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J Biol Chem. 2012 Nov 16; 287(47): 39878–39886. PMCID: PMC3501023
Published online 2012 Oct 2. doi: 10.1074/jbc.M112.411736

Pickpocket1 Is an Ionotropic Molecular Sensory Transducer*

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Received 2012 Aug 20; Revised 2012 Sep 29

Abstract

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The molecular transformation of an external stimulus into changes in sensory neuron activity is incompletely described. Although a number of molecules have been identified that can respond to stimuli, evidence that these molecules can transduce stimulation into useful neural activity is lacking. Here we demonstrate that *pickpocket1* (*ppk1*), a *Drosophila* homolog of mammalian Degenerin/epithelial sodium channels, encodes an acid-sensing sodium channel that conducts a transient depolarizing current in multidendritic sensory neurons of *Drosophila melanogaster*. Stimulation of Ppk1 is sufficient to bring these sensory neurons to threshold, eliciting a burst of action potentials. The transient nature of the neural activity produced by Ppk1 activation is the result of Ppk1 channel gating properties. This model is supported by the observation of enhanced bursting activity in neurons expressing a gain of function *ppk1* mutant harboring the *degenerin* mutation. These findings demonstrate that Ppk1 can function as an ionotropic molecular sensory transducer capable of transforming the perception of a stimulus into phasic neuronal activity in sensory neurons.

Keywords: Acid-sensing Ion Channels (ASIC), ENaC, Mechanotransduction, Membrane Biophysics, Neurons, Degenerin

Introduction

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Peripheral sensory neurons are capable of sensing and responding to a broad range of stimuli including mechanical, chemical, and thermal cues. These neurons are thought to express molecular sensory transducers that transform stimuli into changes in neuronal activity. For a protein to function as a molecular sensory transducer, it must not only be able to sense and respond to a stimulus but also be able to transform the sensing of the stimulus into an electrical signal capable of evoking the firing of action potentials in the sensory neuron.

Ion channel proteins are particularly strong candidates to serve as molecular sensory transducers because their ion channel function enables them to transform the sensing of a stimulus, upon activation, into an electrical current that can alter neuron activity. Although there are many examples of ion channel receptors that can respond to sensory stimuli (1–8), there are few examples where activation of these channels can drive neuronal activity.

The subgroup of mammalian Deg/ENaC[3] channels activated by extracellular acidic pH represents attractive candidates for functioning in mammalian sensory neurons as molecular sensory transducers. These acid-sensing ion channels (ASICs) are expressed in sensory neurons, including in the dorsal root ganglion, and have been implicated in mechanosensation and proprioception (9–15). The *Drosophila* Deg/ENaC homolog, *ppk1*, is expressed in a restrictive manner in class IV multidentritic (md) sensory neurons (14, 16–18). These polymodal sensory neurons form extensive dendritic networks that ramify beneath the epidermis and are required for normal proprioception and nociception (16, 19, 20). In consideration of such findings, we hypothesized that md neurons in *Drosophila*, in which *Ppk1* is restrictively expressed, may harbor acid-sensitive channels that can function as ionotropic molecular sensory transducers.

In the current study, we demonstrate that *ppk1* encodes an acid-sensing ion channel in *Drosophila* class IV md sensory neurons. Activation of the transient depolarizing sodium current conducted by Ppk1 is sufficient to drive md neurons to threshold, eliciting a burst of stimulus-dependent action potentials. Furthermore, this burst of action potentials is sensitive to changes in gating because stimulation of a Ppk1 protein harboring the gain of function *degenerin* mutation results in sustained bursting and loss of normal sensory neuron function. Together, these findings demonstrate that Ppk1 can function as a physiologically relevant ionotropic molecular sensory transducer capable of transforming stimuli into changes in neuronal activity.

EXPERIMENTAL PROCEDURES

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Fly Stocks and Husbandry All fly stocks were maintained on standard food at 25 °C and a 12-h light cycle. Stocks harboring the *f07052* and *d02171* P-elements were obtained from the Bloomington Stock Center (University of Indiana). The *ppk1-Gal4,UAS-mCD8-GFP* fly line was a gift from Darren Williams (King's College, London, UK).

