HETEROLOGOUS DOWNREGULATION OF VASOPRESSIN TYPE 2 RECEPTOR IS INDUCED BY TRANSFERRIN

Bouley, R., Nunes, P. and Brown, D.
Program in Membrane Biology, Nephrology Division, Massachusetts General Hospital, Boston, MA

Vasopressin (VP) binds to the V2R to trigger physiological effects on body fluid homeostasis and blood pressure regulation. These effects are terminated by receptor downregulation involving clathrin-mediated endocytosis and V2R degradation. We now find that green fluorescent protein tagged V2R (V2R-GFP) is internalized from the plasma membrane of LLC-PK1 cells in the presence of another ligand, transferrin (Tfn). The presence of iron-saturated Tfn (holo-Tfn) (4h) reduced V2R binding sites by 40% while iron-unsaturated (apo-Tfn) had no effect. In the presence of partially saturated Tfn-rhodamine (Tfn-Rho), both Tfn-Rho and V2R-GFP accumulated in cytoplasmic vesicles. No change in V2R-GFP distribution was observed in the presence of bovine serum albumin (BSA), atrial natriuretic peptide (ANP) or angiotensin II (AngII). In contrast to the effect of VP, Tfn did not increase intracellular cAMP or modify AQP2 distribution. Co-immunoprecipitation of Tfn receptor and V2R was revealed using an anti-GFP antibody, suggesting that they interact closely, possibly explaining the additive effect of VP (1nM) and Tfn on V2R internalization. This effect was abolished in cells expressing a dominant negative dynamin (K44A) mutant, illustrating the involvement of clathrin coated-pits in this process. Finally, while the initial route of internalization has not yet been clearly visualized, live cell imaging reveals that Tfn-Rho and V2R-GFP are in different vesicle populations after internalization. We conclude that Tfn can induce heterologous downregulation of the V2R and this might desensitize VP target cells without activating downstream V2R signaling events. It also provides new insights on hemochromatosis in which rats expressing abnormally high levels of holo-Tfn are unable to concentrate their urine.