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Intradermal endothelin-1 excites bombesin-responsive superficial dorsal horn neurons in the mouse

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Akiyama T, Nagamine M, Davoodi A, Iodi Carstens M, Cevikbas F, Steinhoff M, Carstens E. Intradermal endothelin-1 excites bombesin-responsive superficial dorsal horn neurons in the mouse. *J Neurophysiol* 114: 2528–2534, 2015. First published August 26, 2015; doi:10.1152/jn.00723.2015.—Endothelin-1 (ET-1) has been implicated in nonhistaminergic itch. Here we used electrophysiological methods to investigate whether mouse superficial dorsal horn neurons respond to intradermal (id) injection of ET-1 and whether ET-1-sensitive neurons additionally respond to other pruritic and algesic stimuli or spinal superfusion of bombesin, a homolog of gastrin-releasing peptide (GRP) that excites spinal itch-signaling neurons. Single-unit recordings were made from lumbar dorsal horn neurons in pentobarbital-anesthetized C57BL/6 mice. We searched for units that exhibited elevated firing after id injection of ET-1 (1 μg/μl). Responsive units were further tested with mechanical stimuli, bombesin (spinal superfusion, 200 $\mu g \cdot ml^{-1} \cdot min^{-1}$), heating, cooling, and additional chemicals [histamine, chloroquine, allyl isothiocyanate (AITC), capsaicin]. Of 40 ET-1-responsive units, 48% responded to brush and pinch [wide dynamic range (WDR)] and 52% to pinch only [high threshold (HT)]. Ninety-three percent responded to noxious heat, 50% to cooling, and >70% to histamine, chloroquine, AITC, and capsaicin. Fifty-seven percent responded to bombesin, suggesting that they participate in spinal itch transmission. That most ET-1-sensitive spinal neurons also responded to pruritic and algesic stimuli is consistent with previous studies of pruritogen-responsive dorsal horn neurons. We previously hypothesized that pruritogen-sensitive neurons signal itch. The observation that ET-1 activates nociceptive neurons suggests that both itch and pain signals may be generated by ET-1 to result in simultaneous sensations of itch and pain, consistent with observations that ET-1 elicits both itch- and pain-related behaviors in animals and burning itch sensations in humans.

endothelin-1; itch; pain; superficial dorsal horn; bombesin

CHRONIC ITCH is a common and costly symptom of many dermatological conditions as well as a variety of systemic diseases. The neurobiological bases of normal and pathophysiological itch transmission are incompletely understood but have recently come under intensive investigation (for recent reviews see Akiyama and Carstens 2013; Bautista et al. 2014; Dhand and Aminoff 2014; Ikoma et al. 2006; Kremer et al. 2014; LaMotte et al. 2014; Ross 2011; Steinhoff et al. 2006; Steinhoff and Ikoma 2011; Twycross et al. 2003). Acute itch can be triggered by numerous agents acting via a variety of receptors including histamine H1 receptors, protease-activated receptor (PAR)-2 and PAR-4, Mas-related G protein-coupled receptor (Mrgpr)A3, MrgprC11, and MrgprD, and many others.

One endogenous mediator that has recently been implicated in itch is endothelin-1 (ET-1). ET-1 is a 21-amino acid peptide that acts at endothelin-A (ET-A) and -B (ET-B) receptors expressed in endothelial and immune cells and in sensory neurons. ET-1 is recognized to be important in mediating acute pain and hyperalgesia (Barr et al. 2011; Hans et al. 2008, 2009; Khodorova et al. 2009). Intraplantar injection of ET-1 elicited pain-related paw flinching and other nocifensive behaviors (Gokin et al. 2001; Kawamata et al. 2009) and activated mechanosensitive nociceptors via the ET-A receptor (Gokin et al. 2001). However, a number of studies implicate ET-1 in itch. Intradermal (id) injection of ET-1 in humans was reported to elicit "burning" itch (Katugampola et al. 2000; Kido-Nakahara et al. 2014; Wenzel et al. 1998) or to elicit pain sometimes accompanied by itch, associated with activation of C-fiber mechanosensitive nociceptors (Namer et al. 2008). In humans ET-1 evoked a brief pain sensation and prolonged itch that was only weakly attenuated by an antihistamine (Kido-Nakahara et al. 2014), and ET-1 was shown to be upregulated in patients with chronic itch due to prurigo nodularis (Kido-Nakahara et al. 2014) or atopic dermatitis (Aktar et al. 2015), suggesting a role for ET-1 in chronic itch. Intradermal injection of ET-1 elicited scratching behavior in rats and mice (Gomes et al. 2012; Imamachi et al. 2009; Kido-Nakahara et al. 2014; Liang et al. 2010a, 2010b, 2011; McQueen et al. 2007; Trentin et al. 2006). With the "cheek" model (Akiyama et al. 2010a; Shimada and LaMotte 2008), id injection of ET-1 elicited hindlimb scratching (indicative of itch) as well as forelimb wiping responses (indicative of pain) (Gomes et al. 2012), suggesting that ET-1 elicits both itch and pain sensations. ET-1 has also been implicated in the scratch-inducing effects elicited by the protease cathepsin E (Andoh et al. 2012). ET-1-evoked scratching was attenuated by the ET-A receptor antagonist BQ123 (Liang et al. 2010a; McQueen et al. 2007; Trentin et al. 2006) but was not affected by an antagonist of the ET-B receptor (McQueen et al. 2007). ET-1-evoked scratching was attenuated by an antagonist of the transient receptor potential ion channel ankyrin-1 (TRPA1) (Liang et al 2010b) and was significantly attenuated in knockout mice lacking TRPA1 (Kido-Nakahara et al. 2014) but not in TRPV1 knockout mice (Imamachi et al. 2009; Kido-Nakahara et al. 2014) or mice lacking phospholipase C β3 (PLCβ3) (Imamachi et al. 2009). Endothelin-converting enzyme-1 (ECE1) regulates ET-1-induced internalization and recycling of the ET-A receptor in

