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Comprehensive Analysis of Protease Cascade Involving Prostasin in Salt-Sensitive Hypertension

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Summary

Identification of gain-of-function mutation in epithelial sodium channel (ENaC) in Liddle's syndrome patients, a hereditary form of salt-sensitive hypertension, indicates the importance of the sodium reabsorption through the kidney in the pathogenesis of salt-sensitive hypertension. In 1997, Vallet et al isolated a channel-activating protease (CAP-1), a trypsin-like serine protease, from A6 cell line and demonstrated that co-expression of CAP-1 and ENaC in *Xenopus* oocytes increased ENaC activity. We isolated a serine protease prostasin, a mammalian CAP-1 homologue, from rat kidney cDNA library and demonstrated that co-expression of prostasin and ENaC increased the amiloride-sensitive sodium current in *Xenopus* oocytes. We also found that aldosterone increases sodium reabsorption through ENaC by increasing the expression of prostasin.

Serine proteases play important roles in human body such as R-A-A system, blood coagulation system, and digestive system. In most cases, the activation of proteases requires proteolytic processing by upstream proteases and the upstream proteases also need to be cleaved by further upstream proteases. Thus, proteases form "a proteolytic cascade" to execute their physiological function. Therefore, we hypothesized that prostasin is involved in a certain proteolytic cascade and in the current studies we tried to identify possible candidates for the processing enzymes for prostasin.

First, we showed that camostat mesilate, a synthetic serine protease inhibitor, inhibited the proteolytic activation prostasin, suggesting that a certain serine protease(s) is/are involved in the activation of prostasin. Then, we performed double-layer fluorescent zymography by using recombinant pro-prostasin and membrane fractions from rat and mouse kidney. We found at least four different candidates for prostasin processing enzymes and we are now in the middle of identifying the amino acid sequences of these proteins.