Interaction between Ca^{2+} tranport regulation and Na^{+} transport in connecting tubule.

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Summary

To characterize Ca^{2+} transport across the apical membrane of rabbit connecting tubule (CNT), we examined the effects of luminal pressure on parathyroid hormone (PTH)-dependent apical Ca^{2+} transport in this segment perfused in vitro. An increase of perfusion pressure (0.2 to 1.2KPa) elevated cytoplasmic Ca^{2+} concentraion ($[Ca^{2+}]_i$) by 42 ±11nM in fura 2 loaded CNT. Basolateral application of 10nM PTH accelerated the response. Addition of 0.1mM chlorphenyl-thio-cAMP (CPT-cAMP) to bath also augmented the response from 36±16nM to 84±26nM. Under steady perfusion pressure at 1.2KPa, 10nM PTH increased $[Ca^{2+}]_i$ by 31±7nM, whereas it did only by 6±2nM at 0.2KPa. The pressure dependent increase of $[Ca^{2+}]_i$ was abolished by removing Ca^{2+} from lumen, and was not affected by adding 0.1 or 10 μM nicardipine into lumen in the presence of 10nM PTH. Both 100 μM nifedipine and benijipine added into lumen suppressed by 23.5± 5.9% and by 30.1±11.0% of $[Ca^{2+}]_i$ increase induced by 50nM PTH, respectively, but luminal 10 μM Gd^{3+} did by 89.5±18.5%. An application of 10nM PTH hyperpolarized transmural voltage (V_t), followed by depolarization which indicated the block of amiloride-sensitive Na^{+} channels. Luminal Gd^{3+} (10 μM) reduced only depolarization from +2.7±0.6mV to +1.3±0.4mV measured at 5min after peak hyperpolarization. Because Ca^{2+} is transported by Na^{+}/Ca^{2+} exchanger in the basolateral membrane, block of apial Na^{+} entry via amiloride-sensitive Na^{+} channel could accelerated Ca^{2+} transport. Cell-attached patch clamp studies on the apical membrane of everted CNT using pipette filled with either 200mM $CaCl_2$ or 140mM NaCl revealed channel activities (42±2pS or 173±7pS, respectively). An application of negative pressure (-4.9KPa) to the patch pipette augmented its mean number of open channels from 0.005±0.001 to 0.022±0.005 in the Ca^{2+}-filled pipette, and was further accelerated to 0.085±0.014 by 0.1mM CPT-cAMP. In the Na^{+}-filled pipette, similar results were obtained, and CPT-cAMP did not activate the stretch-activated channels in the absence of negative pressure. These results suggest that a stretch-activated nonselective cation channel is a major route of Ca^{2+} transport in the apical membrane of rabbit CNT and that it is activated by PTH in the presence of hydrostatic pressure or luminal fluid flow.