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Lidocaine Prevents Referred Hyperalgesia Associated with Cystitis

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Abstract

Aims—Lidocaine produces analgesia by inhibiting excitation of nerve endings or blocking impulse conduction in peripheral nerves. This study was performed to determine whether intrathecal or intravesical administration of lidocaine prior, or subsequent, to induction of chemical cystitis in rats would block referred mechanical hyperalgesia.

Methods—Intrathecal or intravesical lidocaine was administered 15 (intrathecal) or 30 (intravesical) minutes before intravesical instillation of saline or 1mM acrolein (400μl) or 4 hours after saline or acrolein instillation in female Wistar rats. Mechanical sensitivity of hind paws was determined at 24 hours prior to any treatment (baseline) and, 4, 24, and 48 hours after intravesical instillation of acrolein or saline. Also, nerve growth factor (NGF) content was measured in bladder and dorsal root ganglia (DRG).

Results—Pre-treatment with intrathecal or intravesical lidocaine attenuated acrolein-induced referred mechanical hyperalgesia of the hind paws. Lidocaine administered after acrolein instillation did not alter referred hyperalgesia. Lidocaine treatment prior to or after induction of cystitis reduced NGF content in the bladder.

Conclusions—These results indicate that pre-treatment with lidocaine attenuates referred hyperalgesia associated with cystitis. Lidocaine treatment 4 hours after induction of cystitis failed to prevent referred hyperalgesia despite a similar decrease in bladder NGF.

Keywords

cystitis; lidocaine; nerve growth factor; referred hyperalgesia

INTRODUCTION

Local anesthetics have been used extensively to prevent or minimize pain that accompanies cystitis.^{1,2} Lidocaine primarily blocks generation and propagation of action potentials through direct binding of neuronal voltage-gated sodium channels thereby producing analgesia by inhibiting excitation of nerve endings or blocking conduction in peripheral nerves.³ Mechanisms of action of lidocaine may also involve interactions with other ion channels, receptors (such as G protein-coupled receptors), and proteins that modify activity of these channels and receptors, including protein kinase C (PKC) and protein kinase A

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(PKA).^{4,5} These interactions may affect several intracellular signaling pathways involved in pain sensation.⁶

One significant signaling pathway involved in visceral pain is activation of the tyrosine kinase A receptor by nerve growth factor (NGF).⁷⁻⁹ NGF activates trkA receptors through receptor dimerization at the cell surface followed by receptor autophosphorylation. Activation of trkA induces activation of mitogen-activated protein kinase (MAPK) cascades that mediate pain sensation.¹⁰ Little is known about the effects of lidocaine on NGF synthesis and release.

Severity of visceral pain is difficult to assess objectively. Indirect measurements that have been used to assess pain associated with cystitis in rodents include frequency and strength of bladder contractions (determined by cystometry), behavior (reluctance to move or stretch spontaneously), electromyographic (EMG) activity of abdominal muscles, and evaluation of sensitivity of somatic structures (particularly the paws) to mechanical or thermal stimuli.^{9,11,12} Increased sensitivity of somatic structures to nociceptive stimuli is called referred hyperalgesia. Referred hyperalgesia in response to mechanical or thermal stimuli is a well-recognized consequence of experimental cystitis in rodents, and referred hyperalgesia has been shown to accompany cystitis in humans.¹³

It has been reported that NGF is increased by visceral inflammation and contributes to referred hyperalgesia.¹⁴ It has been observed previously that cystitis induced in mice by systemic administration of cyclophosphamide is accompanied by referred hyperalgesia in response to mechanical stimuli that appeared to be dependent upon increased bladder NGF.⁸ NGF was also found to be increased in bladders of patients with idiopathic overactive bladder.¹⁵

This study was performed to assess effects of lidocaine given intrathecally or intravesically on development and persistence of referred mechanical hyperalgesia in rats with experimental cystitis. We hypothesized that: 1) intrathecal or local (intravesical) administration of lidocaine given prior to or after initiation of cystitis would reduce or block referred hyperalgesia that accompanies cystitis; and 2) lidocaine treatment would prevent increased bladder NGF observed during cystitis.

