In this issue of Hypertension, Jeggle et al provide important new insights into the characteristics and functional significance of an amiloride-sensitive sodium channel that is expressed in vascular endothelial cells. This work extends the previous analyses by this group, and using atomic force microscopy and state-of-the-art animal and cell models, provides provocative yet conclusive evidence for the central role of this channel in regulation vascular reactivity and stiffness.

The Figure depicts the heteromeric structure of the amiloride-sensitive sodium channel. Although the overall topology of the channel is well described, there is no general agreement about the subunit composition even within epithelial cells, much less in other tissues like vascular endothelial cells that are the subject of the current report. The bulk of the subunits’ structures are expressed in the intertwined disulfide-rich extracellular domains, the role of which has not been fully explained. The subunits of the epithelial sodium channel (ENaC) were originally defined by now-classical expression cloning studies by Canessa and Rossier. The human genomic clones and the critical insight into the gain of function effect that characterizes Liddle syndrome was based on analogy to defects in the mechanosensor structures of degenerins in Caenorhabditis elegans.

Although longitudinal flow is certainly a feature of many epithelial tubule systems, a direct connection among the putative mechanical transduction function, the extracellular domains, and regulation of apical sodium entry via ENaC is not obvious. In contrast, there are pulsatile pressures and flows in the systemic vascular system. Local regulation of vascular tone in response to changes in pressure and shear stress, an important feature of the microvascular circulation, lending credance to the idea that the extensive extracellular of the vascular endothelial sodium channel (EnNaC) domain could serve as a mechanoforce transducer. Using atomic force spectroscopy and nanoindentation analysis, the current report describes the regulation of cortical stiffness through an important feature of the microvascular circulation, the agency of the EnNaC, either related to apical sodium entry or interactions with the cortical actin cytoskeleton.

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Hypertension is available at http://hyper.ahajournals.org
DOI: 10.1161/HYPERTENSIONAHA.113.00768

In the context of the current work, cortical stiffness is defined as the force needed to indent the individual cell cortex (e.g., apical surface) for a fixed distance, and hence directly reflects the mechanical rigidity of the submembraneous region. The actin cytoskeleton is assumed to organize this subapical structure; dynamic changes in the organization and expression of the act cytoskeleton directly regulates the submembraneous rigidity. The role of ionized calcium, nitric oxide, and other vasoactive agents like angiotensin II in regulating cortical stiffness, and the responsivity to various classes of anti-hypertensive medications are obvious areas for future inquiry.

An important role of aldosterone in regulating cortical stiffness, and the use of knock-down and gain-of-function cellular models strongly supports the critical role of EnNaC in maintaining vascular tone at the individual endothelial cellular level. The current findings take on added relevance with the recent description of vascular endothelial mineralocorticoid receptors, and the appreciation of the importance of local renin-angiotension-aldosterone system in regulating vascular tone. The parallel evolutionary significance of the development of α and β subunits of the sodium channel and the sodium-potassium ATPase has recently been reviewed. With elucidation of the autosomal dominant monogenic gain-of-function defect in Liddle syndrome, the phenotypic extreme of low-renin hypertension was defined, an example for excellence of the Guytonian view of maintaining salt balance and homeostasis. This view emphasizes the importance of salt intake and balance in maintaining the milieu intérieur, and also identifies systemic hypertension with resulting pressure natriuresis as the effector arm of this control loop. What has not been adequately addressed is the mechanism(s) by which dietary salt intake, aldosterone status, and vascular tone participate in the effector arm of the Guytonian servoloop. The new findings reported herein by Jeggle and colleagues provide an approach to defining the effector arm at the level of individual vascular endothelial cell, whereas the overall regulation of salt balance by the classically described ENaC/Na-K-ATPase mechanisms focus at the level of whole-organ physiology.

I introduce novel, in this comment, nomenclature for the EpNaC and the EnNaC in an attempt to stimulate further discussion and experimental effort. ENaC, as a term, would seem to have use in describing the entire family of related amiloride-sensitive sodium channels (even though the E classically refers to epithelial). Although there seem to be many similarities, and both complexes are undoubtedly part of the ENaC/degenerin superfamily, there are important functional distinctions emphasized in the current work that clearly warrant additional investigations and inquiries. The time has come
to clearly distinguish between EpNaC and EnNac as members of the amiloride-sensitive ENaC family.

