PC and PKC: in vivo vs. in vitro

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Principal cells (PC) of the collecting duct are essential both in the regulation of water and electrolyte balance in the kidney and in the regulation of blood pressure. Epithelial Na⁺ channels (ENaC) mediate sodium reabsorption in this cell type. A critical role of protein kinase C (PKC) in the modulation of ENaC activity was previously reported by several groups in vitro and ex vivo. Various pharmacological tools were used to assess regulation of ENaC in PC by PKC. However, as we are all aware, findings in cell culture are not always confirmed in animal models.

In a recent issue of the American Journal of Physiology-Renal Physiology, Bao and colleagues (3) provide direct evidence that PKCα is a kinase that is important in the maintenance of ENaC-mediated sodium balance in PC and regulation of blood pressure. In particular, the authors reported that in the absence of PKCα the active phosphorylated form of ERK1/2 was significantly reduced, and thereby ENaC activity was increased. It was shown that both the number of channels and ENaC open probability were increased in the PKCα−/− knockout mice, and this enhanced channel activity was associated with elevated blood pressure in knockout animals fed a high-salt diet (3). The studies were done to define the role of PKC in the context of whole animals, and the data in the article complement the previous investigations by this group on the mechanisms modulating ENaC activity by PKC in cultured cells (1, 6, 7, 9). This study confirmed initial observations in vitro and provide important information about this critical pathway in vivo. In addition to advancing our scientific knowledge, this is a nicely performed study utilizing many challenging approaches such as single-channel analysis in split-open tubules, confocal microscopy, and in situ biotinylation, and further highlight the importance of in vitro studies, which often guide our work ex vivo and in vivo.

This manuscript presents direct evidence of ENaC regulation by PKC and emphasizes the need for further investigation of mechanisms involved in the control of sodium transport by this kinase. There are a few questions raised by the current study. First, this study is focused on ENaC, but it is not clear whether effects of PKC are specific to this important channel or other channels and transporters in the kidney are also regulated by PKC. Further studies required to test whether PKC is involved in regulation of not only sodium transporters such as NCC and NKCC, but also in the maintenance of potassium, chloride, etc. balance. Moreover, it has been shown that several PKC isoforms are ubiquitously expressed in many organs, such as the lungs, where ENaC also plays a critical role in sodium reabsorption. While current studies provide ample evidence that ENaC is inhibited by PKC in the kidney, we cannot definitely conclude that the same effects and mechanisms will take place in the lungs, due to a different environment, expression of multiple isoforms, and many other factors. Furthermore, in the current studies the authors used global knockout of PKCα; however, use of mice with specific knockout of PKCα in either collecting ducts or renal tubules would provide additional critical knowledge about regulation of sodium transport in the kidney. Another limitation of this study is that blood pressure measurements were performed using the tail-cuff approach, which is relatively insensitive compared with telemetry, so there is a possibility that lack of changes in blood pressure under normal salt conditions were masked by the low sensitivity of this approach.

There are several upstream effectors which could trigger PKC-mediated regulation of ENaC as well as various intracellular signaling mechanisms activated by PKC. In the current study, the authors determined the plasma aldosterone levels in wild-type and PKCα−/− mice on either low- or high-salt diets and did not observe any significant differences between knockout and control animals. However, recent studies by Sun et al. (10) demonstrated that angiotensin II, another critical hormone of the renin-angiotensin-aldosterone system, has effects on ENaC via stimulation of PKC. Interestingly, PKC-mediated effects of angiotensin II on ENaC were Ca²⁺ independent and resulted in activation of the channel (10). Downstream targets of PKC might also vary and depend on many specific factors. In the current study, Bao et al. (3) provide solid evidence that ERK1/2 activity was reduced in knockout animals and speculate that two potential mechanisms explain observed differences in the changes of ENaC gating and number of channels in the plasma membrane. First, it was proposed that active PKC phosphorylates ERK1/2 and subsequent phosphorylation of ENaC promotes Nedd4-2-mediated ubiquitination of ENaC, which results in a reduced number of channels in the plasma membrane. Another proposed mechanism explaining changes in the channel open probability is based on regulation of ENaC by myristoylated alanine-rich C-kinase substrate and phosphatidylinositides (1, 3). However, it is possible that other proteins and signaling molecules could be also involved in regulation of ENaC by PKC.

There are several isoforms of PKC, and while previous studies reported contradictory data about specific localization of PKC isoforms in the kidney, most of them are consistent with high expression of PKCα in the collecting duct. In the current manuscript, Bao et al. (3) demonstrated that PKCα is ubiquitously expressed in the kidney, including PC of collecting duct (as shown by colocalization with AQP2). The specificity of labeling was confirmed in the PKCα knockout mice. Identification of PKCα as a specific isoform important for ENaC-mediated changes in blood pressure could have potential clinical implications. Furthermore, these studies can have even greater clinical relevance since recent genome-wide association studies (GWAS) provided evidence that single-nucleotide polymorphisms in the gene encoding PKCα were associated...
with blood pressure responses to hydrochlorothiazide in European Americans and hypertensive blacks (11). In conclusion, the current manuscript implicates a key role for PKC regulation of ENaC and its relation to blood pressure control. Importantly, this manuscript is one of the first studies providing evidence of a role played by a gene identified in GWAS for ENaC-mediated modulation of blood pressure. Thus manipulating PKC activity or its downstream targets involved in regulation of ENaC could be a promising therapeutic approach, opening a new avenue in the quest for novel drugs to treat hypertension, further emphasizing the need for investigation of this important area of research.

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