MATERIALS AND METHODS

All experiments were performed using 8 week old female Wistar rats (180–250 g). Rats were housed in groups of two per cage and maintained on a 12 hour light/dark cycle, with food and water available ad libitum. Animals were allowed to adapt to their environment for 4 days prior to any testing or treatment. All procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Acrolein-induced Cystitis

Cystitis was induced by a single intravesical instillation of acrolein (1mM, prepared with saline, 400 μ l; Ultra Scientific, Kingstown, RI). Rats were anesthetized by inhalation of isoflurane (2–5%) in oxygen prior to instillation of acrolein. Bladders were catheterized transurethally using lubricated PE 10 tubing with an external diameter of 0.61mm (Intramedic, Sparks, MD). After catheterization, bladders were emptied by light abdominal compression before instillation of acrolein. Rats remained anesthetized, and the catheter was left in place, for 30 minutes after instillation of acrolein. Effects of intravesical instillation of acrolein were compared to intravesical instillation of saline (0.9%; adjusted to pH 6.5, 400 μ l) using the same protocol.

Intravesical and Intrathecal Treatment of Lidocaine

Effects of intravesical or intrathecal lidocaine given prior to or after induction of cystitis were tested using the following treatment groups: intravesical or intrathecal lidocaine prior to intravesical instillation of saline or acrolein (4 groups); and intravesical or intrathecal lidocaine after intravesical instillation of saline or acrolein (4 groups). Results obtained from these groups were compared to those of rats that only received intravesical saline or acrolein without lidocaine treatment. Rats treated with preemptive intravesical lidocaine were anesthetized, catheters were placed transurethraly, bladders were emptied, and lidocaine (2%, pH 6.5, 400 μ l, intravesical; Phoenix Pharmaceutical, St Joseph, MO) was instilled and left in the bladder for 30 minutes. Bladders were then emptied, and acrolein (1mM; 400 μ l, intravesical) or saline (0.9%, pH 6.5, 400 μ l, intravesical) was instilled. Thirty minutes later, catheters were removed, and rats were allowed to recover from anesthesia.

Rats treated with preemptive intrathecal lidocaine were anesthetized by inhalation of isoflurane (2–5%) in oxygen, placed in ventral recumbency on a flat surface, a 27-Ga $\times$ 1/2" needle connected to a 25 μ l Hamilton syringe was passed perpendicular to the skin between L5 and L6 vertebrae into the intrathecal space, and lidocaine (2%, pH 6.5, 20 μ l) was injected. Fifteen minutes after lidocaine administration, a transurethral catheter was passed, and acrolein (1mM; 400 μ l, intravesical) or saline (0.9%, 400 μ l, intravesical) was instilled.

Rats treated with lidocaine after induction of cystitis were anesthetized, transurethral catheters were placed, and acrolein (1mM; 400 μ l) or saline (0.9%, 400 μ l) was instilled intravesically. Thirty minutes later, catheters were removed, and rats were allowed to recover from anesthesia. Four hours after intravesical instillation of acrolein or saline, rats were anesthetized, and lidocaine was injected intravesically or intrathecally as described previously. Preliminary experiments demonstrated that intrathecal or intravesical instillation of saline prior to or after intravesical saline had no effect on any parameters studied, and these results are not presented.

Referred Mechanical Hyperalgesia

Mechanical sensitivity of hind paws was assessed with Von Frey monofilaments using the up-down method.¹⁶ Mechanical sensitivity was determined 24 hours prior to any treatment in all animals (baseline). Animals pre-treated with lidocaine (intravesical or intrathecal) had mechanical sensitivity determined 4, 24, and 48 hours after intravesical instillation of acrolein or saline. Animals treated with intravesical or intrathecal lidocaine after intravesical saline or acrolein had mechanical sensitivity of hind paws tested one hour after lidocaine (5 hours after intravesical instillation of acrolein or saline), 24 and 48 hours after intravesical instillation of acrolein or saline.