Generation of ppk1 Deletion and Transgenic Flies

FRT/flippase recombinase targeted recombination was used to generate flies harboring the *ppk1* gene deletion ($ppk1^{ESB}$) (21, 22). Specifically, the *ppk1* gene was deleted using Flippase-mediated recombination between the PBac insertion lines *f07052* and *d02171*. The *elB* gene was also deleted in the recombination event but ruled out because of functional rescue by the *ppk1* encoding transgene. The *ppk1* transgene used in rescue experiments was generated by cloning the coding sequence of *ppk1* from the cDNA RE19290 (DGRC) into the pUAST vector in front of a C-terminal FLAG epitope. The *degenerin* gain of function mutant, $ppk1^{deg}$, was engineered by incorporating the S551F substitution into this transgene. *UAS-ppk1-FLAG* and *UAS-*$ppk1^{deg}$*-FLAG* flies were generated through P-element-mediated transformation (Rainbow Transgenic). Rescue flies of the genotype $ppk1^{ESB}$*;ppk1-Gal4,UAS-CD8-GFP/UAS-ppk1-FLAG* were generated by crossing homozygotic $ppk1^{ESB}$*;ppk1-Gal4,UAS-CD8-GFP* females to homozygotic $ppk1^{ESB}$*;UAS-ppk1-FLAG* males. All genotypes were verified using PCR.

Genotyping Single fly genomic DNA preparations for the polymerase chain reaction were performed as described previously (23). The *ppk1* gene, including the first two introns, and the *UAS-ppk1-FLAG* (Ppk1-transgene), which contains no introns, were identified with a standard PCR using the forward 5′-GGGAGGATGAGGAGGAAAAG-3′ and reverse 5′-ACTCCATTGCTATCGCAGCT-3′ primers at an annealing temperature of 68 °C with 3 mM Mg^{2+}.

Larvae Crawling Assay Early third instar larvae in the foraging stage were used in all crawling assays (16). Larvae were derived from 10-h-old embryo collections and aged for 80 h at 25 °C and 60% humidity. Prior to the assay, larvae were washed three times with distilled water to remove food particles. Larvae then were placed on 1% agarose plates and allowed to acclimate to testing conditions for 10 min. Larval crawling was assayed at 25 °C. For video capture of movement during the assay, three individual larvae were placed on the center of a fresh 150-mm agarose plate and recorded for 1.5 min or until they moved out of range. Video recordings of larvae crawling were captured at 30 frames/s with a Sony HDV1080i digital high definition video camera and analyzed with DIAS 3.4.2 software (Soll Technologies, Iowa City, IA) (24).

Nociception Assay Nociceptive responses to noxious mechanical stimuli were assayed using a standard protocol (2, 18). In brief, third instar larvae in the wandering stage were exposed to a transient, noxious mechanical stimulus applied to the third, fourth, or fifth segment of the abdominal region with a 46-mN Von Frey filament. Positive responses were scored only when larvae performed at least one 360° rotation around the anterior-posterior axis. Each larva was stimulated only once.

Primary Neuronal Culture Neuronal cultures from midgastrula stage embryos were prepared as described previously (25). Briefly, 3–4-h-old embryos were collected and dechorionated with 50% bleach. The content of three to four embryos was removed and dispersed onto a glass coverslip in a drop of culture medium. Neurons were grown in culture in a defined bicarbonate based medium in 5% CO_2 at 23 °C for 2–3 days. In these midgastrula stage embryo cultures, neurons arise from neuroblast precursors.

