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Invited review

ASICs and mammalian mechanoreceptor function

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ABSTRACT

It is well established that some members of the Deg/ENaC super family of amiloride sensitive ion channels can participate directly in the transduction of mechanical stimuli by sensory neurons in invertebrates. A large body of work has also implicated the acid sensing ion channels family (ASIC1-4) as participants in regulating mechanoreceptor sensitivity in vertebrates. In this review we provide an overview of the physiological and genetic evidence for involvement of ASICs in mechanosensory function. On balance, the available evidence favors the idea that these channels have an important regulatory role in mechanosensory function. It is striking how diverse the consequences of *Asic* gene deletion are on mechanosensory function with both gain and loss of function effects being observed depending on sensory neuron type. We conclude that other, as yet unknown, molecular partners of ASIC proteins may be decisive in determining their precise physiological role in mechanosensory neurons.

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1. Introduction

Almost all the somatic tissues of the body are innervated by sensory neurons that are able to detect mechanical stimuli, be it end-organ stretch, indentation or very light brush. The transduction of a mechanical stimulus, whether it be a change in force or movement, is thought to take place via the direct gating of mechanosensitive channels present at the endings of sensory neurons (Lewin and Moshourab, 2004; Poole et al., 2011; Smith and Lewin, 2009). Somatic sensory neurons with their cell bodies in the dorsal root ganglia (DRG) or trigeminal ganglia also possess mechanosensitive currents that have been recorded in acutely isolated sensory neurons (Coste et al., 2007; Drew et al., 2004, 2002; Hu et al., 2010; Hu and Lewin, 2006; Lechner et al., 2009; Lechner and Lewin, 2009; McCarter et al., 1999). The molecular nature of mechanically gated channels that underpin these mechanosensitive currents has been a subject of intense research activity over the last 20 years.

A major first step in the molecular dissection of mechanoreceptor function came with the discovery that touch transduction in the nematode *Caenorhabditis elegans* requires a two transmembrane, amiloride-sensitive ion channel protein related to epithelial sodium channels (ENaCs) (Driscoll and Chalfie, 1991; Ernstrom and Chalfie, 2002). This protein, called MEC-4, forms a heteromeric Na^+-selective channel together with MEC-10, and a detailed body of work has shown that this channel carries the receptor current, which initiates body touch reception in *C. elegans* (Ernstrom and Chalfie, 2002; O'Hagan et al., 2005; O'Hagan and Chalfie, 2006). Subsequent to the discovery of the *mec-4* gene in *C. elegans*, a number of groups identified related genes in mammals that are expressed in sensory neurons and were shown to be gated by low pH, the first of which was ASIC1a (Waldmann et al., 1997b). These proteins, now designated acid sensing ion channels (ASICs), are coded by four genes that with alternative splicing generate 6 isoforms, each with distinct biophysical properties (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4). The ASICs form Na^+-selective and amiloride-sensitive ion channels and thus were attractive candidates for the mechanosensitive currents in adult sensory neurons that were shown by some workers to be Na^+-selective (Hu and Lewin, 2006; Lechner et al., 2009). Other groups have claimed that the RA-mechanosensitive current is non-selective (Coste et al., 2007; McCarter et al., 1999), but there are methodological differences between studies that may confound their interpretation (see Poole et al., 2014). Indeed some workers have reported that mechanosensitive currents in isolated sensory neurons can be

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inhibited by high concentrations of amiloride or benzamil (Coste et al., 2007; McCarter et al., 1999), but this finding has not been reproduced by other workers (Drew et al., 2002; Hu and Lewin, 2006). It is at present unclear why some groups see a block of mechanosensitive currents with amiloride or its analogs and others do not. Here we will review the available evidence that implicates ASIC proteins in the regulation of mechanosensitivity in different types of somatic sensory neurons.

2. Mechanoreceptor function in the absence of single or multiple ASIC proteins

If ASICs are directly involved in the transduction of mechanical stimuli by sensory neurons then deletion of single or multiple *Asic* genes in the mouse should have a significant impact on sensory neuron function. Indeed, multiple studies have shown that mechanoreceptor function is altered in the absence of different ASIC subunits. Below we have summarized the published literature starting with those channels for which the most robust changes have been observed after gene targeting.

