The (F)low Down on the Endothelial Epithelial Sodium Channel

Epithelial Sodium Channel as a Brake on Flow-Mediated Vasodilation

Heather A. Drummond

The epithelial sodium channel (ENaC) contributes to blood pressure homeostasis through renal salt and water transport; increases in ENaC activity increase salt and water reabsorption along the distal nephron, increasing extracellular fluid volume and blood pressure. The importance of tubular ENaC in cardiovascular homeostasis is manifested in Liddle’s disease and type I pseudohypoaldosteronism, diseases associated with the gain and loss of ENaC function, respectively. However, several recent studies suggest that ENaC proteins may play a more ubiquitous role in blood flow control as mechanosensors. Because ENaC proteins are closely related to a family of mechanosensitive proteins in the nematode termed “degenerins,” several laboratories have suggested that ENaC proteins may act as sensors of pressure-induced vascular stretch and laminar flow. The role of ENaC as a mediator of pressure-induced constriction in certain vessels is supported by several investigations; however, the role of ENaC as a flow sensor remains unclear.

ENaC as an Endothelial Flow Sensor

Because of their evolutionary relationship to mechanotransduction in the nematode, several laboratories have suggested that ENaC channels may act as flow sensors. Several lines of evidence suggest that ENaC channels are activated by flow. First, increases in laminar flow increase ENaC-dependent Na⁺ transport in the distal collecting duct. Second, laminar flow increases ENaC whole-cell current and channel open probability in oocytes expressing ENaC channels. Third, laminar flow increases the open-probability ENaC channels in endothelial cells. These studies suggest that ENaC channel activity can be regulated by laminar flow and, thus, that ENaC channels are candidates for transducing shear force in endothelial cells; however, the functional significance of ENaC in flow-mediated vascular reactivity is not clear.

The current report by Pérez et al5 demonstrates that ENaC expression in mesenteric endothelial cells regulates vascular tone. ENaC inhibition with amiloride (0.5 μmol/L) and benzamil (1 μmol/L) blunts agonist-induced vasoconstriction in an NO-dependent manner and increases NO production, a finding that suggests that basal ENaC activity in endothelial cells maintains vasoconstrictor reactivity by suppressing NO production (Figure). Furthermore, ENaC inhibition enhanced flow-mediated vasodilation, demonstrated by a upward shift of the flow-diameter relationship (Figure 7C in Reference 5), suggesting that basal-level ENaC activity acts as a brake on flow-mediated vasodilation in mesenteric arteries (resting diameter: ≈200 μm). The findings by Pérez et al5 demonstrate a functional role for endothelial ENaC in flow-mediated dilation but suggest that endothelial ENaC negatively modulates, but does not mediate, flow-mediated dilation in the mesenteric circulation.

Interpreting the importance of ENaC channels in this study is heavily dependent on the specificity of amiloride and its analogs. Although amiloride can block other channels and transporters, eg, voltage-gated Ca²⁺ and Na⁺ channels, Na⁺/H⁺ transporter, or the Na⁺/Ca²⁺ exchanger, amiloride can be highly selective for ENaC. The caveat is that the concentration must be submicromolar or very low micromolar. The study by Pérez et al5 used a submicromolar concentration of 0.5 μmol/L. Pérez et al5 also found a similar effect of benzamil, a lipophilic amiloride analog with increased selectivity for ENaC, and a lack of effect of ethyl isopropyl amiloride (0.5 μmol/L; Figure S2 in Reference 5), an amiloride analogue selective for the Na⁺/H⁺ transporter. Thus, as far as we know, the doses of amiloride and benzamil used in the study by Pérez et al5 are relatively specific for ENaC. However, because there is no “silver bullet,” and lingering doubt regarding the amiloride specificity remains, future studies using alternative methods to inhibit ENaC expression, eg, transient gene silencing, and genetically modified animals are needed to confirm the results of Pérez et al5.

Although the findings of Pérez et al5 suggest that ENaC is probably not a flow sensor in mesenteric arteries, a direct role for ENaC as a mediator of flow-induced dilation in other circulatory beds cannot be ruled out. Heterogeneity in signaling mechanisms in vessels of different sizes and locations is common, particularly as it relates to the role of ENaC in vascular smooth muscle cells in mesenteric, renal, and cere-

DOI: 10.1161/HYPERTENSIONAHA.109.128868

Hypertension is available at http://hyper.ahajournals.org

© 2009 American Heart Association, Inc.
bral arteries.1,7 Thus, it is possible that the negative modulatory role of endothelial ENaC in the current report by Pérez et al may be specific to the specific size (150 to 200 μm) and/or location (mesenteric) of arteries.

**How Might ENaC Mediate Flow-Induced Dilation?**

The mechanism of flow-induced vasodilation is thought to involve activation of K⁺ channels, membrane hyperpolarization, and subsequent NO release.8 Given that ENaC activation would be expected to depolarize the endothelial cell membrane, it seems unlikely, at first consideration, that ENaC would be the mediator. However, if ENaC channels were “coupled” to a K⁺ channel or if ENaC proteins were components of flow-sensitive channels with different ion selectivity, then it would be possible for ENaC proteins to mediate flow-induced dilation. Although there is no evidence that ENaC channels are linked/coupled/interact with K⁺ channels, there is evidence that the closely related ASIC channels directly regulate certain K⁺ channels.9 Thus, a heteromeric ENaC/ASIC channel could potentially regulate a K⁺ channel. Alternatively, ENaC proteins may interact with other ASIC proteins or putative endothelial mechanosensitive proteins (caveolin, integrins, etc) to form a multimeric flow-sensitive receptor complex that has altered amiloride sensitivity and more selectivity for K⁺ than Na⁺. This is a possibility because amiloride sensitivity and ionic selectivity of ENaC channels can be modified with single amino acid mutations, and ASIC/ENaC heteromers have varying ion selectivity.10,11 Although these scenarios may seem unlikely, before we drive the stake into the heart of the “ENaCs-as-endothelial-flow-sensors” hypothesis, the role of ENaC proteins as endothelial flow sensors should be evaluated further using multiple approaches, including in vitro and whole-animal approaches in genetically modified animals.

An important finding of Pérez et al is that this study provides evidence that the endothelial ENaC channels are active and participate in flow-induced dilation. Future studies are needed to define the role of ENaC channels as shear stress sensors in endothelial cells in mesenteric and other circulatory beds.

**Source of Funding**

This work was supported by National Heart, Lung, and Blood Institute grant HL086996.

**Disclosures**

None.

**References**

The (F)low Down on the Endothelial Epithelial Sodium Channel: Epithelial Sodium Channel as a Brake on Flow-Mediated Vasodilation
Heather A. Drummond

Hypertension. 2009;53:903-904; originally published online April 27, 2009;
doi: 10.1161/HYPERTENSIONAHA.109.128868

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/53/6/903

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/