Electrophysiology Class IV md sensory neurons were identified in primary midgastrula neuronal cultures with epifluorescence for GFP expression, as driven by *ppk1-Gal4,UAS-mCD8-GFP*. Acid-sensing macroscopic currents from these neurons were recorded in voltage clamp experiments using the whole cell patch clamp configuration. The neurons were clamped to −60 mV (unless noted otherwise), and currents were activated by rapid solution exchange from control (pH 7.4) to acidic pH. For most experiments, the extracellular bath solution contained 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM D-glucose, 10 mM HEPES, and 10 mM MES (pH was adjusted with HCl before each experiment). The pipette solution contained 120 mM cesium gluconate, 20 mM NaCl, 0.1 mM $CaCl_2$, 1 mM EGTA, and 10 mM HEPES (pH was adjusted to 7.2 with CsOH). In paired patch clamp experiments examining P_K/P_{Na} selectivity, the extracellular bath solution initially presented to the neuron contained 110 mM NaCl plus 40 mM TEA-Cl. The NaCl was subsequently replaced with 110 mM KCl. Under both conditions, the pipette contained 140 mM cesium gluconate with current evoked with pH 4.5.

The currents were filtered at 1 kHz and acquired at 2 kHz and analyzed with an Axopatch 200B interfaced via a Digidata 1440A to a PC running the pClamp 10.2 software suite (Molecular Devices). Recording pipettes had resistances of 10–13 MΩ. Acidic pH was applied using a computer-controlled fast perfusion system (AutoMate Scientific). All of the electrophysiological experiments were performed at room temperature.

Action potentials from class IV md neurons were recorded using the whole cell current clamp configuration. Action potentials were evoked with 600-ms intracellular injections of suprathreshold depolarizing current pulses or by relief from amiloride blockade subsequent to an acid challenge. For these experiments, the extracellular bath solution was 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM D-glucose, and 10 mM HEPES, (pH was adjusted to 7.4 with NaOH). The pipette solution was 120 mM potassium gluconate, 20 mM NaCl, 0.1 mM $CaCl_2$, 1 mM EGTA, and 10 mM HEPES (pH was adjusted to 7.2 with KOH). The first action potentials evoked with minimal current excitation were used to determine action potential threshold, amplitude, half-width, and maximal rates of depolarization and repolarization. Firing frequencies were compared at 2 pA/pF current injection. Cell capacitance was determined by integrating the area of capacitive transients from the average of 10 ramps from −60 to −100 mV.

Data Analysis and Statistics For the analytical description of block by amiloride, the dose response curve was fitted with the following Hill equation:

$$\delta = \frac{1}{1 + \left(\frac{IC_{50}}{[X]}\right)^{n}} \qquad \text{(Eq. 1)}$$

where δ is the blocked fraction of the current in the presence of amiloride at the concentration [X], IC_{50} is the half-maximal inhibitory concentration of amiloride, and *n* is the Hill coefficient. The pH response curve for the sustained acid-sensing current was fitted with the alternative

formulation of the following Hill equation,

$$\theta = \frac{1}{1 + 10^{n \cdot pH - pK_a}} \qquad \text{(Eq. 2)}$$

where θ is the fraction of current activated at *pH*, and *n* is the Hill coefficient. All of the summarized data are reported as the means ± S.E. The summarized data were compared with that of a two-tailed Student's *t* test. The proportions were compared with a *z* test. $p \leq 0.05$ was considered significant.

RESULTS

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Class IV md Sensory Neurons Contain Multiple Acid-sensing Currents The mammalian homologs of *ppk1* that are expressed in sensory receptors of the peripheral nervous system, the ASICs, are activated by acidic pH (9–15). Thus, we voltage-clamped Ppk1-expressing md sensory neurons and probed the effects of an acid stimulus on macroscopic current. For these studies, primary neuronal cultures were generated from embryos expressing GFP in class IV md neurons (Fig. 1*a*). As shown in Fig. 1, decreasing extracellular pH rapidly evoked a sustained inward Na^+ current (I_s) in md sensory neurons. A distinct transient inward Na^+ current (I_t) was also observed in these neurons. This transient current, however, was only observed following relief from amiloride blockade subsequent to an activating acid challenge with application of amiloride alone in the absence of an acid stimulus not provoking I_t. These data demonstrate for the first time the existence of acid-sensitive currents in *Drosophila* sensory neurons.

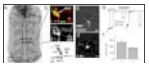

FIGURE 1.