Further work to precisely define the subunit structures and assembly, the possibility of alternative transcription start sites or splicing, and even other subunits need to be fully explored. As an example, in the original clinical phenotype of the autosomal dominant gain-of-function condition by Liddle et al., there was a striking effect of increased dietary sodium intake (and presumably increased urinary sodium concentration) to reduce the effectiveness of sodium channel blockade observed with triamterene. In contrast, EnNac activity as described in the current report by Jeggle and colleagues was fully inhibited by amiloride in the presence of normal extracellular sodium concentrations of 140 mmol/L. A recent report by Kleyman and colleagues describes a novel point mutation in the ENaC subunit (γL511Q) that increases amiloride-sensitive currents and largely eliminates the Na+ self-inhibition response, which reflects downregulation of ENaC open probability by higher extracellular Na+ concentrations. This sort of downregulation would be appropriate for the amiloride-sensitive EpNaC in the distal nephron but would not be relevant for EnNac in the systemic circulation that is continuously bathed in relative higher extracellular Na+ concentrations. Hence, sequence variations in the γ ENaC subunit at the single nucleotide level could have important consequences for understanding the regulation of EnNaC activity.

It is well recognized that renin-angiotension-aldosterone system blockade is an important avenue for treating chronic kidney disease and reducing mortality in cardiovascular disease. Major efforts have demonstrated the clinical benefit of using mineralocorticoid receptor antagonism in cardiovascular disease, with novel new approaches coming to the fore. Unfortunately, the cardioprotective effects associated with mineralocorticoid receptor blockade nearly 15 years ago are not readily available to patients with moderate-to-severe chronic kidney disease who can develop dose-limiting hyperkalemia in the setting of aggressive renin-angiotension-aldosterone system blockade, such as described in the recent ALTITUDE Trial.

Previous consideration has been given to trying to separate the effects of Na-channel blockers like amiloride or triamterene from the parallel decreases in K+ secretion that invariably accompany EpNaC blockade. Furthermore, the currently available EpNaC blockers do not have a substantial effect on reduction of systemic blood pressure, at least in the currently used dosing regimens. An exception to this overall analysis is provided by the salutary responses to amiloride in adolescents with Liddle syndrome, who presumably have not developed the long-term vascular changes associated with chronic hypertension, although this effect may represent a primary renal effect rather than an effect on the vascular endothelium in Liddle syndrome and is clearly predicated on control of dietary salt intake.

EnNaC may present a specific target for therapeutic intervention if its inhibition can be functionally separated from EpNaC, and thus avoiding or at least minimizing impairment of renal and intestinal K+ secretion. Recent developments of novel 2-acyl-amiloride derivatives may provide a potential avenue for further development. An interesting analogue would have reasonable bioavailability (oral, sublingual, or transdermal), with hepatobiliary rather than renal clearance, and effectiveness as a noncompetitive inhibitor against EnNaC at subnanomolar concentrations. Amiloride, at usual oral dosing, achieves plasma levels that are well below the IC50 for EnNaC, with nearly 100-fold greater concentrations present in the final urine. Appropriate preclinical and clinical models and trials will need to be carried out to determine whether amiloride analogues can be developed that are renal sparing, minimize hyperkalemia, and also provide effective antihypertensive effects as well as longer term cardioprotective effects such as has been described with mineralocorticoid receptor blockade.

Sources of Funding
Preparation of this comment was supported by the Hilda B. Anderson Endowed Chair in Nephrology at the University of Alabama at Birmingham.

Disclosures
D.G. Warnock serves as a consultant to Parion Sciences Inc and Gilhead Sciences Inc on the clinical development of amiloride analogues.

References


The Amiloride-Sensitive Endothelial Sodium Channel and Vascular Tone

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*Hypertension*. 2013;61:952-954; originally published online March 4, 2013;
doi: 10.1161/HYPERTENSIONAHA.113.00768

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/61/5/952

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