Amplifying effect of a release mechanism for fast adaptation in the hair bundle (L)

Bora Sul[a] and Kuni H. Iwasa
Section on Biophysics, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, 5 Research Court, Room 1B03, Rockville, Maryland 20850-3211

(Received 17 March 2009; revised 4 May 2009; accepted 4 May 2009)

A "release" mechanism, which has been experimentally observed as the fast component in the hair bundle's response to mechanical stimulation, appears similar to common mechanical relaxation with a damping effect. This observation is puzzling because such a response is expected to have an amplifying role in the mechanoelectrical transduction process in hair cells. Here it is shown that a release mechanism can indeed have a role in amplification, if it is associated with negative stiffness due to the gating of the mechonoelectric transducer channel.
 [DOI: 10.1121/1.3143782]

PACS number(s): 43.64.Bt, 43.64.Ld, 43.64.Nf, 43.64.Kc [BLM] Pages: 4–6

I. INTRODUCTION

Fast adaptation of the mechonoelectric transducer (MET) channel in hair bundle has been a focus of recent hair cell physiology because it is considered to be a reverse transduction mechanism with an amplifying effect (LeMasurier and Gillespie, 2005; Fettiplace, 2006; Hudspeth, 2008; Vollrath *et al.*, 2007). However, experimental examinations tend to show that the partial closure of the MET channel is accompanied by reduction in tension at the tip-link (Stauffer *et al.*, 2005; LeMasurier and Gillespie, 2005), which is attributed to elongation of the link between the MET and an unconventional myosin that is responsible for adaptation (Gillespie *et al.*, 1993; Holt *et al.*, 2002; Bozovic and Hudspeth, 2003; Martin *et al.*, 2003). These observations are puzzling in view of its presumed biological role because such a phase delayed elongation is the property of a damper and not of an amplifier.

In this report, we show that such a mechanism can have indeed an amplifying effect if it is associated with negative stiffness of the MET. In the following, we examine a specific example, which is called a "release model" (Stauffer *et al.*, 2005; LeMasurier and Gillespie, 2005) for fast adaptation. However, the applicability of the conclusion is not limited to this particular model as it will become clear by the analysis.

II. RELEASE MECHANISM

Here we give a brief description of a release mechanism. Let x_r be the length of the link, which serves as a release element that connects the MET and the slow adaptation motor. In response to displacement x at tip-link, the MET responds with force F, given by

$$F = -k_g(x - x_g P_o - x_r), \tag{1}$$

where k_g is the stiffness of the gating spring and x_g is the gating distance. The open probability P_o of the channel is determined by gating energy if its gating is much faster than relaxation,

$$P_o = \frac{1}{1 + \exp[-\beta k_g x_g (x - x_g - x_r)]}, \tag{2}$$

where $\beta = 1/k_B T$ with Boltzmann's constant k_B and the temperature T. We assume the distance x_r obeys a relaxation equation with time constant τ,

$$\frac{d}{dt}x_r = (x_{\max}P_r - x_r)/\tau. \tag{3}$$

Here $x_{\max}P_r$ is the equilibrium distance for the intracellular Ca^{2+} concentration that corresponds to open probability P_o. If this release element has one Ca^{2+}-binding site, P_r may be expressed as

$$P_r = \frac{P_o}{\nu + P_o}, \tag{4}$$

where ν is a constant.

III. RESPONSE TO SMALL DISPLACEMENTS

Let us assume that displacement x has a time-dependent component Δx,

$$x = \bar{x} + \Delta x.$$

If this displacement Δx is small, it elicits small responses ΔP_o, ΔP_r, and Δx_r in the open probability, Ca^{2+}-binding, and the release distance, respectively. Equations (2) and (4) respectively lead to

$$\Delta P_o = \beta k_g x_g \bar{P}_o(1 - \bar{P}_o)(\Delta x - \Delta x_r), \tag{5}$$