Acid-sensing currents in class IV md neurons. *a*, inverted fluorescent image of dissected ppk1-Gal4, UAS-mCD*-GFP larvae stained for GFP expression. The ventral nerve cord (*VNC*) and a GFP-expressing class IV md neuron (*ddaC*) are indicated (*circle*). *b*, **...**

Further analysis of the sustained current (I_s) in voltage-clamped class IV md sensory neurons determined, as shown in Fig. 2, that the half-activation pH (pH_{50}) for I_s is 4.1 ± 0.08. The majority of this sustained, acid-sensing current, moreover, is sensitive to the Deg/ENaC channel inhibitor amiloride, IC_{50} = 230 ± 20 μM, with ~20% residual current remaining in the presence of a saturating dose of amiloride. In contrast, I_s is refractory to the broad spectrum TRP channel inhibitor ruthenium red. I_s is also equally selective for Na^+ and K^+. This pharmacological and biophysical fingerprint is consistent with a Deg/ENaC ion channel mediating the bulk of this current. Similar to I_s, the transient acid-sensing current I_t identified in class IV md neurons, as shown in Fig. 3, is sensitive to amiloride, refractory to ruthenium red and carried by inward Na^+ flux. Thus, I_s and I_t are acid-activated depolarizing currents, likely conducted by Deg/ENaC but not TRP channels.

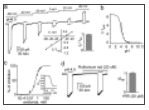

FIGURE 2.

Characterization of I_s. *a*, representative macroscopic current trace of I_s repetitively evoked with pH 4.5 in a voltage-clamped class IV md neuron at different test potentials (as indicated above) and the corresponding *I-V* relation (shown in the *inset* **...**

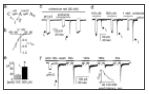

FIGURE 3.

Characterization of I_t. *a*, this simple kinetic scheme rationalizes activation of I_t by relief from blockade following removal of blocker subsequent to activation by an acid challenge and explains why I_t is transient. *C* indicates closed channels; *A* indicates **...**

The Drosophila Deg/ENaC Protein, Ppk1, Contributes to an Acid-sensing Ion Channel That Conducts a Transient Depolarizing Current in Class IV md Sensory Neurons

The simplified kinetic scheme shown in Fig. 3*a* can be used to rationalize the transient nature of I_t and the appearance of this current only after relief from blockade following an activating acid challenge, with the condition that the open channel blocker, amiloride, is able to protect the activated channel from inactivation. Although open channel blockers do not always function in this manner, this is the most reasonable explanation of these results and is consistent with additional experiments designed to test this possibility. For example, this model predicts that the magnitude of I_t should depend on the concentration of blocker because it controls the capture of channels in the activated but blocked state. (Conversely, I_s decreases with increasing [amiloride] as described for Fig. 2*c*.) Moreover, the magnitude of I_t is expected to decline as a function of the latency period separating the removal of blocker from the activating acid pulse. As shown in Fig. 3 (*d–f*), both expectations are met by I_t. Because I_t increases as a function of the [amiloride] used to capture the channel in the activated but blocked state and is evoked by relief from inhibition, this current must be amiloride-sensitive.

A *ppk1* null mutant ($ppk1^{ESB}$) and rescue flies were used to determine the relation between the gene product of *ppk1* and the acid-sensing currents identified above in class IV md neurons. To verify our *ppk1* genetic approaches, we first confirmed the effects of our *ppk1* loss of function ($ppk1^{ESB}$) mutants on previously published behavioral phenotypes (16, 18). As shown in Fig. 4, $ppk1^{ESB}$ null mutant larvae have a defect in larval locomotion and a reduction in a characteristic motor response to mechanical nociception similar to what has been previously published for *ppk1* mutants. Both defects are rescued by the expression of wild type Ppk1 in md neurons of $ppk1^{ESB}$ mutants consistent with Ppk1 function being disrupted in the $ppk1^{ESB}$ mutant md neuron.

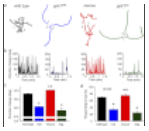

FIGURE 4.

