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Sensing Mechanism of Cytosolic Cl^- as a Signal Molecule in Hypotonic Stress

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Summary

In renal epithelium, plasma hypotonic stress stimulates Na^+ reabsorption to recover normal plasma osmolality. However, it is still under investigation how dose cell sense the changes in extracellular osmolality and stimulate Na^+ reabsorption. We have previously indicated in renal epithelia that: 1) the hypotonic stress causes a biphasic reduction of cytosolic Cl^- concentration ($[Cl^-]_c$); a decrease in $[Cl^-]_c$ during initial cell swelling followed by that during a subsequent regulatory volume decrease, and 2) the hypotonic stress modulates tyrosine phosphorylation of src kinase playing a crucial role in signal transduction. Based on these observations, we hypothesized that cytosolic Cl^- acts as a signal molecule to regulate tyrosine phosphorylation in hypotonic stress in renal epithelia. To study if the cytosolic Cl^- acts as a signal molecule for the hypotonic stress, we studied the effects of $[Cl^-]_c$ on phosphorylation state of src kinase at Tyr416. Generally src kinase autophosphorylates Tyr416 to show its enzymatic activation. A reduction of $[Cl^-]_c$ increased phosphorylation of src kinase at Tyr416. On the other hand, the treatment of vanadate (a protein tyrosine phosphatase (PTP) inhibitor) was more effective to an increase in phospshorylation of src kinase at Tyr416 under high $[Cl^-]_c$ condition. These observations suggest that: 1) both activities of PTP and src kinase decrease at lowered $[Cl^-]_c$, and 2) the activity of src kinase is larger than that PTP at lowered $[Cl^-]_c$. Furthermore, $[Cl^-]_c$ -dependent activation of src kinase stimulated Na^+ reabsorption through induction of beta- and gamma-ENaC mRNA expression in renal epithelium.