$$\Delta P_r = \frac{\nu}{(\nu + \bar{P}_o)^2}\Delta P_o, \tag{6}$$

where $\bar{P}_o$ is the steady state open probability. Equation (3) turns into

[a]Author to whom correspondence should be addressed. Electronic mail: sulb@nidcd.nih.gov

$$\frac{d}{dt}\Delta x_r = (x_{\max}\Delta P_r - \Delta x_r)/\tau = (C\Delta x - (1+C)\Delta x_r)/\tau \quad (7)$$

with $C = \beta\nu x_{\max} k_g x_g \bar{P}_o(1-\bar{P}_o)/(\nu+\bar{P}_o)^2$.

If Δx is sinusoidal and we let $\Delta x = \delta x \cos \omega t$ and $\Delta x_r = \delta x_r \cos(\omega t + \phi_r)$, Eq. (7) leads to (see Appendix)

$$\delta x_r \sin \phi_r = -\frac{\omega\tau C \delta x}{(1+C)^2 + (\omega\tau)^2}. \quad (8)$$

IV. WORK DONE DURING ONE CYCLE

For a given hair bundle displacement $x = \bar{x} + \delta x \cos \omega t$, the work W done by force F [Eq. (1)] at the tip-link during one cycle is

$$W = -k_g \int (x - x_g P_o - x_r) \cdot d\Delta x.$$

By using Eqs. (5) and (6), the integrand can be expressed by a sum of terms proportional to either Δx or Δx_r. Of these terms, only the ones proportional to Δx_r contribute, leading to

$$W = k_g(1 - \beta k_g x_g^2 \bar{P}_o(1-\bar{P}_o)) \int \Delta x_r \cdot d\Delta x.$$

Here $k_g(1-\beta k_g x_g^2 \bar{P}_o(1-\bar{P}_o))$ is known as *gating stiffness* (Howard and Hudspeth, 1988; Martin *et al.*, 2000) and will be denoted by $\tilde{k}_g$. This stiffness is reduced by the gating of the MET channel and can take negative values. Because the phase difference between Δx_r and Δx is ϕ_r, the integration over a cycle results in $\pi \sin \phi_r$. With the aid of Eq. (8), we then obtain

$$W = -\pi \tilde{k}_g \frac{\omega\tau C}{(1+C)^2 + (\omega\tau)^2} \delta x^2.$$

This result shows that the work done by the MET is negative as long as gating stiffness $\tilde{k}_g$ remains positive, implying that the MET functions as a damper for periodic stimuli. However, it should also be noted that the work done is positive, if gating stiffness is negative. Under this condition, the MET functions as an amplifier.

How can this be explained? Negative stiffness proves a 180° delay. An additional phase delay introduced by the release mechanism, in effect, gives a phase advance between 0° and 180°, providing amplification. A 90° phase delay due to the relaxation process, the condition for maximal damping, is also optimal for amplification if it is combined with negative stiffness. This observation is applicable to any relaxation process and is not specific to our model.

To take advantage of negative stiffness to do mechanical work, the system must spend energy to maintain itself in a state with negative stiffness. One such energy source is the Ca^{2+} concentration gradient across the plasma membrane and another is adenosine-5′-triphosphate (ATP) for the myosin motor.

V. DISCUSSION

We showed that the release model that we examined provides amplification when it is associated with negative stiffness. However, it is clear that this property is not specific to this particular release model but is generic to any relaxation mechanism. One such example is the model proposed by Tinevez *et al.* (2007), which posits that fast adaptation is an epiphenomenon that arises from an interplay between gating of the MET channel and the myosin motor that is responsible for slow adaptation. It includes viscoelastic relaxation and relaxation involving the movement of the myosin motor.

Here we have treated linearized response for small stimuli to obtain some insight into the issue. For this reason, we have not analyzed the stability of the system, specifically how the operating point of the MET channel, which makes gating stiffness negative can be maintained. It appears to us that the previously reported analysis (Camalet *et al.*, 2000) on the stability of the operating point of the MET would be applicable to our model.