Normal *ppk1* function in class IV md neurons is necessary for typical larval locomotion and nociceptive responses to stimuli. *a*, the centroid paths of three representative wild type (*black*, *left*), *ppk1*^{ESB} null mutant (*blue*, *middle left*), *ppk1*^{ESB} rescued **...**

As shown by the representative current traces from voltage-clamped class IV md neurons and the corresponding summary graphs in Fig. 5, deletion of *ppk1* abolishes I_t but is without effect on I_s. Moreover, targeted expression of wild type Ppk1 in class IV md neurons of *ppk1*^{ESB} mutant flies rescues I_t, having no effect on I_s. These results demonstrate that *ppk1* encodes a nonredundant component of an acid-sensing Na^+ channel in md neurons responsible for I_t but not I_s.

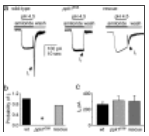

FIGURE 5.

Ppk1 conducts I_t but not I_s. *a*, typical acid-evoked macroscopic Na^+ currents in voltage-clamped (holding potential −60 mV) class IV md neurons from wild type (*left*; $n = 4$), *ppk1*^{ESB} mutant (*middle*; $n = 3$), and *ppk1*^{ESB} mutant rescued with expression **...**

Importantly, it also identifies a paradigm by which relief from blockade following activation by an acid stimulus can be used as a means for the targeted manipulation of the transient current conducted by Ppk1 channels in md neurons. Such targeted activation is critical for testing whether a certain channel is capable of driving stimulus-dependent action potential firing: a requirement for establishing the channel as an ionotropic molecular sensory transducer. Use of relief from inhibition following an acid stimulus to specifically activate Ppk1 *versus* applying a mechanical stimulus, which is known to activate many mechanosensitive ion channels in md and other sensory neurons, allowed us to focus specifically on the cellular function of Ppk1. The relationship of this heterotypic stimulation to the endogenous stimuli remains to be determined; nonetheless this approach is valid for determining Ppk1 channel properties.

Additional support for the conclusion that Ppk1 is a channel protein responsible for I_t comes from study of md neurons expressing the gain of function mutant *ppk1*^{deg}. The gain of function *degenerin* mutation increases the activity of Deg/ENaC channels (26–29). As shown in the representative current trace and summary graphs in Fig. 6 from voltage-clamped class IV md neurons, expression of the *ppk1*^{deg} mutant significantly increases the magnitude of the amiloride-sensitive (depolarizing) leak current and I_t and also slows inactivation of I_t. These results are consistent with the *ppk1*^{deg} mutant acting as a gain of function *ppk1* mutation.

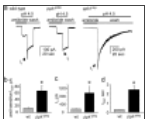

FIGURE 6.

The gain of function *ppk1*^{deg} mutation increases the amiloride-sensitive leak current in md neurons at rest and I_t. *a*, representative acid-evoked macroscopic Na^+ currents in voltage-clamped (holding potential −60 mV) class IV md neurons from wild **...**

The mechanosensory behavior of larvae harboring the *ppk1*^{deg} mutation is similarly compromised to what was observed in loss of function *ppk1*^{ESB} mutants (Fig. 4). Our behavioral results support that manipulation of Ppk1 activity in md neurons is sufficient to alter normal sensory function.

Ppk1 Does Not Influence the Inherent Excitability of Class IV md Sensory Neurons Electrical and cellular ablation studies demonstrate that complete loss of neuronal activity results in similar effects on behavior as the *ppk1*^{ESB} mutation (16, 19, 30). Therefore it is possible that the intrinsic activity of class IV md neurons could be dramatically altered in *ppk1*^{ESB} null mutants. This was tested in current clamp experiments on class IV md neurons as shown in Fig. 7. Surprisingly, the intrinsic characteristics and firing frequency of action potentials, evoked by supra-threshold depolarizing currents, in current-clamped *ppk1*-expressing class IV md sensory neurons is similar in wild type and *ppk1*^{ESB} flies. As expected, the voltage-gated Na^+ channel inhibitor tetrodotoxin abolished all action potential firing. These observations are consistent with Ppk1 not contributing to the shape of the action potential or being required for the inherent excitability of md neuron but rather being involved in the sensing and/or transformation of a stimulus into changes in neuronal activity.

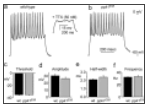

FIGURE 7.