2.1. ASIC3

Originally cloned from cDNA library derived from rat DRG, ASIC3 (originally known as DRASIC, Dorsal Root Acid Sensing Ion Channel) (Waldmann et al., 1997a) was found to be expressed in many large-diameter and small-diameter DRG neurons (Babinski et al., 1999; Price et al., 2001). Consistent with a role in sensory transduction that takes place at the peripheral endings of sensory neurons, ASIC3 immunoreactivity was detected in Meissner's corpuscles, palisades of the hair shaft lanceolate endings, Merkel cells and free nerve endings (Price et al., 2001), all end-organs innervated by distinct mechanoreceptor types. Mutant mice with a targeted deletion of the *Asic3* gene have been examined for deficits in the function of sensory afferents innervating the hairy skin. Interestingly, the absence of ASIC3 was associated with both reductions and enhancements of sensory afferent function (Moshourab et al., 2013; Price et al., 2001). Specifically amongst low threshold mechanoreceptors there was a highly specific increase in the sensitivity of rapidly-adapting mechanoreceptors (RAMs) to moving stimuli in the absence of effects on slowly-adapting mechanoreceptors (SAMs) or D-hair receptors (Moshourab et al., 2013; Price et al., 2001). In the hairy skin RAMs are associated with hair follicles and with Meissner's corpuscles in glabrous skin (Lechner and Lewin, 2013) (Fig. 1). The increased velocity sensitivity of RAMs in the absence of ASIC3 was entirely due to reduced adaptation during a constant velocity movement and could be largely mimicked by acute application of Apetx2, a toxin which blocks ASIC3 homomeric channels and most ASIC3 heteromeric channels (Diochot et al., 2004; Moshourab et al., 2013). In contrast, A-fiber mechanonociceptors (AMs) that are involved in signaling fast mechanical pain (Lewin and Moshourab, 2004) showed reduced sensitivity in the absence of ASIC3 protein (Moshourab et al., 2013; Price et al., 2001). No changes in the mechanosensitivity of C-fiber nociceptors were noted although some of these fibers may require ASIC3 to signal sustained drops in extracellular pH in the skin (Price et al., 2001). Pressure applied to the skin leads to local vasodilatation, probably via activation of the axon reflex (Jänig and Lisney, 1989; Lewin et al., 1992). Recent evidence supports a role for ASIC3 in detecting pressure-induced vasodilatation in both humans and mice, a response that protects against pressure-induced ulceration (Fromy et al., 2012). Unlike in wild type mice, pressure-induced vasodilatation was never observed in ASIC3 null mice, leading to an early decrease of the cutaneous blood flow upon application of very low pressures (Fromy et al., 2012). This data is consistent with the idea that the relatively modest reductions in AM fiber sensitivity to mechanical stimuli observed in *Asic3* mutant mice (Moshourab et al., 2013; Price et al., 2001) are physiologically relevant for pressure-induced vasodilatation. Behavioral tests carried out with *Asic3* as well as *Asic1a* and *Asic2* null mice, showed normal paw withdrawal behaviors in the punctate von Frey filament test (Borzan et al., 2010; Chen et al., 2002; Page et al., 2004; Staniland and McMahon, 2009). However, *Asic3* null mice had greater sensitivity to stroking with a von Frey hair when compared to wild