Histological Analysis of Bladder

Animals were euthanized (pentobarbital 100 mg/kg, intraperitoneal) 48 hours after infusion of acrolein or saline into the bladder. A cannula was inserted into the left ventricle, and each rat was perfused with saline (0.9 %) for 7 minutes. Bladders were removed and weighed. Bladder dome was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological evaluation. Three transverse sections of each bladder were obtained at 100 mm intervals and analyzed for evidence of edema and inflammatory cell infiltration. An edema score was determined as: 0 = no edema evident; 1 = edema present, limited to submucosal region; 2 = edema present in bladder wall but not detrusor; 3 = edema present in bladder wall, possibly including occasional areas of detrusor; and 4 = edema present throughout bladder wall, including entire detrusor, and urothelium was frequently lost due to severity of injury. Inflammatory cell infiltration was determined by dividing bladder sections into quadrants and assigning a score to each quadrant as: 0 = no

inflammatory cells observed or 1 = leukocytic infiltration. Inflammatory cell score (leukocyte infiltration score) was calculated as the average of total score for each of 3 sections examined (ranging from a possible minimum of 0 to a possible maximum of 4 for averaged scores).

Measurement of NGF Using Enzyme-linked Immunosorbent Assay (ELISA)

Forty-eight hours after intravesical instillation of acrolein or saline, bladders were collected as described above. L6/S1 DRGs are associated with afferent bladder innervation. These were exposed by careful dissection and collected. Bladder and L6/S1 DRGs were stored at -80°C until processed for protein measurement.

Bladder and L6/S1 DRG were homogenized in cold lysis buffer (137 mM NaCl, 20 mM Tris HCl, 1% NP₄₀, 10% glycerol, 1mM PMSF, 10 $\mu\text{g}/\text{ml}$ aprotinin, 1 $\mu\text{g}/\text{ml}$ lupeptin, 0.5 mM sodium vanadate, pH 8.0). Homogenates were centrifuged for 30 minutes at 15,000 rpm at 4°C , and supernatants were acidified with HCl (1M, 1 $\mu\text{l}/50\text{ }\mu\text{l}$ of homogenates) to pH 2.0 – 3.3 for 15 minutes at room temperature, followed by neutralization with NaOH (1M, 1 $\mu\text{l}/50\text{ }\mu\text{l}$ of homogenates) to pH 7.6. Protein concentrations of samples were determined using the BCA Protein Assay (Pierce Biotechnology, Rockford, IL). NGF content was measured by commercially-available NGF Emax ImmunoAssay Systems ELISA kits according to manufacturer's instructions (Promega, Madison, WI). NGF content was normalized to total tissue protein content and expressed as pg/mg protein.

Statistical analysis

All data were expressed as mean $\pm$ standard error of the mean (S.E.M.) and analyzed using GraphPad Prism 4.01 statistical software (Graph-Pad Software, San Diego, CA). Mechanical nociception (referred hyperalgesia) data were analyzed with two-way ANOVA, and protein data were analyzed with one-way ANOVA. Tukey's test for multiple comparisons was performed when $p < 0.05$, and a value of $p < 0.05$ was considered indicative of significant difference.

RESULTS

Pre-treatment with Lidocaine Attenuates Referred Mechanical Hyperalgesia

Referred mechanical hyperalgesia was observed 4 and 24 hours after intravesical instillation of acrolein, but the threshold to peripheral mechanical stimuli returned to baseline values by 48 hours after acrolein instillation (Figure 1). Intravesical instillation of saline alone had no effect on referred hyperalgesia at any time point (data not shown). A single intrathecal administration of lidocaine prior to instillation of acrolein prevented referred hyperalgesia (Figure 1A). Intravesical instillation of lidocaine prior to acrolein prevented referred hyperalgesia at 4 hours after acrolein, but as inflammation persisted, referred hyperalgesia was observed in these rats 24 hours after instillation of acrolein (Figure 1B). Peripheral mechanical sensitivity returned to baseline levels 48 hours after instillation of acrolein.

Lidocaine Administered after Acrolein did not Prevent Referred Mechanical Hyperalgesia

Treatment of rats with intravesical or intrathecal lidocaine 4 hours after instillation of acrolein had no effect on referred hyperalgesia measured 5 hours (1 hour after lidocaine treatment) after treatment with acrolein (Figure 2). Intrathecal lidocaine had no effect on mechanical sensitivity in animals treated with intravesical saline (Figure 2A). Rats that received intravesical acrolein followed by intravesical lidocaine showed the same pattern of referred hyperalgesia as rats treated with acrolein only (Figure 2B).