***ppk1* does not influence the inherent excitability or the intrinsic characteristics of action potentials in class IV md neurons.** *a* and *b*, representative action potentials are shown from wild type (*a*) and *ppk1*^{ESB} (*b*) current-clamped class IV md neurons. **...**

Ppk1 Containing Channels Can Function as an Ionotropic Molecular Sensory Transducer To determine whether Ppk1 functions as an ionotropic molecular sensory transducer in md neurons, we tested the ability of I_t to depolarize md neurons, resulting in the generation of action potentials. This was accomplished by selectively activating I_t using the relief from blockade following an acid challenge paradigm. As shown in Fig. 8, targeted activation of I_t drove current-clamped md neurons to threshold, resulting in a burst of action potentials. This movement to threshold and the dependent train of action potentials is absent in md neurons from *ppk1*^{ESB} flies but is rescued to wild type by the targeted expression of the wild type *ppk1* transgene in these neurons of *ppk1*^{ESB} flies. These results are consistent with the activation of Ppk1 in md neurons being sufficient to transiently depolarize membrane potential to threshold, evoking a burst of action potentials. That the move to threshold and associated firing of action potentials is transient reflects the transient nature of I_t. Moreover, these results are consistent with Ppk1 being capable of transforming external stimuli into neuronal activity.

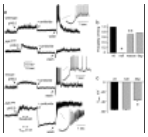

FIGURE 8.

Ppk1 is an ionotropic molecular sensory transducer capable of bringing sensory neurons to threshold. *a*, representative action potentials evoked by relief from amiloride inhibition following an acid stimulus from current-clamped class IV md neurons from **...**

Normally, Ppk1 contributes little to the resting membrane potential (Fig. 8*c*) of md neurons, allowing this channel to function as an incidence sensor. This is not the case in md neurons expressing $ppk1^{deg}$, where the constitutively active Ppk1 makes a notable contribution to leak current at rest (Fig. 6*b*). As shown in Fig. 8, this robust depolarizing leak current in md neurons containing the $ppk1^{deg}$ mutant results in marked depolarization of the resting membrane potential, causing these neurons to sit near threshold (which is −35 mV; Fig. 7*c*). This change in the resting membrane potential combined with a larger and more prolonged I_t (Fig. 6) results in md neurons expressing $ppk1^{deg}$ having abnormal electrical activity and responses to relief from amiloride blockade following an acid stimulus. As shown in the example current trace from a representative md neuron expressing $ppk1^{deg}$ in Fig. 8*a* (*bottom panel*), membrane potential is depolarized near threshold at rest. The addition of amiloride drives membrane potential below threshold nearer the normal resting membrane potential for (wild type) class IV md neurons by blocking the robust depolarizing leak current arising from the constitutively active $Ppk1^{deg}$ channel. This enables the firing of action potentials upon relief from amiloride-blockade subsequent to an acid challenge. Activation of the robust and prolonged I_t following wash rapidly drives membrane potential back toward E_{Na}, evoking a sustained (instead of a transient) train of action potentials in md neurons from $ppk1^{deg}$ flies; there is no adaptation but rather action potentials continue firing until membrane potential reaches E_{Na}.

DISCUSSION

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The current results demonstrate that the *Drosophila* Deg/ENaC protein, Ppk1, is a key component of an acid-sensing ion channel expressed in class IV md sensory neurons. Activated Ppk1 channels conduct a transient depolarizing sodium current in md neurons. Ppk1 is not active at rest, however, and consequently contributes little to the resting membrane potential, the shape of the action potential, and the inherent excitability of md neurons. Rather, stimulus-dependent activation of Ppk1 channels drives md neurons to threshold evoking a phasic burst of action potentials. In this regard, Ppk1 channels serve as ionotropic molecular sensory transducers functioning as incidence detectors capable of transforming an external stimulus into an electrical signal able to evoke action potential firing in class IV md neurons. This function of Ppk1 (in md neurons) is critical for normal sensory perception.

