Published in final edited form as:
J Pain. 2010 March ; 11(3): 210–218. doi:10.1016/j.jpain.2009.07.004.

ASIC1 and ASIC3 Play Different Roles in the Development of Hyperalgesia Following Inflammatory Muscle Injury

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Abstract

Acid-sensing ion channels (ASICs) respond to acidosis that normally occurs after inflammation. We examined the expression of *ASIC1*, *ASIC2*, and *ASIC3* mRNAs in lumbar DRG neurons before and 24h after carrageenan-induced muscle inflammation. Muscle inflammation causes bilateral increases of *ASIC2* and *ASIC3*, but not *ASIC1* (neither *ASIC1a* nor *ASIC1b*) mRNA, suggesting differential regulation of *ASIC1* versus *ASIC2* and *ASIC3* mRNA. Similar mRNA increases were observed following inflammation in knockout mice: *ASIC2* mRNA increases in *ASIC3*−/− mice; *ASIC2* and *ASIC3* mRNAs increase in *ASIC1*−/− mice. Prior behavioral studies in *ASIC3*−/− mice showed deficits in secondary hyperalgesia (increased response to noxious stimuli outside the site of injury), but not primary hyperalgesia (increased response to noxious stimuli at the site of injury). In this study, we show that *ASIC1*−/− mice surprisingly do not develop primary muscle hyperalgesia, but develop secondary paw hyperalgesia. In contrast and as expected, *ASIC3*−/− mice develop primary muscle hyperalgesia, but do not develop secondary paw hyperalgesia. The pharmacological utility of the non-selective ASIC inhibitor A-317567, given locally, was tested. A-317567 reverses both primary and the secondary hyperalgesia induced by carrageenan muscle inflammation. Thus, peripherally located ASIC1 and ASIC3 play different roles in the development of hyperalgesia after muscle inflammation.

Keywords

ASIC; muscle inflammation; hyperalgesia; DRG neurons; carrageenan; A-317567

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Introduction

Acid-sensing ion channels (ASICs) are proton-gated voltage-independent ion channels located on neurons in the peripheral and central nervous systems. ASICs belong to the epithelial sodium channel/degenerin (ENaC/DEG) family of amiloride-sensitive transmembrane ion channel proteins (see (37,41,78)). Four genes within mammalian genomes encode seven subunits to date – ASIC1a, ASIC1b, ASIC1b2, ASIC2a, ASIC2b, ASIC3 and ASIC4.(1,15,26,27,40,54, 69,73) Homomeric and heteromeric ASIC subunits combine to form trimeric ASICs,(13,34) which depending on the subunit composition in the DRG display differences in pH sensitivity, current kinetics and ion selectivity.(11,20,29,53) ASICs respond to acidosis, play a significant role in nociceptive processing of hyperalgesia both peripherally and centrally.(1,3,6,8–[10],[15], [16],[21],[27],[28],[37],[40]–[42],[54],[65],[66],[68],70,72–74,76)

Despite a number of well-designed and controlled studies, the role of ASICs in nociception has led to conflicting results. Some prior behavioral studies generated from ASIC knockout or dominant negative mutant mice show no differences or increases in hyperalgesia of the paw after intraplantar inflammation.(16,45,56,68) Previous studies show increases in *ASIC1*, *ASIC2*, and *ASIC3* mRNAs after cutaneous paw inflammation (30,71). Our laboratory, however, has consistently shown that ASIC3 plays a critical role in the development of inflammatory and non-inflammatory hyperalgesia induced by deep tissue insult,(30,65,66) which Yen et al. also reported recently.(81) We have suggested that these differences could be related to differences between cutaneous and deep tissue injury.

However, differences between pain at the site of injury, termed primary hyperalgesia, and pain outside the site of injury, termed secondary hyperalgesia, could also explain these differences. Animal models of cutaneous inflammation typically measure primary hyperalgesia at the paw; while those of deep tissue insult have typically measured secondary hyperalgesia at the paw. One purpose of this study was to determine if there were differences in expression of ASICs with deep tissue inflammation, and behavioral deficits in ASIC1 and ASIC3 knockout mice with regard to primary and secondary hyperalgesia after muscle insult.

Animal models of pain have not sorted out whether ASICs play a peripheral or central role in the continued manifestation of the hyperalgesia after the inflammation is established. Previous literature suggests that peripherally located ASIC3 is important at the site of muscle inflammation since re-expression of ASIC3 into muscle of *ASIC3*−/− mice prior to induction of inflammation restores the hyperalgesia.(66) Co-administration of the ASIC3 antagonist, APETx2 with CFA into the paw prevents the development of cutaneous hyperalgesia 4h later in rats, whereas blockade of peripheral ASIC1 with Psalmotoxin 1(PcTx1) at the time of intraplantar CFA injection has no effect on cutaneous hyperalgesia.(21) Intrathecal or intracerebroventricular blockade of ASIC1 induces analgesia and reverses hyperalgesia after nerve injury.(21,44) Systemic application of the non-selective antagonists A-317567 and amiloride reduce primary hyperalgesia induced by CFA and skin incision.(24) In this study, we therefore used a non-selective inhibitor of ASICs, A-317567, injected directly into the muscle 24h after muscle inflammation to test if activation of peripheral ASICs were important in maintaining hyperalgesia. We further tested the effectiveness of this drug in *ASIC1*−/− and *ASIC3*−/− mice to examine whether ASIC1 or ASIC3 were critical for the behavioral effects.