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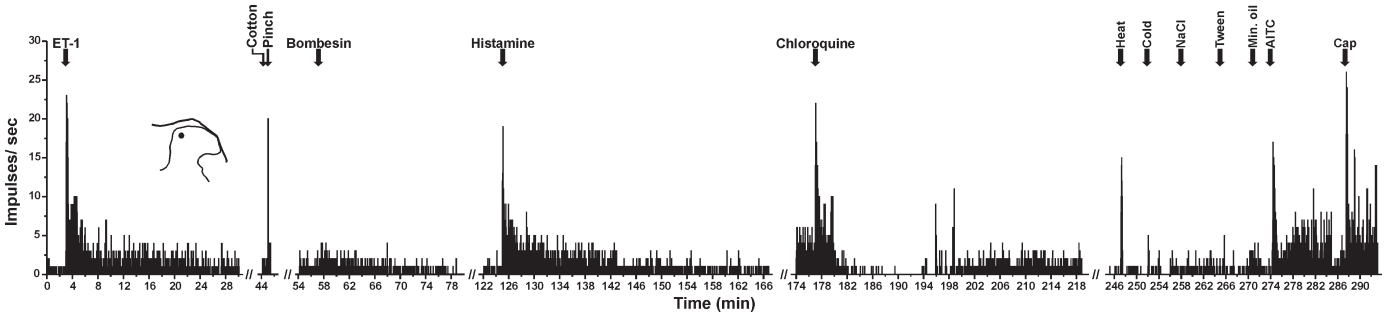

Fig. 1. Example of endothelin-1 (ET-1)-responsive unit. Shown are peristimulus time histograms (PSTHs; bins: 1 s) of, from *left* to *right*, the unit's response to intradermal (id) injection of ET-1, cotton brush, pinch, id histamine, id chloroquine, heat and cold stimuli, id saline and Tween (vehicle controls), topical mineral oil, topical allyl isothiocyanate (AITC), and id capsaicin. *Inset*: spinal recording site (dot) on drawing of lumbar spinal cord section.

dorsal root ganglion (DRG) neurons, and inhibition of ECE1 substantially enhanced ET-1-evoked scratching behavior (Kido-Nakahara et al. 2014).

ET-1 injections in skin elicited Fos expression in neurons in the superficial spinal dorsal horn (Imamachi et al. 2009; Kawamata et al. 2009), an area containing neurons responsive to pruritogenic stimulation (Akiyama and Carstens 2013). One aim of the present study was to determine whether neurons in the superficial dorsal horn respond to id injection of ET-1 in a manner consistent with itch and/or pain. We thus investigated whether ET-1-responsive dorsal horn neurons additionally responded to other pruritic and algesic stimuli. We additionally tested whether ET-1-responsive neurons responded to spinal superfusion with bombesin, an agonist of the gastrin-releasing peptide (GRP) receptor that has been implicated in spinal transmission of itch (Akiyama et al. 2013b, 2014a, 2014b; Sun et al. 2009; Sun and Chen 2007). A preliminary report of this study was presented in abstract format (Akiyama et al. 2013a).

METHODS

The procedures used in this study were approved by the University of California Davis Animal Care and Use Committee. Methods were similar to those described in our recent study (Akiyama et al. 2014a) and are summarized here. A total of 46 male C57BL/6 mice (25–29 g) were anesthetized with pentobarbital sodium (60 mg/kg ip), and a laminectomy was performed to expose the lumbar spinal cord for extracellular single-unit recording. The spinal cord was continually superfused with artificial cerebrospinal fluid (ACSF) consisting of (in mM) 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose, equilibrated with 95% O_2-5% CO_2 at 37°C. Extracellular action potentials were amplified and displayed with PowerLab (AD Instruments, Colorado Springs, CO) and Spike2 (CED, Cambridge, UK) software. Only one unit was recorded in a given animal. Action potentials were continually monitored during recording to ensure that the unit was still present, sorted by spike size and waveform, quantified as number of action potentials per second, and displayed in peristimulus time histogram (PSTH) format with 1-s bins.

