AKT and SGK-1 regulate albumin endocytosis *via* separate signalling pathways

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Albumin endocytosis in the renal proximal tubule occurs *via* a receptor medicated process that requires the coordinate regulation of a number of transmembrane and accessory proteins. Previously, we have demonstrated an essential role in albumin uptake for the Cl^- channel, ClC-5 as well as accessory proteins NHERF-2, Nedd4-2 and cofilin. The Serum Glucocorticoid Kinase 1 (SGK-1) is a member of the AGC kinase family of proteins that associates with both NHERF-2 and Nedd4-2. In addition, another AGC family member, AKT (v-Akt Murine Thymoma Viral Oncogene or Protein Kinase B), plays a role in the initial stages of albumin uptake *via* an association with the albumin receptor megalin. The roles of both these AGC kinases in regulating constitutive albumin uptake have yet to be determined.

In this study we investigated the roles of AKT and SGK-1 in albumin endocytosis using the well characterized opossum proximal tubule (OK) cell line. We then investigated whether pathophysiological concentrations of albumin regulated the levels of these kinases. To measure albumin uptake we used our standard protocol with Texas Red conjugated albumin (TR-albumin). Overexpression of dominant negative kinases or siRNA were used to manipulate the activity of the kinases in OK cells. We found that treatment of OK cells with AKT inhibitor I (Invitrogen) significantly reduced albumin uptake (75 ± 5% compared to control; $n = 4$; $p < 0.05$), indicating a role for AKT in constitutive endocytosis in proximal tubule cells. Inhibition of the downstream ERK1/2 kinases with U1026 did not further reduce uptake (70 ± 2 % compared to cells with AKT silenced; $n = 3$), suggesting that AKT was upstream of ERK in this pathway. When the concentrations of albumin were increased to either 100 or 1000 μg/ml, Western blot analysis showed that total AKT was significantly reduced (49 ± 9% and 62 ± 13%, respectively, $n = 3$, $p < 0.05$). However, there was no change in the level of phosphorylated AKT indicating that the level of activity is maintained.

To investigate the role of SGK-1, we used both silencing and dominant negative strategies. When SGK-1 was silenced albumin uptake was reduced to 68 ± 8% ($n = 4$) of control levels. Similarly the kinase dead mutant of SGK-1 also reduced uptake to 81 ± 3% ($n = 4$). Both treatments significantly reduced the level of TR-albumin endocytosis ($n = 4$, $P < 0.05$) compared to control. Further, exposure of proximal tubule cells to increased levels of albumin (100 μg/ml or 1000 μg/ml) caused significant decreases in the levels of SGK protein in the cells (87 ± 3% and 84 ± 5%, respectively, $n = 3$, $p < 0.05$).

SGK and AKT can act *via* the same pathways. In order to determine if these two kinases were using the same pathway to inhibit albumin uptake, we treated cells silenced for SGK-1 with AKT inhibitor I (Invitrogen). This treatment resulted in a further significant reduction in the level of albumin uptake to (50 ± 7%, $n = 3$, $p < 0.05$) compared to cells silenced for SGK-1 (68 ± 8%, $n = 3$). This important result indicated that the effects of SGK-1 and AKT on albumin uptake are mediated by different signalling pathways. The activity of the sodium hydrogen exchanger NHE3 is required for albumin uptake. NHE3 is also inhibited by SGK-1. We therefore investigated whether SGK-1 was inhibiting albumin uptake by inhibiting NHE3. In control cells, the NHE exchange inhibitor EIPA (50 μM) reduced albumin uptake by 61 ± 3% ($n = 3$). When these experiments were repeated in cells in which SGK-1 had been silenced, there was no further inhibition of albumin uptake (66 ± 6%, $n = 3$, $p < 0.05$). This result suggests that SGK may inhibit albumin uptake by inhibiting NHE3.

This study shows that AGC kinases differentially regulate constitutive albumin uptake by using different signalling pathways. The alterations in protein levels in the presence of high albumin suggest that these proteins may be key players in mediating the changes in albumin handling in proteinuric renal disease.