We therefore hypothesized that there would be differential expression of ASICs after inflammation, and that ASIC3, but not ASIC1, would mediate the secondary hyperalgesia associated with muscle inflammation. Since there were behavioral deficits in both *ASIC1*−/− and *ASIC3*−/− mice, we tested the ability of A-317567 to reverse both primary and secondary hyperalgesia in WT and in *ASIC3*−/− and *ASIC1*−/− mice with muscle inflammation.

Methods

Animal Care and Use

All animal experiments were approved by the University of Iowa Animal Care and Use Committee and were conducted in accordance with National Institutes of Health guidelines. Congenic *ASIC1−/−* and *ASIC3−/−* on a C57Bl/6 background and congenic *C57Bl/6* (WT) mice were bred at the University of Iowa Animal Care Facility.(56,76) New breeding pairs of mice are started every six months. The congenic *ASIC1−/−* is a knockout of ASIC1a, and not ASIC1b.(76) Male and female mice, 6–10 weeks of age, WT (n=64), *ASIC3−/−* (n=32), *ASIC1−/−* (n=24) were used in these studies.

Induction of Inflammation

Mice were briefly anesthetized with 4% isoflurane and one gastrocnemius muscle (left) was injected with 20 μl of 3% carrageenan dissolved in sterile isotonic saline, which has a slightly acidic pH (6.0), typical of commercially available saline preparations. The pH of the final 3% carrageenan solution was 6.0. Behavior measurements were made before and 24 hours after carrageenan injection, and after administration of drug, A-317567.

A-317567

Mice were given one dose of 0.025 μmol A-317567 (C-{6-[2-(1-isopropyl-2-methyl-1,2,3,4-tetrahydro-isoquinolin-7-yl)-cyclopropyl]-naphthalen-2-yl}-methanediamine) (10μl) injected into the left gastrocnemius muscle 24 hours after induction of inflammation. As a control for systemic effects one group of WT mice (n=8) received 0.025 μmol A-317567 (10μl) in the contralateral muscle. Isotonic saline (10 μl) was used as the vehicle control in separate mice (n=45). Animals were tested 15 minutes after administration of the drug or vehicle.

Behavioral Testing

Mice were acclimated for 2 days before testing for muscle sensitivity and cutaneous mechanical sensitivity, as described previously.(30) **Muscle sensitivity** was tested by squeezing the gastrocnemius muscle of the mice with a calibrated pair of tweezers until the mouse withdrew from the stimulus. The force at which the mouse withdrew was measured in mN. A decrease in threshold was interpreted as primary muscle hyperalgesia. Sensitivity in both the ipsilateral and contralateral muscles was measured. Muscle sensitivity was tested as follows: before carrageenan injection of the muscle, 24h after the injection, and 15 min after A-317567 injection. Baseline responses for muscle sensitivity for WT, *ASIC1−/−*, and *ASIC3−/−* mice did not differ significantly, and baseline responses were similar for left and right sides. **Cutaneous mechanical sensitivity** was tested bilaterally by assessing the number of responses to repeated application of a 0.4 mN von Frey filament to the plantar surface of the paw. The number of withdrawals out of 5 was assessed in 10 trials and an average of all 10 trials was determined for each time period. A significant increase in the number of responses was interpreted as secondary mechanical hyperalgesia. Cutaneous mechanical sensitivity was tested as follows: before carrageenan injection of the muscle, 24h after the injection, 15 min after A-317567 injection. Testing was blinded for genotype and drug status of the animals. WT, *ASIC1−/−*, and *ASIC3−/−* mice did not differ in baseline cutaneous mechanical sensitivity responses.

Quantitative RT-PCR

RNA was purified from ipsilateral and contralateral lumbar (L4, L5, L6) DRGs using the Trizol reagent (Invitrogen, Carlsbad, CA). DRGs were collected 24h after carrageenan injection or from control mice. All of the mice were subjected to behavioral testing for muscle and cutaneous mechanical sensitivity 1–2 hours prior to collecting the DRGs. RNA concentration

and purity was assessed by spectrophotometric measurement at 260 and 280nm. First strand cDNA was synthesized from 0.2-1ug of each RNA sample using Superscript III or VILO reverse transcriptase (Invitrogen, Carlsbad, CA). Taqman PCR was carried out using an ABI prism 7900 sequence detector (Applied Biosystems, Inc., Foster City, CA) on diluted cDNA samples (University of Iowa, DNA Facility, Iowa City, IA). Reactions were carried out for 40 cycles in triplicate. ASIC1 (ASIC1a and ASIC1b) (Mm01305997_m1), ASIC2 (Mm00475691_m1), ASIC3 (Mm00805460_m1) and the mouse control assay for glyceraldehyde-3-P-dehydrogenase (GAPDH) were obtained from Applied Biosystems, Inc. (Foster City, CA). All of the assays would generate a single PCR product which spans the boundary of two exons, thus reducing the possibility of genomic DNA contamination in the results. Control samples without reverse transcriptase did not produce any background PCR products. Quantitative RT-PCR data were normalized with GAPDH mRNA levels and relative amounts of mRNA were determined by using the comparative cycle thresholds (C_T).

Statistical Analysis

Data are represented as the mean ± S.E.M. Behavioral data were analyzed with a repeated measures ANOVA followed by post-hoc testing with a Tukey's test. Differences were considered significant at $p< 0.05$. Quantitative RT-PCR was analyzed with a two-way ANOVA for group (WT, WT-inflamed, *ASIC1−/−*, *ASIC3−/−*) and side (ipsilateral vs. contralateral).

