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# Increased response of muscle sensory neurons to decreases in pH after muscle inflammation

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## Abstract

Acid sensing ion channels (ASIC) are found in sensory neurons, including those that innervate muscle tissue. After peripheral inflammation there is an increase in proton concentration in the inflamed tissue, which likely activates ASICs. Previous studies from our laboratory in an animal model of muscle inflammation show that hyperalgesia does not occur in ASIC3 and ASIC1 knockout mice. Therefore, in the present study we investigated if pH activated currents in sensory neurons innervating muscle are altered after induction of muscle inflammation. Sensory neurons innervating mouse (C57/Bl6) muscle were retrogradely labeled with DiI. Two weeks after injection of DiI, mice were injected with 3% carrageenan to induce inflammation (n=8; 74 neurons) or pH 7.2 saline (n=5; 40 neurons, control) into the gastrocnemius muscle. 24h later sensory neurons from L4–L6 DRG were isolated and cultured. The following day the DRG neuron cultures were tested for responses to pH by whole-cell patch-clamp technique. Approximately 40% of neurons responded to pH 5 with an inward rapidly desensitizing current consistent with ASIC channels in both groups. The mean pH-evoked current amplitudes were significantly increased in muscle sensory neurons from inflamed mice (pH 5.0, 3602 ± 470 pA) in comparison to controls (pH 5.0, 1964 ± 370 pA). In addition, the biophysical properties of ASIC-like currents were altered after inflammation. Changes in ASIC channels result in enhanced responsiveness to decreases in pH, and likely contribute to the increased hyperalgesia observed after muscle inflammation.

### Keywords

inflammation; protons; DRG neurons; carrageenan; pain; voltage clamp

## INTRODUCTION

Acid sensing ion channels (ASICs) are voltage-insensitive $Na^+$-permeating channels activated by increases in extracellular proton. ASICs belong to the ENaC/DEG (epithelial



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amiloride-sensitive $Na^+$ channel/degenerin) family of ion channels (Kellenberger and Schild, 2002) and are expressed by four genes encoding seven different subunits. Functional channels form as a trimer complex of the same or different subunits (Benson et al., 2002, Jasti et al., 2007, Carnally et al., 2008). ASICs are present in both the central and peripheral nervous systems. Specifically, ASIC1, ASIC2 and ASIC3 are expressed in primary sensory neurons in trigeminal, nodose, and dorsal root ganglia (Lingueglia, 2007).

Molecular and behavioral studies suggest that ASICs are important in processing nociceptive information during inflammation. Decreases in pH occur in a variety of inflammatory exudates, including those from people with inflammatory arthritis. These decreases in pH are generally between 6.9 and 6.0 and correlate with the severity of the disease and pain (Habler, 1929a,b, Jebens and Monk-Jones, 1959, Goldie and Nachemson, 1969, 1970, Geborek et al., 1989). Decreasing pH in muscle, experimentally, produces pain and hyperalgesia in human subjects (Issberner et al., 1996, Frey Law et al., 2008). Furthermore, ASIC1, ASIC2 and ASIC3 mRNA levels are increased up to 15 fold after paw inflammation (Voilley et al., 2001) and ASIC2 and ASIC3 mRNA levels increased 10–12 fold after muscle inflammation (Walder et al., 2010). In parallel ASIC3 protein expression is increased in animal models of joint inflammation (Ikeuchi et al., 2008, 2009) and compression of the lumbar nerve root from spinal disk herniation (Ohtori et al., 2006). These increases in ASIC mRNA and protein could result in changes in pH-induced current in DRG. Previous reports show that ASIC current density is increased in rat DRG neurons in models of nerve injury, stomach ulcers, and after hindpaw inflammation (Sugiura et al., 2005, Poirot et al., 2006, Deval et al., 2008).

Studies suggest that ASICs play different roles in muscle pain compared to cutaneous pain. *ASIC1*−/− mice show loss of muscle hyperalgesia associated with muscle inflammation (Walder et al., 2010) but enhanced pain behavior after cutaneous inflammation with formalin test (Staniland and McMahon, 2009). *ASIC3*−/− mice show a loss of secondary hyperalgesia of the paw after deep tissue injury, however they have enhanced or no change in primary mechanical sensitivity of injured skin, paw or joint (Chen et al., 1998, Price et al., 2001, Mogil et al., 2005, Ikeuchi et al., 2008, Walder et al., 2010). Thus, ASICs play varied roles in pain, which is probably dependent upon the specific tissue injured, the specific tissue tested, and the specific ASIC subunit isoforms.

The purpose of the present study was to investigate if there were functional changes in ASICs in mouse sensory neurons innervating inflamed muscle. Using whole-cell patch-clamp analysis of pH-evoked currents we examined if inflammation caused: 1) ASICs expression in muscle DRG neurons that previously did not express ASICs, 2) increased ASIC expression in the cells that expressed them prior to inflammation, or 3) changes in ASIC properties.

## EXPERIMENTAL PROCEDURES

### Animals

For the present study we used C57BL/6 male mice (age 2–4 months; n=18) (Jackson Laboratories, Bar Harbour, Maine). The Animal Care and Use Committee at the University of Iowa approved all experiments.