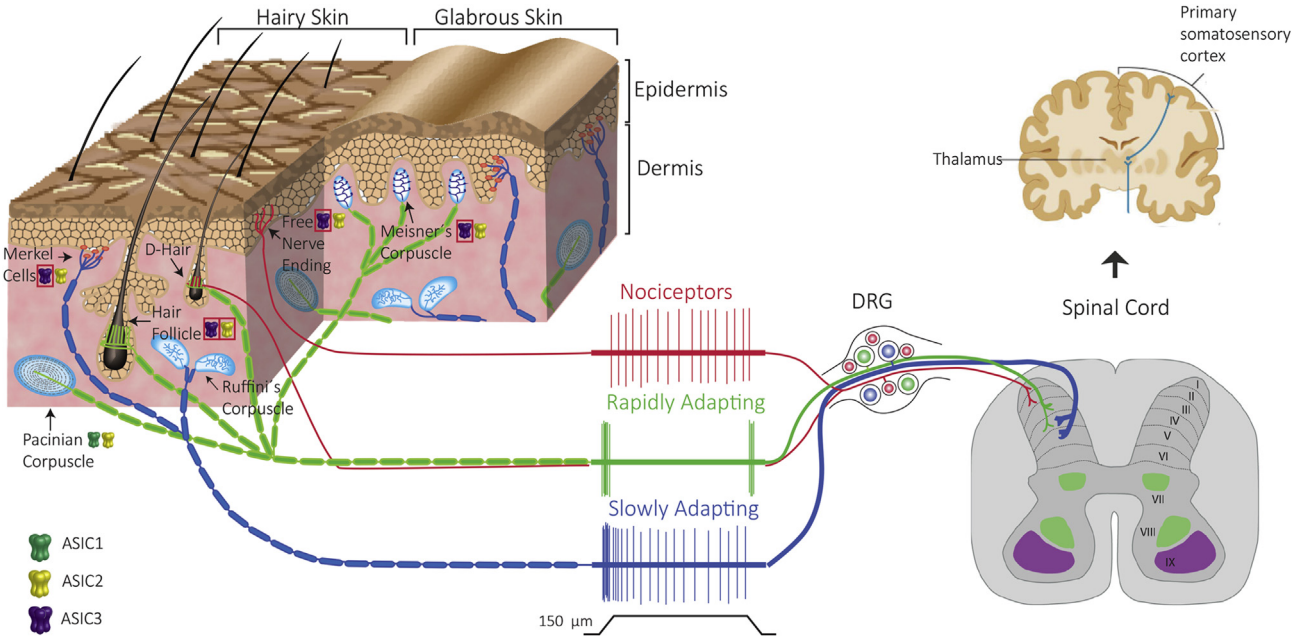

Fig. 1. ASIC channels in mechanoreceptor end-organs. Dorsal root ganglia afferents terminate in glabrous and hairy skin as distinct sensory organs while central termini project to distinct dorsal horn laminae. Expression of ASIC1, ASIC2 and ASIC3 subunits in sensory organs is shown. Red rectangles represent where absence of staining has been observed in designated organs in knock-out mice.

type littermates, an effect that was not observed in *Asic2* null mice (Borzan et al., 2010). The alteration in stroking-induced behavior might conceivably be a behavioral correlate of increased RAM sensitivity in *Asic3* mutant mice.

Intramuscular acid injection activates nociceptors and evokes central sensitization and mechanical hyperalgesia to stimulation of the paw (Sluka et al., 2001). Interestingly, the development of secondary mechanical hyperalgesia was largely abolished in *Asic3* null mice, but not in *Asic1a* mutant mice (Sluka et al., 2003). In a follow-up study it was found that *Asic3* mutant mice develop primary hyperalgesia at the site of the injury, but not secondary hyperalgesia, in contrast, *Asic1a* mutant mice did not develop primary hyperalgesia but did develop secondary hyperalgesia (Walder et al., 2010). Interestingly, mechanical hyperalgesia induced by cutaneous injection of carrageenan was slightly more pronounced in *Asic3* null mice (Price et al., 2001), supporting the view that this channel subserves distinct functions in afferents innervating muscle and skin. Chen and colleagues also generated an *Asic3* mutant mouse and observed reduced latency for the onset of pain behaviors when stimuli of moderate to high intensity were used (Chen et al., 2002). The cellular basis of this altered behavior was not investigated in their mouse model.

A marked impairment of gastrointestinal mechanosensory function was observed in *Asic3* null mice – sensitivity of all the afferent classes innervating the upper and the lower gut was reduced, with the exception of gastroesophagal mucosal receptors (Page et al., 2005). However, gastric emptying and fecal pellet output was unaltered in these mice. Similarly, loss of ASIC3 was shown to decrease the sensitivity to phasic colon distention and afferent fiber sensitivity to stretch of the colon was reduced while inflammatory mediators failed to enhance afferent fiber responses (Jones et al., 2005). Bielefeldt and Davis found that *Asic3* gene deletion is associated with impaired mechanosensitivity of vagal afferents upon gastric distention only at lower stimulus intensities, suggesting a relatively subtle effect of ASIC3 on gastroesophagal mechanosensation (Bielefeldt and Davis, 2008).

