

ALBUMIN INDUCED DOWN-REGULATION OF P GLYCOPROTEIN IN HK-2 HUMAN TUBULAR CELLS: INFLUENCE OF VARIOUS CHEMICALS



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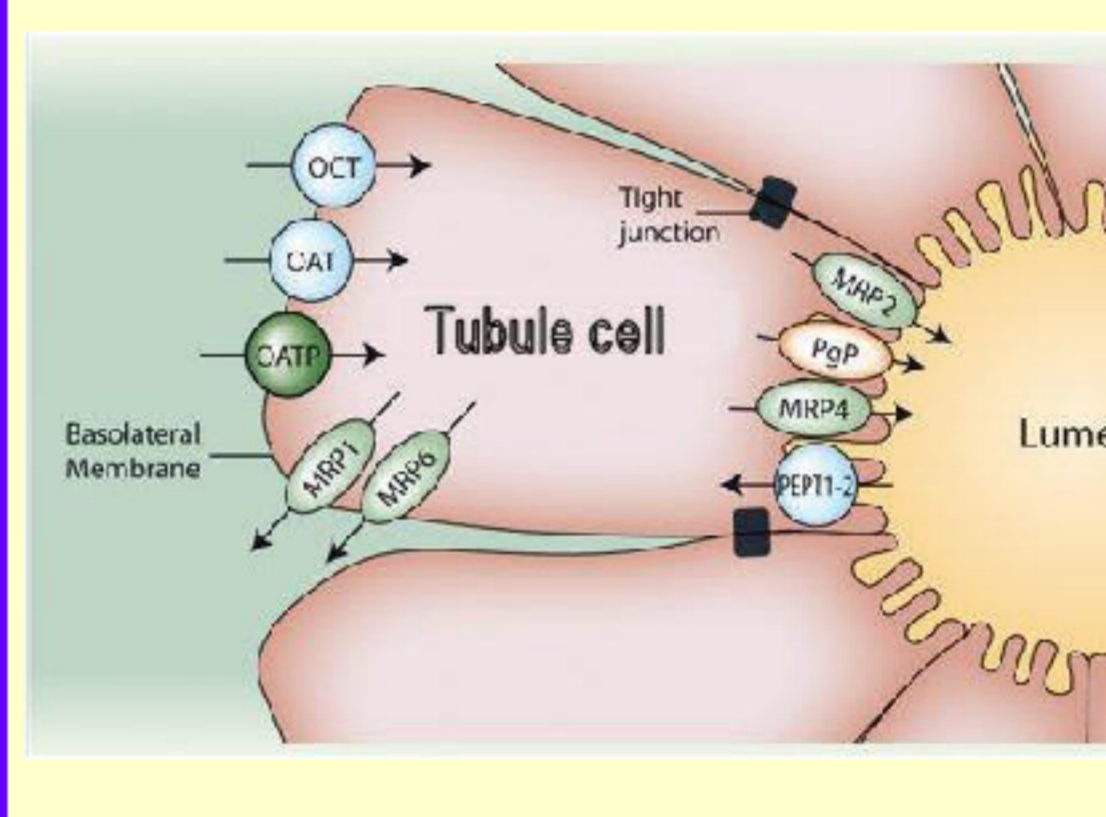
Introduction:

- Progressive renal failure has been closely linked to proteinuria and tubulointerstitial involvement
- Tubular reabsorption of filtered albumin may contribute to the structural interstitial injury that has been associated with albuminuria
- Upon protein endocytosis, the tubules that reabsorb albumin demonstrate increased expression of pro-inflammatory cytokines that promote inflammation, tubular degeneration, and fibrosis
- A large number of genes encoding membrane transporter proteins of tubular cells was found to be up- or down-regulated by proteinuria
- One membrane transporter is P-glycoprotein (Pgp), a glycoprotein involved in the ATP-dependent transmembrane efflux of a wide range of compounds leading to a decrease in drug concentration within the cell
- In the kidney Pgp is mainly expressed in the proximal tubule
- The influence of albumin on expression and function of Pgp in HK-2 proximal tubular cells has previously been demonstrated

