Epithelial Na\(^+\) Channel (ENaC), Hormones, and Hypertension*

Published, JBC Papers in Press, May 11, 2010, DOI 10.1074/jbc.R109.025049
James K. Bubien

From the Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama 35294

This minireview examines both the basic science and clinical observations over the past 20 years to show how and why overstimulation of the amiloride-sensitive epithelial Na\(^+\) channel (ENaC) expressed by epithelial principal cells of the renal collecting duct may be responsible for a large portion of hypertension in modern society. This idea is based on the finding that, in Liddle syndrome, a mutation of the \(\beta\)- and/or \(\gamma\)-subunits of ENaC produces an activated ion channel, in turn resulting in severe hypertension that is resistant to most forms of conventional antihypertensive therapy. ENaC can also be stimulated to conduct sodium by two hormones: aldosterone and insulin. These hormones are both often elevated in obese individuals with therapy-resistant hypertension. Thus, overstimulation of ENaC by metabolic abnormalities in obese individuals may be a likely cause of the hypertension that accompanies obesity. The molecular mechanisms underlying both Liddle syndrome and obesity-related hypertension are different (i.e. genetic and hormonal, respectively), but both have the same end result, namely increased ENaC activity.

In 1994, Shimkets et al. (1) found a mutation in the amiloride-sensitive epithelial Na\(^+\) channel (ENaC)\(^2\) that is expressed on the apical membrane of epithelial cells in the renal cortical collecting duct. This mutation was present in members of a family afflicted with the genetic disease known as Liddle syndrome (2–4). This discovery was important for several reasons. One reason was that it spawned a large number of studies that elucidated the mechanisms of channel insertion and retrieval from the plasma membrane (5–14). Physiologically, it was important because it demonstrated that improper function or regulation (unhindered by any complicating factors such as metabolic and hormonal processes) of ENaC was independently capable of producing sustained severe hypertension. The mutation that is responsible for Liddle syndrome produces constitutively hyperactive ENaC (15–18), and this abnormality is the biochemical basis for the pathophysiology of Liddle syndrome. Liddle syndrome is characterized by severe hypertension and low plasma potassium concentrations in combination with low levels of renin and aldosterone (2–4, 19). Two other notable characteristics of Liddle syndrome are that the hypertension is resistant to conventional antihypertensive therapies, even in high doses and combinations,\(^3\) and that individuals with Liddle syndrome are not always volume-expanded in proportion to the severity of the elevated blood pressure (20). These pathophysiological features highlight some potential deficiencies in our understanding of the underlying physiological mechanism that involves ENaC. The renal collecting duct reabsorbs salt and water and is under hormonal regulation (13, 17). In Liddle hypertension, the presumption is that increased NaCl retention leads to volume expansion with increased mean circulatory filling pressure and a concomitant increase in blood pressure. In turn, Na\(^+\) excretion is increased by pressure natriuresis, so a new steady state of Na\(^+\) balance is achieved with an elevated blood pressure. However, to my knowledge, an extracellular fluid volume expansion has not been observed or reported in any Liddle patient using precise fluid compartment measurements. As indicated previously, many of these individuals do not appear to be volume-expanded or, in some cases, even volume-contracted (20, 21). In other words, the high blood pressure appeared to be independent of volume status. In cases in which volume expansion is noted, furosemide (commonly used to reduce volume) would be counterindicated (undiagnosed) in Liddle hypertension because this drug would exacerbate the hypokalemia already typical in this disease (3, 4, 22, 23). To understand the pathology of Liddle syndrome, another interpretation of the physiological and biochemical involvement of ENaC may be needed.

Physiological Mechanism

ENaC participates in a process that involves transepithelial ionic transport between the lumen of the renal collecting duct and the blood. ENaC is expressed in the apical membrane of renal tubule principal cells (24–30). When ENaC opens, sodium flows from the lumen into the cell powered by the negative electrical potential lumen-to-intracellular and steep Na\(^+\) concentration gradient across the apical membrane. Subsequently, Na\(^+\) is actively transported across the basolateral membrane by the Na\(^+\)/K\(^+\)-ATPase, in exchange for potassium. In turn, potassium exits the apical membrane via potassium channels (24, 25) and recycles across the basolateral membrane through a different set of potassium channels. When the apical membrane potential becomes depolarized, e.g. by increased sodium entry through activated ENaC, potassium secretion across this membrane increases, and K\(^+\) is lost to the urine. A primary function of this process is the reabsorption of salt (NaCl). However, this may not be the only function. Consider that the normal serum potassium concentration is between 3.5 and 5 mm, whereas the sodium concentration ranges from 135 to 145 mm (25). It is true that all cells have a high potassium concentration, so the intracellular component acts as a large

---

*This is the fourth article in the “Biochemistry in Medicine: Hypertension Minireview Series.” J. K. B. has an equity interest in Hemodynamic Therapeutics, a subsidiary of Cato Biosciences. This minireview will be reprinted in the 2010 Minireview Compendium, which will be available in January, 2011.

