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Inhibitory effect of lidocaine on pain and itch using formalin-induced nociception and 5'-guanidinonaltrindole-induced scratching models in mice: behavioral and neuroanatomical evidence

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Abstract

The aim of this study was to establish the effect of lidocaine, a local anesthetic, on pain and itch using formalin-induced nociception and kappa opioid antagonist-induced scratching models in mice. We investigated if local intradermal pretreatment (at –10 min) with lidocaine N-ethyl bromide (lidocaine, 2%, 0.1 ml) antagonizes behavioral responses and prevents c-fos expression induced by pain and itch. Male, Swiss Webster mice (25–30 g, n=6–10) were used. Formalin (5%, 20 µl, s.c.) or saline was administered to the right dorsal hindpaw and the time spent licking this paw was recorded at 0–10 min and 20–35 min. For itching, mice were challenged with 5'-guanidinonaltrindole (GNTI, 0.3 mg/kg, s.c., behind the neck) or saline and the number of neck-directed scratches with hindpaws was counted for 30 min. C-fos immunohistochemistry was performed in lumbar (for pain) and cervical (for scratching) spinal sections 2 h after respective treatments. We found that lidocaine (a) antagonizes both formalin-induced pain and GNTI-induced scratching and (b) prevents c-fos expression evoked by pain (medial side of the superficial layer and deeper layers of the dorsal horn) and itch (lateral side of the superficial layer of the dorsal horn). Additionally, GNTI caused c-fos activation in mice wearing an Elizabethan collar (to prevent scratching of the neck) suggesting that GNTI provokes c-fos expression by inducing an itch sensation. Our results highlight the antipruritic properties of lidocaine and argue for its comprehensive clinical testing against pruritic states.

Keywords

itch; pain; formalin; GNTI; c-fos expression

1. Introduction

There are similarities and differences as well as interactions between pain and itch. While the pain sensation evokes withdrawal behavior, itch is a helpful warning of potential hazards and

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evokes scratching as a reflex or conscious mechanical stimulation to relieve the hazardous stimulant. Primary afferents for pain sensation are mechano-sensitive and mechano-insensitive A δ - and polymodal C-nociceptors with fast conduction velocities (Schmidt et al., 2000; Andrew and Craig, 2001) whereas primary afferents for itch sensation (defined in human skin to date) are non-nociceptive, histamine-activated mechano and heat-insensitive C-fibers with a slow conduction velocity (Schmelz et al., 1997) and cowhage-activated mechano-sensitive fibers (Namer et al., 2008). Both stimuli follow the spinothalamic pathway, however, neurons stimulated by histamine project to the posterior part of the ventromedial nucleus of the thalamus (Andrew and Craig, 2001) whereas neurons stimulated by pain project to the ventroposterior lateral nucleus of the thalamus (Craig, 2002).

In the present study, we compared the effects of locally administered lidocaine N-ethyl bromide (peripherally restricted lidocaine) on pain and itch stimuli using formalin-induced nociception and kappa opioid receptor antagonist-induced excessive scratching models in mice. Formalin nociception is, of course, one of the most commonly utilized murine pain models (Hunskar et al., 1985; Vaccarino et al., 1989; Staniland and McMahon, 2008). Previously, we reported that a highly selective kappa opioid receptor antagonist, 5'-guanidinonaltrindole (GNTI) (Jones and Portoghese, 2000; Black et al., 2003), precipitates immediate excessive scratching in mice (Cowan and Inan, 2009) using the standard model described by Kuraishi et al. (1995). In this procedure, scratching behavior is used as a quantitative index for the study of itch. For example, a submaximal dose of GNTI (0.3 mg/kg, s.c.) can cause an impressive 605 ± 69 scratching bouts in 30 min. In the present work, we used two different approaches: first, we studied if pretreatment with lidocaine antagonizes the behavioral response elicited by either formalin-induced nociception or GNTI-induced excessive scratching and second, we examined if pretreatment with lidocaine inhibits spinal cord c-fos expression evoked by the pain and scratch stimuli. Preemptive use of local anesthetics in humans aims to prevent activation of peripheral nociceptors (by tissue damage) which causes hyperexcitability in the dorsal horn. Our key question was whether lidocaine, administered before a scratch stimulus, would prevent activation of peripheral nerve fibers. Here, we show for the first time, the suppressant effect of lidocaine on behavioral and neuronal responses evoked by a scratch stimulus in mice.