Because negative stiffness is intrinsically unstable, a relatively large stimulus used for experiments would shift the system into a condition with positive gating stiffness. Such a large stimulus is outside the validity of our treatment. Together with the difficulty of achieving extremely high time resolution in stimulation and recording, it may not be surprising to record only relaxation components during experiments (Stauffer *et al.*, 2005; LeMasurier and Gillespie, 2005).

ACKNOWLEDGMENT

This research was supported by the Intramural Research Program of the NIDCD, NIH.

APPENDIX: DERIVATION OF EQUATION (8)

Since $\Delta x = \mathrm{Re}[\delta x e^{(i\omega t)}]$ and $\Delta x_r = \mathrm{Re}[\delta x_r e^{i(\omega t+\phi_r)}]$, Eq. (7) can be expressed as

$$i\omega\tau \delta x_r e^{i(\omega t+\phi_r)} = C\delta x e^{i\omega t} - (1+C)\delta x_r e^{i(\omega t+\phi_r)},$$

which leads to

$$\delta x_r e^{i\phi_r} = \frac{C}{(1+C)+i\omega\tau}\delta x.$$

The imaginary part of this equation is Eq. (8).

Bozovic, D., and Hudspeth, A. J. (**2003**). "Hair-bundle movements elicited by transepithelial electrical stimulation of hair cells in the sacculus of the bullfrog," Proc. Natl. Acad. Sci. U.S.A. **100**, 958–963.

Camalet, S., Duke, T., Jülicher, F., and Prost, J. (**2000**). "Auditory sensitivity provided by self-tuned critical oscillations of hair cells," Proc. Natl. Acad. Sci. U.S.A. **97**, 3183–3188.

Fettiplace, R. (**2006**). "Active hair bundle movements in auditory hair cells," J. Physiol. **576**, 29–36.

Gillespie, P. G., Wagner, M. C., and Hudspeth, A. J. (**1993**). "Identification of a 120 kD hair-bundle myosin located near stereociliary tips," Neuron **11**, 581–594.

Holt, J. R., Gillespie, S. K. H., Provance, D. W., Shah, K., Shokat, K. M., Corey, D. P., Mercer, J. A., and Gillespie, P. G. (**2002**). "A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells," Cell **108**, 371–381.

Howard, J., and Hudspeth, A. J. (**1988**). "Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the

bullfrog's saccular hair cell," Neuron **1**, 189–199.
Hudspeth, A. J. (**2008**). "Making an effort to listen: Mechanical amplification in the ear," Neuron **59**, 530–545.
LeMasurier, M., and Gillespie, P. G. (**2005**). "Hair-cell mechanotransduction and cochlear amplification," Neuron **48**, 403–415.
Martin, P., Bozovic, D., Choe, Y., and Hudspeth, A. J. (**2003**). "Spontaneous oscillation by hair bundles of the bullfrog's sacculus," J. Neurosci. **23**, 4533–4548.
Martin, P., Mehta, A. D., and Hudspeth, A. J. (**2000**). "Negative hair-bundle stiffness betrays a mechanism for mechanical amplification by the hair cell," Proc. Natl. Acad. Sci. U.S.A. **97**, 12026–12031.
Stauffer, E. A., Scarborough, J. D., Hirono, M., Miller, E. D., Shah, K., Mercer, J. A., Holt, J. R., and Gillespie, P. G. (**2005**). "Fast adaptation in vestibular hair cells requires myosin-1c activity," Neuron **47**, 541–553.
Tinevez, J.-Y., Jülicher, F., and Martin, P. (**2007**). "Unifying the various incarnations of active hair-bundle motility by the vertebrate hair cell," Biophys. J. **93**, 4053–4067.
Vollrath, M. A., Kwan, K. Y., and Corey, D. P. (**2007**). "The micromachinery of mechanotransduction in hair cells," Annu. Rev. Neurosci. **30**, 339–365.