2.2. ASIC1

Targeting the *Asic1a* gene in the mouse was shown to be associated with quite marked changes in visceral mechanoreceptor function (Page et al., 2004). Both colonic and gastroesophagal afferents showed increased sensitivity to mechanical stimuli in *Asic1a* mutant mice compared to controls. The gastric emptying time in *Asic1a* null mice was increased compared to wild type littermates, but the fecal output was unaffected (Page et al., 2005, 2004). The ex vivo skin-nerve preparation was used to test the functional properties of cutaneous afferents in *Asic1a* mutant mice. However, no effects of *Asic1a* gene deletion were observed in the sensitivity of five distinct populations of cutaneous mechanoreceptors and the behavioral responses of these mice to mechanical and thermal stimuli were similarly unaffected by *Asic1a* gene disruption (Page et al., 2004). It is noteworthy that the first exon of the *Asic1* gene was targeted in the *Asic1* knock-out model used in the aforementioned studies, thus deleting the ASIC1a splice variant, while not affecting the expression of the ASIC1b isoform (Wemmie et al., 2002).

2.3. ASIC2

Targeted disruption of the *Asic2* gene, which resulted in ablation of the ASIC2a and ASIC2b isoforms, was found to be primarily associated with a reduced sensitivity of low-threshold rapidly adapting (RA) mechanoreceptors (Price et al., 2000). No effects of *Asic2* gene deletion were observed on the functional properties of myelinated nociceptors (AM) or non-myelinated nociceptive C-fibers. Consistent with this moderate functional change, ASIC2-immunostaining was found in the lanceolate endings that surround hair follicles, the end-organs of RAMs in the hairy skin (Lechner and Lewin, 2013; Price et al., 2000). Another study reported immunostaining of Meissner's and Merkel's corpuscles, penicillate, reticular and lanceolate endings, and hair follicle palisades, as well as some intraepidermal and free myelinated nerve endings, with an ASIC2a-specific antibody in rat and mouse skin (Fig. 1) (García-Añoveros et al., 2001). Roza and colleagues also generated an *Asic2* knock-out mouse and did not observe any change in cutaneous RAM properties when compared to the wild type littermates (Roza et al., 2004). It is possible that the velocity sensitivity of RAMs is primarily affected in the absence of the ASIC2 channel, but neither study used stimulus protocols suitable to test this hypothesis.

Clear alterations in the mechanosensitivity of visceral afferents were observed in *Asic2* mutants, but the absence of this protein led to differential effects in distinct sub-populations. Thus there were reductions in the sensitivity of lower gut afferents to mechanical stimuli, but upper gut afferents showed enhanced sensitivity in *Asic2* mutant mice. Furthermore, the disruption of *Asic2* led to increased sensitivity of vagal gastroesophagal mucosal receptors, but was associated with a decrease in tension-receptor sensitivity. On the other hand, colonic mesenteric afferents were unaffected in *Asic2* mutant mice, however, there was a concurrent increase in the sensitivity of serosal mechanoreceptors (Page et al., 2005). In contrast to *Asic1a* deletion, loss of *Asic2* did not affect gastric emptying time, but changed lower bowel function by decreasing the number of fecal pellets. The visceral neurosecretory nociceptive fibers release neuropeptides such as calcitonin gene-related peptide (CGRP) and tachykinins upon stimulation and this process is thought to be involved in signaling discomfort and pain upon excessive distention in the gastrointestinal tract and urinary bladder (Roza and Reeh, 2001). However, in *Asic2* mutant mice, pressure-stimulated CGRP release from the colon was the same as in the littermate controls, suggesting that ASIC2 has no effect on visceral mechanonociception, although it should be noted that no electrophysiological recordings were reported (Roza et al., 2004).

More recently, ASIC2 was shown to be an important determinant of arterial baroreceptor sensitivity (Lu et al., 2009). Aortic baroreceptor neurons of the nodose ganglia and their terminals express ASIC2 protein and disruption of this channel in mice was associated with hypertension and a decreased gain of the baroreflex. Furthermore, the mice had exaggerated sympathetic and reduced parasympathetic control of the circulation, suggesting that mechanosensitivity is diminished in *Asic2* null mice (Lu et al., 2009). In this context it is interesting that a recent study in humans has shown that touch sensation may have a common genetic basis with baroreflex sensitivity (Frenzel et al., 2012).

