, however, that mechanical sensitivity was always tested after application of chemical stimuli, which may have altered neuron mechanically evoked responses.

Unit recording sites were typically located superficially in the dorsal aspect of Vc at or near the level of the pyramidal decussation in the caudal medulla. The mean depth of recordings was $148.7 \pm 110.4$ (SE) $\mu$m from the medullary surface. Histologically recovered lesion sites are shown in Figs. 4 and 5.

HISTAMINE SEARCH. Twenty-five units isolated using the histamine search strategy were studied. An example is shown in Fig. 4A. This superficially located NS unit responded to intradermal histamine and pinch but not brush and was generally unresponsive to other stimuli. Averaged responses of the 25 histamine-sensitive units are shown in Fig. 5A. The mean histamine-evoked response was significantly greater than the preceding baseline out to 9 min after injection. Of these, 4/14 tested also responded to intradermal cheek injection of the PAR-2 agonist, 8/11 to 5-HT, 8/17 to capsaicin, and 12/14 to AITC. None of the 14 units tested responded to intradermal saline or Tween-80 (vehicle controls). Recordings sites in superficial Vc are shown in the inset to Fig. 5B.

AITC SEARCH. Eighteen units were isolated using an AITC search stimulus. An example is shown in Fig. 4C. This superficial Vc unit responded to intradermal cheek injection of AITC and also gave response to histamine and a multiphasic response to capsaicin. Averaged responses of the 18 units are shown in Fig. 5C. Of units tested, 7/17 responded to histamine, 2/15 to SLIGRL-NH2, 3/13 to 5-HT, and 12/14 to capsaicin. Units were unresponsive to vehicle (saline or Tween-80). Recordings sites are shown in the inset to Fig. 5C.

CROSS-INTERACTIONS. We recorded from a sufficient number of AITC-responsive neurons to study the effect of prior pruritogenic stimulation on neuronal responses to this algogen. The mean baseline-corrected response when AITC was tested after application of histamine, SLIGRL-NH2, and 5-HT (Fig. 5, A and B; mean $184.5 \pm 53.5$ impulses/min; $n = 17$) was significantly greater ($P = 0.049$, unpaired t-test) compared with the response to AITC when tested first ($50.7 \pm 12.9$; $n = 12$). This suggests that one or a combination of the pruritogens sensitized neuronal responses to subsequent application of AITC.

COMPARISON OF TG AND VC CELLS. Table 2 lists the percentages of histamine-, SLIGRL-NH2-, and AITC-responsive TG and Vc cells that additionally responded to other agents. The Vc cells are sorted according to the chemical search stimulus (which activated 100% of the cells). In general, TG and Vc cells exhibited similar coincidences of activation by different agents, with no significant difference between TG and Vc cells. The only exception was that histamine-responsive Vc neurons exhibited a significantly higher percentage of coactivation by 5-HT and AITC compared with histamine-responsive TG cells.

TABLE 2. Co-incident activation of TG and Vc cells by pruritogens and algogens

<table>
<thead>
<tr>
<th>Excited by</th>
<th>Histamine</th>
<th>SLIGRL</th>
<th>5-HT</th>
<th>AITC</th>
<th>Capsaicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>Vc</td>
<td>TG</td>
<td>Vc</td>
<td>TG</td>
<td>Vc</td>
</tr>
<tr>
<td>Histamine</td>
<td>100</td>
<td>13.8</td>
<td>28.6</td>
<td>23.3</td>
<td>72.7*</td>
</tr>
<tr>
<td>SLIGRL</td>
<td>35.6</td>
<td>100</td>
<td>100</td>
<td>35.6</td>
<td>40</td>
</tr>
<tr>
<td>AITC</td>
<td>34.2</td>
<td>21.2</td>
<td>13.3</td>
<td>26.9</td>
<td>23.1</td>
</tr>
</tbody>
</table>

