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Poly-L-arginine enhances paracellular permeability via serine/threonine phosphorylation of ZO-1 and tyrosine dephosphorylation of occludin in rabbit nasal epithelium

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PURPOSE: The purpose of the present study is to explore whether a poly-L-arginine (poly-L-Arg)-induced increase in tight junctions (TJ) permeability of fluorescein isothiocyanate-labeled dextran (MW 4.4 kDa, FD-4) is associated with the Ca^{2+}-dependent signaling and occurs following the phosphorylation/dephosphorylation of TJ proteins.
METHODS: Excised rabbit nasal epithelium was mounted in an Ussing-type chamber for measurement of FD-4 transport and membrane conductance (Gt) in the presence of various inhibitors that are involved in the Ca^{2+}-dependent pathway and the phosphorylation/dephosphorylation of TJ proteins. The resultant distribution of TJ proteins was observed using confocal laser scanning microscopy (CLSM) in an immunostaining.
RESULTS: The increase in TJ permeability of FD-4 induced by 0.2 mg/ml poly-L-Arg was not altered by treatment with inhibitors of possible Ca^{2+} mobilization pathways followed by exposure of poly-L-Arg, suggesting that the promoting effect of poly-L-Arg is independent of Ca^{2+}-related signaling. On the other hand, the protein kinase C (PKC) and tyrosine phosphatase inhibitors suppress the increase in TJ permeability by poly-L-Arg, indicating that serine/threonine phosphorylation by way of Ca^{2+}-independent PKC and tyrosine dephosphorylation of junction proteins may have occurred. Furthermore, immunofluorescent monitoring of ZO-1, a TJ associated protein, and occludin, an

integral membrane protein localizing at TJ, after preincubation with PKC and tyrosine phosphatase inhibitors followed by poly-L-Arg treatment has shown that the internalization of ZO-1 and occludin occurred by way of serine/threonine phosphorylation by PKC activation and by way of tyrosine dephosphorylation, respectively, providing TJ disassembly.

CONCLUSIONS: We conclude that poly-L-Arg enhances the paracellular permeability of FD-4 (i.e., macromolecules), at least, by way of both serine/threonine phosphorylation of ZO-1 and tyrosine dephosphorylation of occludin in rabbit nasal epithelium.