Regulation of NaCl transport by hormone and drug in renal tubule.

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Summary

To clarify the mechanism of natriuresis by acetylcholine (ACh), we examined the effect of ACh on the amiloride sensitive Na^+ conductance in the apical membrane of the isolated rabbit cortical collecting duct (CCD) perfused in vitro using the conventinal microelectrode and the microscopic fluorescence spectro-photometry techniques. Basolateral application of ACh positively shifted transepithelial voltage (V_T) and triggered a transient increase of cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) followed by a sustained increase in a dose-dependent manner (10^{-8} – 10^{-5} M). Both actions of ACh were blocked by atropine and pirenzepine, muscarinic receptor antagonists. On the basis of cable analysis, we found ACh to increase fractional resistance of apical membrane (fR_A) of the collecting duct (CD) cells, but not β-intercalated (β-IC) cells, accompanied by a positive deflection of V_T and an increase of transpithelial resistance (R_T). Luminal application of 10^{-5} M amiloride, a Na^+ channel blocker, almost completely abolished the electrophysiological effects of ACh. ACh-induced increase of $[Ca^{2+}]_i$ was not changed by removing luminal Ca^{2+}, while ACh evoked only a transient increase of $[Ca^{2+}]_i$, after removal of basolateral Ca^{2+}. This observation indicated that ACh triggered a release of Ca^{2+} from intracellular store site and an influx of Ca^{2+} via basolateral membrane. Both phorbol-12-myristate-13-acetate (PMA) and phorbol-12, 13-dibutylate (PDBu), protein kinase C (PKC) activators, inhibited apical Na^+ conductance, and prevented ACh to show further inhibition. 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), an inhibitor of PKC, partially attenuated the inhibitory effect. These results support the view that ACh inhibits the apical Na^+ conductance in the CD cells of rabbit CCD by both increase of $[Ca^{2+}]_i$ and activation of PKC. This view partly explains the natriuretic effect of ACh.