Acrolein Induced Histological Evidence of Cystitis

Intravesical instillation of acrolein caused cystitis as indicated by increased bladder weight and histological evidence of edema and leukocyte infiltration within bladder walls 48 hours after infusion of acrolein (Table I). Treatment with saline or lidocaine prior to, or after, instillation of acrolein had no effect on severity of cystitis. Intrathecal or Intravesical lidocaine alone did not induce histological evidence of cystitis.

Lidocaine Prevented Cystitis-Induced Increase of Bladder NGF Content

Intravesical instillation of acrolein caused a significant increase in bladder NGF content relative to that observed in bladders infused with saline (217 ± 10 and 44 ± 9 pg/mg protein, respectively; Table II). Treatment with intravesical or intrathecal lidocaine prior to or after instillation of acrolein significantly reduced bladder NGF content relative to the effects of acrolein alone. Acrolein-induced cystitis had no effect on NGF content of DRG. Intrathecal or intravesical lidocaine had no effect on NGF content of DRG in the presence or absence of cystitis.

DISCUSSION

Results of the present study indicate that referred mechanical hyperalgesia that accompanies acrolein-induced cystitis is blocked by treatment with lidocaine prior to intravesical instillation of acrolein, but not by lidocaine administered after initiation of cystitis. Cystitis increased NGF content within bladders, but had no effect on NGF content in DRGs.

Visceral pain originating from inflamed bladder can be relieved by use of local anesthetics such as lidocaine.^{2,3} The present study evaluated the effects of lidocaine on referred mechanical hyperalgesia (as opposed to visceral pain per se), and results indicate that pre-treatment with lidocaine prevents referred hyperalgesia that accompanies cystitis. A single intrathecal injection of lidocaine completely blocked referred hyperalgesia after induction of cystitis. Treatment with intravesical lidocaine prior to infusion of acrolein induced a transient reduction in referred hyperalgesia that was observed 4 after acrolein instillation. However, referred hyperalgesia was observed 24 hours after acrolein instillation, indicating that the effects of intravesical lidocaine given prior to acrolein were less durable than intrathecal lidocaine. No treatments reduced histological severity of cystitis. Therefore, effects of intravesical lidocaine in preventing referred hyperalgesia were transient, while those of intrathecal lidocaine were more persistent.

Previous studies have demonstrated prolonged effects of spinal infusion of lidocaine in reversing thermal allodynia in neuropathic models.^{17,18} Furthermore, intravenous administration of lidocaine provided durable relief of pain after experimental sciatic nerve ligation and in humans with peripheral neuropathic pain.¹⁹ Mechanisms responsible for long-lasting effects of lidocaine are currently unknown, although it may involve peripheral processes such as changes in activity and expression of voltage-gated Na^+ channels in sensory neurons after inflammation and nerve injury.¹⁷ Inflammatory mediators such as prostaglandins, adenosine, serotonin/5-hydroxytryptamine (5-HT), and epinephrine can modulate activity of voltage-gated Na^+ currents sensitizing primary afferent neurons.^{10,14} Thus, exposure of the spinal cord and DRG to lidocaine prior to onset of inflammation may inhibit changes in abundance or activity of inflammatory molecules that result in referred hyperalgesia.

Patients with painful bladder syndromes are sometimes treated with intravesical administration of lidocaine to relieve pain. We therefore compared effects of intrathecal and intravesical administration of lidocaine on referred hyperalgesia after cystitis was established. Neither intrathecal or intravesical lidocaine reversed referred hyperalgesia once

cystitis was established. Mechanisms resulting in referred hyperalgesia remain largely unknown but likely involve sensitization of pathways that mediate pain perception in the central nervous system.¹² In fact, chemical cystitis induced by intraperitoneal injection of cyclophosphamide increases expression of c-Fos and Krox-24 in the spinal cord and hindbrain.¹² Peripheral sensitization of primary afferents may also occur and alter synaptic functions in the CNS to increase pain sensation.¹² Our findings suggest that lidocaine can prevent development of referred hyperalgesia but cannot reverse the condition once it is established.

