

Supplemental Material

Supplemental Figure S1. Preliminary experiments to optimize timing and doses.

Supplemental Spreadsheet 1. Ranking of kinases that could phosphorylate Aqp2 at S269 and their Bayes' Probability.

Figure S1. Preliminary experiments to optimize timing and doses. In the absence of dDAVP, IMCD suspensions treated with 0.1 μ M EGF alone for 30-120 minutes had no effect on phosphorylation of AQP2 at S261, whereas IMCD treated with dDAVP had reduced phosphorylation as compared with vehicle controls as previously seen (27). Figure S1B shows a dose-response experiment testing the ability of EGF to reverse the dDAVP-mediated downregulation of S261-AQP2 phosphorylation at different dDAVP concentrations. IMCD samples were first treated with dDAVP (1-1000 pm) for 30 min followed by another 30 min of incubation with or without 0.1 μ M EGF with continuous presence of dDAVP. Again, there was no detectable effect of EGF on S261-AQP2 phosphorylation. Thus, S261-AQP2 phosphorylation did not change in response to EGF in normal rat IMCD either in the absence or presence of dDAVP. Note, however, that in samples treated with dDAVP at 100 pmol, the same concentration that was used in *in vitro* perfusion experiments (Figure 2), EGF reduced phosphorylation of S264-AQP2 and S269-AQP2 (Figure S1C), although dDAVP caused the expected dose-dependent increase in phosphorylation of S256, S264, and S269 (Figure S1C).