3. Disruption of multiple *Asic* genes

The study of *Asic* gene knock-out mice has clearly implicated this ion channel family in mechanosensation, however, the apparent increase in certain mechanosensitive properties upon deletion of ASICs suggests that these channels function as regulators and modulators of mechanosensation, rather than transducers. Another possible explanation for the complex phenotypes observed in the knock-out studies would be functional redundancy of the remaining ASICs and/or compensatory effects. Given that ASICs assemble as trimers (Carnally et al., 2008; Jasti et al., 2007) and that ASIC1-4 are expressed in DRG neurons, evidence suggests that different ASIC subunits assemble as heteromers in order to form proton-gated channels in mouse sensory neurons (Benson

et al., 2002). Therefore, deletion of one *Asic* gene could merely change the subunit composition of the remaining trimeric channels, the composition of which will of course depend on expression of ASIC subunits in each sensory neuron sub-type. As such, it might be expected that deletion of multiple *Asic* genes would reveal more profound mechanosensory phenotypes. A triple knock-out mouse model in which *Asic1a*, *-2* and *-3* genes were deleted was generated and is viable. Behavioral assessments showed that the animals were hypersensitive to mechanical stimuli with an increased paw withdrawal frequency observed after stimulation of the paw with von Frey filaments. Recordings from hairy skin afferents in these triple mutants revealed that AMs displayed increased activity to mechanical stimuli. No alteration in the mechanosensitivity of any other receptor type was observed compared to the wild type littermates (Kang et al., 2012). This was a particularly surprising finding as it suggests that the increased responsiveness of RAMs in *Asic3* mutant mice (Moshourab et al., 2013; Price et al., 2001) was rescued by deletion of other *Asic* genes. Unfortunately, the authors did not compare their data obtained from triple mutants with data from single *Asic* gene deletions recorded at the same time with the same methodology. Another approach to address the function of ASICs in mechanosensation was developed by Seguela and colleagues. Here, a transgenic knock-in mouse was made in which a dominant-negative form of ASIC3 that inactivates all the neuronal ASIC-like currents was expressed (Mogil et al., 2005). Interestingly, these transgenic mice exhibited hypersensitivity in a battery of behavioral nociceptive assays, including mechanical pain, chemical/inflammatory pain, mechanical hypersensitivity after zymosan inflammation and mechanical hyperalgesia after intramuscular injection of hypotonic saline. It is noteworthy that the authors used the FVB/J mouse strain for the majority of electrophysiological and behavioral tests, while Price and colleagues used mice on a $(129 \times C57BL/6)F_2$ background (Mogil et al., 2005; Price et al., 2001). No electrophysiological investigations of the mechanosensitivity of sensory neurons innervating viscera in this transgenic model have to our knowledge been published.

Consistent with the lack of change in mechanoreceptor properties in *Asic* triple mutant mice, Drew and colleagues had already reported that the amplitude and incidence of mechanically-activated currents recorded from isolated DRG neurons from *Asic2*/*Asic3* double mutant as well as *Asic2* and *Asic3* single mutant mice were unchanged compared to controls (Drew et al., 2004). Interestingly, the expression of *Asic2b* and *Asic3* were dramatically up-regulated during a developmental period in the embryo where large diameter sensory neurons acquire mechanosensitive currents (Lechner et al., 2009). However, deletion of the *Asic2* or *Asic3* genes did not alter the amplitude or kinetics of mechanosensitive currents in DRG neurons (Lechner et al., 2009). Taken together, data from double and triple knock-out studies strongly suggest that heteromeric ASICs are not required for the function of mechanosensitive channels *per se*, but do modulate the mechanosensitivity of sensory neurons. Rapidly-activating and inactivating mechanically gated currents, often termed RA-mechanosensitve currents, are found in DRG neurons with a mechanoreceptor function (Coste et al., 2007; Hu et al., 2010; Hu and Lewin, 2006; Lechner et al., 2009; Lechner and Lewin, 2009; Poole et al., 2014). There is increasing evidence that the physiologically relevant channel here is Piezo2 which forms a non-selective cation channel gated by mechanical stimuli (Coste et al., 2012, 2010; Ikeda et al., 2014; Maksimovic et al., 2014; Poole et al., 2014; Ranade et al., 2014; Woo et al., 2014). Nevertheless, the native RA-mechanosensitive current in mouse mechanoreceptors is sodium selective, insensitive to membrane voltage and as such resembles an ASIC channel (Hu and Lewin, 2006; Lechner et al., 2009). It is tempting to speculate that ASIC proteins may play a role in conferring sodium selectivity on native mechanosensitive currents in sensory neurons.