### Labeling of muscle sensory neurons

Muscle sensory neurons were fluorescently labeled using the retrograde tracer DiI (1,1-dioctadecyl-3,3,3,3 tetramethylindocarbocyanine perchlorate). Animals were anesthetized with 2–5% isoflurane, and a small incision was made in skin over the left gastrocnemius muscle. 10μl DiI (17 mg/ml dissolved in 20% v/v ethanol and suspended in 80% v/v sterile

saline) was injected into the left gastrocnemius muscle. After injection, saline soaked sterile gauze was placed on the open incision for 10 min to prevent the dye from leaking to the overlying skin. The skin was then sutured closed and mice were allowed to recover for approximately 2 weeks.

### Induction of inflammation

To induce inflammation, mice were injected 2 weeks after DiI injection into the same gastrocnemius muscle with 20 μl of 3% carrageenan dissolved in sterile saline while the mice were deeply anesthetized with isoflurane (5%). As a control, a separate group of mice were injected with 20 μl pH 7.2 sterile saline into the left gastrocnemius muscle. 24 h after saline or carrageenan injection, mice were euthanized and the L4–L6 DRG neurons were isolated and cultured.

### Isolation of DRG neurons

L4–L6 DRG were collected and dissociated as previously described (Benson et al., 2002). In brief, the DRG were treated with papain and collagenase/dispase, and then gently triturated to isolate neurons. Neuron suspensions were then plated on 35mm Petri dishes coated with poly L-lysine and laminin. Cells were cultured in F12 medium supplemented with 10% heat inactivated serum, penicillin- streptomycin and 50ng/ml NGF. 24 hours after plating we examined cells with whole-cell patch-clamp.

### Electrophysiology of cultured DRG neurons

Whole-cell patch-clamp recordings of DiI labeled DRG neurons were performed at room temperature at a holding potential of 70mV. Currents were filtered at 1 kHz and sampled at 2 kHz using the Axopatch 200B amplifier, Digidata 1200 and Clampex 8.2 (Axon instruments, Union city, CA). Micropipettes (3–5 MΩ) were filled with internal solution (mM): 100 KCl, 10 EGTA, 40 HEPES, and 5 $MgCl_2$, pH 7.4 with KOH. External solutions contained (mM) 120 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 10 MES; pH was adjusted with tetramethylammonium hydroxide, and osmolarity was adjusted with tetramethylammonium chloride. Extracellular pH solutions (pH 7.4 (control)), 6.8, 6.5, 6.0, 5.0) were used to study ASIC currents. Whole cell capacitance was compensated. Solutions with different pH were applied directly to the cell by using a perfusion system BPS 8 (ALA scientific, Westbury NY), which was controlled by Digidata 1200 and Clampex 8 software (pClamp8).

To measure pH dose responses, pH currents activated by pH 5, 6, 6.5 and 6.8 solutions were normalized to pH 5 induced currents. Time constants (τ) for desensitization were measured from single exponential fit to the falling phase of the current evoked by pH application. The time course of recovery from desensitization was measured by completely desensitizing the ASIC current at pH 6 by a long desensitizing pulse followed by bathing in pH 7.4 for a defined time followed by a second stimulation at pH 6. Recovery is percentage of recovery of current evoked by second pulse by first pulse.

### Experimental Design

DRG from three groups of mice were used for the present study as follows: a control group that did not receive injections into the gastrocnemius muscle (n=7), a control group injected with pH 7.2 saline (n=6), and the experimental group injected with 3% carrageenan (n=8).

### Statistical analysis

Patch clamp data were analyzed using Clampfit (Axon instruments), Microsoft Excel and Origin 7 software (Northampton MA). A two-way ANOVA was used to study differences

between groups and differences between pH using SPSS 17. Post-hoc testing between groups was performed with a Tukey's test. $p<0.05$ were considered significant. Recovery from desensitization data was fit with single exponentials, and the rate constants ($\tau$) were assessed for statistical significance between groups using unpaired Student's *t* test. Data are represented as mean ± SEM.

## RESULTS

### ASIC-like acid-evoked currents in muscle DRG neurons

To study sensory neurons from muscle, we injected a retrograde tracer dye (DiI) into the gastrocnemius muscle of C57Bl/6 mice. 2 weeks after injection, dissociation and culture of the DRG (L4–L6) on the side ipsilateral to the muscle injection typically yielded 15–20 neurons labeled with DiI, whereas there were no labeled neurons on the contralateral side (Figure 1A). Only those with strong fluorescence labeling were subsequently studied for their acid responsiveness. Figure 1B shows representative pH-evoked currents recorded from a labeled muscle DRG neuron. In response to acidic pH, a transient $H^+$-gated current is activated which desensitizes within milliseconds. In some neurons, pH 5 and pH 6 activated a sustained current along with a transient component. These current properties are characteristic of ASIC channels (Benson et al.,2002, Hesselager et al.,2004). However, high proton concentrations (below pH 6.0) also activate TRPV1 channels in DRG neurons (Catarina et al., 1997). To better characterize the contribution of ASICs and TRPV1 to the pH-activated currents in muscle DRG neurons, we studied pH 5-evoked currents in the presence of an ASIC inhibitor (1mM amiloride) or TRPV1 inhibitor (30μM capsazepine) (Seabrook et al., 2002). Currents were blocked ~80% in the presence of amiloride ($p<0.05$) (Figure 1C), whereas capsazepine had minimal effect on pH 5-activated currents. These data suggest that ASICs are the primary channels that underlie pH-evoked currents in muscle DRG neurons.