1 To whom correspondence should be addressed. E-mail: jbubien@bellsouth.net.

2 The abbreviation used is: ENaC, epithelial Na\(^+\) channel.

3 Individuals with high blood pressure are not routinely screened for genetic abnormalities. Consequently, they are not typically given amiloride or triamterene (drugs that block ENaC) as part of their conventional antihypertensive therapy.
Repository for potassium. However, this intracellular potassium concentration must be maintained to produce the electrical chemical gradient that is vital to many cellular functions. Thus, a continuous drain of the body potassium stores would eventually be fatal. Also, the plasma potassium must be maintained in a narrow range for the proper functioning of nerve and muscle cells, including the heart. Therefore, a critical homeostatic function is maintenance of the plasma potassium concentration in its narrow range. It follows that plasma [K\(^+\)] requires precise regulation. It is probably not regulated by the autonomic nervous system because transplanted kidneys with no nerve connections function normally. Therefore, the regulation must be chemical and, most likely, hormonal.

**Role of Aldosterone in the Regulation of ENaC**

The mineralocorticoid hormone aldosterone functions in the regulation of salt and water balance. The question is, which salt: sodium or potassium? For the reasons explained above, it is likely that the regulation is aimed at potassium. As plasma potassium concentrations rise, plasma aldosterone concentrations rise concurrently. In fact, elevation of serum potassium is considered the most powerful agonist for aldosterone secretion (24). Pratt et al. (31) showed that, over time, plasma potassium concentrations and plasma aldosterone levels precisely matched, with aldosterone concentrations rising and falling with increases and decreases in the serum potassium concentration.

Much of what we know about the effects of aldosterone comes from studying animal models, especially rodents (32, 33). There are advantages and drawbacks to the use of these models. One advantage is that experimental organs and tissues are readily available. One potential drawback is the possibility that information learned from animal models may not be applicable to humans because findings obtained for one species may not apply to other. In 2001, Zhou and Bubien (34) demonstrated such species differences directly using whole cell patch-clamped cells from five different species. One such example is the effect of aldosterone on ENaC activity. It is well known that aldosterone binds intracellularly to the mineralocorticoid receptor and that this interaction causes genomic activation and increased expression of the Na\(^+/K^+\)-ATPase. This process occurs in all species and can increase ENaC-mediated reabsorption due to increased removal of sodium from the cells by pumping it into the blood as well as increasing the number of active ENaCs in the apical membrane (29). In whole cell patch clamp experiments using freshly isolated collecting duct principal cells from rats and mice, aldosterone had no direct effect on ENaCl itself (34). Thus, these findings demonstrate a major difference between rat and mouse models and the other species. Also, spironolactone (a compound used to inhibit the mineralocorticoid receptor) does not inhibit the acute aldosterone-stimulated current in these rodent tissues.

**Biochemical Mechanism**

The biochemical effects of aldosterone on the mechanism of sodium reabsorption and potassium excretion are illustrated in Fig. 1. The genomic effects and biochemical pathways of aldosterone have been extensively studied, but the non-genomic effects of aldosterone have been underappreciated. Not only has the non-genomic acute activation of ENaC by aldosterone been observed, but a number of other non-genomic effects of aldosterone have been observed and documented. These effects have been reviewed recently by Sowers et al. (35). With respect to blood pressure regulation, this non-genomic effect of aldosterone on ENaC may be of considerable importance. Currently, no plasma membrane aldosterone receptor has been identified. Also, the intracellular signaling pathways that have been linked to this postulated receptor are unknown. Thus, no biochemical process exclusive to non-genomic aldosterone-mediated cellular signaling has been identified. Nonetheless, the non-genomic cellular responses to aldosterone are numerous and include activation of ENaC.