To isolate ET-1-sensitive neurons, a small microinjection of ET-1 (~0.25 μl, 0.1 μg/μl) was made in the hindpaw and the microelectrode was driven into the superficial dorsal horn to isolate ongoing action potential firing. After activity subsided, a second id microinjection of ET-1 (1 μg in 1-μl volume) was made. Units were considered responsive if they exhibited a >30% increase in firing rate. Subsequent analysis revealed that the response of these units to ET-1 was >30% greater than their response to vehicle. Next, a variety of additional stimuli were tested. The stimulus order was as follows. First, the cutaneous receptive field was mapped with cotton, brush, and pinch stimuli. Units were categorized as wide dynamic range (WDR) type if they differentially responded to innocuous brush and noxious pinch or as high threshold (HT) if they responded to pinch but not brush. After this, in many experiments the unit was next tested with spinal superfusion of bombesin as previously described (Akiyama et al. 2014a). The ACSF superfusion solution was replaced with ACSF containing bombesin (40 μg/ml; 10 ml/min) delivered to the spinal cord for 1 min, followed by switching back to ACSF alone. Regardless of whether bombesin was tested or not, the unit was next tested with id histamine (50 μg/1 μl), followed in some experiments by id chloroquine (100 μg/1 μl), thermal testing with noxious heat (to 54°C) and cooling (to 4°C) delivered by a computer-controlled Peltier thermode, vehicles (id saline, id 7% Tween 80, topical mineral oil), topical application of allyl isothiocyanate (AITC; 75% in 2 μl), and finally id capsaicin (30 μg/1 μl).

For ET-1, histamine, chloroquine, and bombesin, each unit's activity was summed over a 3-min period before the stimulus and again in 3-min epochs after stimulus application. Each 3-min epoch after stimulus out to 30 min was compared with the prestimulus baseline by paired *t*-test, with $P < 0.05$ set as significant. Responses to thermal stimuli, AITC, and capsaicin were similarly analyzed by summing

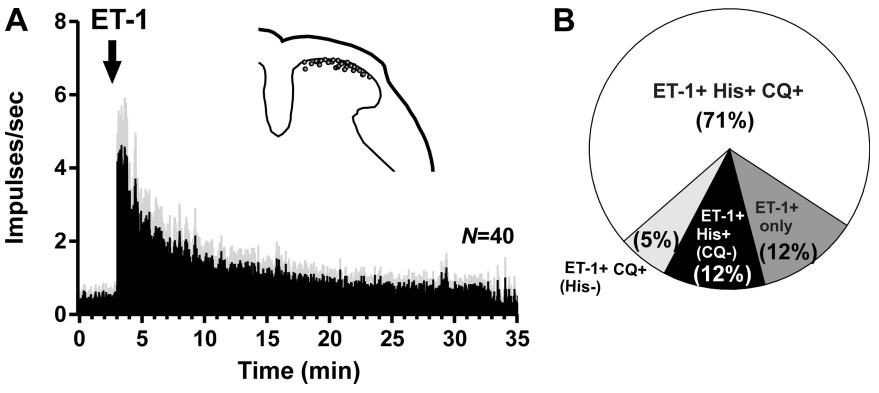

Fig. 2. Mean response to ET-1. *A*: averaged PSTH (bins: 1 s) of responses of 40 units to id injection of ET-1. Gray error bars are SE. *Inset*: histologically recovered recording sites (dots) compiled on a representative lumbar spinal cord section. *B*: % of ET-1-responsive units ($n = 40$) that also responded to histamine and/or chloroquine.