Results

Increased expression of *ASIC2* and *ASIC3* but not *ASIC1* mRNA in DRG after muscle inflammation

There is a significant induction (approximately 10-fold increases) of *ASIC2* and *ASIC3*, but not *ASIC1* (*ASIC1a* and *ASIC1b*) mRNA in the lumbar DRG innervating muscle 24h following carrageenan-induced muscle inflammation when compared to DRG from mice without muscle inflammation (Figure 1). Statistical analysis showed a significant effect for inflammation for *ASIC2* ($F_{1,40}$=13.7, p=0.001) and *ASIC3* ($F_{1,32}$=13.7, p=0.001) but not *ASIC1*. However there was no effect for side (ipsilateral or contralateral) indicating a bilateral increase in mRNA in the DRG after unilateral muscle inflammation. Since there was not a significant difference between the results for the ipsilateral and contralateral mRNAs, the data for the two sides were pooled graphically. Twenty-four hours after carrageenan-induced muscle inflammation, *ASIC3 −/−* mice showed increases in *ASIC2* mRNA (6-fold) and *ASIC1−/−* mice showed increases in *ASIC2* and *ASIC3* mRNAs, at 12- and 6-fold, respectively; these increases were not significantly different from WT mice (Figure 2). Thus, *ASIC2* and *ASIC3* increased with inflammation and *ASIC1* did not, suggesting a differential regulation of ASICs.

Hyperalgesia of the muscle does not develop in *ASIC1−/−* mice

C57Bl/6 (WT) mice showed a significant bilateral decrease in the withdrawal threshold of the muscle 24h after carrageenan-induced muscle inflammation; the decrease was greater for the ipsilateral inflamed muscle compared to the contralateral muscle ($p<0.05$) (Figure 3A and B). A similar decrease in withdrawal threshold of the muscle was observed in *ASIC3−/−* mice 24h after muscle inflammation (Figure 3A and B). Muscle withdrawal thresholds in *ASIC1−/−* mice, however, were unchanged 24h after carrageenan-induced muscle inflammation, on both the ipsilateral or contralateral sides, and the withdrawal thresholds were significantly greater than WT mice ($P<0.05$).

Hyperalgesia of the paw does not develop in *ASIC3−/−* mice

C57Bl/6 (WT) mice showed a significant bilateral increase in the number of withdrawals to a 0.4 mN force applied to the paw 24h after carrageenan-induced muscle inflammation (Figure

3C and D). There was a similar increase in the number of withdrawals for both the ipsilateral and contralateral paws of *ASIC1*−/− mice 24h after carrageenan-induced muscle inflammation (Figure 3C and D). However, *ASIC3*−/− mice showed a significant reduction ($p<0.05$, compared to *C57Bl/6* (WT) mice) in the number of withdrawals on both the ipsilateral and contralateral sides 24h after muscle inflammation (Figure 3C and D) in agreement with a prior study.(66)

A-317567 reverses muscle and paw hyperalgesia

To determine if ASICs are important for maintaining hyperalgesia once developed, we next tested if peripheral blockade of ASICs would reverse the hyperalgesia in *C57Bl/6* mice. Injection of 0.025 μmole A-317567, a non-selective ASIC antagonist, into the inflamed gastrocnemius muscle (24h after inflammation) reversed the decrease in muscle withdrawal threshold in *C57Bl/6* (WT)(Figure 4A and B). Surprisingly, the unilateral injection of A-317567 reversed the hyperalgesia on both the ipsilateral and the contralateral sides. To confirm that this dose did not produce a systemic effect, we injected 0.025 μmol A-317567 into the contralateral gastrocnemius muscle of animals with carrageenan induced inflammation of the ipsilateral muscle. The withdrawal thresholds of both sides remained unchanged 15 min after injection of the drug into the contralateral gastrocnemius muscle (798 ± 31 mN ipsilateral; 990 ± 50 mN contralateral), which is comparable to the withdrawal thresholds prior to drug administration (880 ± 24 mN ipsilateral; 1128 ± 40 mN contralateral) in WT mice with muscle inflammation of the ipsilateral gastrocnemius muscle.

To further confirm the role of ASIC1 in muscle sensitivity and ASIC3 in cutaneous sensitivity we tested the effect of A-317567 in *ASIC3*−/− and *ASIC1*−/− mice, respectively. In *ASIC1*−/− mice where there is an increased number of withdrawals to mechanical stimulation of the paw, unilateral intramuscular injection of 0.025 μmol A-317567, 24h after carrageenan induced muscle inflammation, decreases the number of withdrawals bilaterally (Figure 4D), However, in *ASIC1*−/− mice that do not show a decrease in muscle withdrawal threshold after inflammation, A-317567 had no effect (Figure 4C). In contrast, in *ASIC3*−/− mice intramuscular injection of 0.025 μmole A-317567 reduces the inflammation-induced decrease in withdrawal threshold of the muscle (Figure 4E). However, in *ASIC3*−/− mice, where there is no change in the number of withdrawal thresholds of the paw to mechanical stimulation, at 24h after carrageenan induced muscle inflammation, A-317567 has no effect (Figure 4F).