2. Materials and methods

2.1. Animals

Male, Swiss Webster mice (25–30 g, n = 6–10, Ace Laboratories, Boyertown, PA) were used. Animals were housed under a 12 h light/dark cycle with food and water available ad libitum. Experiments were carried out between 10:00 AM and 5:00 PM. Experimental procedures were approved by the Temple University Institutional Animal Care and Use Committee, in accordance with the 1996 NIH Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral studies

Mice, brought to the laboratory on the morning of the experimental day for acclimation, were placed in individual observation boxes (18 cm $\times$ 23 cm $\times$ 25 cm).

2.2.1. Formalin-induced nociception—Mice were injected i.d. to the dorsal side of the right hind leg (to block sensation on the dorsal side of the hindpaw) with saline or 2% lidocaine (0.1 ml) using a 30-gauge needle fitted to a 50 μ l Hamilton syringe. Ten min later, the animals received saline or formalin (5%, 20 μ l, s.c.) to the dorsal side of the right hindpaw and the time spent licking this paw was recorded at 0–10 min and 20–35 min using a stopwatch. Mice were perfused 2 h after the saline or formalin injection for c-fos immunohistochemistry (IHC).

2.2.2. Kappa opioid antagonist-induced scratching—After acclimation in their observation box for at least 2 h, mice were injected i.d. to the back of the neck with saline or 2% lidocaine (0.1 ml) using a 30-gauge needle fitted to a 50 µl Hamilton syringe. Ten min later, they were given saline or GNTI (0.3 mg/kg, s.c.) behind the neck and the number of hindleg scratches directed to the neck was counted for 30 min. Two h after the saline or GNTI injection, the mice were perfused for c-fos IHC.

2.2.3. Measurement of locomotor activity—Mice were placed in individual cages (27 cm × 48 cm × 20 cm) and acclimated for 1 h. They were injected i.d. behind the neck with either saline or 2% lidocaine (0.1 ml) and monitored for total distance traveled over 30 min using a Digiscan D Micro System (AccuScan, Columbus, OH).

2.3. Determination of c-fos immunoreactivity in spinal cord sections

Two h after administration of saline, GNTI or formalin, the mice were deeply anesthetized with urethane (1.2 g/kg, i.p.) and perfused intracardially with ice-cold 0.1 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS and 0.2% picric acid. The fixed cervical spinal cords (for scratching) and lumbar spinal cords (for pain) were removed and post fixed in 4% paraformaldehyde solution overnight at 4°C. Tissue samples were transferred to 30% sucrose solution for at least 3–4 days before sectioning. Cervical spinal cord (C5–C7) and lumbar spinal cord (L3–L5) were cut at 35 µm thickness at –19°C using a cryostat. Free floating sections were kept in PBS solution at 4°C until IHC was performed. Ten cervical or ten lumbar spinal cord sections from each mouse were randomly chosen to conduct IHC.

To establish if c-fos activation results from a GNTI-induced itch sensation, rather than from direct scratching behavior, additional mice were each fitted with an Elizabethan collar (Kent Scientific Corp., Torrington, CT) and allowed to acclimate for 6 h. The animals (n=3) were then injected s.c. with either saline or GNTI (or not injected) and, 2 h later, perfused intracardially and cervical spinal cord sections removed as mentioned above.