Ppk1 Is a Critical Component of an Acid-sensing Deg/ENaC Channel The results from the current electrophysiology studies provide compelling evidence that Ppk1 is a key component of an ion channel in md neurons: normal *ppk1* expression and function are required for a transient, acid-sensing, amiloride-sensitive depolarizing sodium current in these sensory neurons. In this regard, this *Drosophila* homolog mirrors the ion channel function of other members of the Deg/ENaC channel family, in particular, the mammalian ASIC and *Caenorhabditis elegans degenerin* channel proteins also expressed in sensory neurons (9, 11, 31). Sequence homology and structural similarity with ASIC proteins, which contribute to a conductive pore and for which the structure has been resolved (32, 33), are consistent with Ppk1 being a pore-forming subunit. This is supported by our findings that a gain of function mutation in Ppk1 changes the biophysical properties of the transient current associated with the expression and function of this channel protein.

Importantly, the alteration in gating by the gain of function mutation was sufficient to alter sensory responses, consistent with Ppk1 functioning during signal transduction in sensory neurons. Moreover, the observation that the behavioral phenotype of larvae harboring a gain of function *ppk1* mutation in their md neurons phenocopies larvae harboring loss of function *ppk1* mutations is similar to observations made for loss and gain of function mutations in Deg/ENaC channels expressed in *C. elegans* touch receptors (26–29). These results support that any change in normal Ppk1 activity, be it an increase or a decrease, disrupts the ability of md neurons to transform sensory information appropriately, compromising normal behavioral responses. This emphasizes the importance of the appropriate activation of Deg/ENaC channels to the process of changing a stimulus into a properly graded electrical signal during sensory transduction.

Deg/ENaC Channels Function as Molecular Signal Transducers The current studies demonstrate that decreases in pH activate and then quickly inactivate Ppk1 channels. This is similar to the effects of decreases in pH on mammalian ASIC channels (9, 10, 15) with the exception that the inactivation of Ppk1 is particularly rapid. Importantly, the ability to relieve activated Ppk1 from blockade following stimulation by an acid challenge allowed us to test whether the activation of Deg/ENaC proteins is sufficient to drive sensory neurons to threshold and thus function as ionotropic molecular sensory transducers. These results help define the role that Deg/ENaC channels play in the mechanistic and ionic basis of sensory transduction. They serve two discrete functions: 1) during the initial phase of sensory transduction, they act as protein sensors involved in the direct perception of the stimulus, and 2) through their ability to conduct a depolarizing current upon stimulus-dependent activation capable of bringing the membrane potential to threshold, they transform the sensing of a stimulus into an electrical signal that results in changes in neuronal activity. Interestingly, the neuronal activity resulting from the activation of Ppk1 is shaped by the gating properties of the channel, suggesting that these channels can also play an important role in the encoding of the stimuli. This idea is supported by the observation of the deleterious effects of the *degenerin* mutation on mechanosensory behavior.

The current results provide support for the idea that Deg/ENaC channels function as ionotropic molecular sensory transducers important for sensory transduction in peripheral sensory neurons. This solidly places Deg/ENaC channels, along with TRP and Piezo channels (1–3, 7, 8, 34, 35), into a small group of ion channels capable of sensing and transforming stimuli into electrical signals. The current results extend understanding significantly by identifying Deg/ENaC channel proteins as essential elements in sensory transduction, being important in both the early formative events encoding sensory stimuli and the transformative events resulting in changes in sensory neuron activity upon channel activation. The mechanistic paradigm emerging from the current studies, in consideration of earlier findings regarding the function of Deg/ENaC channels, has stimulus-dependent activation of Deg/ENaC channels in sensory dendrites, resulting in a depolarization of the plasma membrane via a transient

inward sodium current. The resulting transient depolarization to threshold evokes a burst of action potentials, defined by channel gating properties, resulting in the transformation of the stimulus into an electrical signal in sensory neurons that carries instructive information into the nervous system, allowing for the appropriate behavioral response.

*This work was supported, in whole or in part, by National Institutes of Health Grant RO1 NS062811 (to B. A. E.). This work was also supported by American Heart Association Established Investigator Award 0640054N (to J. D. S.).

[3]The abbreviations used are:

Deg Degenerin
ENaC epithelial sodium channel
ASIC acid-sensing ion channel
md multidendritic
TTX tetrodotoxin.

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Go to:

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