Discussion

Bilateral changes in ASIC expression after injury

The current study shows an upregulation of *ASIC2* and *ASIC3* mRNAs in lumbar DRGs following muscle inflammation, not only ipsilateral to the inflamed muscle, but also contralaterally. Surprisingly, the bilateral increases in mRNAs were of similar magnitude, despite a unilateral muscle inflammation, and a greater hyperalgesia ipsilaterally. This is a uniquely different pattern to that observed after cutaneous inflammation induced by CFA injection where increases were observed unilaterally for *ASIC1a*, *ASIC1b*, *ASIC2b*, and *ASIC3*.(71) The differences between the magnitude of hyperalgesia and mRNA levels could be the result of protein modification and interactions ipsilaterally in the inflamed tissue. Inflammatory mediators and protein phosphorylation can modulate and enhance ASIC currents.(2,31,38,71) Thus, although mRNA levels are similar bilaterally, there could be enhanced ASIC activity ipsilaterally from the inflamed muscle that would be manifested as a greater degree of hyperalgesia.

Similar to the results with mRNA expression, local blockade of ASICs with A-317567 reversed the hyperalgesia not only on the injected side, but also on the contralateral side. The reversal

of hyperalgesia in knockout mice was specific for ASIC1 and muscle hyperalgesia, and for ASIC3 and paw hyperalgesia, whether ipsilateral or contralateral to the inflamed muscle. The bilateral effect was unexpected but suggests activation of ASICs in inflamed muscle is critical for maintaining hyperalgesia through enhancing central excitability. In support of a role for ASICs in central sensitization, dorsal horn neurons from *ASIC3−/−* mice do not develop enhanced central excitability that normally occurs after repeated intramuscular acid injection.(65)

The bilateral increases in expression of mRNA for ASIC in the lumbar DRG following muscle inflammation could result from generation of dorsal root reflexes (DRR) bilaterally. Dorsal root reflexes are antidromic action potentials generated at the central terminals of primary afferent fibers, which then result in peripheral release of inflammatory mediators to enhance inflammation and pain.(67) Previously we showed that carrageenan-induced inflammation generates DRRs at the level of the spinal cord that enhance the inflammatory process ipsilaterally.(57,63,64) Unilateral inflammation also produces DRRs not only on the ipsilateral side, but also the contralateral side.(12,36,58) There are also measurable bilateral effects indicative of inflammation such as edema, vasodilation and plasma extravasation.(36,58) Thus, we conclude that unilateral muscle inflammation results in the generation of DRRs ipsilaterally and contralaterally that then increases expression of ASIC mRNAs in both the ipsilateral and contralateral DRGs. Bilaterally increased expression of ASIC mRNAs in the DRGs could result in increased sensitivity to peripherally applied stimuli manifested as hyperalgesia. Blocking input from the inflamed muscle, with the local injection of A-317567 into the ipsilateral side, would prevent this input from reaching the spinal cord to generate the central excitability and dorsal root reflexes.

With muscle inflammation, WT, *ASIC1−/−*, and *ASIC3−/−* mice showed a significant upregulation of *ASIC2* mRNA. We propose that ASIC2 might modulate channel activity. In cell culture experiments, ASIC2/ASIC3 heteromeric channels demonstrate increased responses to decreased pH over that of ASIC3 homomeric channels(7) and ASIC2a enhances ASIC1a's response to the regulatory neuropeptide FMRF-amide.(5) In our studies, the *ASIC1* mRNA levels in the lumbar DRGs before and after inflammation remain unchanged. Our mRNA data suggest that *ASIC1*, *ASIC2*, and *ASIC3* are differentially regulated with inflammation.

ASIC1 and ASIC3 play distinct roles in muscle and cutaneous hyperalgesia

Genetically modified knockout mice or transgenic animals for ASICs have not always demonstrated a positive result for their role in nociception after peripheral inflammation or acid injections.(22,45,49,55,56,59,68) Some prior literature focusing on *ASIC3−/−* mice show no difference or even enhanced hyperalgesia of the paw after paw inflammation, i.e. primary hyperalgesia.(16,56,68) However, the current study, and our prior studies consistently show deficits in secondary hyperalgesia after muscle or joint insult in *ASIC3−/−* mice,(65,66) again suggesting differences between deep tissue hyperalgesia and cutaneous hyperalgesia.

We also show for the first time that muscle hyperalgesia at both the site of inflammation and the contralateral hindlimb does not develop in *ASIC1−/−* mice. This result is distinctly different from prior work showing that there is still increased mechanical hyperalgesia of the paw after carrageenan paw inflammation, and after repeated intramuscular acid injection.(65,68,81) We suggest the differences between the prior reports and the current study are directly related to differences between processing of cutaneous and muscle nociceptive information. Muscle afferents express more ASIC3, calcitonin gene-related peptide (CGRP), and substance P and less isolectin B4 and somatostatin when compared to cutaneous afferents.(46,47,52) Injection of neuropeptides into skin or muscle results in different responses. Substance P produces spontaneous pain when injected into skin and a decrease in the pressure pain threshold, without

spontaneous pain, when injected into muscle.(35) CGRP does not produce pain when injected alone into either skin or muscle but produces pain when injected with substance P into muscle.(51) Centrally there are also differences in processing of nociceptive information from muscle when compared to skin. For example, stimulation of C-fibers innervating muscle produces prolonged discharges in flexor motor neurons that outlasts the stimulus, is longer lasting than cutaneous stimulation of C-fibers, and increases response to noxious stimuli bilaterally.(75, 79) Formalin injected into the skin of the lower back increases c-fos expression throughout laminae I–V, but when injected into the muscles of the lower back there is no labeling in laminae II.(48) Thus, differences in processing of nociceptive stimulation from skin and muscle likely underlie the differences between prior studies utilizing cutaneous models of inflammation and the current study using a model of muscle inflammation.

