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Poly-L-arginine predominantly increases the paracellular permeability of hydrophilic macromolecules across rabbit nasal epithelium in vitro

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PURPOSE: The purpose of this work was to characterize the main transport pathway of hydrophilic macromolecules induced by poly-L-arginine (poly-L-Arg; molecular weight 42.4 kDa) across the excised rabbit nasal epithelium.
METHODS: Excised rabbit nasal epithelium was mounted in an Ussing-type chamber for measurement of fluorescein isothiocyanate-labeled dextran (FD-4; molecular weight 4.4 kDa) transport and transepithelial electrical resistance (TEER). The main transport pathway of FD-4 enhanced by poly-L-Arg was evaluated using confocal laser scanning microscopy. Immunolocalization of junction proteins (ZO-1, occludin, and E-cadherin) after treatment with poly-L-Arg was also observed.
RESULTS: After apical application of a poly-L-Arg (0.05, 0.5, and 5 mg/mL), the permeability coefficient of FD-4 increased by 1.6-, 2.9-, and 5.2-fold, respectively, compared with the control of 5.2 +/- 1.3 x 10^{-7} cm/s. Consistent with the increase in transport, there was a concurrent reduction in TEER. At a concentration of 0.05 mg/mL poly-L-Arg. both FD-4 transport and TEER returned to the control level. A good correlation was obtained between the FD-4 permeability coefficient and 1/TEER. Basolateral application of poly-L-Arg at 5 mg/mL, however, did not increase FD-4 transport. Marked FD-4 fluorescence was located in the paracellular spaces after treatment with apical poly-L-Arg compared with that in the absence of poly-L-Arg. Immunofluorescence of ZO-1, occludin, and E-cadherin in cell-to-cell junctions was reduced and distributed into the cytoplasm by apical application of poly-L-Arg, suggesting that poly-L-Arg regulates the junction proteins to enhance

paracellular permeability across the nasal epithelium. After pretreatment with either 2,4-dinitrophenol or ouabain, the enhancing effect of apical poly-L-Arg was abolished, indicating the contribution of metabolic energy (cell viability) to the poly-L-Arg-mediated enhancing effect.

CONCLUSION: In the nasal epithelium, apical poly-L-Arg appears to increase predominantly the paracellular transport of hydrophilic macromolecules via disorganization of tight- and adherens-junction proteins. The regulatory mechanism of the poly-L-Arg effect is likely to be dependent on energy-requiring cellular processes.