Pickpocket1 Is an Ionotropic Molecular Sensory Transducer

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Received 2012 Aug 20; Revised 2012 Sep 29

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Abstract

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 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

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 phasic neuronal activity. Although there are many examples of ion channel receptors that can respond to sensory stimuli, there are few examples where activation of these channels can drive neuronal activity.

The subgroup of mammalian Deg/ENaC-\(^2\)-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 multidendritic (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

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
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(PH was adjusted to 7.2 with KOH). The first action potentials evoked with minimal current excitation were used to determine action potential

(PH was adjusted to 7.4 with NaOH). The pipette solution was 120 mM potassium gluconate, 20 mM NaCl, 0.1 mM CaCl₂, 10 mM EGTA, and 10 mM HEPES

For these experiments, the extracellular bath solution was 140 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 10 mM D-glucose, and 10 mM HEPES, (PH was

°C with 3 mM Mg²⁺.

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

Acidic pH was applied using a computer-

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Electrophysiology Class IV md sensory neurons were identified in primary midgastrula neuronal cultures with epifluorescence for GFP expression, as

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

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

\[ \delta = \frac{1}{1 + \left( \frac{IC_{50}}{[X]} \right)^n} \]  

(Eq. 1)

where \( \delta \) 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,

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

where $\theta$ 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**

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 $pH$ ($\sim 5–15$). 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. 1a]). 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 ($daaC$) 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 $4.1 \pm 0.08$. The majority of this sustained, acid-sensitive current, moreover, is sensitive to the Deg/ENaC channel inhibitor amiloride, $IC_{50} = 230 \pm 20 \mu M$, with ~20% residual current remaining in the presence of a saturating dose of amiloride. In contrast, $I_t$ is refractory to the broad spectrum TRP channel inhibitor ruthenium red. $I_t$ 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 $I_t$.** $a$, representative macroscopic current trace of $I_t$ 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. 3a] 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, $I_t$ decreases with increasing [amiloride] as described for [Fig. 2c].) 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 noiceception 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.
Pickpocket1 is an ionotropic molecular sensory transducer. 

The mechanosensory behavior of larvae harboring the pickpocket1 mutant is similarly compromised to what was observed in loss of function pickpocket1 mutants (Fig. 4). Our behavioral results support that manipulation of Ppk1 activity in md neurons is sufficient to alter normal sensory function.

**FIGURE 4.** Normal pickpocket1 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), pickpocket1ESB null mutant (blue, middle left), pickpocket1ESB 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 pickpocket1 abolishes I\textsubscript{t} but is without effect on I\textsubscript{c}. Moreover, targeted expression of wild type Ppk1 in class IV md neurons of pickpocket1ESB mutant fly rescues I\textsubscript{t} having no effect on I\textsubscript{c}. These results demonstrate that pickpocket1 encodes a nonredundant component of an acid-sensing Na\textsuperscript{+} channel in md neurons responsible for I\textsubscript{t} but not I\textsubscript{c}.

**FIGURE 5.** Ppk1 conducts I\textsubscript{t} but not I\textsubscript{c}. a, typical acid-evoked macroscopic Na\textsuperscript{+} currents in voltage-clamped (holding potential −60 mV) class IV md neurons from wild type (left; n = 4), pickpocket1ESB mutant (middle; n = 3), and pickpocket1ESB 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 molecular sensory transducer. Use of relief from inhibition following an acid stimulus to specifically activate Ppk1 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 heterotopic stimulation to the endogenous stimuli remains to be determined; nonetheless this approach is valid for determining Ppk1 channel properties.

**FIGURE 6.** The gain of function pickpocket1deg mutation increases the amiloride-sensitive leak current in md neurons at rest and I\textsubscript{c}. a, representative acid-evoked macroscopic Na\textsuperscript{+} currents in voltage-clamped (holding potential −60 mV) class IV md neurons from wild ...

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**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 pickpocket1ESB mutation (16, 19, 30). Therefore it is possible that the intrinsic activity of class IV md neurons could be dramatically altered in pickpocket1ESB 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 pickpocket1-expressing class IV md sensory neurons is similar in wild type and pickpocket1ESB flies. As expected, the voltage-gated Na\textsuperscript{+} 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.** pickpocket1 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 pickpocket1ESB (b) current-clamped class IV md neurons. ...

To determine whether Ppk1 functions as an ionotropic molecular sensory transducer in md neurons, we tested the ability of I\textsubscript{t} to depolarize md neurons, resulting in the generation of action potentials. This was accomplished by selectively activating I\textsubscript{t} using the relief from blockade following an acid challenge paradigm. As shown in Fig. 8, targeted activation of I\textsubscript{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 pickpocket1ESB flies but is rescued to wild type by the targeted expression of the wild type pickpocket1 transgene in these neurons of pickpocket1ESB 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\textsubscript{t}. Moreover, these results are consistent with Ppk1 being capable of transforming external stimuli into neuronal activity.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3501023/?report=classic
Ppkl is an ionotropic molecular sensory transducer capable of bringing sensory neurons to threshold. A representative action potential follows relief from amiloride inhibition following an acid stimulus from current-clamped class IV md neurons from Drosophila. Normally, Ppkl contributes little to the resting membrane potential (Fig. 8c) of md neurons, allowing this channel to function as an incidence sensor. This is not the case in md neurons expressing *ppk* 1*deg*, where the constitutively active Ppkl makes a notable contribution to leak current at rest (Fig. 6h). As shown in Fig. 8, this robust depolarizing leak current in md neurons containing the *ppk* 1*deg* mutant results in marked depolarization of the resting membrane potential, causing these neurons to sit near threshold (which is +15 mV; Fig. 7c). This change in the resting membrane potential combined with a larger and more prolonged *I*  

Potential to threshold, they transform the sensing of a stimulus into an electrical signal that results in changes in neuronal activity. Interestingly, the activation of Ppkl depolarizes membrane potential below threshold near the normal resting membrane potential for (wild type) class IV md neurons by blocking the robust depolarizing leak current arising from the constitutively active Ppkl*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*  

They serve two discrete functions: 1) during the initial phase of sensory transduction, they act as protein sensors involved in the direct perception 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 Ppkl being a pore-forming subunit. This is supported by our findings that a gain of function mutation in Ppkl changes the biophysical properties of the transient current associated with the expression and function of this channel protein.

For normal sensory perception, Ppkl is critical for normal sensory perception.

Ppkl is a Critical Component of an Acid-sensing Deg/ENaC Channel The results from the current electrophysiology studies provide compelling evidence that Ppkl is the key component of an ion channel in class IV md sensory neurons. Activated Ppkl channels conduct a transient depolarizing sodium current in md neurons. Ppkl 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 Ppkl1 channels drives md neurons to threshold evoking a phasic burst of action potentials. In this regard, Ppkl1 channels serve as ionotropic molecular sensory transducers functioning as incense 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 Ppkl (in md neurons) is critical for normal sensory perception.

Importantly, the alteration in gating by the gain of function mutation was sufficient to alter sensory responses, consistent with Ppkl1 functioning during signal transduction in sensory neurons. Moreover, the observation that the behavioral phenotype of larval harboring a gain of function *ppk* 1 mutation in their md neurons phenocopies larvae harboring loss of function *deg* mutations in sensory neurons (26–29). These results support that any change in normal Ppkl 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 Ppkl1 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 Ppkl1 is particularly rapid. Importantly, the ability to relieve activated Ppkl1 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 Ppkl1 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 *deg* 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.).

The abbreviations used are:

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

REFERENCES