The mechanosensory gain-of-function phenotypes observed with *Asic* gene deletions are difficult to explain if ASIC channel subunits form part of a mechanosensitive channel. It is theoretically possible that ASIC channels could function as inhibitory subunits of a mechanotransduction complex, but there is no precedent for such a mechanism. Detailed analysis of mechanoreceptor function in *Asic3* mutant mice suggests that enhanced receptor activity has little to do with gain of function, but is instead due to specific changes in the adaptation behavior of mechanoreceptors in the absence of the ASIC3 subunit. In general, the term adaptation refers to decreasing receptor response to a constant adequate stimulus. The adequate stimulus for rapidly adapting mechanoreceptors is velocity (Milenkovic et al., 2008) so that a moving stimulus of constant velocity will under ideal circumstances evoke a constant receptor output, measured as spikes per second. In fact RAMs adapt during a constant velocity stimulus so that firing rate decreases towards the end of moving stimulus (Milenkovic et al., 2008). This adaptation behavior was dramatically reduced in RAMs from *Asic3* mutant mice meaning that more spikes were evoked for each stimulus regardless of its velocity (Moshourab et al., 2013). Interestingly, it has recently been shown that voltage-gated K^+ channels may actually regulate the process of adaptation in mechanoreceptors (Heidenreich et al., 2012; Lechner and Lewin, 2013; Wende et al., 2012). Early studies have shown that the $K_v1.1$ channels are expressed by large sensory neurons and their expression is controlled by neurotrophins (Shin et al., 2003), and recent published data suggests that $K_v1.1$ may participate in regulating the adaptation behavior of mechanoreceptors (Hao et al., 2013). In addition, the M-current K^+ channel KCNQ4 was shown to specifically regulate RAM receptor adaptation, and consequently pharmacological blockade of this channel or its genetic ablation leads to an increase in RAM mechanosensitivity (Heidenreich et al., 2012). The expression of KCNQ4 as well as a variety of other K^+ channels was also shown to be regulated by the transcription factor c-Maf (Wende et al., 2012). Mice lacking c-Maf have RAMs with dramatically reduced adaptation and enhanced receptor firing similar to that observed in *Asic3* mutant mice (Moshourab et al., 2013; Wende et al., 2012). It has been suggested that ASICs can directly inhibit the activity of high-conductance Ca^{2+}- and voltage-activated (BK) K^+ channels in a heterologous system (Petroff et al., 2008). It is thus conceivable that loss of ASICs could lead to disinhibition of K^+ channels, but increased activity of a K^+ channel might be expected to enhance RAM adaptation. It is, nevertheless, possible that ASICs participate in protein complexes present at sensory nerve endings that are involved in regulating receptor excitability.

4. Role of stomatin-like protein regulation

From the evidence discussed above, it is clear that ASICs have a role in mechanosensation even if they do not participate directly in the transduction event. In *C. elegans*, the mechanotransduction ion channels are, like ASICs, members of the Deg/ENaC protein family and form the center of a large mechanosensory complex (Driscoll and Chalfie, 1991; Ernstrom and Chalfie, 2002; Poole et al., 2011; Smith and Lewin, 2009). Some of the proteins within this mechanosensory complex are members of the stomatin family of proteins. Stomatin and stomatin-like (STOML) proteins are integral membrane proteins, which are characterized by a core domain termed the stomatin domain, which has also been found in members of the prohibitin, flotillin and HflK/HflC protein families and thus is also called the SPFH domain (Lapatsina et al., 2012a). In *C. elegans*, worms are touch insensitive when the stomatin protein MEC-2 is mutated (Huang et al., 1995). However, MEC-2 does not itself form a