### Inflammation does not alter the number of muscle DRG neurons expressing ASIC-like currents

We produced muscle inflammation by injecting either carrageenan or saline into the same gastrocnemius muscle that had previously been injected with DiI, and then collected and cultured DRG neurons 24 hr later. To begin to test if inflammation alters ASIC-like currents in muscle DRG neurons, we recorded the percentage of labeled neurons that responded to a pH 5 application (a neuron was defined as acid-responsive if pH 5 evoked an inward current greater than 100pA). Comparison of data from 3 groups of mice (carrageenan injected, saline injected and uninjected) found no statistical difference in the percentage of labeled neurons that respond to pH (Figure 2A). In addition, after carrageenan injection, pH 5-evoked currents from muscle afferents were inhibited by capsazepine or amiloride to a similar degree as currents from uninjected mice (Figure 1C), indicating that the contribution of ASICs and TRPV1 to pH-evoked currents was not altered after inflammation.

ASICs are expressed in small and medium sized neurons that primarily respond to noxious stimuli, and are also expressed in larger neurons that correspond to low threshold mechanoreceptors (Linquelia, 2007). We found acid-responsive muscle sensory neurons were slightly larger in diameter than unresponsive neurons (Figure 2B)($F_{1, 150} = 35.6$, $P = 0.001$). However, inflammation did not cause a shift in the size of acid-responsive or unresponsive neurons ($F_{2,150} = 0.34$, $P = 0.72$), suggesting that inflammation did not produce a shift in ASIC expression from one cell population to another. On the whole, these data suggest muscle inflammation did not recruit new expression of ASICs in muscle afferents that did not already express ASICs.

### Mean current amplitudes of pH-evoked currents are increased after inflammation

We next examined if inflammation alters ASIC-like current properties. By measuring the peak amplitudes of the transient pH-evoked currents, we found a significant difference between groups ($F_{2,285}$= 5.4, P =0.005)(Figure 3A). In the carrageenan group the mean maximal current amplitude, evoked by pH 5.0, was increased (3602 ± 470 pA) when compared to uninjected (2127 ± 470 pA) or saline injected (1964 ± 370 pA) controls. There was no interaction between the pH tested and group (pH*group, $F_{8,285}$ = 0.84, P= 0.57), indicating that there were increases in the carrageenan group at all pH values when compared to the controls. Thus, while inflammation did not increase the percent of DRG neurons that express ASIC-like currents, it did increase the amplitude of the currents in responsive cells.

### ASIC channel properties are altered by inflammation

Sensory neurons have been found to express ASIC1, ASIC2, and ASIC3 isoforms, and ASIC channels in DRG neurons generally form as heteromers (Benson et al., 2002). Each of the different heteromeric channels displays distinct biophysical properties (Benson et al., 2002, Hesselager et al., 2004). We hypothesized that inflammation might change the composition of the ASIC channels in muscle DRG neurons. In addition, post-translational modification of the channels can alter ASIC properties (Leonard et al., 2003, Bashari et al., 2008). To test for these possibilities we studied specific biophysical properties of pH-evoked currents that have been shown to distinguish different ASIC channels – including pH sensitivity, desensitization kinetics, and recovery from desensitization – to see if they were altered in muscle DRG neurons after inflammation.

Different ASIC channels possess markedly different pH sensitivity. By normalizing the current amplitude recorded at varying pH (6.8–6.0) to the pH 5 current amplitude, figure 3B shows a slight, but significant, shift in the pH sensitivity of ASIC-like currents after carrageenan-induced inflammation when compared to uninjected group. However, there was no difference when compared to saline-injected group.

As evident from the current traces in Figure 1B, ASIC currents desensitize in the continued presence of acidic pH. By fitting the desensitizing phase of the current to the single exponential, the rate ($\tau$) of desensitization can be measured (Figure 4A). The fast rates suggest the composition of ASIC channels in these cells are primarily heteromers since ASIC heteromers generally desensitize faster than ASIC homomers, and in fact, pH 6-evoked currents with rate constants less than 200 ms only occur when ASIC3 is present in a heteromeric complex with ASIC2a and/or ASIC1 subunits (Benson et al., 2002; Hesselager et al., 2004). However, inflammation did not change the rate of desensitization compared to the control groups.

After ASIC channels desensitize, they need to be exposed again to a more alkaline pH for some period of time to allow the channels to “recover”, before they can be activated again (see methods for protocol of how recovery was measured). Figure 4B shows that carrageenan-induced inflammation produced a significant shift in the rate of recovery from desensitization compared to the uninjected control group. Although there was a trend for the saline-injected group to recover more slowly that the uninjected group, this shift was not statistically significant.

In summary, inflammation produced an increase in ASIC-like current amplitudes in muscle sensory neurons. In addition, carrageenan-induced inflammation caused changes in the biophysical properties of ASIC-like currents when compared to uninjected control data.