Left column indicates the chemical that excited the TG or Vc cells. For Vc cells, the chemical was used as a search stimulus to identify responsive units. The 2nd through 11th columns indicate the percentages of TG and Vc cells that were additionally excited by the given chemical. 100%: all cells responded to that chemical. *Significant difference between TG and Vc incidence ($P < 0.05$, Fisher’s exact test).
This study was predicated on the observation that intradermal cheek injections of pruritogens or algogens elicit distinct behavioral responses, i.e., hindlimb scratches or forelimb wipes, respectively (Shimada and LaMotte 2008). We recently extended this by showing that other pruritogens (PAR-2 and PAR-4 agonists; 5-HT; chloroquine) also elicit facial scratching but little wiping, whereas algogens (AITC, bradykinin) elicit mainly forelimb wiping with little hindlimb scratching (Akiyama et al. 2010a). The scratching elicited by cheek injection of histamine or the PAR-2 agonist SLIGRL-NH2 was significantly attenuated by naltrexone but not morphine, whereas capsaicin-evoked wiping was dose-dependently reduced by morphine but unaffected by naltrexone (Akiyama et al. 2010a), supporting the view that scratching and wiping reflect itch and pain, respectively.

The PAR-2 and -4 agonists and chloroquine elicited a significant increase in number of hindlimb scratch bouts, whereas the number of ipsilateral forelimb wipes was not different from vehicle controls (Fig. 1). This supports the argument that these agents are selective pruritogens. In contrast, capsaicin, AITC, and bradykinin elicited significant wiping but did not elicit scratching above vehicle control levels (Fig. 1), supporting the argument that these agents are selective algogens. 5-HT elicited significant scratching, but also more forelimb wipes compared with vehicle controls. This suggests that 5-HT may elicit pain in addition to itch, consistent with 5-HT

**DISCUSSION**

This study was predicated on the observation that intradermal cheek injections of pruritogens or algogens elicit distinct behavioral responses, i.e., hindlimb scratches or forelimb wipes, respectively (Shimada and LaMotte 2008). We recently extended this by showing that other pruritogens (PAR-2 and PAR-4 agonists; 5-HT; chloroquine) also elicit facial scratching but little wiping, whereas algogens (AITC, bradykinin) elicit mainly forelimb wiping with little hindlimb scratching (Akiyama et al. 2010a). The scratching elicited by cheek injection of histamine or the PAR-2 agonist SLIGRL-NH2 was significantly attenuated by naltrexone but not morphine, whereas capsaicin-evoked wiping was dose-dependently reduced by morphine but unaffected by naltrexone (Akiyama et al. 2010a), supporting the view that scratching and wiping reflect itch and pain, respectively.

The PAR-2 and -4 agonists and chloroquine elicited a significant increase in number of hindlimb scratch bouts, whereas the number of ipsilateral forelimb wipes was not different from vehicle controls (Fig. 1). This supports the argument that these agents are selective pruritogens. In contrast, capsaicin, AITC, and bradykinin elicited significant wiping but did not elicit scratching above vehicle control levels (Fig. 1), supporting the argument that these agents are selective algogens. 5-HT elicited significant scratching, but also more forelimb wipes compared with vehicle controls. This suggests that 5-HT may elicit pain in addition to itch, consistent with 5-HT
being an inflammatory mediator (Hong and Abbott 1994) and pruritogen. Formalin also elicited more scratching than wiping, suggesting the possibility that it may elicit itch and pain in rodents. Cowhage spicules elicited equivalent numbers of scratch bouts and wipes (Fig. 1), consistent with recent human psychophysical studies showing that intradermal insertion of a single cowhage spicule simultaneously elicits itch and nociceptive (stinging, burning) sensations (LaMotte et al. 2009; Sikand et al. 2009).