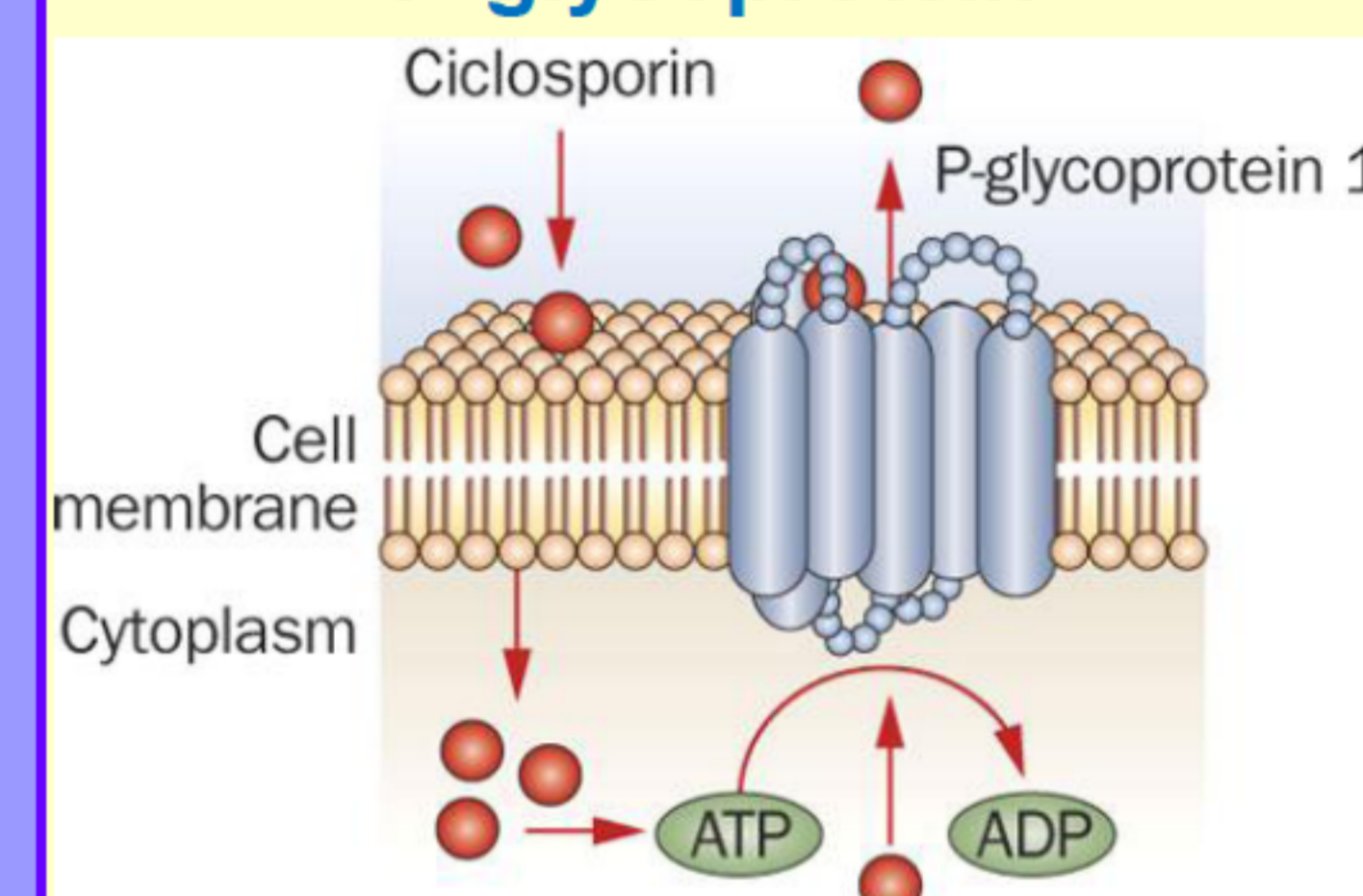
Aim:

- In this study we investigated
- Expression and function of Pgp in HK-2 proximal tubular cells exposed to albumin
 - The influence of different chemicals on the effect of albumin on HK-2 cells