## DISCUSSION

Our data demonstrates that muscle inflammation caused DRG neurons innervating muscle to become more responsive to acidic pH, without changing the number of cells that respond to decreases in pH. The enhanced response to acidic pH after inflammation was observed as an increased current amplitude to decreases in pH, and is consistent with prior studies showing increased ASIC current density after nerve injury (Poirot et al., 2006) or gastric ulcers (Sugiura et al., 2005). In addition, we found changes in the biophysical properties of the pH-evoked currents after inflammation; including a shift in the pH dose-response, such that the currents were more pH sensitive, and a slight slowing in the rate of recovery from desensitization. The increase in current amplitude is consistent with our prior work showing increased mRNA expression of ASIC2 and ASIC3 in DRG after muscle inflammation (Walder et al., 2010), and increased ASIC protein expression after inflammation (Ikeuchi et al., 2009). Moreover, we show here that the enhanced expression occurs in those muscle sensory neurons that previously express ASICs. Together these studies suggest increased mRNA and protein expression of ASICs underlies enhanced responsiveness to acidic pH in DRG neurons.

The interpretation of the alterations in biophysical properties is less straight forward. Changes in properties of ASIC channels can occur by altering the composition of the channels. Our previous work found that ASIC2 and ASIC3 mRNA were upregulated after muscle inflammation, but ASIC1 was unchanged (Walder et al. et al., 2010). Such a relative shift in ASIC subunit expression could change the subunit composition of the channels, and account for the changes in acid-evoked current properties that we observed. Alternatively, ASIC properties can be modulated by other post-translational mechanisms. Moreover, whereas carrageenan-induced inflammation caused changes in ASIC properties when compared to the uninjected group, these were not different when compared to data from the saline-injected group. We speculate that injection of saline into the gastrocnemius muscle might have induced a mild injury that caused a slight, but statistically insignificant, change in the biophysical properties, but did not increase ASIC expression to generate an increase in current amplitude. Consistent with this, we previously have shown that carrageenan, but not pH 7.2 saline, injected into muscle causes hyperalgesia (Sluka et al., 2001). Compared to the saline-injected group, carrageenan produced a more definitive shift in current properties, and increased ASIC expression to cause an increase in pH-evoked current amplitudes.

### ASIC channel composition in muscle DRG neurons

Although our studies do not directly test the contribution of various ASIC isoforms to pH-evoked currents, a comparison of our data to previous characterizations of ASIC channel properties provides clues regarding the composition of the ASIC channels in muscle afferents. Most muscle DRG neurons displayed pH-evoked currents with fast desensitization kinetics ($\tau \leq 200$ ms at pH 6). Previous studies of heterologously expressed ASICs have shown that only heteromeric channels that consist of ASIC3 with ASIC2a and/or ASIC1 subunits can reproduce these fast kinetics (Benson et al., 2002; Hesselager et al., 2004). This suggests that most ASICs in muscle DRG neurons are heteromeric channels comprised of multiple different subunits, and ASIC3 is a necessary component. This is consistent with our previous findings that ASIC3 null mice do not develop secondary mechanical hyperalgesia after muscle inflammation (Sluka et al., 2007; Walder et al., 2010), and ASIC3 mRNA is upregulated in DRG after muscle inflammation (Walder et al., 2010). In addition, we have shown that ASIC1a null mice show a reduction in hyperalgesia of the inflamed muscle (Walder et al., 2010), suggesting ASIC1a is also a contributor to the channel composition in muscle DRG neurons. Finally, we have previously found that ASIC2 mRNA is upregulated in DRG after muscle inflammation (Walder et al., 2010), which suggests that ASIC2 might also a be a component of the channels, at least after inflammation. In a related population of

muscle afferents, we previously described that ASIC2a/ASIC3 heteromeric channels are the principle pH sensors in DRG neurons that innervate the heart (Sutherland et al., 2001, Hattori et al., 2009). In sum, our data suggests that the channel composition in muscle afferents is likely to be heteromeric channels consisting of ASIC3, and some combination of ASIC2 and ASIC1 subunits. Further studies will be necessary to decipher the specific contributions of various ASIC subunits in muscle DRG neurons under normal conditions and after muscle inflammation.

### Significance of increased ASIC expression after muscle inflammation

Inflammation results in a decrease in tissue pH, which activates nociceptors. Moreover, inflammation causes an increase in ASIC expression and an increase in ASIC function (Walder et al., 2010; Voilley et al., 2001; Ikeuchi et al., 2009), which would further enhance responsiveness to acidic pH, and manifest as an increase in neuronal excitability. This enhanced excitability could be the driving force for maintaining hyperalgesia observed after muscle inflammation and is consistent with our prior studies show that blockade of ASICs reverses existing muscle hyperalgesia (Walder et al., 2010). This increased sensitivity of nociceptors to peripherally applied stimuli, termed peripheral sensitization, has been previously observed after muscle inflammation (Diehl et al., 1993), and our data suggests that ASICs are part of the underlying molecular mechanism.

It should be noted that TRPV1 also mediates acid-sensitivity in cutaneous and muscle tissue and is upregulated after cutaneous inflammation (Voilley et al., 2001, Breese et al., 2005) and therefore may contribute to this pH effect we observed. However, our studies show that the observed pH-evoked currents are not inhibited by blockade of TRPV1 and the channel kinetics we observed are similar to ASICs.