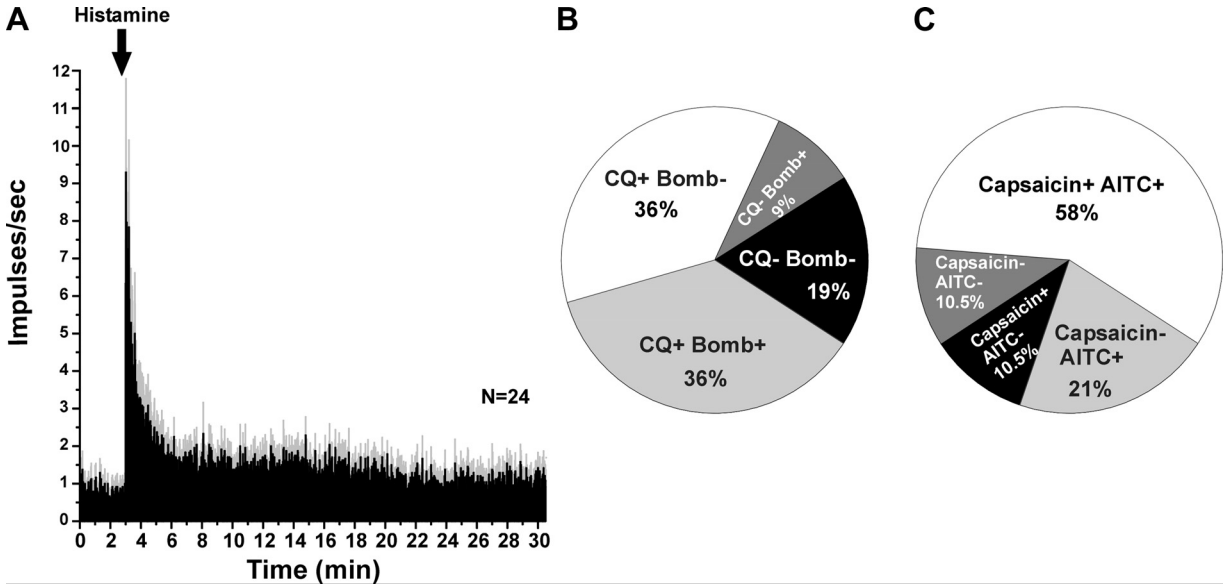

Fig. 3. Responses of ET-1-sensitive units to id histamine and other pruritogens. *A*: averaged PSTH of response of 24 units to id injection of histamine (format as in Fig. 2*A*). For the 24 histamine-responsive units, action potential firing summed over 3 min after histamine was significantly greater compared with firing summed over the 3 min before histamine ($P < 0.005$, paired *t*-test). *B*: most (81%) of the 24 histamine-responsive units also responded to id injection of chloroquine and/or spinal superfusion with bombesin. *C*: most (90.5%) histamine-responsive units also responded to id capsaicin and/or topical AITC.

action potential activity over a 60-s period prior to and again after the stimulus and compared by *t*-test. Cotton brush and noxious pinch stimuli were applied for 10 s; baseline firing was recorded over a 30-s period before and after each stimulus and compared by paired *t*-test. The poststimulus analysis period was longer than the stimulus duration to capture afterdischarges of the units to the mechanical stimuli.

At the end of each experiment, an electrolytic lesion was made at the recording site and the spinal cord was postfixed in 10% buffered formalin. Spinal cord sections were cut and examined by light microscope to identify lesion sites.

RESULTS

Unit sample. A total of 46 units responsive to the ET-1 search stimulus were identified. Of these, 40 units exhibited increased firing after id injection of 1 μg/μl ET-1. All unit recordings were in the superficial dorsal horn at a mean depth of 132.8 $\pm$ 12.7 (SE) μm below the surface of the lumbar spinal cord. For most units the location was confirmed by post hoc histological identification of lesion sites (see Fig. 2*A*, *inset*).

Response to ET-1 and other chemical stimuli. An example of an ET-1-responsive unit is shown in Fig. 1. This unit was localized to the superficial dorsal horn and responded robustly to id injection of ET-1 with a discharge lasting >30 min (Fig. 1, *left*). It responded to noxious pinch but not innocuous brush and was classified as HT type. It additionally responded to spinal superfusion with bombesin, id histamine, id chloroquine (with a postexcitatory reduction in firing), noxious heat, AITC, and capsaicin, with a weak response to cooling. This unit did not respond to id injections of the vehicles isotonic saline or 7% Tween 80 and responded weakly to topical application of mineral oil.

Figure 2*A* shows the averaged response of 40 units to id injection of ET-1. A peak response was achieved during the first minute after injection and persisted for >30 min (Fig. 2*A*). The summed action potential firing over the 30-min period