P-glycoprotein



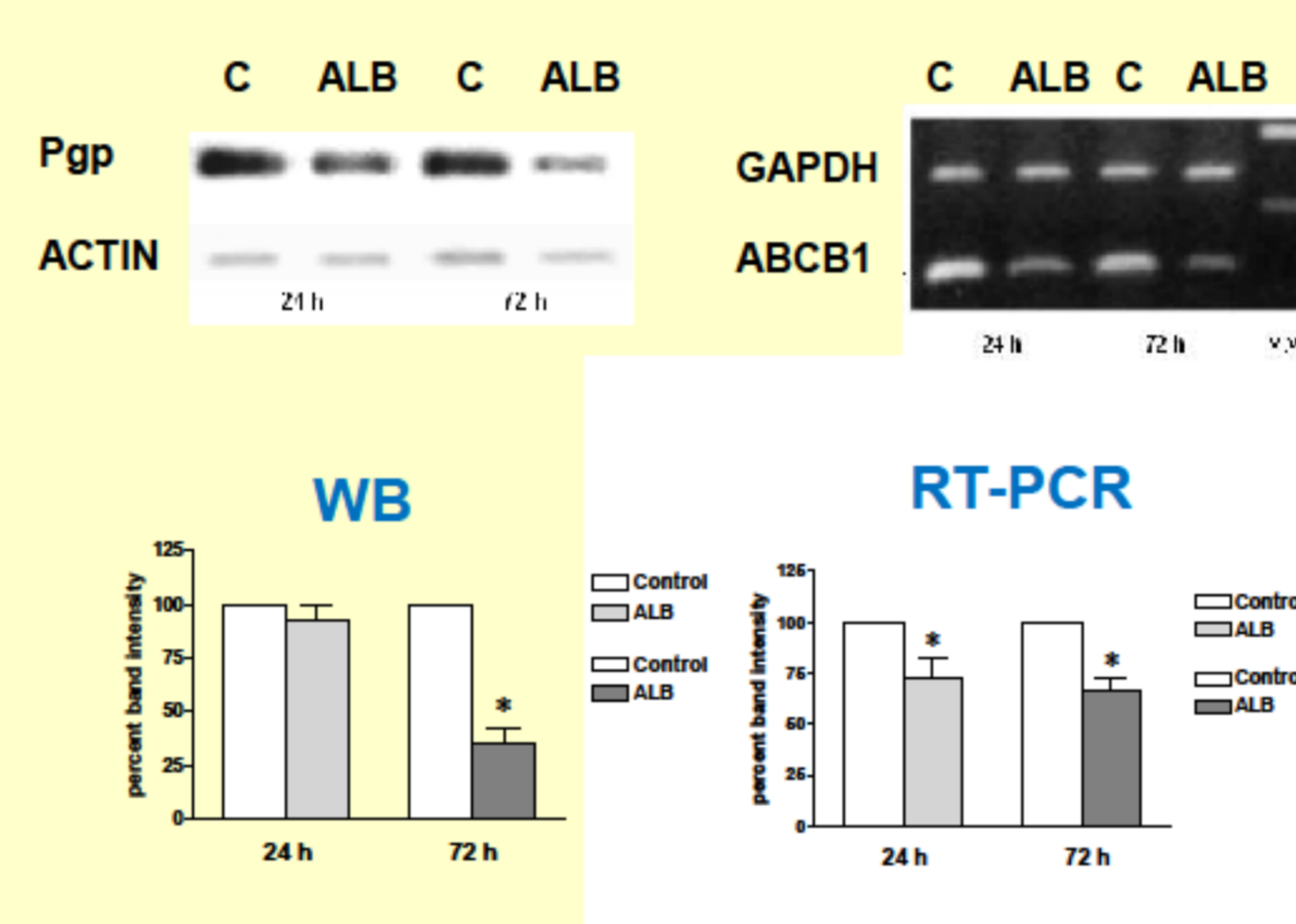
P-glycoprotein



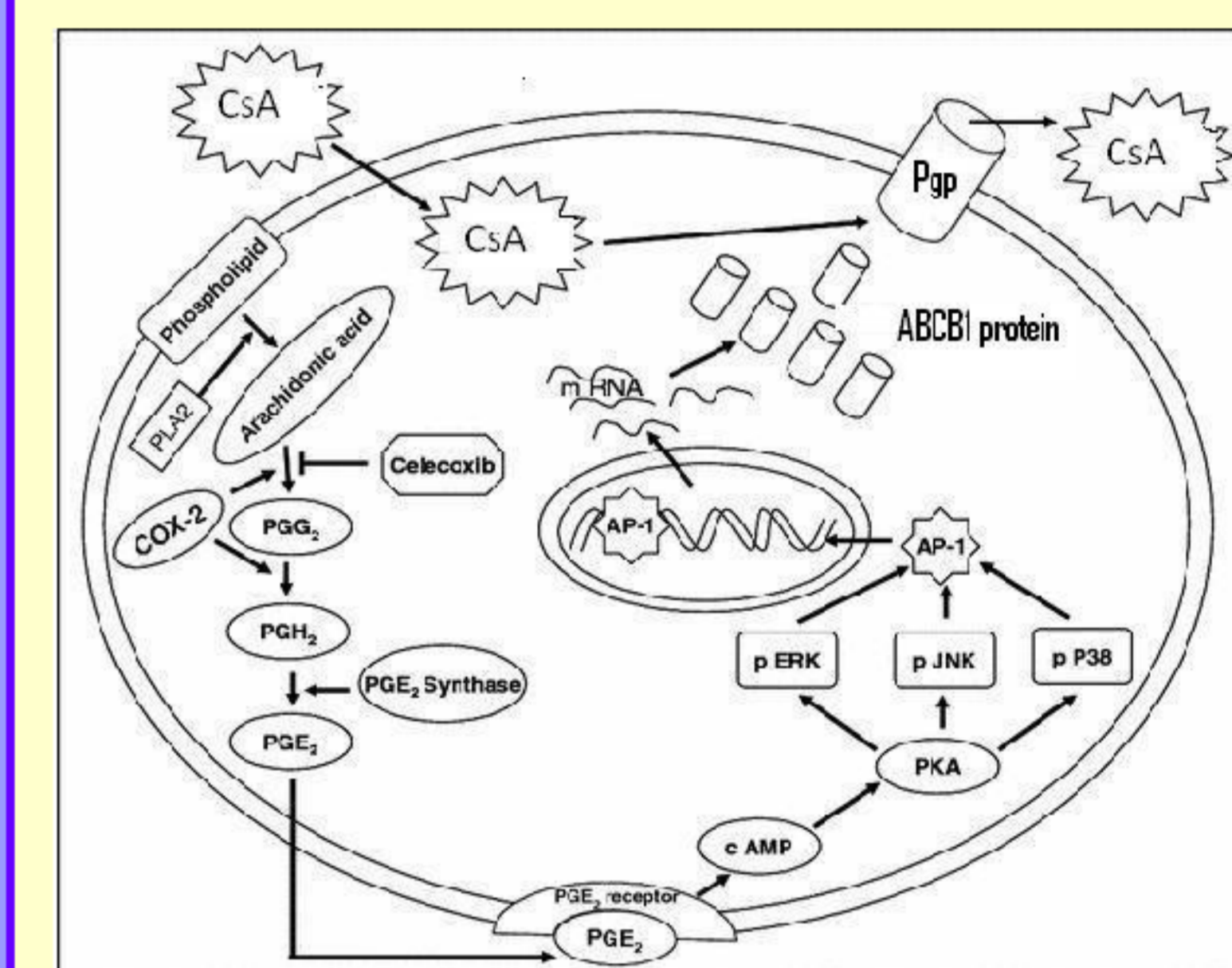
Methods:

- Tubular cells were cultured in presence of albumin (15 mg/ml) for 72 hours
- To culture medium were added:
 - mycophenolic acid (MPA, 100 mM)
 - paracalcitol (PAR, 100 mM)
 - celecoxib (CEL, 20 mM)
- Pgp protein expression was assessed by Western Blot (WB)
- To study ABCB1 gene expression semi-quantitative RT-PCR was employed
- Pgp transport function was evaluated by intracellular accumulation of rodhamine-123 test