**Insulin-mediated ENaC Activation**

It has long been known that insulin contributes to hypertension by its effects on renal Na\(^+\) retention (36–40). In 1998, Blazer-Yost et al. (41) demonstrated that insulin also has the ability to activate ENaC. In patch clamp experiments, Bubien et al. (58) demonstrated that insulin in physiological concentrations was a potent acute activator of ENaC in human lymphocytes (as a proxy for renal principal cells), with an IC\(_{50}\) of 27
This IC₅₀ falls well within the normal range of serum insulin concentrations, which is ~6 μIU/ml (fasting) and can exceed 100 μIU/ml minutes after ingestion of sugar (41–43). This information is important for two reasons. First, it demonstrates directly that the renal collecting duct reabsorptive mechanism can be activated indirectly by dietary sugar intake. Second, insulin imbalance is a common disorder. Thus, if the serum insulin concentration remains elevated or receives constant stimulation, the reabsorptive mechanism will be overstimulated, with concomitant pathophysiological consequences.

Hence, both aldosterone and insulin stimulate ENaC. Aldosterone can function as an acute ENaC agonist in human, rabbit, and canine cells but not in rat and mouse principal cells (34). Also, aldosterone can act via the mineralocorticoid receptor to produce genomic effects. Of particular interest is aldosterone-mediated stimulation of expression of the Na⁺/K⁺-ATPase because this transporter plays in integral role in collecting duct reabsorption on the basolateral membrane of renal collecting duct principal cells. The end result is that both insulin and aldosterone act on ENaC to produce excessive and physiologically inappropriate reabsorption of K⁺ with the concurrent loss of potassium from the extracellular fluid.

**Obesity and Hypertension**

More Americans are becoming obese daily. National health statistics for 1999 state that 26% of the entire United States population was obese, having a body mass index of >30. Obesity has increased since 1999. Obesity is the most visually obvious symptom of a serious medical situation. Obesity is often accompanied by significantly elevated blood pressure. As obese individuals progress into the fourth and fifth decades of life, hypertension becomes an overriding medical concern. One not-so-obvious problem with obesity-related hypertension is that it is often resistant to conventional antihypertensive therapy, primarily consisting of a beta blocker, a diuretic (usually hydrochlorothiazide), and an angiotensin-convert-
heart attack, stroke, and/or renal failure, not to mention non-insulin-dependent diabetes mellitus, which adds a whole other dimension to the pathophysiological state and medical treatment problems for these individuals.

**What Molecular Similarities Link Liddle Syndrome and Obesity-related Hypertension?**

The question then is, what physiological mechanisms are operating that cause blood pressure to rise concurrently with weight gain in obese individuals and in those individuals with Liddle syndrome? The standard answer to this question is that blood pressure regulation is a multifactorial complex process, and therefore, there is no single underlying cause. Thus, it is said that a large fraction of all hypertension is essential hypertension, meaning that there is no identifiable etiology. However, in many ways, individuals with obesity-related hypertension share a pathophysiological profile similar to individuals with Liddle syndrome. Notably, the hypertension in both groups is not well controlled with standard medical treatments (43, 44). Also, obesity in dogs and humans produces the same low serum potassium levels as seen in individuals with Liddle syndrome (45, 46), although studies in humans are confounded by other factors. There are, however, some differences. Individuals with Liddle syndrome have low circulating aldosterone levels. In contrast, many obese individuals have elevated levels of circulating aldosterone (43–47). Also, individuals with Liddle syndrome have normal insulin and blood glucose levels. Obese individuals often have elevated blood sugar and insulin levels. Thus, it is likely that, in many instances, overactivated ENaC may be the underlying mechanism responsible for the elevated blood pressure in both sets of individuals. In the case of Liddle syndrome, the cause is a mutation of ENaC itself; in the case of obese individuals, the overstimulation of ENaC occurs because of the hormonal imbalances (i.e. elevated insulin and aldosterone) that are common in obese individuals.

The common features of both hypertensive clinical situations are that (a) ENaC is active, and (b) in many (but not all) cases, patients display lowered serum [K⁺]. How then can chronically low serum potassium result in hypertension in the context of normal volume? The answer is not known, but it may be due in part to lowered production of nitric oxide (NO) in vascular smooth muscle cells. A relationship exists between serum K⁺ and NO production: NO production drops as ambient K⁺ decreases because of a decrease in endothelial NO synthase activity (48, 49). Thus, as NO levels decrease, vascular smooth muscle tone increases with a decrease in vascular compliance and a rise in central blood pressure. By fostering a preoxidative state, hypokalemia results in endothelial and renal tubular dysfunction, contributing to the hypertensive state (50). Chronic hypokalemia seen in Liddle syndrome, obesity, and other conditions like Cushing syndrome, primary aldosteronism, and pheochromocytoma, all with associated hypertension, may be a prime (but certainly not the only) contributor to the process (51–54).