Differences in the tissue expression of ASIC subunits in peripheral neurons or peripheral tissues could influence nociceptive behavior. The current data shows that blockade of peripheral receptors with A-317567 at the site of inflammation reverses the muscle and cutaneous hyperalgesia once developed suggesting peripheral receptor activation is critical to the development of hyperalgesia. In addition to expression in sensory neurons and brain, ASIC3 is located in non-neuronal tissues such as testis, muscle, lung, bone and synovium.(6,30,32, 33) The role of ASIC3 in these non-neuronal cells is unclear but ASIC3 may also modulate nociception indirectly. ASIC1 is abundantly expressed in spinal cord neurons and brain,(1,3, 6,8–[10,15,19,27,40,54,73],74,76–78,80) and its action is thought to primarily be through central mechanisms. It is unclear if centrally expressed ASIC1a plays a distinct role in the processing of hyperalgesia after muscle inflammation. This study highlights the action of ASIC1 in the peripheral sensory neurons, since blockade of peripheral ASICs completely reverses the hyperalgesia once developed.

Inhibition of ASICs reduces nociceptive behaviors

The pharmacology of ASICs has been extensively studied with non-selective drugs such as amiloride and A-317567.(4,14,17,18,[21,24,43],50,60,70,71) A-317567 produces a concentration dependent inhibition of acid-evoked ASIC currents in DRG neurons(24,39). Intramuscular injection of amiloride also prevents the onset of hyperalgesia induced by repeated intramuscular acid injections suggesting ASIC3 and ASIC1 are important for mediating peripheral nociception at the site of injury.(65,66) Re-expression of ASIC3 in primary afferent fibers innervating muscle of *ASIC3*−/− mice restores the mechanical hyperalgesia of the paw, (66) supporting a peripheral role for ASICs in deep tissue hyperalgesia.

In this study, blockade of ASICs in the inflamed muscle reverses the inflammation-induced hyperalgesia bilaterally. In contrast, we previously showed that intramuscular lidocaine in rats reversed carrageenan-induced muscle hyperalgesia ipsilaterally, but not contralaterally.(61) This difference is surprising since lidocaine should reduce all afferent input to the spinal cord. However, differences could be related to the mode of action of the drug (specific blockade of ASICs vs. sodium channels), differences in hyperalgesia between mice and rats, or the magnitude of the change and power in the previous study (3 vehicle and 7 lidocaine) compared to the current study (8 vehicle and 8 A-317567).

Prior reports suggest that ASIC1 plays a role in the generation of hyperalgesia through the central nervous system.(44) Intrathecal application of amiloride or the ASIC1 antagonist PcTx1, however, prevents development of nocifensive behaviors that develop after formalin, acetic acid, glutamate, nerve injury, and repeated intramuscular acid injections.(25,44,62) Intrathecal delivery of siRNA to ASIC1a or ASIC3 prevents development of hyperalgesia induced by paw inflammation.(21,23,24) However our data show a reversal of muscle hyperalgesia with local injection of A-317567 in WT and *ASIC3*−/− mice, suggesting that ASIC1 at the site of muscle inflammation is important in the generation of muscle

inflammation. While prior data support a central role for ASIC1, our data for the first time show a peripheral role of ASIC1 in the generation of hyperalgesia.

In summary, growing bodies of evidence indicate that ASICs play a role in the sensory signaling associated with inflammatory pain. In this study, we find that ASIC1 and ASIC3 play distinct roles in the development of muscle inflammatory hyperalgesia. Analgesics targeted to specific subunits of ASICs may be useful in alleviating inflammatory muscle pain. Pharmaceuticals designed to reduce ASIC1a could result in decreases in pain at the site of muscle inflammation. Inhibitors of ASIC3 could reduce the development of secondary hyperalgesia, and referred pain following inflammation.

Perspective

This study shows changes in *ASIC* mRNA expression and behavioral hyperalgesia of *C57Bl/6* (wild type), *ASIC1−/−*, and *ASIC3−/−* mice before and after the induction of muscle inflammation. A-317567 was effective in reversing hyperalgesia in these animals, suggesting the potential of ASICs as therapeutic targets for muscle inflammatory pain.

Acknowledgments

Supported by National Institutes of Health AR053509 (KAS), NS057821 (ARL), Grant from Department of Anesthesiology, University of Utah (ARL).