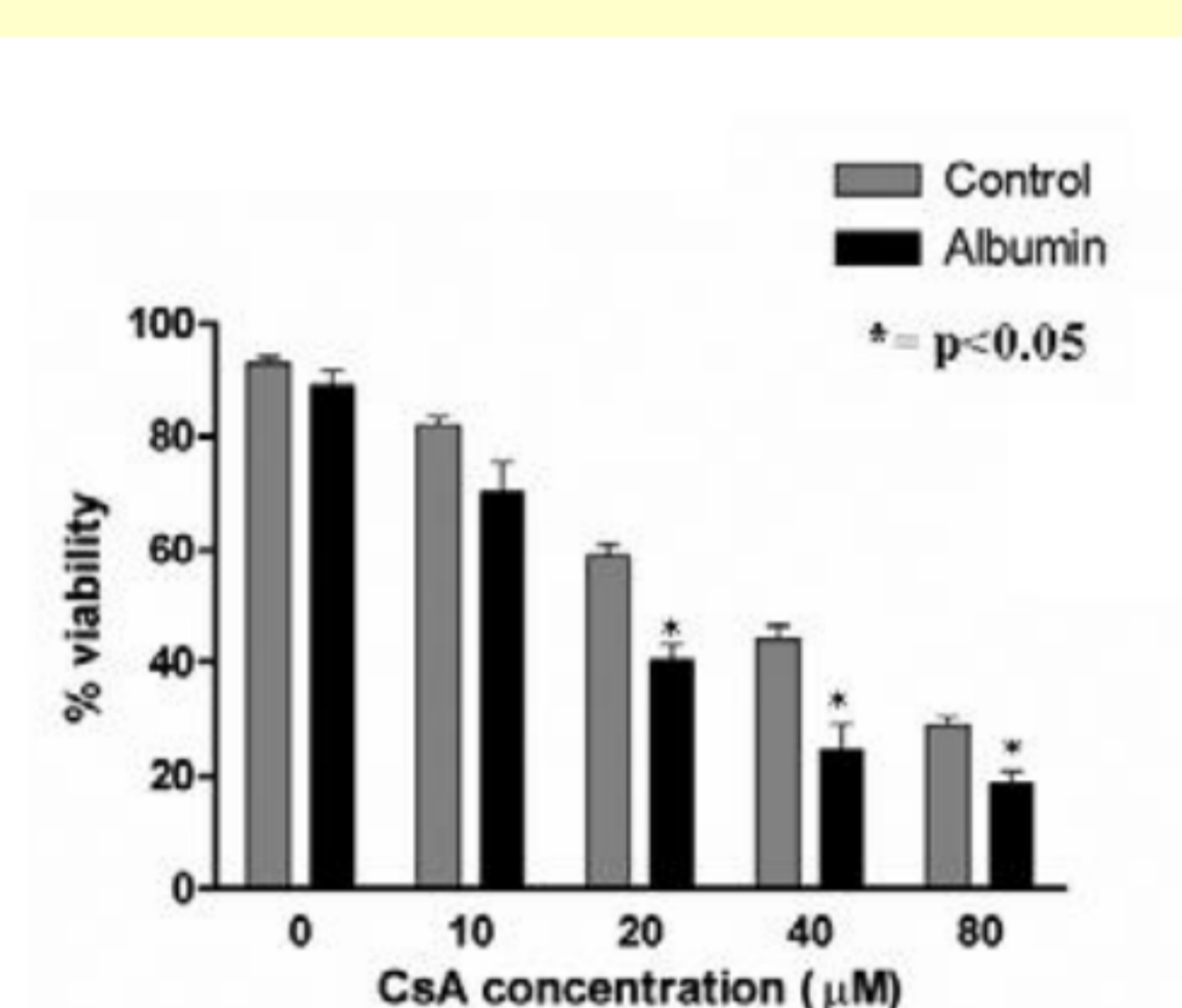
PROTEIN AND ABCB1 GENE EXPRESSION IN HK-2 CELLS CULTURED IN PRESENCE OF ALBUMIN (15 mg/mL)