### Muscle pain is processed differently from cutaneous pain

Musculoskeletal pain is uniquely different from cutaneous pain; in general, it is more diffuse and longer-lasting (Mense, 2008). For example, injection of the irritant capsaicin into the skin of the rat hindpaw produces a short-lived hyperalgesia (hours), whereas the same injection into the muscle or joint causes a much longer hyperalgesia (days)(Sluka, 2002). Similarly, electrical stimulation of a cutaneous sensory nerve produces a much shorter excitation of the ventral root than electrical stimulation of a muscle sensory nerve (Wall and Woolf, 1984). In human subjects, activation of cutaneous nociceptors produces a sharp, well-defined, localized pain (Torebjork, 1985); in contrast, activation of muscle nociceptor produces a more diffuse and aching or cramping pain (Simone et al., 1994). Moreover, infusion of acidic buffer into the skin of human subjects produces a localized pain only, while infusion of acid into muscle produces an additional referred pain (Steen and Reeh, 1993, Frey Law et al., 2008).

Differences in nociceptive processing from muscle and skin are found anatomically as well as behaviorally. Nociceptors innervating muscle express more substance P and CGRP, and less isolectin B4 and somatostatin, when compared to nociceptors innervating skin (O'Brien et al., 1989). Relevant to our work, small muscle afferents express more ASIC3 when compared to cutaneous afferents (Molliver et al., 2005). Accordingly, accumulating evidence suggest a differential role of ASICs in musculoskeletal pain compared to cutaneous pain. *ASIC3*−/− mice responses to noxious stimuli are unchanged, or even enhanced, after cutaneous paw inflammation (Chen et al., 1998, Price et al., 2001, Staniland and McMahon, 2009), while they show complete loss of hyperalgesia after muscle or joint inflammation (Sluka et al., 2007, Ikeuchi et al., 2009). Similarly, *ASIC1*−/− mice demonstrate no difference in hyperalgesia after cutaneous paw inflammation (Staniland and McMahon, 2009), but there is a loss of hyperalgesia after muscle inflammation (Walder et al., 2010).

Furthermore, after hyperalgesia associated with muscle inflammation has developed in wild-type mice, it can then be attenuated by non-selective blockade of ASICs (Walder et al., 2010). This suggests that activation of ASICs is important for the development and continued maintenance of the hyperalgesia associated with deep tissue inflammation.

In summary, our results indicate that inflammation causes increases in expression of ASIC subunits, which manifest as increases in acid-evoked current amplitudes in muscle sensory neurons. In addition, alteration in expression might underlie changes in current properties. Our work suggests that these changes in ASICs likely contribute to the development of hyperalgesia after muscle inflammation.

## Abbreviations

| | |
|---|---|
| **ASIC** | Acid sensing ion channel |
| **ANOVA** | Analysis of variance |
| **DRG** | dorsal root ganglia |

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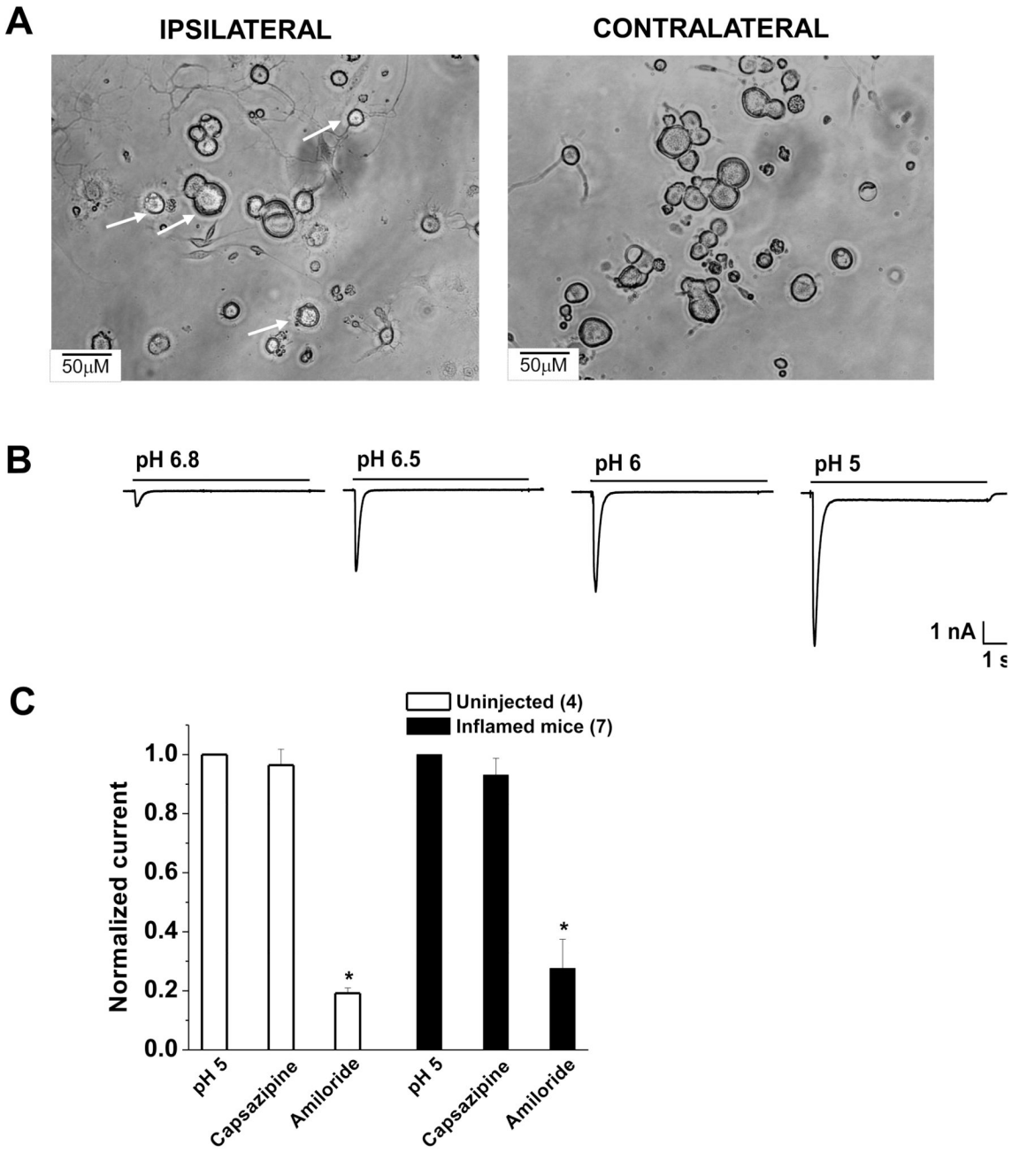