**FIG. 5.** Averaged responses of Vc units isolated by chemical search strategy. A: histamine. Averaged PSTHs (bin width: 1 s) of 25 units isolated using histamine search strategy. Gray error bars: SE. Average firing following intradermal histamine (left PSTH) was significantly greater than prestimulus baseline out to 9 min after injection ($P < 0.05$, paired t-test). Right PSTHs show averaged responses of units that responded to indicated stimuli. Numbers in parentheses indicate the number that responded (>30% increase) of all units tested. The mean response of the 4 units to SLIGRL-NH2 1 min after injection did not reach statistically significant difference compared with preinjection baseline ($P = 0.052$). The same was true for the mean response of the 8 units to 5-HT ($P = 0.053$) and the 8 units to brush ($P = 0.057$). The mean response to pinch was significantly different compared with prestimulus baseline ($P < 0.05$). The mean response to capsaicin was significantly different from baseline at the 1st minute after injection ($P < 0.05$), whereas the mean response to AITC was significantly different out to the 6th minute after injection ($P < 0.05$). The mean firing rate after saline injection was not significantly different from prestimulus baseline at any time ($P > 0.5$). Inset: histologically identified lesion sites plotted on representative drawing of caudal medulla at the level of the pyramidal decussation. NTS, nucleus of the solitary tract; PX, pyramidal decussation. B: PAR-2 agonist SLIGRH-NH2 (format as in A). Mean response to SLIGRL-NH2 was significantly greater than prestimulus baseline out to minute 3 ($P < 0.05$). The mean response to histamine was significantly different for 1st minute after injection ($P < 0.05$). The mean response 1 min after 5-HT was not significantly different from baseline ($P = 0.07$). The mean brush-evoked response was significantly different from baseline ($P < 0.01$). The mean pinch-evoked response was significantly different from baseline ($P < 0.001$). The mean response to AITC was significantly different from baseline at minute 2 ($P < 0.05$) but not at minute 1 ($P = 0.054$) after injection. The mean response to capsaicin was significantly different from baseline at the 1st minute ($P < 0.01$). Mean responses to saline and Tween-80 were not significantly different from baseline ($P > 0.4$ for both). Inset: recording sites (format and abbreviations as in A). C: AITC (format as in A and B). Mean response to AITC significant at the 1st minute after injection ($P < 0.05$). The mean response to histamine was significantly different from baseline out to the 11th minute after injection ($P < 0.05$ for minutes 1–11). The mean responses of the few units to SLIGRL-NH2 or 5-HT were not significantly different from baseline ($P > 0.3$ for both). The mean response to brush was significantly greater than baseline ($P < 0.01$), as was the mean response to pinch ($P < 0.05$). The mean response to capsaicin was also not significantly different from baseline ($P = 0.15$). Neither mean responses to saline nor Tween-80 were significantly different from baseline ($P > 0.5$ for both). Inset: recording sites (format and abbreviations as in A).
TG cell imaging

We observed that 15.6% of TG cells responded to histamine. This is similar to reported proportions of histamine-sensitive dorsal root ganglia (DRG) cells (13.4 and 15.9%) from mice (Akiyama et al. 2010b; Han et al. 2006) and rats (15%; Nicolson et al. 2002). Oh’s group has reported higher percentages of histamine-sensitive DRG cells (62.7%; Kim et al. 2004; Shim et al. 2007). Presently, only a small percentage (5.8%) of TG cells responded to the PAR-2 agonist, similar to the small percentage (2.7%) of mouse DRG cells that were activated by SLIGRL-NH2 (Akiyama et al. 2010b). In contrast, much higher proportions (32–50%) of rat DRG cells responded to PAR-2 agonists (Amadesi et al. 2004; Steinhoff et al. 2000). Eleven percent of TG cells responded to 5-HT, a value intermediate between that of mouse (6.5%; Ohta et al. 2006) and rat (21%; Nicolson et al. 2002) DRG cells. The majority (64–70%) of histamine- and SLIGRL-NH2–sensitive TG cells also responded to capsaicin, similar to proportions of histamine-sensitive (40–66%) and PAR-2 agonist–sensitive (79.3%) DRG cells that also responded to capsaicin (Akiyama et al. 2010b; Amadesi et al. 2004; Nicolson et al. 2002; Shim et al. 2007).