A novel finding in the present study was that lidocaine (intravesical or intrathecal) treatment reduced bladder NGF concentrations within 48 hours after acrolein instillation. The mechanism(s) underlying this effect remain unclear. Processes that affect abundance of any protein in a specific tissue include translation and transcription of relevant mRNA, protein degradation, and protein uptake or translocation. Lidocaine has been demonstrated to inhibit neurite outgrowth of PC12 cells in culture, and this effect was attributed to inhibition of TrkA phosphorylation.²⁰ Inhibition of trkA phosphorylation by lidocaine would seem to be inconsistent with a decrease in bladder NGF induced by acrolein given subsequent to intravesical lidocaine. We reported previously that intravesical instillation of k252a (an inhibitor of NGF binding to trkA) was associated with increased bladder concentrations of NGF subsequent to acute cystitis,²¹ and it has been suggested that tissue concentrations of NGF may be in part dependent upon NGF binding to trkA and retrograde transport of homodimers to the dorsal root ganglia.²²

Very little is known about the effects of lidocaine on NGF synthesis or degradation, but one could hypothesize that, in the presence of bladder inflammation, prior treatment with lidocaine may inhibit neural feedback in response to inflammation that under other circumstances stimulates further synthesis and release of NGF. Voltage-gated sodium channels and tyrosine kinase A (trkA, the high-affinity receptor for NGF) are colocalized on sensory neurons.²³⁻²⁴ While NGF has been reported to increase expression of voltage-dependent sodium channels by sensory neurons in the presence of chronic pain,²⁵ and brief exposure to NGF has been demonstrated to activate sodium channels and other cation currents,²⁶ there is no apparent evidence that inhibition of sodium channels by local anesthetics affects NGF synthesis or release. Bladder NGF was increased in a model of neurogenic inflammation,²⁷ and it is theoretically possible that inhibition of voltage-gated sodium (or other cation) channels by lidocaine prevents increased NGF that might be the result of activation of sensory nerves. As mentioned previously, local anesthetics have been shown to interact with other ion channels and receptors, such as voltage-gated Ca^{+2} and K^{+} channels, ionotropic and metabotropic receptors, G protein-coupled receptors, and TRPV receptors.⁴⁻⁶ These effects could be at least in part related to changes in synthesis, release, and uptake of NGF in peripheral tissues.

CONCLUSIONS

Data presented here show that referred hyperalgesia associated with experimental cystitis was blocked by treatment with lidocaine prior to induction of cystitis. Intrathecal administration of lidocaine had a more durable effect on referred hyperalgesia than intravesical instillation of lidocaine. Since referred hyperalgesia accompanying cystitis persisted in animals that received lidocaine after initiation of cystitis despite attenuation of increased bladder NGF, reduction in bladder NGF alone may be insufficient to block referred hyperalgesia after cystitis is established. These results are relevant to clinical observations in patients with bladder pain and suggest that the use of regional anesthesia should be considered when patients with pelvic pain undergo painful medical procedures such as cystoscopy, hydrodistention, biopsy and/or fulguration of the bladder.

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Figure 1. Response to application of mechanical stimuli to hind paws of rats with cystitis or with treatment with lidocaine prior to acrolein or saline

(A) Intravesical (ives) instillation of acrolein was accompanied by decreased threshold of response to mechanical stimuli 4 and 24 hours after acrolein instillation. Intrathecal (it) administration of lidocaine prior to acrolein prevented reduction of the threshold of response to mechanical stimuli 4 and 24 hours after treatment with acrolein. (B) Intravesical administration of lidocaine prior to acrolein prevented reduction of the threshold to mechanical stimulation 4 hours, but not 24 hours, after acrolein instillation. Data are presented as mean $\pm$ S.E.M. * $p < 0.05$, and ** $p < 0.01$ compared to baseline of each group. (n = 5–7 for each group)

Figure 2. Response to application of mechanical stimuli to hind paws of rats with cystitis or with treatment with lidocaine after acrolein

(A) Intrathecal (it) administration of lidocaine after acrolein failed to prevent reduction of the threshold of response to mechanical stimuli 4 and 24 hours after cystitis. (B) Intravesical (ives) administration of lidocaine after instillation of acrolein had no significant effect on the threshold of response to mechanical stimuli at any time points after infusion of acrolein.