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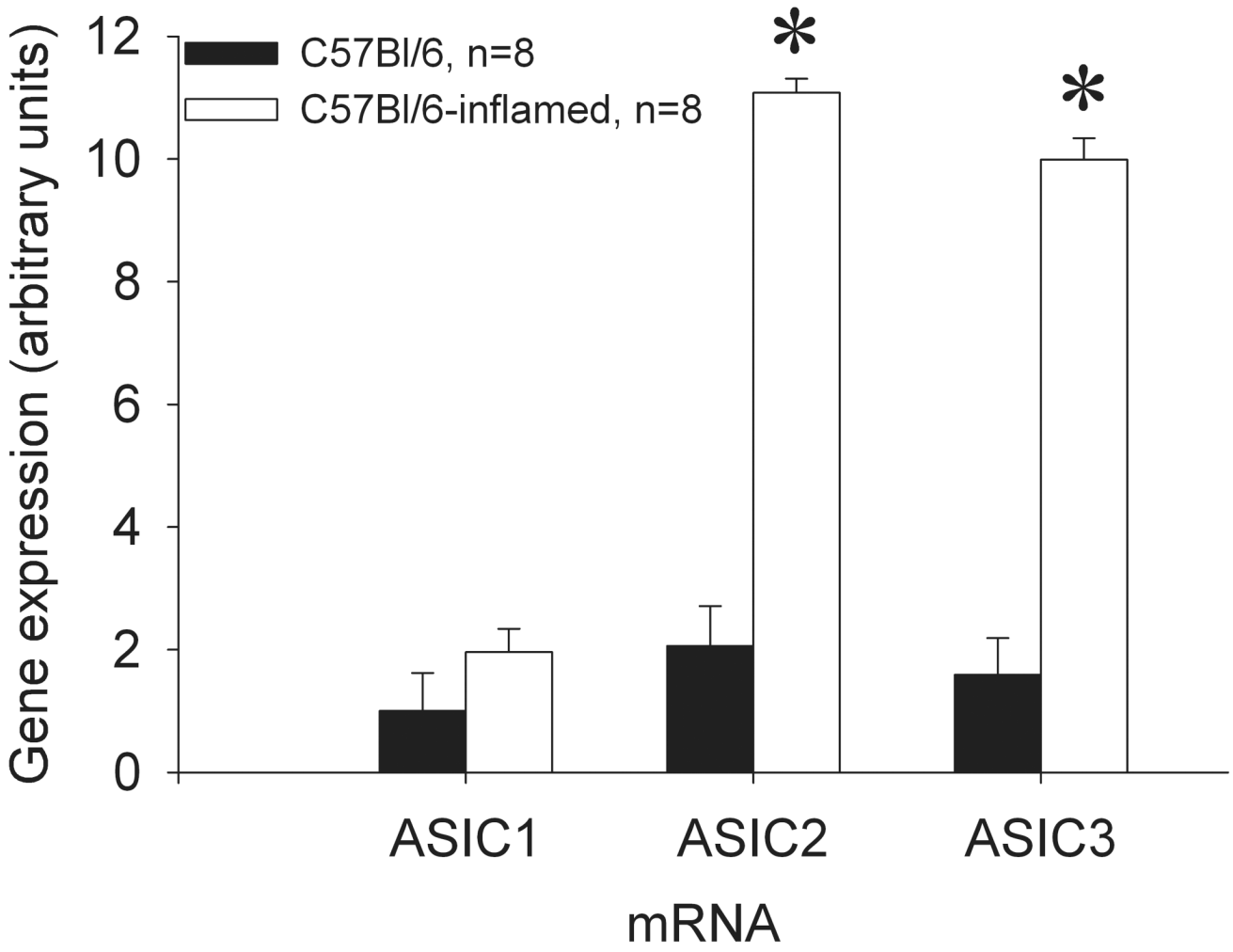

Figure 1.
Comparative qRT-PCR analysis for *ASIC1*, *ASIC2*, and *ASIC3* mRNAs of L4, L5, and L6 DRGs from *C57Bl/6* versus *C57Bl/6*-inflamed mice. There are bilateral increases in *ASIC2* and *ASIC3*, and not *ASIC1* mRNA levels, 24 hours after muscle inflammation. No differences were measured between the ipsilateral and contralateral mRNAs. (* = significantly greater than uninflamed mice, $P<0.05$).