Overall, <5% of TG cells responded selectively to pruritogens, whereas the vast majority additionally responded to capsaicin and/or AITC. This is consistent with human micro-neurographic studies showing that histamine-sensitive, mechanically insensitive C-fiber afferents, as well as cowhage-sensitive C-fiber polymodal nociceptors, also responded to capsaicin (Namer et al. 2008; Schmelz et al. 1997), although capsaicin excited a much lower percentage of monkey C-fiber polymodal nociceptors (Johanek et al. 2008). About one third of SLIGRL-NH2–sensitive TG cells also responded to histamine, whereas 14% of histamine-sensitive TG cells responded to SLIGRL-NH2, indicating that the majority of TG cells are selective for one but not both pruritogens. This is consistent with a recent study showing that histamine but not cowhage preferentially excited a subpopulation of mechanically insensitive C-fiber afferents, whereas cowhage but not histamine excited mechano-sensitive C-fiber polymodal nociceptors in humans (Namer et al. 2008). In monkeys, a higher percentage of C-fiber polymodal nociceptors responded to both cowhage and histamine, although cowhage-evoked responses were considerably larger (Johanek et al. 2008).

Vc neurons

Our chemical search strategy maximized the chances of finding chemoresponsive neurons in superficial laminae of Vc. Only 28.6% of histamine- and 36% of SLIGRL-NH2–responsive Vc neurons also responded to the other pruritogen. This differs from superficial lumbar dorsal horn neurons identified using a similar hindpaw chemical search strategy; these neurons exhibited a much higher incidence of responsiveness (76–85%) to both pruritogens (Akiyama et al. 2009a). The segregation of histamine- and PAR-2 agonist–responsive Vc neurons is consistent with lumbar primate spinthalamic tract neurons that have been shown to respond either to histamine or to cowhage, but rarely both (Davidson et al. 2007). Many histamine- and SLIGRL-NH2–responsive Vc units also responded to 5-HT. Interestingly, Vc neurons identified by the histamine search stimulus exhibited a significantly greater incidence of responsiveness to 5-HT and AITC compared with histamine-sensitive TG cells (Table 2), suggesting that these Vc neurons receive convergent input from histamine-, 5-HT-, and/or AITC-sensitive trigeminal afferents. The incidence of responses of SLIGRL-NH2– and AITC-sensitive Vc and TG cells to other agents did not differ (Table 2). This suggests that input from SLIGRL-NH2–sensitive primary afferents fully accounts for the chemical tuning of the SLIGRL-NH2–sensitive Vc neurons. The same logic applies to AITC-sensitive TG and Vc neurons.

Given that AITC enhances responses of nociceptive trigeminal neurons (Hu et al. 1992), it is possible that our use of an AITC search stimulus may have sensitized Vc neurons to increase the proportion that responded to subsequent chemical stimuli. Nevertheless, only a minority of Vc neurons identified by the AITC search stimulus responded to pruritogens, in proportions similar to TG cells (Table 2). This argues against the possibility that AITC markedly enhanced Vc neuronal sensitivity to pruritogens. On the other hand, the mean response of AITC-sensitive Vc neurons was smaller when AITC was tested first compared with when it was tested following sequential application of pruritogens. This suggests that one or more of the pruritogens may have sensitized responses to AITC, consistent with previous reports of hyperalgesia induced by histamine, 5-HT (Hong and Abbott 1994), and PAR-2 agonists (Vergnolle et al. 2001). Finally, several of the present Vc units were mechanically insensitive. The present Vc units were isolated using a chemical search strategy, disallowing assessment of their mechanical responsiveness before chemical application. We thus cannot rule out the possibility that prior chemical stimuli might have attenuated or otherwise affected unit mechanosensitivity. It should be noted, however, that many histamine-responsive feline lamina I spinothalamic tract neurons were mechanically insensitive (Andrew and Craig 2001), suggesting a role for such units in signaling itch.

