Essential role of CPI-17 for muscle force generation in urinary bladder using CPI-17 genetically modified mice

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Back ground: Detrusor smooth muscle contraction depends on myosin light chain (MLC) phosphorylation, which is regulated by a balance between Ca^{2+} /calmodulin-dependent myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) activities. Biochemical investigations revealed that CPI-17, MLCP inhibitory protein, inhibited MLCP activity via PKC-dependent phosphorylation of CPI-17 at threonine 38 (T38) resulted in increment of MLC phosphorylation, which in turn enhanced muscle force. However, there is no direct evidence to clarify essential role of CPI-17 on force generation in detrusor smooth muscle using CPI-17 genetically modified mice. Aim: We try to demonstrate essential role of CPI-17 for force generation in urinary bladder using CPI-17 deficient mice (CPI-KO) and phospho-inactive mutant CPI-17 at T38 to alanine knocked-in mice (CPI[T38A]-KI). Methods: We measured isometric force of detrusor smooth muscle tissues isolated from Wild type mice (WT), CPI-KO and CPI[T38A]-KI. We measured MLC-phosphorylation and CPI-17 phosphorylation at T38 by Western blot. Results: High concentration of KCl (High K^{+})-induced contractions are not different between WT, CPI-KO and CPI[T38A]-KI. MLC-phosphorylation level stimulated with High K^{+} at 30s were also same in these three animals. Carbachol (CCh; 5 μM) induced phasic contraction 30 s after the stimulation, followed by a sustained contraction over 10 min. The phasic contractions by CCh in CPI-KO and CPI[T38A]-KI were significantly decreased compared with that in WT in accordance with changes in MLC-phosphorylation. The sustained tonic components of CCh-induced contraction in both CPI-17 mutant mice were also tended to decrease than WT. CPI-17 was phosphorylated when the muscle was stimulated with CCh but not with High K^{+} . Conclusion: CPI-17 played an important role for force generation by CCh but not High K^{+} . Phosphorylation of CPI-17 at T38 may be essential to inhibit MLCP activity, because CCh-induced contraction in CPI-KO was exactly same with in CPI[T38A]-KI. CPI-KO and CPI[T38A]-KI could be powerful tools to identify physiological and pathophysiological roles of CPI-17 in urinary drainage mechanisms.