mechanosensitive ion channel, but instead dramatically increases the amplitude of currents formed by the Deg/ENaC proteins MEC-4 and MEC-10 (Goodman et al., 2002). The amino acid sequence for mammalian STOML3 shows 77% similarity with MEC-2 and our work showed that like MEC-2, STOML3 plays an important role in mechanosensation. In *Stoml3* mutant mice, we observed that approximately 40% of skin mechanoreceptors were insensitive to mechanical stimuli, an effect that was associated with reduced tactile-driven behavior and also resulted in impaired development of touch evoked pain behavior after a neuropathic injury (Wetzel et al., 2007). Considering that stomatin proteins modulate mechanosensory *C. elegans* Deg/ENaC ion channels, it is perhaps unsurprising that stomatin proteins have been found to modulate ASICs. Stomatin was first shown to co-precipitate with ASIC1a, ASIC2a and ASIC3, but functional effects of this interaction were only observed for ASIC2a and ASIC3: ASIC2a inactivates more rapidly when co-expressed with stomatin and ASIC3 current amplitude is inhibited when co-expressed with stomatin (Price et al., 2004). We recently solved a high-resolution crystal structure for the mouse stomatin (amino acids 86 – 213) and observed that it forms dimers (Brand et al., 2012), which is in contrast to the trimer structure observed for the stomatin homologue isolated from *Pyrococcus horikoshii* (Yokoyama et al., 2008); moreover, mutated stomatin, which is unable to form dimers, no longer functionally inhibits ASIC2a or ASIC3 (Brand et al., 2012).

As noted above, mice lacking STOML3 apparently have fewer DRG neurons with a mechanosensitive current and in the same study it was shown that proton-gated currents were of larger magnitude in DRG neurons isolated from *Stoml3* null mice compared to those in wild-type mice (Wetzel et al., 2007). Furthermore, we showed that STOML3 immunoprecipitated with ASIC2a, ASIC2b and ASIC3 and that overexpression of STOML3 in HEK cells suppressed the endogenous proton-gated current. Our follow-up study used fluorescence resonance energy transfer (FRET) microscopy to demonstrate that STOML3 is present in highly mobile vesicular structures that also contain stomatin (Lapatsina et al., 2012b). STOML3 was further shown to co-immunoprecipitate with all six ASIC subunits and truncation studies showed that a hydrophobic region (amino acids 21 – 50) is necessary for vesicular distribution and interaction with ASICs. As for stomatin, STOML3 inhibits both ASIC2a and ASIC3, but both effects were upon current amplitude and no effect was seen upon the inactivation time constant (Lapatsina et al., 2012b). As had been previously observed for the effects of stomatin upon ASIC3 (Price et al., 2004), the inhibition of ASIC2a by STOML3 was not due to reduced surface membrane expression (Lapatsina et al., 2012b). The third member of the stomatin family to be analyzed in terms of its interactions with ASICs was STOML1. We have recently shown that STOML1 also modulates ASIC-mediated currents: ASIC1a current amplitude is inhibited and the inactivation time constant of ASIC3 is accelerated (Kozlenkov et al., 2014). ASIC1b, the ASIC1 splice variant, is not inhibited by STOML1, suggesting that the N-terminal region of ASIC1a may be the site of STOML1 interaction with ASIC1a, moreover, STOML1 is unique amongst the stomatin proteins in containing a sterol carrier protein-2 domain, removal of which prevented the inhibition of ASIC1a-mediated currents (Kozlenkov et al., 2014).

In view of the mechanosensory deficits observed in mice lacking either ASIC or stomatin proteins and the modulatory effects of stomatin proteins upon proton-gated ASIC-mediated currents, we recently interrogated the phenotypes of sensory neurons from different double mutant mouse strains, in which a combination of a stomatin and ASIC proteins were missing. Thinly myelinated Aδ-fibers can be either AMs or D-hair mechanoreceptors (Lechner and Lewin, 2013; Smith and Lewin, 2009) and AMs from *Asic3* mutant mice are less sensitive to mechanical stimulation (Moshourab et al., 2013; Price et al., 2001), whereas mechanically sensitive AMs from *Stoml3* mutant mice display normal mechanosensitivity (Wetzel et al., 2007). However, AMs recorded from *Asic3:Stoml3* double mutant mice displayed a very profound insensitivity to mechanical stimuli compared to AMs from *Asic3* single mutant mice (Moshourab et al., 2013). Even more profound effects was observed in *Asic2:stomatin* double mutant mice in that 25% of Aδ-fibers displayed no mechanosensitivity (determined using an electrical search protocol), a phenotype not observed in either *Asic2* or *stomatin* single mutant mice (Martinez-Salgado et al., 2007; Moshourab et al., 2013). Finally, C-mechanoheat fibers from *Asic3:stomatin* double mutant mice were observed to be less sensitive to mechanical stimuli than C-mechanoheat fibers recorded from *Asic3* single mutant mice (Moshourab et al., 2013). This study demonstrates that the modulation of ASIC-mediated proton-gated currents by stomatin proteins (Brand et al., 2012; Lapatsina et al., 2012b; Price et al., 2004; Wetzel et al., 2007), is not limited to neuronal acid-sensitivity, but that interaction between ASICs and stomatin proteins also has an impact upon neuronal mechanosensitivity. However, as discussed previously in this review, ASICs have at most a minor role in the initial mechanotransduction event, which contrasts with evidence demonstrating that the recently identified Piezo proteins are themselves mechanosensitive ion channels and are responsible for some, but not all, of the mechanosensitive currents in sensory neurons. Using a novel elastomeric pillar array technique, we have recently shown that stomatin proteins modulate Piezos, such that STOML3 greatly increases the mechanosensitivity of both Piezo1 and Piezo2, an effect that requires the stomatin domain of STOML3, as such STOML3 is the first modulator of Piezos to have been identified (Poole et al., 2014).