**Figure 1.**

Acid-evoked currents from muscle DRG neurons. **A**, Fluorescent/phase micrographs of cultured L4–L6 DRG neurons 2 weeks after injection of DiI into the gastrocnemius muscle demonstrating that labeled neurons (arrows) were seen only in DRG preparations from the ipsilateral, and not the contralateral side. **B**, Representative currents evoked by applications of pH 6.8, 6.5, 6, or 5 in a labeled muscle DRG neuron. **C**, pH 5-evoked current amplitudes from muscle DRG neurons in the presence of capsazepine (30μM) or amiloride (1mM) normalized to pH 5-evoked currents without blockers. In DRG neurons from both uninjected and carrageenan-injected (inflamed) there was a significant decrease in the pH 5.0-evoked

current in the presence of amiloride, but not in the presence of capsazepine (* $p<0.05$ by paired Student's *t* test).

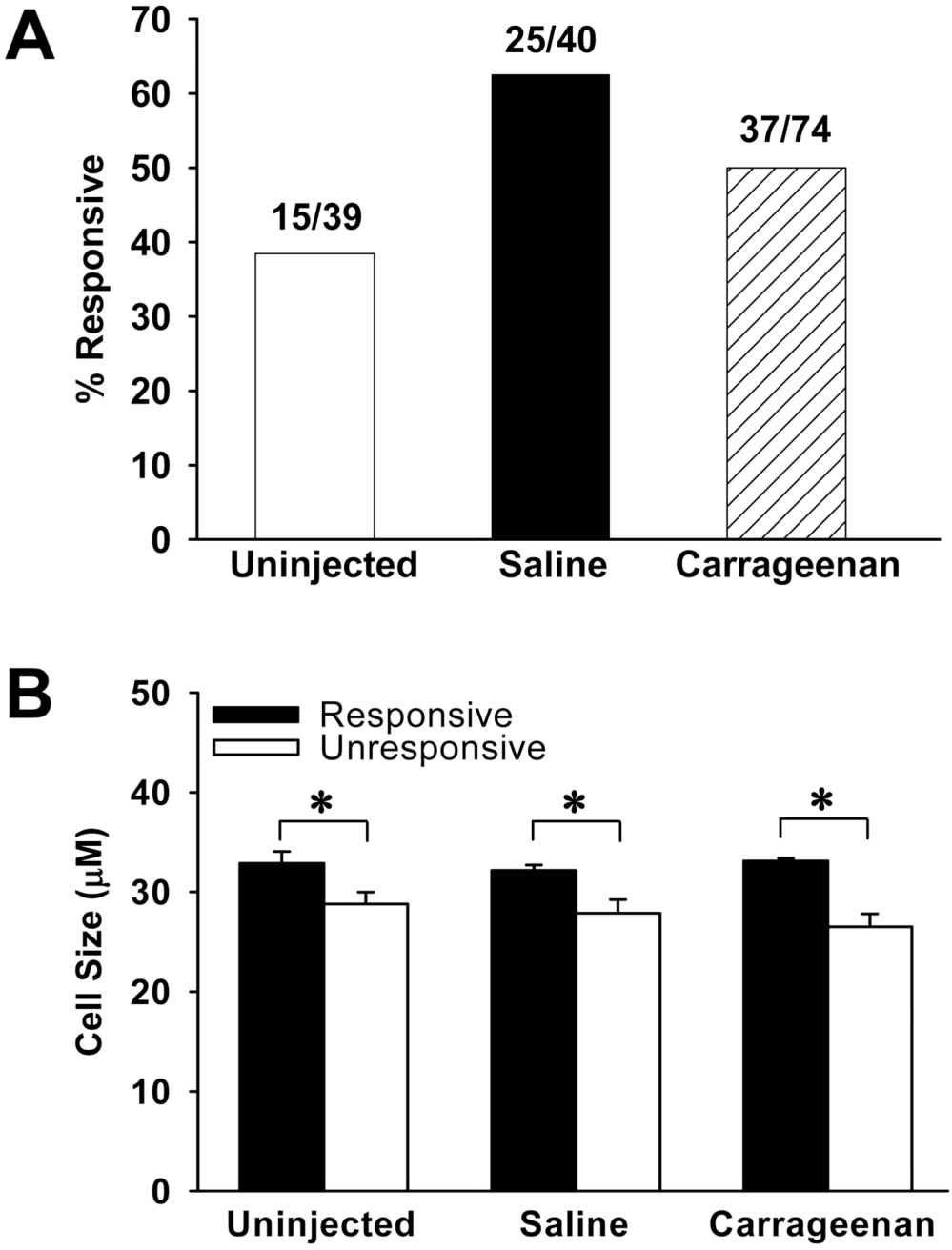


**Figure 2.**

The percentage and cell diameter of muscle DRG neurons that respond to acidic pH is unchanged by inflammation. **A**, The percentage of muscle DRG neurons that responded to pH 5 application with a current that was greater than 100 pA was not different between the three study groups. The numbers above bars are the number of responsive cells per total studied. **B**, The mean cell diameter of the muscle DRG neurons that respond to acidic solution (pH 5) was 32.9±1.2 μm for uninjected controls (n=15), 32.2±0.6 μm for saline-injected group (n=25) and 33.1±0.3 μm for carrageenan-injected mice (n=39). The neurons that did not respond to pH 5.0, termed unresponsive, were on average smaller in size:

28.8±1.2 μm for uninjected controls (n=19), 27.9 ±0.5 μm for saline-injected group (n=15) and 26.5±1.3 μm for carrageenan-injected mice (n=37).

