No. 1645

Functional Analysis Myosin Phosphatase Regulator in High Salt Diet-Mediated Hypertension

Masatoshi Hori, Hirokazu Tsubone, Shigeru Kakuta

Graduate School of Agriculture and Life Sciences, The University of Tokyo

Summary

Vascular smooth muscle contraction is regulated by balance between MLC kinase (MLCK) and myosin light chain phosphatase (MLCP) activities. MLCP activity is negatively regulated by rhoA/rho-kinase signaling and PKC/CPI-17 signaling (CPI-17; a protein kinase C potentiated phosphorylation-dependent inhibitory protein of MLCP). These negative regulatory pathways are thought to modulate blood pressure in physiological and/or pathophysiological condition, such as hypertension and cerebrovascular spasm. We here generated CPI-17-deficient mouse (CPI-KO) and mutant CPI knock in mice in which phosphorylatable T38 was replaced with unphosphorylatable alanine (CPI-TA) by using CRSPR/Cas9 system, then investigated the effect of CPI-17 on vascular contractility and mean blood pressure (MBP).

In wild type mice (WT) aorta, phorbol ester (PDBu) potentiated high K^+-induced contraction although PDBu alone did not induce contraction. In contrast, in aorta isolated from CPI-KO and CPI-TA, the PDBu-mediated potentiation of high K^+-induced contraction was completely abolished, indicating that CPI-17 is functionally knocked out in both CPI-17 mutant mice. Phenylephrine-induced contractions in aorta of CPI-KO and CPI-TA were also smaller than that of WT in accordance with decrease in MLC-phosphorylation. In radio-telemetory system, MBP in CPI-KO and CPI-TA were significantly decreased than that in WT. In contrast, heart rates (HR) in CPI-KO and CPI-TA were increased rather than that in WT. This may be compensative action to maintain blood pressure. In high salt diet-induce hypertension model of WT, MBP was significantly increased within 1 week later after starting high salt diet then maintained the high level MBP over 3 weeks. In contrast in CPI-KO and CPI-TA, increment of MBP induced by high salt diet was significantly lower and maintained lower level over 3 weeks.

In conclusion, we, for the first time, succeeded to generate CPI-17 deficient mice and phospho-inactive mutant CPI-17 knock-in mice. We established that PKC/CPI-17 signaling pathway is important to regulate MBP not only in physiological condition but also in pathophysiological condition of high salt diet-induced hypertension.