Neural mechanisms of itch and pain

These data indicate that largely overlapping populations of primary and second-order trigeminal sensory are activated by pruritogens versus algogens, raising questions as to the central transmission of itch and pain.

The gastrin-releasing peptide receptor (GRPR) is important for the spinal transmission of itch but not pain (Sun and Chen 2007). Neurotoxic ablation of GRPR-expressing neurons drastically attenuated prurito-gen-evoked scratching with no effect on pain sensitivity (Sun et al. 2009), suggesting neurochemically distinct central pathways for itch versus pain. Neuro-
toxic ablation of substance P NK-1 receptor–expressing neurons in the superficial dorsal horn also significantly attenuated pruritogen-evoked scratching in rats (Carstens et al. 2010). This treatment reduced hyperalgesia but had little effect on acute nocifensive behavior (Mantyh et al. 1997), suggesting that substance P may also function as a spinal itch-transmitting neuropeptide transmitter. Conceivably, itch may be signaled by spinal neurons co-expressing GRPR and NK-1 receptors.

We presently identified small subpopulations of TG and Vc sensory neurons that selectively responded to either pruritic or algesic stimuli, providing limited evidence for itch- and pain-selective pathways. However, the large majority of pruritogen-responsive TG and Vc neurons responded to algogens. This is partly consistent with previous studies of pruriprissive primary afferents and fully consistent with reports that a majority of pruriprissive lumbar dorsal horn neurons also responds to capsaicin and/or AITC (Akiyama et al. 2009a,b; Andrew and Craig 2001; Davidson et al. 2007; Jinks and Carstens 2002; Simone et al. 2004). However, this raises the question as to how the nervous system discriminates between itch and pain. One possibility is that a small subpopulation of TG and Vc neurons that responds selectively to pruritogens is sufficient to signal itch. Likewise, the subpopulation of TG and Vc neurons responsive to algogens but not pruritogens may signal pain. A second possibility involves a population code. ICH mediators activate pruritogen-selective TG and Vc neurons (Fig. 6, white) and a larger population that responds to both pruritogens and algogens (Fig. 6, light gray), with activity in both neuronal populations postulated to signal itch. Noxious chemicals activate algogen-selective neurons (Fig. 6, dark gray) that are proposed to signal pain. It is possible that both mechanisms, i.e., selectivity and population coding, contribute to itch and pain signaling.

A third possibility is that the population of TG and Vc neurons that responds to both pruritogens and algogens (Fig. 6, light gray) signals both itch and pain simultaneously. This concept is supported by recent psychophysical evidence that intradermal insertion of spicules of cowhage, or inactivated cowhage spicules loaded with histamine or capsaicin, all elicit similar complex sensations consisting of itch accompanied by nociceptive sensations of pricking-stinging and/or burning (LaMotte et al. 2009; Sikand et al. 2009). The degree to which itch or nociceptive sensation dominates may depend on the degree of activation of pruritogen- or algogen-selective neural populations in combination with the nonselective neurons. Figure 6 presents a model for the differential behavioral effects of pruritogens and algogens. Pruritogens elicited hindlimb scratches directed to the cheek injection site, implying that the trigeminal sensory stimulus ultimately engages networks in the lumboesacral spinal cord that govern extensor and flexor motoneurons driving the rhythmic back-and-forth hindlimb scratch movements. In contrast, algogens elicited ipsilateral forelimb cheek wipes, implying that the sensory stimulus ultimately engages a neural circuit in the ipsilateral cervical enlargement that governs motoneurons driving the forelimb motion. Future studies are needed to elucidate the detailed neural circuitry that underlies the ability of pruritic and algogenic trigeminal stimulation to differentially elicit these distinct motor responses.