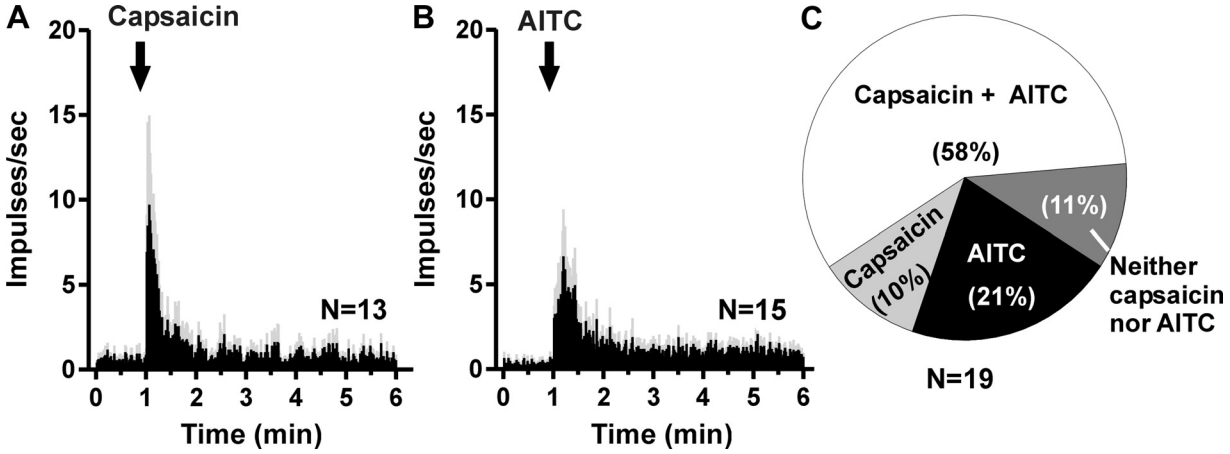

Fig. 4. Responses to algogens. *A*: mean response of ET-1-responsive units to capsaicin. *B*: mean response of ET-1-responsive units to AITC. *C*: % of ET-1-responsive units that additionally responded to capsaicin and/or AITC or neither.

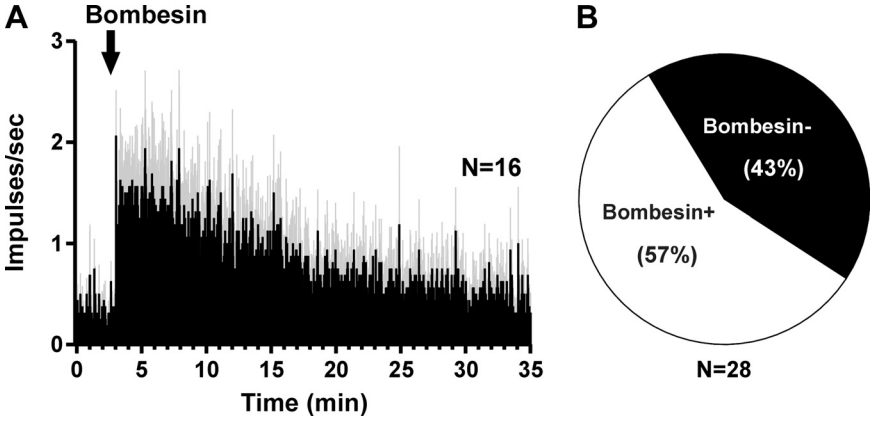

Fig. 5. Mean response to spinal superfusion of bombesin (format as in Fig. 2*A*). *A*: averaged PSTH of responses of 16 ET-1-sensitive units to spinal superfusion of bombesin. *B*: % of ET-1-responsive units that did (bombesin+) or did not (bombesin−) respond to spinal superfusion of bombesin.

after ET-1 injection was significantly greater compared with the 3-min period preceding ET-1 injection [$P < 0.05$ to <0.001 for all 3-min intervals after ET-1, paired *t*-test; pre: 69.5 ± 18.9 (SE) impulses/3 min vs. post-3 min: 508.6 ± 106.7]. Of units tested subsequently for responses to the pruritogens histamine and chloroquine, the vast majority responded to both, while some responded to one but not the other; only 12% of ET-1-sensitive units did not respond to either histamine or chloroquine (Fig. 2*B*).

Eighty-three percent (24/29) of the ET-1-responsive units tested responded to id injection of histamine, the averaged response of which is shown in Fig. 3*A*. The response to histamine peaked within the first minute after injection. Unit activity summed over a 3-min period was significantly greater after vs. before histamine ($P < 0.005$, paired *t*-test; pre: 127 ± 29.9 impulses/3 min vs. post-3 min: 497.1 ± 115.8). Figure 3*B* shows that the majority of ET-1- and histamine-sensitive units additionally responded to id injection of chloroquine (16/21 units tested; 76%) and/or spinal superfusion of bombesin (16/28 units tested; 57%). Thus many ET-1-responsive units additionally respond to other pruritic stimuli.

Because previous studies have shown that prurisponsive spinal neurons also respond to the algogens capsaicin and AITC, we tested whether ET-1-responsive units responded to these agents. Figure 4*A* shows the averaged response of ET-1-sensitive units to id injection of capsaicin. Of 19 tested units, 13 (68.4%) responded to capsaicin and 15 (79%) responded to AITC. Unit responses summed over a 1-min period after capsaicin were significantly greater compared with activity summed over the corresponding period preceding capsaicin ($P < 0.05$, paired *t*-test; pre: 32.7 ± 9.7 impulses/60 s vs. post-60 s: 180.3 ± 68.9). Similarly, Fig. 4*B* shows the averaged response to AITC, which was significantly greater during the first minute after injection ($P < 0.05$, paired *t*-test; pre: 19.8 ± 5.6 impulses/60 s vs. post-60 s: 176 ± 56.9). Figure 4*C* shows that 89% of the ET-1-sensitive units responded to capsaicin and/or AITC. Similarly, >89% of ET-1- and histamine-sensitive units responded to capsaicin and/or AITC (Fig. 3*C*). Thus the vast majority of ET-1-responsive units also respond to algogenic stimuli, confirming previous studies of spinal neurons responsive to other pruritogens.

