EFFECTS OF N-WASP181-200 AND ITS MUTANT PEPTIDE ON RENAL BINDING AND UPTAKE OF GENTAMICIN
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Nephrotoxicity is one of the dose-limiting factors in the use of aminoglycoside antibiotics (AGs). Megalin, a multiligand endocytic receptor abundantly expressed in the apical membrane of the renal proximal tubular cells, plays an important role in renal accumulation of AGs such as gentamicin and amikacin. Our previous studies showed that coadministration of megalin ligands such as cytochrome c with gentamicin decreases the renal accumulation and toxicity of gentamicin. In addition, we found that renal accumulation of gentamicin was significantly inhibited by coadministration of a 20-residue peptide, N-WASP181-200 (NISHTKEKKKGAKKKRLTK). However, the molecular mechanism(s) by which N-WASP181-200 decreases renal accumulation of gentamicin is not understood. In this study, we investigated the role of lysine residues of N-WASP181-200 in the inhibitory effect on gentamicin accumulation in the kidney. N-WASP181-200 and N-W(M1), a mutant peptide in which two lysines at 189 and 195 were substituted for glycine, were prepared by solid-phase synthesis. The peptide sequences of synthesized peptides were confirmed by mass spectrometry. Both N-WASP181-200 ($p_I=10.87$) and N-W(M1) ($p_I=10.75$) inhibited the binding of $[^3$H]gentamicin to rat renal brush-border membranes in a concentration-dependent manner. However, the half-maximal inhibitory concentration of N-W(M1) was about 3-fold higher than that of N-WASP181-200. In addition, the inhibitory potency of N-W(M1) for uptake of $[^3$H]gentamicin in OK kidney epithelial cells expressing megalin was lower than that of N-WASP181-200. These observations suggest that lysine 189 and/or lysine 195 in N-WASP181-200 may be partly responsible for the inhibition of renal accumulation of gentamicin by N-WASP181-200.