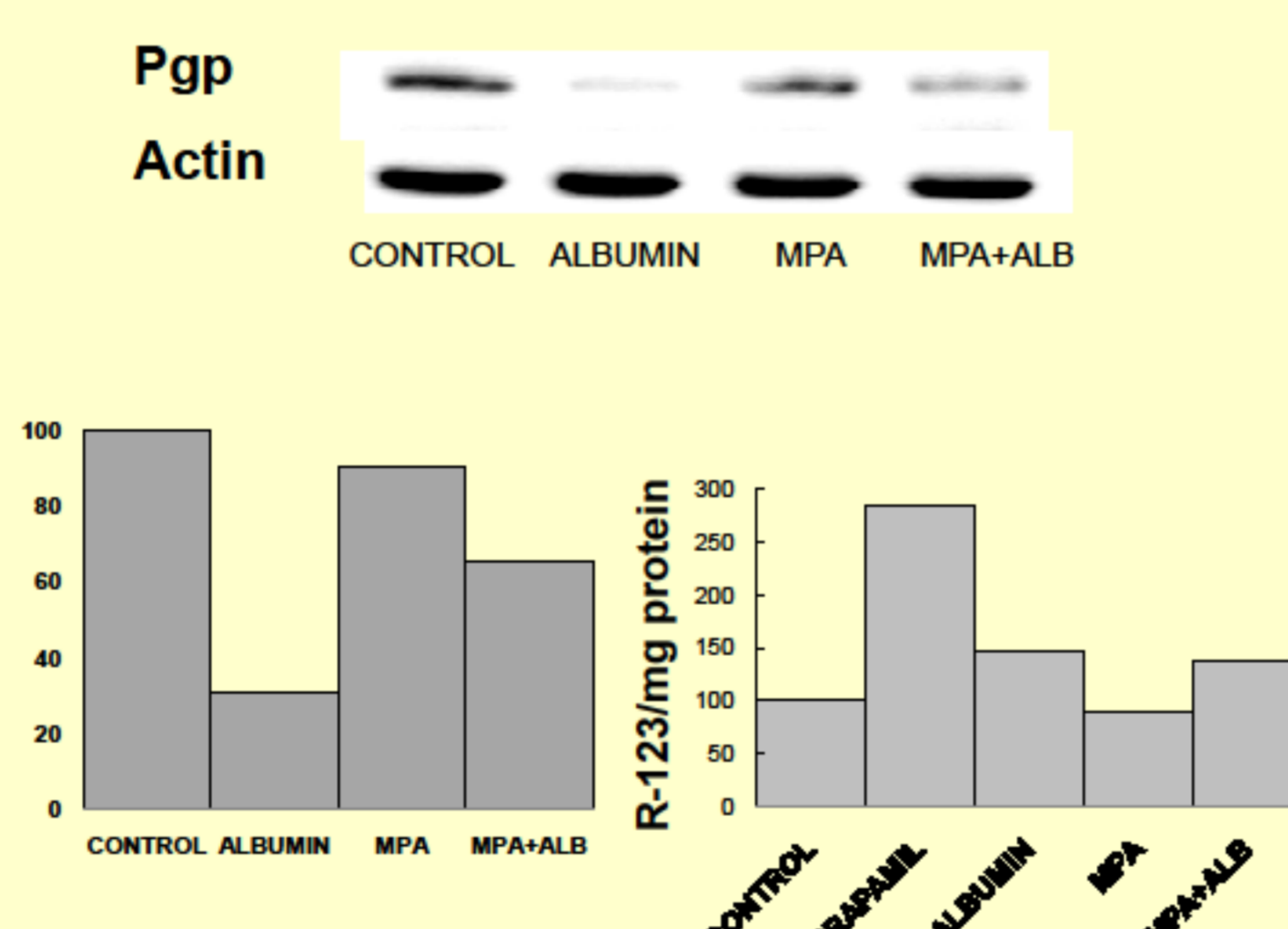
Pgp CsA TOXICITY



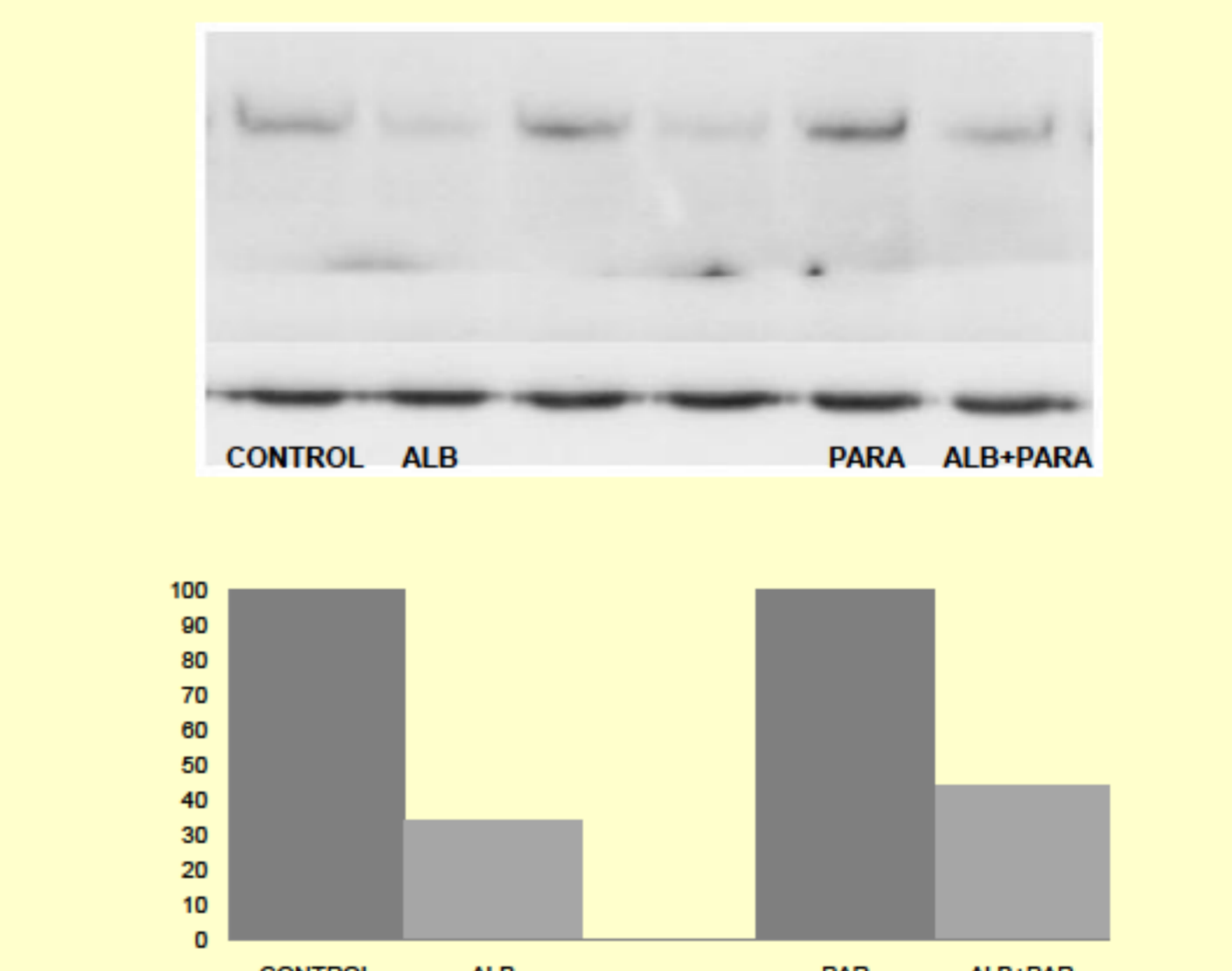
CYCLOSPORIN A TOXICITY IN HK-2 CELLS EXPOSED TO ALBUMIN (20 mg/mL)