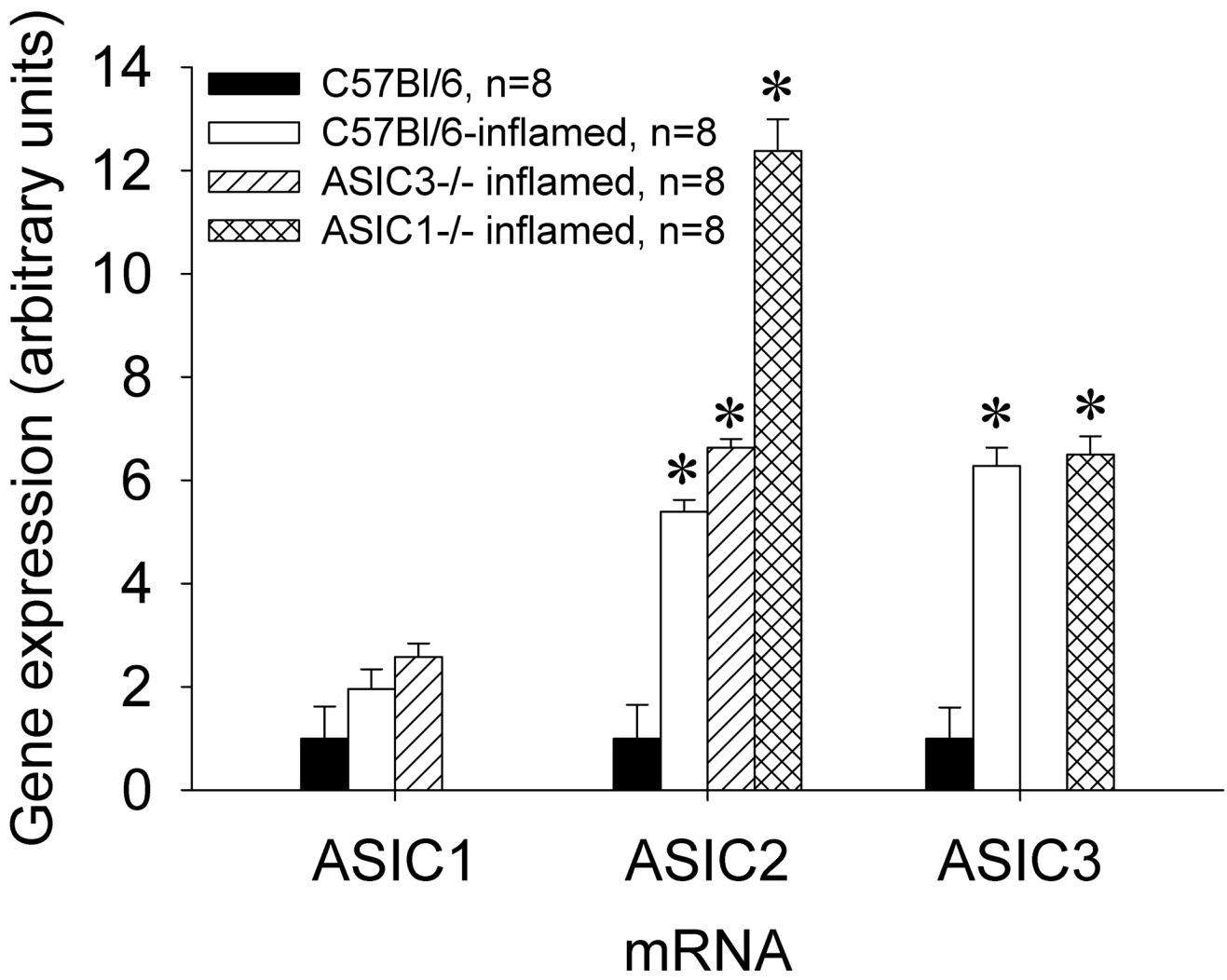

Figure 2.

Comparative qRT-PCR analysis for *ASIC1*, *ASIC2*, and *ASIC3* mRNAs in L4, L5, and L6 DRGs from *C57Bl/6*, *C57Bl/6*-inflamed, *ASIC3*−/− inflamed, and *ASIC1*−/− inflamed mice. *ASIC2* mRNA was increased in *C57Bl/6*-inflamed, *ASIC3*−/− inflamed and *ASIC1*−/− inflamed mice. *ASIC3* mRNA increased in *C57Bl/6*-inflamed and in *ASIC1*−/− inflamed mice. *ASIC1* mRNA levels were not significantly different between the groups. (* = significantly increased from *C57Bl/6* uninflamed mice, $P<0.05$).