Tissues were processed for c-fos immunoreactivity by the avidin-biotin complex procedure as described previously (Brailoiu et al., 2005). They were first treated with 3% H₂O₂ to reduce endogenous peroxidase, washed two times for 10 min each with PBS, and blocked with 20% normal goat serum (1:20) at room temperature for 2 h. The sections were then incubated on a shaker for 2 days at 4°C with rabbit c-fos antibody 1:4000 dilution (F7799, Sigma, St. Louis, MO). After thorough rinsing, sections were incubated in biotinylated anti-rabbit immunoglobulin G secondary antibody (1:300 dilution, Vector Laboratories, Burlingame, CA) for 2 h at room temperature. Following two 10 min rinses with PBS, sections were incubated in avidin-biotin-peroxidase complex at room temperature for 90 min (1:150 dilution, Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA). Following three 10 min washes in Tris-buffered saline, sections were reacted with 0.05% diaminobenzidine (Sigma, St. Louis, MO)/ 0.001% H₂O₂ solution for 5–7 min and washed 3 times for 10 min with Tris-buffered saline. Sections were mounted on slides with 0.25% gel alcohol, air-dried, dehydrated with absolute alcohol (50%, 70%, 95%, 100%, 100% for 10 min each) followed by xylene (two times for 10 min each) and coverslipped with Permount. C-fos positive nuclei were observed under a light microscope and counted at 40x magnification.

2.4. Compounds

GNTI (Tocris, Ellisville, MO) and lidocaine N-ethyl bromide (Sigma, St. Louis, MO) were dissolved in saline.

2.5. Data analysis

Statistical comparisons were performed using a one-way analysis of variance (ANOVA), followed by the Newman-Keuls test to compare groups, and the unpaired Student's t-test to compare two groups. The ANOVA was followed by Newman-Keuls test for data in figures 1 and 2 and followed by Student's t-test for data in figures 3 and 7. All data are expressed as the mean $\pm$ standard error of the mean (S.E.M.). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Lidocaine antagonizes formalin-induced nociception

Mice pretreated with saline and then challenged with formalin produced a typical biphasic licking response (Murray et al., 1988; Choi et al., 2001), whereas mice challenged with saline rarely licked their paws ($P < 0.001$). Pretreating animals with lidocaine antagonized significantly ($P < 0.001$) formalin-induced licking in the first phase of the test. Licking in the second phase was also diminished by lidocaine but not to a statistically significant extent (Fig. 1).

3.2. Lidocaine antagonizes GNTI-induced scratching

Mice pretreated with saline and then injected with GNTI scratched 595 ± 71 times in 30 min. Pretreating mice with lidocaine significantly antagonized this excessive scratching (63.6 ± 33.7 ; $P < 0.001$) (Fig. 2). Mice pretreated with either saline or lidocaine and then challenged with saline did not scratch.

3.3. Lidocaine has no marked effect on locomotion

Locomotor activities of saline-injected and lidocaine-injected mice were similar (Fig. 3), suggesting that lidocaine caused no marked behavioral depression.

3.4. Lidocaine prevents pain-evoked c-fos expression

A representative picture of c-fos positive neurons activated by a pain stimulus and inhibition of activation of these neurons by lidocaine pretreatment is shown in Fig. 4. Formalin induced an increase in c-fos expression on the medial side of the superficial and deeper layers of the dorsal horn of the lumbar spinal cord in mice pretreated with saline. C-fos expression was not observed in sections obtained from mice pretreated with saline and then injected with saline. Similarly, no c-fos activation was detected in sections from mice pretreated with lidocaine and then challenged with saline (picture not shown). Pretreating animals with lidocaine significantly reduced the number of c-fos positive nuclei induced by formalin (Fig. 7) ($P < 0.001$).

3.5. GNTI-provoked c-fos expression is a result of itch sensation

GNTI induced c-fos expression in mice wearing an Elizabethan collar. C-fos expressing neurons were located on the lateral aspect of the superficial layer of the dorsal horn (Fig. 5).

3.6. Lidocaine reduces itch-evoked c-fos expression

A representative picture of c-fos positive nuclei activated by an itch stimulus and prevention of activation of these neurons by lidocaine pretreatment is shown in figure 6. C-fos positive nuclei were detected on the lateral side of the superficial lamina of the dorsal horn of the cervical spinal cord in mice treated with saline followed by GNTI. The number of c-fos positive nuclei induced by an itch stimulus significantly decreased in mice pretreated with lidocaine (Fig. 7) ($P < 0.001$).