We tested responses of ET-1-sensitive units to spinal superfusion of bombesin, reasoning that itch-signaling neurons express the GRP receptor and should be activated by bombesin. Of the 28 units tested, 16 (57.1%) responded. Figure 5*A* shows the averaged response of the 16 bombesin-responsive units. The mean response peaked within the first minute of bombesin superfusion, and summed activity was significantly greater during the initial 3 min after bombesin compared with the 3-min period before bombesin ($P < 0.05$, paired *t*-test; pre: 34.3 ± 7.8 impulses/3 min vs. post-3 min: 208.3 ± 64.6). Overall, the majority of ET-1-sensitive units responded to bombesin (Fig. 5*B*), consistent with a role in signaling itch (Akiyama et al. 2014a).

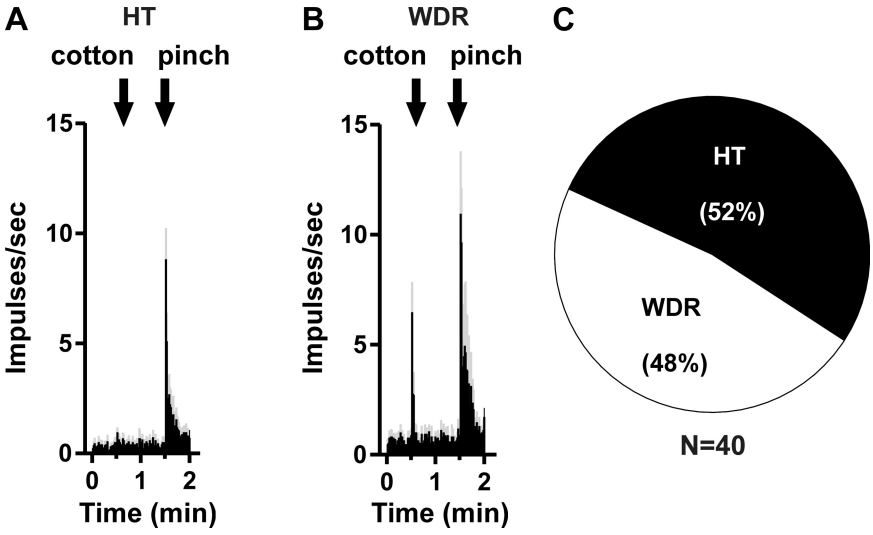

Fig. 6. Mean responses of ET-1-responsive high-threshold (HT) and wide-dynamic range (WDR) units to light brush with cotton and noxious pinch stimuli. *A*: averaged PSTH of responses of HT units to cotton brush and pinch. The mean firing over 30 s after pinch was significantly greater compared with that before pinch ($P < 0.005$, paired *t*-test). *B*: averaged PSTH of WDR unit responses to brushing with a cotton wisp and to noxious pinch. The mean firing was significantly different over the 30-s periods before compared with after cotton brush ($P = 0.001$, paired *t*-test) and between before and after pinch ($P < 0.001$, paired *t*-test). *C*: % of ET-1-responsive units that were classified as HT or WDR.

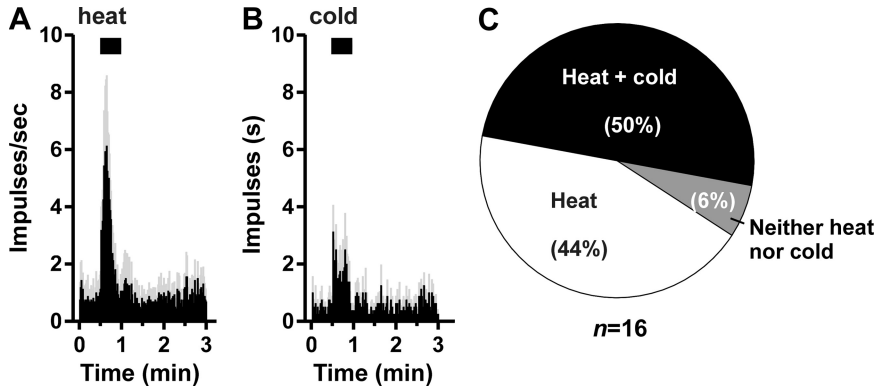

Fig. 7. Thermosensitivity of ET-1-responsive units. *A*: PSTH of mean responses of ET-1-responsive units to noxious heat. The summed firing over 30 s was significantly greater before vs. after heat ($P < 0.005$, paired *t*-test). *B*: PSTH of mean response to cooling. Summed activity over 30 s was significantly greater before vs. after cooling ($P = 0.05$, paired *t*-test). *C*: % of ET-1-responsive units that responded to noxious heat and/or cold or neither.