**Summary**

Resistant hypertension is operationally defined as blood pressure that remains high (>140/90 mm Hg) despite the simultaneous use of three or more antihypertensive drugs of different mechanistic classes (43). Although the prevalence is unknown, resistant hypertension is a common clinical disorder (43). Because genetic screening of people with resistant hypertension is infrequent, many patients with Liddle syndrome or obesity-related hypertension fall into this group. The similarities between the hypertension observed in Liddle syndrome and obese individuals suggest the possibility that the underlying etiology is the same (overactive ENaC) but produced by different sources: mutation of ENaC in Liddle syndrome and a hormonal imbalance in obese individuals. To control blood pressure in these individuals, the activity of ENaC must be controlled (55). Carter et al. (56) demonstrated that individuals with hyperactive ENaC responded positively to therapy using the ENaC inhibitor amiloride. Saha et al. (57) subsequently showed that blocking ENaC reduced blood pressure in African Americans (all of whom were clinically obese). This simple therapy may significantly reduce the severity and occurrence of obesity-related hypertension because is not that the hypertension is resistant to therapy, but rather the standard therapy does not address the underlying etiology of the high blood pressure.

**REFERENCES**

1. Shimkets, R. A., Warnock, D. G., Bositis, C. M., Nelson-Williams, C., Hanson, J. H., Schambelan, M., Gill, J. R., Jr., Ulick, S., Milora, R. V., and Findling, J. W. (1994) *Cell* **79**, 407–414
2. Botero-Velez, M., Curtis, J. J., and Warnock, D. G. (1994) *N. Engl. J. Med.* **330**, 178–181
3. Warnock, D. G. (2001) *Am. J. Med. Sci.* **322**, 302–307
4. Garovic, V. D., Hilliard, A. A., and Turner, S. T. (2006) *Nat. Clin. Pract. Nephrol.* **2**, 624–630
5. Weisz, O. A., and Johnson, J. P. (2003) *Am. J. Physiol. Renal Physiol.* **285**, F833–F842
6. Staub, O., and Verrey, F. (2005) *J. Am. Soc. Nephrol.* **16**, 3167–3174
7. Knight, K. K., Olson, D. R., Zhou, R., and Snyder, P. M. (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103**, 2805–2808
8. Zhou, R., Patel, S. V., and Snyder, P. M. (2007) *J. Biol. Chem.* **282**, 20207–20212
9. Lu, C., Pribanic, S., Debonneville, A., Jiang, C., and Rotin, D. (2007) *Traffic* **8**, 1246–1264
10. Kabra, R., Knight, K. K., Zhou, R., and Snyder, P. M. (2008) *J. Biol. Chem.* **283**, 6033–6039
11. Shi, P. P., Cao, X. R., Sweezer, E. M., Kinney, T. S., Williams, N. R., Husted, R. F., Nair, R., Weiss, R. M., Williamson, R. A., Sigmund, C. D., Snyder, P. M., Staub, O., Stokes, J. B., and Yang, B. (2008) *Am. J. Physiol. Renal Physiol.* **295**, F462–F470
12. Bruce, M. C., Kanellis, V., Fouladkou, F., Debonneville, A., Staub, O., and Rotin, D. (2008) *Biochim. J.* **415**, 155–163
13. Butterworth, M. B., Edinger, R. S., Frizzell, R. A., and Johnson, J. P. (2009) *Am. J. Physiol. Renal Physiol.* **296**, F10–F24
14. Bueck, T. M., Kolb, A. R., Boyd, C. R., Kleyman, T. R., and Brodsky, J. L. (2010) *Mol. Biol. Cell* **21**, 1047–1058
15. Bubien, J. K., Ismaelov, I. L., Berdiev, B. K., Cornwell, T., Lifton, R. P., Fuller, C. M., Achard, J. M., Benos, D. J., and Warnock, D. G. (1996) *Am. J. Physiol. Cell Physiol.* **270**, C208–C213
16. Bubien, J. K., Jope, R. S., and Warnock, D. G. (1994) *J. Biol. Chem.* **269**, 17780–17783
17. Warnock, D. G., and Bubien, J. K. (1994) *Hosp. Pract.* **29**, 95–105
18. Bubien, J. K., Watson, B., Khan, M. A., Langloh, A. L., Fuller, C. M., Berdiev, B., Tousson, A., and Benos, D. J. (2001) *J. Biol. Chem.* **276**, 8557–8566
19. Liddle, G. W., Bledsoe, T., and Copping, W. S. (1963) *Trans. Assoc. Am. Phys. 76*, 199–213
20. Rekalld, L., and Borra, S. (2000) *Clin. Nephrol.* **53**, 66–70
21. Mutoh, S., Hirayama, H., Ueda, S., Tsuruta, K., Imafujii, M., and Ikegami, K.