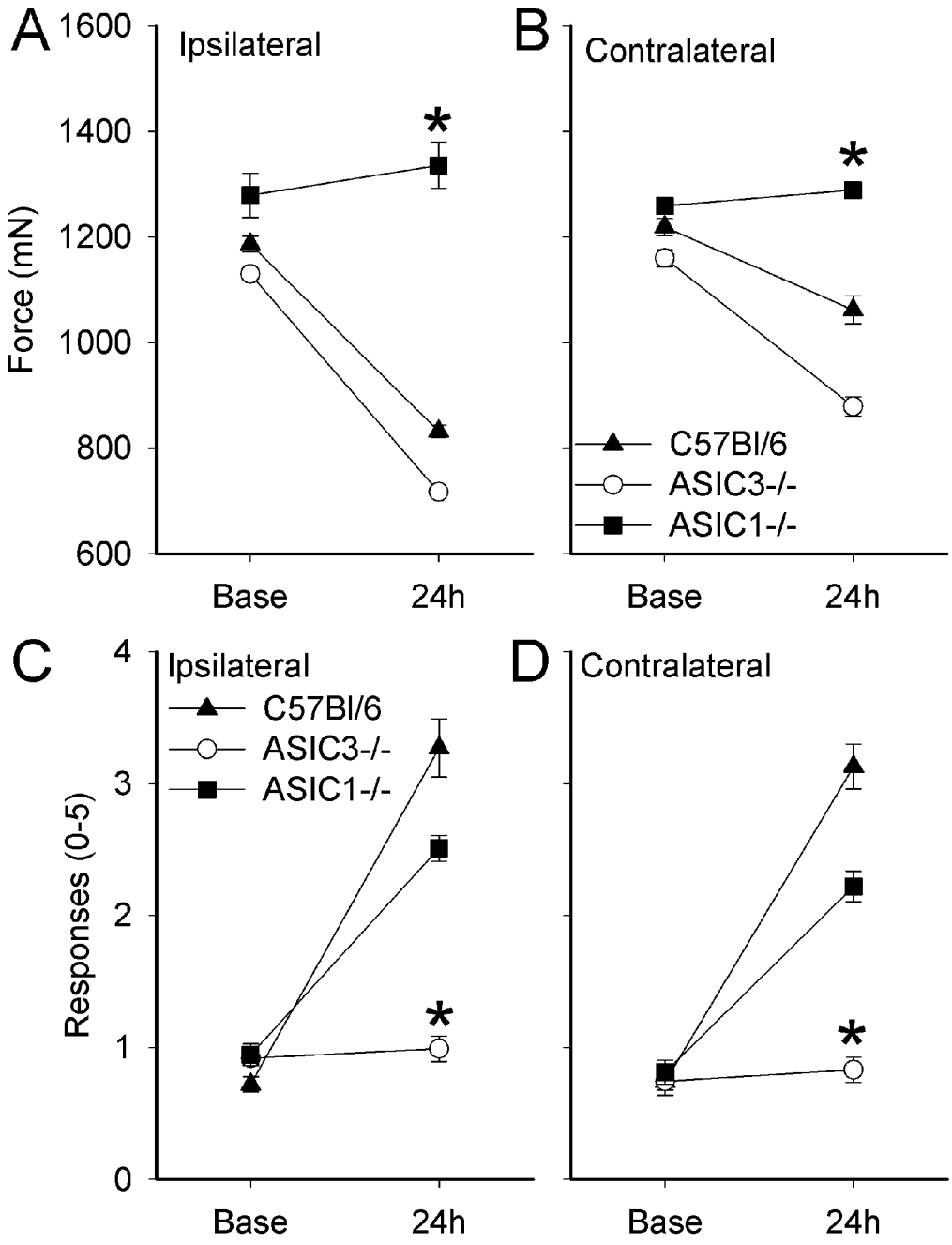

Figure 3.

A and B) Muscle hyperalgesia (tweezers) develops similarly in both *C57Bl/6* (WT) and *ASIC3−/−* mice after muscle inflammation on both ipsilateral and contralateral sides. *ASIC1−/−* mice do not develop muscle hyperalgesia after muscle inflammation. **C and D)** Cutaneous mechanical hyperalgesia (von Frey) develops similarly in both *C57Bl/6* (WT) and *ASIC1−/−* mice on both sides. Cutaneous hyperalgesia does not develop in *ASIC3−/−* mice after muscle inflammation. (* = $P<0.05$).

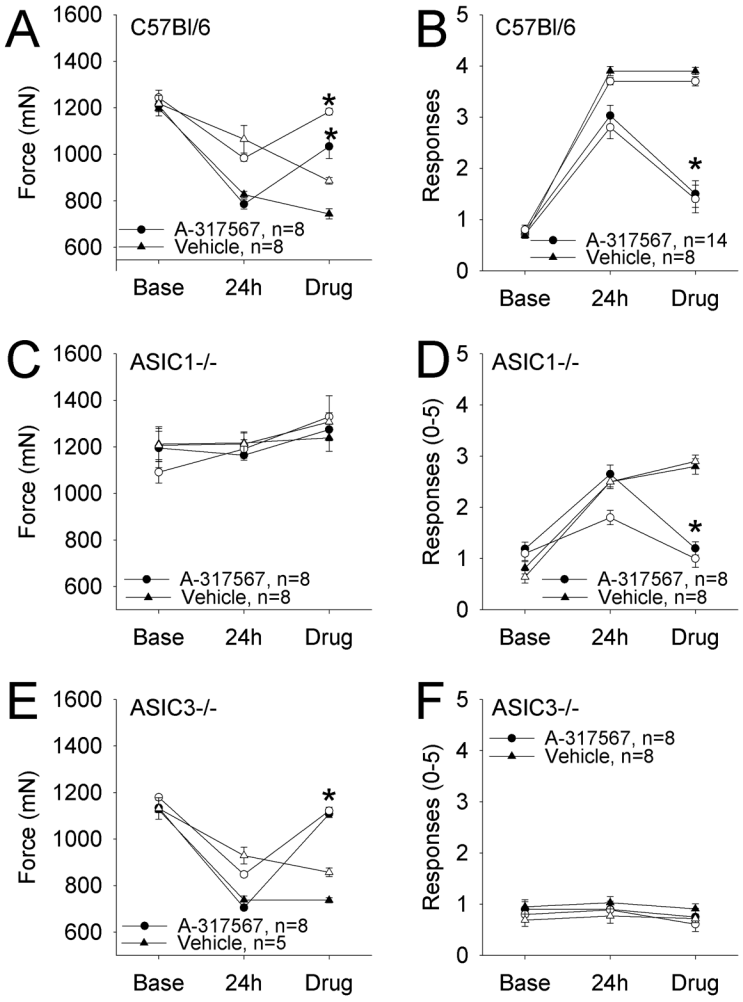

Figure 4. Effect of A-317567 on muscle inflammation in *C57Bl/6* (WT), *ASIC1*−/− and *ASIC3*−/− mice

A, C and E Muscle sensitivity (tweezers) in mice before and 24 h after carrageenan-induced muscle inflammation, and after A-317567 treatment. **B, D and F** Cutaneous mechanical sensitivity before and after muscle inflammation, and after A-317567 treatment. Closed symbols = ipsilateral, open symbol = contralateral sides. *C57Bl/6* (WT) mice **(A and B)** develop muscle hyperalgesia (tweezers) **(A)** and cutaneous mechanical hyperalgesia **(B)** 24 h after carrageenan-induced muscle inflammation and is reversed by A-317567. Hyperalgesia and reversal with A-317567 is seen in both the ipsilateral and contralateral sides. *ASIC1*−/− mice **(C and D)**, do not develop muscle hyperalgesia after muscle inflammation and A-317567 has no effect on muscle withdrawal thresholds **(C)**. Cutaneous mechanical hyperalgesia develops in *ASIC1*−/− mice after muscle inflammation and A-317567 reverses the hyperalgesia on both sides **(D)**. *ASIC3*−/− mice **(E and F)** develop muscle hyperalgesia after muscle inflammation and A-317567 reverses the effect on both ipsilateral and contralateral sides **(E)**. A-317567 has no effect on mechanical responsiveness in *ASIC3*−/− mice that do not develop mechanical hyperalgesia after muscle inflammation **(F)**. (* = P<0.05 when compared to the vehicle).