Characterization of a long-term rat mTAL cell line

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Abstract

A medullary thick ascending limb (mTAL) cell line, termed raTAL, has been established from freshly isolated rat mTAL tubules and cultured continuously for up to 75 passages; it retains characteristics of mTAL cells even after retrieval from storage in liquid nitrogen for several months. The cells express Tamm-Horsfall glycoprotein (THP), a TAL-specific marker, grow to confluence, exhibit a polygonal morphology characteristic of epithelial cells, and form “domes.” Detection of THP, Na^+-K^+-$2Cl^-$ cotransporter (NKCC2), Na^+-K^+-ATPase, and renal outer medullary K^+ channel (ROMK) was achieved using indirect immunofluorescence and confocal microscopy. Western blot analysis of NKCC2 expression using two different antibodies revealed a band of ~160 kDa, and RT-PCR analysis demonstrated the presence of NKCC2 isoforms A and F, which was confirmed by DNA sequencing; transport of Cl^- into raTAL cells was inhibited by furosemide. Ouabain- and bumetanide-sensitive oxygen consumption, an index of ion transport activity in the mTAL, was observed in raTAL cells, and the number of domes present was reduced significantly when cells were incubated in the presence of ouabain or bumetanide. The specific activity of Na^+-K^+-ATPase activity was determined in raTAL cells (0.67 ± 0.18 nmol P_i·μg protein^{-1}·min^{-1}), primary cultures of mTAL cells (0.39 ± 0.08 nmol P_i·μg protein^{-1}·min^{-1}), and freshly isolated mTAL tubules (1.10 ± 0.29 nmol P_i·μg protein^{-1}·min^{-1}), and ~30–50% of total cellular ATPase activity was inhibited by ouabain, in accord with other mTAL preparations. This cell line will be used in studies that address biochemical, molecular, and physiological mechanisms in the mTAL.