Discussion

The major findings of the present study are 1) lidocaine antagonizes GNTI-induced scratching behavior in mice, and 2) lidocaine prevents neuronal activity evoked by both pain and scratch stimuli in the dorsal horn of the spinal cord by blocking excitatory input to the neurons.

To our knowledge, this is the first study to demonstrate anti-itch activity after local injection of lidocaine in an animal model of pruritus. Previously, Wheeler and colleagues (1988) reported that the topical application of 4% lidocaine to the neck of mice diminished peripherally, but not centrally, given bombesin-induced scratching. In our study, lidocaine almost completely inhibited scratching induced by GNTI. Lidocaine blocks voltage-gated sodium channels (VGSCs) which are responsible for the increase in sodium permeability during the rapid phase of the action potential in excitable cells like neurons, cardiac and muscle cells (Strichartz 1976; Sandtner et al., 2004). VGSCs are important for pain sensation. From nine distinct sodium channels, animal studies indicate that $\text{Na}_v1.7$, $\text{Na}_v1.8$ and $\text{Na}_v1.9$ are crucial for transmission of inflammatory pain and $\text{Na}_v1.3$ may play a role in neuropathic pain (Wood et al., 2004; Cummins et al., 2007). In addition, the present study shows prevention by lidocaine of c-fos expression which is a consequence of itching in mice. The demonstration of c-fos immunoreactivity in mice wearing an Elizabethian collar indicates that c-fos activation is a consequence of itch sensation induced by GNTI. C-fos expression is commonly used as a marker for neuronal activation. Taken together, antagonism of the GNTI-induced behavioral and neuronal responses by locally administered lidocaine is a result of suppression of peripheral nerve firing. This antagonistic effect is through inhibition of VGSCs, which are required for action potentials.

After a bolus intravenous injection of lidocaine to patients suffering from pruritus due to chronic liver disease, there is a decrease in the severity of this behavior (Fishman et al., 1997; Villamil et al., 2005). Also, a 1:1 mixture of lidocaine and prilocaine (EMLA cream) relieves cowhage and papain-induced pruritus (Shuttleworth et al., 1988) and is reportedly a safe and effective treatment of postburn pruritus in pediatric patients (Kopecky et al., 2001). This cream may relieve the itch of notalgia paresthetica, which is a chronic sensory neuropathy causing itch, pain and paresthesia (Layton and Cotterill, 1991). Transient drowsiness during i.v. injection of lidocaine to counter neuropathic pain has been reported as a side effect (Tremont-Lukats et al., 2005). We studied the peripherally restricted analog of lidocaine (QX-314) (Taylor et al., 1995; Abbadie et al., 1997; Binshtok et al., 2007; Lim et al., 2007). Nevertheless, we measured locomotion to discover if the anti-itch activity of lidocaine is a consequence of behavioral depression. Lidocaine did not markedly affect the spontaneous locomotion of mice suggesting that the anti-itch activity of lidocaine is not linked to behavioral depression.

The patch form of lidocaine has been found effective in neuropathic pain conditions such as post herpetic neuralgia and carpal tunnel syndrome in humans (Wasner et al., 2005; Nalamachu et al., 2006; Vadalouca et al., 2006; Geha et al., 2008). In the present study, pretreating mice with lidocaine antagonized licking in the first phase of the formalin test and decreased (but not to a significant extent) this behavioral response in the second phase. The first phase is caused by activation of peripheral nociceptors. The second phase is associated with ongoing activity in primary afferents and increased sensitivity of dorsal horn neurons (Murray et al., 1988; Taylor et al., 1995). In some previous reports, pretreatment with lidocaine (10–30 mg/kg, i.p.) antagonized the behavioral response in both phases of the test in mice (e.g., Bittencourt and Takahashi, 1997; Sahebgharani et al., 2006). However, Hitosugi et al. (1999) found that pretreating mice with the same doses of lidocaine (i.p.) only inhibited the behavioral response in the second phase. The choice of route of administration of lidocaine may be critical; i.e., intraperitoneal vs. intradermal. We also described an inhibitory effect of lidocaine on c-fos

expression evoked by the nociceptive stimulus. Yashpal et al. (1998) reported that lidocaine, given intrathecally before formalin, inhibits c-fos expression on the dorsal horn of the spinal cord in rats. In our study, formalin-induced nociception activated neurons located on the medial side of the superficial and deeper layers of the dorsal horn. Similar c-fos expression patterns were reported following the formalin test in mice (Bon et al., 2002; Zhao et al., 2003) as well as in different pain models in mice (acetic acid writhing) and rats (plantar incision) (de los Santos-Artega et al., 2003; Sun et al., 2004).

