

Analysis of Structure and Function of Water channel Proteins

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Summary

Among aquaporins AQP2 is the vasopressin regulated water channel. Vasopressin causes cAMP production in renal collecting duct cells, this is believed to initiate a series of events leading to an dramatic increase in water permeability of the apical membrane of this cell. AQP2 contains a consensus sequences for cAMP dependent protein kinase. To determine the role of this site, Ser256 was substituted for Ala, Leu, Thr, Asp, or Gly by site-directed mutagenesis. Incubation with cAMP plus forskolin or direct cAMP injection into the oocytes increased Pf of wild-type, but not mutated, AQP2-expressing oocytes, whereas the amount of AQP2 expression were similar in wild and mutated types as identified by Western blot analysis. In vitro phosphorylation studies showed that cAMP dependent protein kinase phosphorylated wild-type, but not mutated AQP2 proteins. Phosphoamino acid analysis revealed that this phosphorylation occurred at the serine residue. These data suggest that cAMP stimulates water permeability of AQP2 by phosphorylation. This process should contribute to the vasopressin-regulated water permeability of kidney collecting ducts.