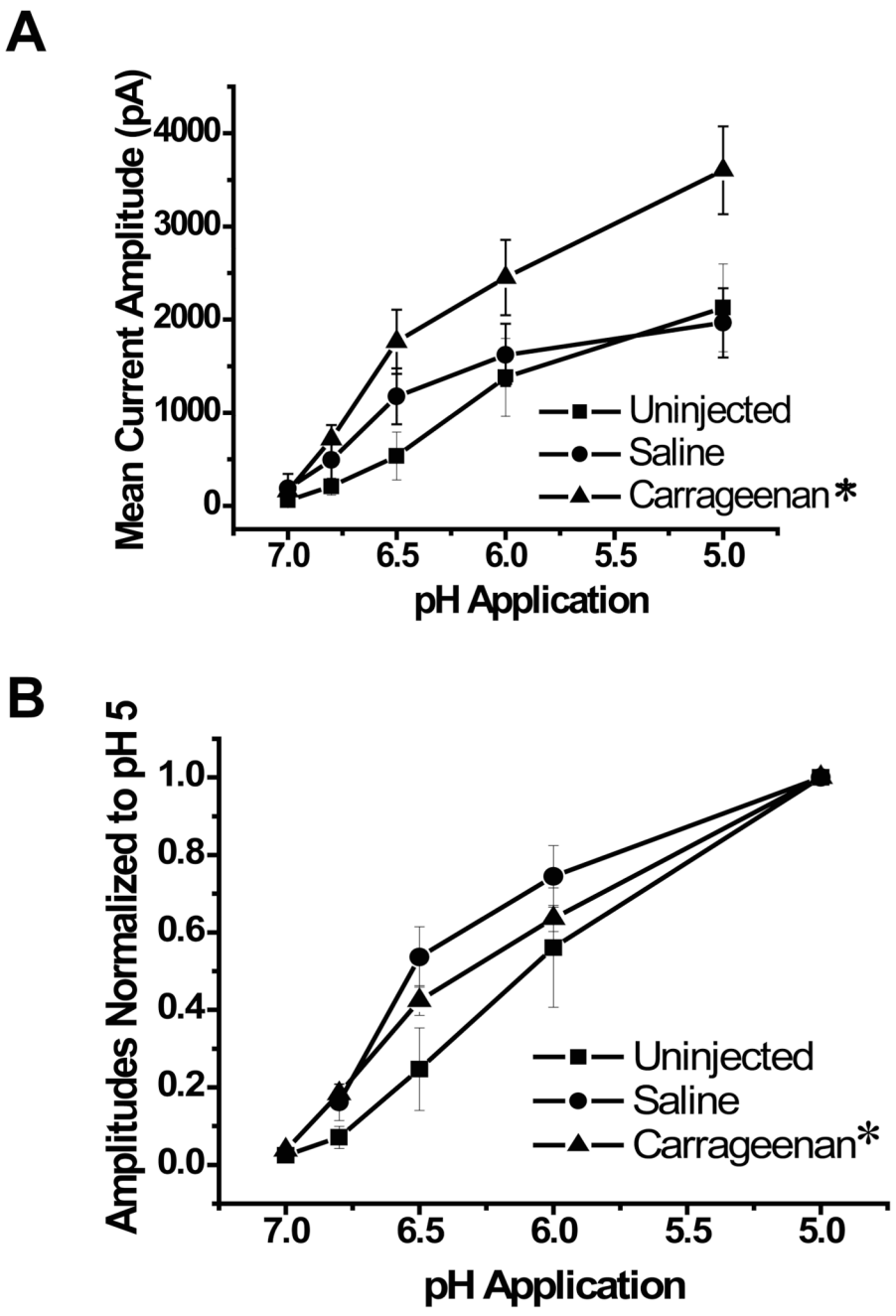


**Figure 3.**

The acid-evoked current amplitudes and pH sensitivity are altered by inflammation. **A**, The mean amplitudes of pH currents at pH 5, 6, 6.5, 6.8 and 7.0 are significant increased in muscle DRG neurons after carrageenan injection (n= 38), compared to the uninjected (n=15) or saline-injected groups (n=24). (* $p<0.05$). **B**. Data from A was normalized to the peak transient currents evoked by pH 5 to analyze pH dose-responses. The carrageenan-injected group showed a significant increase in pH sensitivity compared to the uninjected group (* $p<0.05$). Note the asymmetry of the curves; thus they did not fit well to the Hill equation.