In summary, we report here, for the first time, that pretreating mice with locally administered lidocaine (a) antagonizes excessive scratching induced by GNTI and (b) prevents c-fos expression evoked by both pain and scratching, indicating that lidocaine blocks peripheral neuronal activity. Our results argue for the comprehensive clinical testing of lidocaine as an antipruritic against itch in regional skin conditions caused by poison ivy, notalgia paresthetica, bites and sunburn.

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Fig. 1.

Mice (n=10) were pretreated with either saline or lidocaine (i.d., hind leg at -10 min) and then injected with either saline or 5% formalin solution (s.c., i.paw). The time spent licking the injected paw was measured using a stop watch for 0–10 min and 20–35 min. Formalin induced licking behavior during both phases compared to saline (###P<0.001). Paw licking was significantly decreased (***P<0.001) in mice pretreated with lidocaine (i.d.) compared to mice pretreated with saline over 0–10 min, but not over 20–35 min (i.paw; intrapaw). (ANOVA followed by Newman-Keuls test).

Fig. 2.

Mice (n=8) were injected (i.d., behind the neck) with either saline or lidocaine and then, 10 min later, they were administered (s.c., behind the neck) either saline or GNTI (0.3 mg/kg). The number of scratches was counted for 30 min. Mice injected with saline then GNTI scratched significantly more often than mice injected with saline then saline ($P<0.001$). Pretreating mice with lidocaine significantly inhibited scratching behavior elicited by GNTI ($***P<0.001$). (ANOVA followed by Newman-Keuls test).

Fig. 3. Lidocaine has no marked effect on spontaneous locomotion. Distance traveled in 30 min in mice injected with lidocaine was similar to that in mice given saline (n=8) (Student's t-test).

Fig. 4.

Mice (n=6) were pretreated with either saline or lidocaine (i.d., hind leg) and then treated with either saline or 5% formalin (s.c., i.paw). Two h later, animals were perfused intracardially and lumbar spinal cords were removed. C-fos positive nuclei were detected on the medial side of the superficial and deeper layers of the dorsal horn of the mice treated with formalin. Markedly fewer c-fos expressed nuclei were observed in the lumbar spinal cord sections obtained from mice pretreated with lidocaine compared to mice pretreated with saline (scale bars: for A, C and E 100 μ m; B, D and F 50 μ m). (i.paw; intrapaw).

Fig. 5. GNTI-provokes c-fos expression on the lateral side of the superficial layer of the dorsal horn in mice wearing an Elizabethan collar suggesting that GNTI-induced c-fos activation is a result of itch sensation (scale bars: A, C and E 100 μm; B, D and F 50 μm) (n=3).

Fig. 6.

Mice (n=6) were injected with either saline or lidocaine (i.d., behind the neck) and 10 min later they were administered either saline or GNTI (0.3 mg/kg, s.c., behind the neck). Two h later animals were perfused intracardially and cervical spinal cords were removed. C-fos positive nuclei were localized on the lateral side of the superficial layer of the dorsal horn in mice injected with saline and GNTI. Markedly fewer c-fos expressed nuclei were observed in cervical spinal cord sections obtained from mice pretreated with lidocaine compared to mice pretreated with saline (scale bars: A, C and E 100 μ m; B, D and F 50 μ m).

Fig. 7.

The number of c-fos positive nuclei was counted and averaged from 10 randomly chosen cervical (for scratch) and lumbar (for nociception) sections from each animal in each group (saline + GNTI; lidocaine + GNTI; saline + formalin and lidocaine + formalin, n=6). Lidocaine administration before either GNTI or formalin significantly decreased the number of c-fos positive nuclei (**P<0.001, Student's t-test).