Data are presented as mean latency for withdrawal $\pm$ S.E.M. * $p < 0.05$, and ** $p < 0.01$ compared to baseline of each group. ($n = 5-7$ for each group)

Table I

Bladder Weight and Histology 48 hours after Intravesical Saline or Acrolein.

Control Groups	Bladder/Body Weight (mg/g)	Edema Score	Leukocyte Infiltration Score
Saline ives (n=5)	0.4 ± 0.04	0.3 ± 0.1	0 ± 0.0
Lidocaine treatment prior to saline			
lidocaine it + saline ives (n=5)	0.5 ± 0.03	0.3 ± 0.3	0.06 ± 0.0
lidocaine ives + saline ives (n=5)	0.5 ± 0.06	0.9 ± 0.4	0.06 ± 0.0
Lidocaine treatment after saline			
saline ives + lidocaine it (n=5)	0.4 ± 0.03	0.7 ± 0.1	0.06 ± 0.0
saline ives + lidocaine ives (n=5)	0.5 ± 0.05	0.3 ± 0.2	0 ± 0.0
Cystitis Groups	Bladder/Body Weight (mg/g)	Edema Score	Leukocyte Infiltration Score
acrolein ives (n=12)	1 ± 0.03 *	3.5 ± 0.1 *	3.7 ± 0.1 *
Lidocaine treatment prior to acrolein			
lidocaine it + acrolein ives (n=7)	0.9 ± 0.05 *	4 ± 0.0 *	3 ± 0.2 *
lidocaine ives+ acrolein ives (n=7)	0.9 ± 0.03 *	3.6 ± 0.1 *	3.6 ± 0.2 *
Lidocaine treatment after acrolein			
acrolein ives+ lidocaine it (n=7)	1.1 ± 0.01 *	3.3 ± 0.3 *	3 ± 0.2 *
acrolein ives + lidocaine ives (n=7)	1.1 ± 0.06 *	3.1 ± 0.2 *	3.6 ± 0.2 *

All values are expressed as mean ± S.E.M. No differences were observed within control or cystitis groups.

* p<0.001 for comparison of cystitis groups to control groups. (ives = intravesical; it = intrathecal)

Table II

NGF Protein Content in Bladder and L6/S1 DRG at 48 hours after Intravesical Saline or Acrolein.

Control Groups	Bladder NGF (pg/mg)	L6/S1 DRG NGF (pg/mg)
Saline ives (n=5)	44 ± 9	154 ± 34
Lidocaine treatment prior to saline		
lidocaine it + saline ives (n=5)	63 ± 17	256 ± 40
lidocaine ives + saline ives (n=5)	92 ± 15	162 ± 13
Lidocaine treatment after saline		
saline ives + lidocaine it (n=5)	100 ± 9	214 ± 26
saline ives + lidocaine ives (n=5)	93 ± 6	180 ± 35
Cystitis Groups	Bladder NGF (pg/mg)	L6/S1 DRG NGF (pg/mg)
acrolein ives (n=12)	217 ± 10 [#]	184 ± 14
Lidocaine treatment prior to acrolein		
Lidocaine it + acrolein ives (n=7)	105 ± 16 [*]	203 ± 24
Lidocaine ives + acrolein ives (n=7)	118 ± 15 [*]	158 ± 13
Lidocaine treatment after acrolein		
acrolein ives + lidocaine it (n=7)	180 ± 13 [*]	250 ± 25
acrolein ives + lidocaine ives (n=7)	140 ± 13 [*]	187 ± 32

All values are expressed as mean ± S.E.M.

[#] p<0.05 for comparison of cystitis groups to control groups.^{*} p<0.05 for comparison of ives acrolein alone to other cystitis groups. (ives = intravesical; it = intrathecal)