ET-1-sensitive units were generally unresponsive to vehicles. Thus of 19 units tested with id injection of saline, there was no significant difference ($P > 0.1$, paired *t*-test) in the summed activity 1 min after compared with before injection. Similarly, neither id injection of Tween 80 nor topical application of mineral oil had any significant effect on firing during the 1-min period after vs. before application ($P > 0.1$ for both, paired *t*-test, $n = 19$ for Tween 80 and mineral oil groups).

Mechanically evoked responses. ET-1-sensitive units were tested for mechanosensitivity. Figure 6*A* shows the averaged response of units that responded to noxious pinch but not innocuous brush with a cotton wisp. These units were classified as HT and constituted just over half of the sample (Fig. 6*C*). Figure 6*B* shows averaged responses of units that responded to both the innocuous cotton brush stimulus and noxious pinch. These units were classified as WDR and constituted 48% of the sample (Fig. 6*C*).

Thermally evoked responses. Of those ET-1-sensitive units tested, 94% responded to noxious heating of the cutaneous receptive field and 50% responded to cooling (Fig. 7). Figure 7, *A* and *B*, show averaged responses of heat- and cold-evoked responses, respectively. Both heat- and cold-evoked responses were significantly greater during the 30-s period after stimulus compared with prestimulus baseline ($P < 0.05$, $P = 0.05$, respectively, paired *t*-test; heat: pre 13.5 ± 5.1 impulses/60 s vs. post: 64.6 ± 11/1; cold: pre 12.9 ± 6.2 impulses/60 s vs. post: 47.6 ± 18.3). Figure 7*C* shows the percentages of units responsive to heat and/or cold stimuli.

DISCUSSION

Because ET-1 elicits itch-related scratching behavior in rodents (Gomes et al. 2012; Imamachi et al. 2009; Kido-Nakahara et al. 2014; Liang et al. 2010a, 2010b, 2011; McQueen et al. 2007; Trentin et al. 2006) and itch sensation in humans (Katugampola et al. 2000; Kido-Nakahara et al. 2014; Wenzel et al. 1998), we here investigated whether superficial dorsal horn neurons in mice respond to id injection of ET-1 in a manner consistent with a role in signaling itch. Our search strategy revealed a population of superficial dorsal horn neurons that responded to id injection of ET-1. These were classified as HT and WDR, and their responses to id ET-1 peaked within the first few minutes after injection and persisted for at least 30 min, consistent with the time course of ET-1-evoked scratching behavior. Most ET-1-sensitive neurons additionally responded to the itch mediators histamine and chloroquine, to spinal superfusion of bombesin that is thought to target itch-signaling spinal neurons (Akiyama et al. 2014a), as well as to algogenic stimuli, consistent with many previous studies of pruritogen-sensitive superficial dorsal horn neurons (Akiyama et al. 2009a, 2009b, 2010b; Davidson et al. 2012; Jinks and Carstens 2002; Moser and Giesler 2014; Simone et al. 2004). Thus our data are consistent with the hypothesis that ET-1-responsive superficial dorsal horn neurons signal itch sensation.

Given our use of id injection of ET-1 into naive skin as a search stimulus, it was not possible to determine whether the ET-1 may have sensitized neuronal responses to subsequent mechanical or other stimuli. A previous study from our laboratory reported that id injection of histamine sensitized subsequent mechanically evoked responses of histamine-responsive (but not histamine insensitive) dorsal horn neurons (Akiyama et al. 2014c), providing a potential mechanism for alloknesis. Further studies are needed to determine whether ET-1 elicits alloknesis and sensitizes spinal itch-signaling neurons.

We previously reported that most superficial dorsal horn neurons identified by their response to id injection of chloroquine also responded to spinal superfusion with bombesin (Akiyama et al. 2014a). Conversely, most units identified by their response to spinal superfusion of bombesin also responded to id injections of the itch mediators chloroquine, histamine, and/or SLIGRL, an agonist of PAR-2 and MrgprC11 (Liu et al. 2011). In contrast, very few pruritogen-insensitive nociceptive neurons responded to spinal superfusion of bombesin. It was argued that spinal bombesin acts at GRP receptors expressed by spinal neurons that are essential for the central transmission of itch (Akiyama et al.

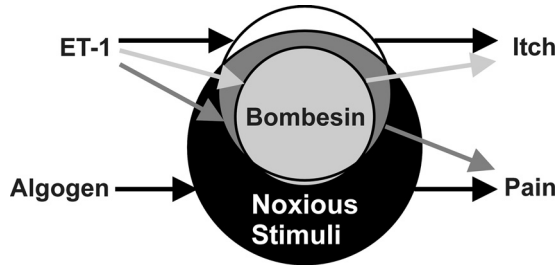

Fig. 8. Summary. Venn diagram shows that most, but not all, ET-1-responsive units (white) also responded to algogens (dark gray overlap) and bombesin (light gray overlap). A larger population responded to algogens and other noxious stimuli but not pruritogens (black); this subpopulation is proposed to signal pain. It is speculated that the bombesin- and ET-1-sensitive subpopulation (light gray) signals itch, while the ET-1-sensitive, bombesin-insensitive subpopulation (dark gray) signals pain. This would explain why ET-1 simultaneously evokes both itch- and pain-related behaviors.