5. Proton sensing by ASICs: a link to mechanotransduction?

Proton sensing and mechanotransduction by sensory neurons may have common underlying mechanisms. For example, under inflammatory conditions which are often characterized by tissue acidosis, the high proton concentration may directly influence the mechanosensitivity of sensory afferents. Using an ex vivo skin-nerve preparation, mild acidification (pH 6.1) reduced the mechanical thresholds of polymodal rat nociceptors; interestingly this appeared to be independent of acid-induced action potential firing because in some cases sensitization occurred in neurons that did not show acid-induced action potential firing (Steen et al., 1992). Similarly, in mice the majority of C-fiber nociceptors displayed an increased firing in response to a mechanical stimulus at pH 6.0 compared to pH neutral control conditions (Smith et al., 2011). Thus mechanical hyperalgesia associated with inflammation may partially be due to the sensitizing effect of protons upon sensory neurons. Further evidence for this idea comes from the demonstration that in a joint inflammation model secondary mechanical hyperalgesia is reduced in *Asic3* mutant mice (Ikeuchi et al., 2008) and similarly, mechanical hyperalgesia is reduced in a model of muscle pain in mice lacking ASIC3 (Price et al., 2001; Sluka et al., 2003). Thus the ASIC3 protein could be a primary sensor of protons necessary to initiate mechanical sensitization or it may actually mediate such sensitization.

We recently observed that at more acidic pH (pH 4.0), the number of mechanically induced action potentials in C-fiber nociceptors was dramatically reduced reflecting the opposite of sensitization. The inhibitory effects of protons on mechanosensitivity were significantly more prominent in naked mole-rats, a rodent species that does not experience acid-pain (Park et al., 2008; Smith et al., 2011). Voltage-gated sodium channels (Na_V) are inhibited by acid (Khan et al., 2002) and we demonstrated that naked mole-rat

DRG neuron Na_V currents are more potently inhibited by acid than those in mouse DRG neurons. We subsequently identified a 3 amino acid motif in the naked mole-rat $Na_V1.7$ channel that, when transferred to human $Na_V1.7$, conferred enhanced proton block (Smith et al., 2011). It is likely that the naked mole-rat evolved acid insensitivity because it lives in a hypercapnic environment and carbon dioxide can evoke tissue acidosis. Interestingly, a similarly charged motif in the same position of $Na_V1.7$ that controls the proton sensitivity of the channel was found in a number of species that are likely adapted to hypercapnic conditions. These species included other African mole-rat species, the distantly related blind mole-rat *Spalax galili* and numerous hibernating species (Fang et al., 2014a, 2014b; Liu et al., 2014). Species in the same taxa that are likely not exposed to hypercapnic conditions (e.g. tree dwelling bats or non-hibernating related species) exhibited a motif at the same position more similar to the human or mouse $Na_V1.7$ sequence that is associated with less proton sensitivity (Liu et al., 2014; Smith et al., 2011).

6. Conclusions

It is now clear that ASICs participate in regulating mechanoreceptor and nociceptor mechanosensory function. Genetic loss-of-function models in mice have shown that ASICs play important and neuron-specific roles in regulating receptor function. However, the mode of their molecular interaction at the peripheral endings of sensory neurons remains largely a mystery. It is clear that ASIC subunits probably interact with multiple unknown membrane proteins in different sensory neurons. One family of interaction partners that have been shown to be functionally relevant are stomatin-domain proteins. Further work is needed to clarify how ASIC proteins are assembled and function to regulate mechanosensitivity at a molecular level.

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