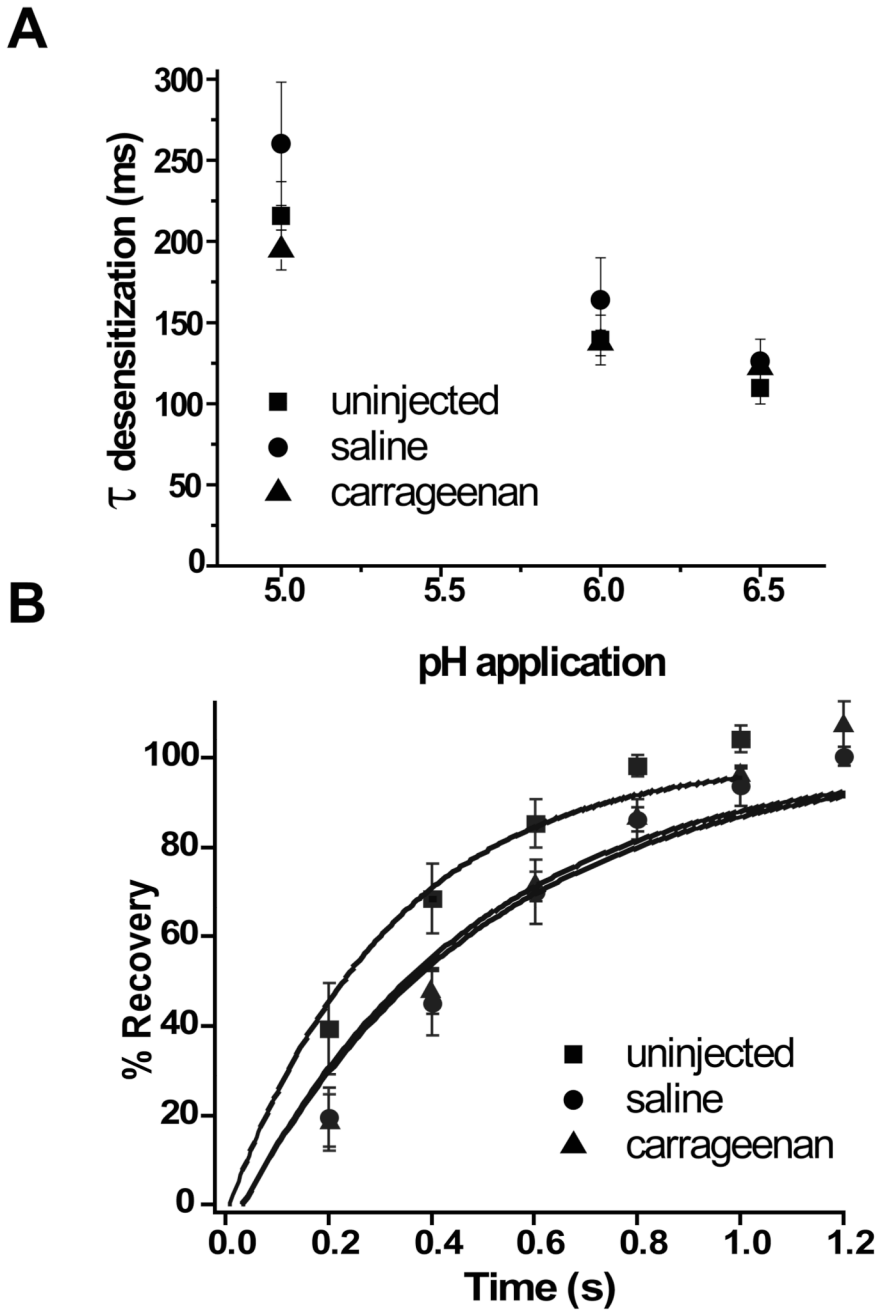


**Figure 4.**

The recovery from desensitization, but not the kinetics of desensitization, of acid-evoked currents from muscle DRG neurons is altered by inflammation. **A**, The mean time constants (τ) of desensitization of the transient currents evoked by the indicated pH solutions were similar between uninjected (n=12), saline-injected (n=22), and carrageenan-injected groups (n=31). **B**. The rate of recovery from desensitization (see methods) was slower in muscle DRG neurons after carrageenan injection (n=7) compared to the uninjected group (n=6) (* $p<0.05$), but was not different than the saline-injected group (n=5).