2014a). In the present study, the majority of ET-1-responsive neurons also responded to spinal superfusion with bombesin, consistent with our prior study and shown schematically in Fig. 8. There is considerable overlap of pruritogen- and bombesin-sensitive neurons that are proposed to signal itch, with much less overlap between bombesin-sensitive neurons and pruritogen-insensitive nociceptive neurons that are proposed to signal pain (Fig. 8).

It is important to note that ET-1 elicits behavioral signs of pain as well as itch, and ET-1 has been associated with pain and hyperalgesia (Barr et al. 2011; Hans et al. 2008, 2009). Using the cheek model, Gomes et al. (2012) showed that id injection of ET-1 elicited significant, dose-dependent increases in both hindlimb scratch bouts (indicative of itch) and forelimb wipes (indicative of pain) directed to the injection site, interpreted to indicate that ET-1 elicits a mixed itch and pain sensation. Our present data are potentially consistent with this, since ET-1-sensitive neurons also responded to noxious pinch and most responded to application of the algogens capsaicin and/or AITC. We have previously hypothesized that such pruritogen-sensitive neurons signal itch, even though they also respond to noxious stimuli. Given that ET-1 elicits both itch and pain sensations and associated behavioral responses, our hypothesis requires reconsideration. One possibility is that the subpopulation of neurons responsive to ET-1 and the GRP receptor agonist bombesin (light gray overlap area, Fig. 8) signals itch. This is consistent with previous studies reporting that pruritogen-evoked scratching behavior is significantly attenuated in animals lacking the GRP receptor (Sun and Chen 2007) or in animals in which GRP receptor-expressing spinal neurons were neurotoxically ablated (Mishra and Hoon 2013; Sun et al. 2009). We further speculate that the subpopulation of neurons activated by ET-1 but not bombesin (dark gray overlap area, Fig. 8) signals pain rather than itch. The approximate 60:40 split in bombesin-sensitive vs. -insensitive neurons that were excited by ET-1 implies that ET-1 coactivates both itch- and pain-signaling spinal neurons. This would explain the ability of ET-1 to elicit signs of both itch and pain in behavioral studies (Gomes et al. 2012). In addition to ET-1, we previously reported that cheek application of cowhage spicules and id injection of serotonin or formalin also elicit forelimb wipes as well as hindlimb scratch bouts (Akiyama et al. 2010a). Thus these stimuli along with ET-1 appear to be capable of eliciting dual itch and pain sensations.

ET-1 presumably elicits itch and pain via activation of nociceptive afferent fibers. In rats, intraplantar injection of ET-1 elicited hindpaw flinching and other nocifensive behaviors (Gokin et al 2001; Kawamata et al. 2009) and activated C- and Aδ-fiber nociceptors in a dose-dependent manner that was blocked by an ET-A receptor antagonist (Gokin et al. 2001). Interestingly, most responses exhibited a bursting pattern, as also observed for responses of monkey C-fiber nociceptors to cowhage spicules that elicit itch (Johanek et al 2008). In humans, id injection of ET-1 activated mechanosensitive but not mechanoinsensitive C-fiber nociceptors, often with prolonged (>15 min) discharges, and usually evoked a sensation of pain that was accompanied by itch in three subjects, with one subject only reporting itch (Namer et al. 2008). We reported more recently that superficial id prick testing with ET-1 in human subjects resulted in a brief pain sensation (seconds), followed by a sustained itch sensation persisting for >5 min (Kido-Nakahara et al. 2014). These data indicate that ET-1 is predominantly a pruritogen in humans but also has brief algogenic activity, probably via activation of ET-A and/or ET-B receptors on C- and/or A-delta nociceptors. Future experiments are required to clarify the role of ET-1 in chronic pruritic human skin.

Using calcium imaging, we recently reported that ~3% of cultured mouse DRG cells responded to application of ET-1; of these, many also responded to histamine (69%), chloroquine (24%), capsaicin (85.5%), and/or AITC (73.5%) (Kido-Nakahara et al. 2014). We presently observed that ET-1-sensitive dorsal horn neurons also frequently responded to histamine (83%), chloroquine (76%), capsaicin (69%), and/or AITC (79%). Thus ET-1-sensitive dorsal horn neurons and the primary sensory afferents activating them exhibit broad tuning to a variety of chemonociceptive agents.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.A., M.S., and E.C. conception and design of research; T.A., M.N., A.D., M.I.C., and F.C. performed experiments; T.A. and E.C. analyzed data; T.A. and E.C. interpreted results of experiments; T.A. and E.C. prepared figures; T.A., M.S., and E.C. edited and revised manuscript; T.A. and E.C. approved final version of manuscript; E.C. drafted manuscript.

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