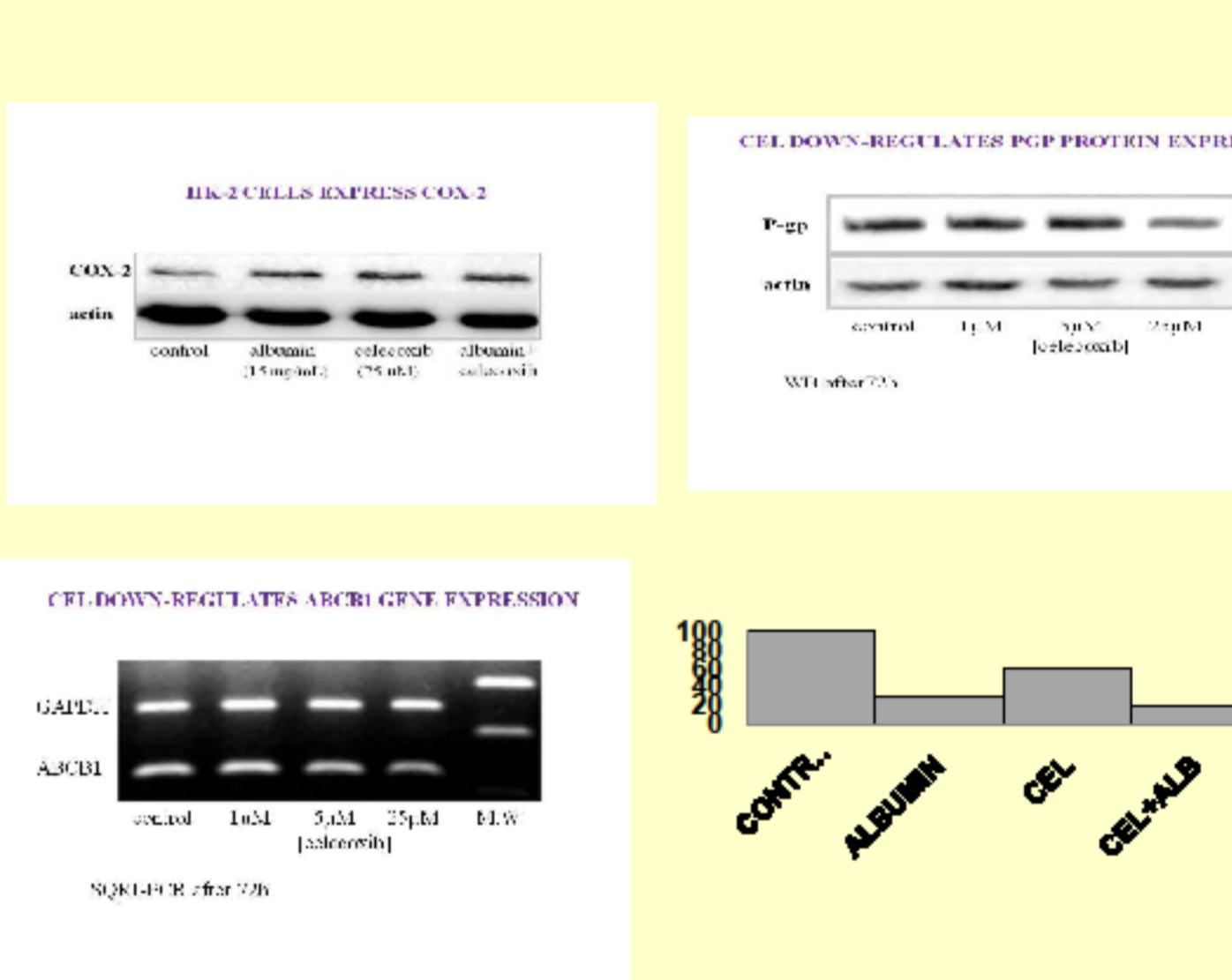
INFLUENCE OF MPA (100 mM) ON Pgp EXPRESSION AND TRANSPORT FUNCTION IN HK-2 CELLS EXPOSED TO ALBUMIN



INFLUENCE OF PAR (100 mM) ON Pgp EXPRESSION (WB) IN HK-2 CELLS EXPOSED TO ALBUMIN



INFLUENCE OF CEL (25 mM) ON Pgp EXPRESSION (WB) IN HK-2 CELLS EXPOSED TO ALBUMIN



Results:

- After 24 hrs albumin exposure a slight reduction in Pgp expression, evaluated by Western blot, was found (-7 %) while after 72 hrs the protein expression resulted 37 % respect to controls ($p < 0.001$ t-test and 1-way ANOVA)
- ABCB1 gene expression showed a reduction to 66 % of controls ($p < 0.02$)
- Pgp mediated transport assessed by the R-123 test was also impaired. In fact, the fluorescence of HK-2 cells resulted 2.4-fold higher than that of controls ($p < 0.05$)
- HK-2 cells exposed to albumin were more sensitive to CsA toxicity
- MPA improved the Pgp down regulation induced by albumin (to 56% of controls)
- PAR added to the medium partially restored Pgp expression and function (to 44% of controls)
- The COX-2 inhibitor CEL further reduced in presence of albumin Pgp expression (WB) to 19.6% of controls
- Cell viability resulted unmodified by albumin

Comments:

- Tubular cells exposed to albumin present a reduction in both protein and gene expression of Pgp with ensuing impairment in the membrane transport function
- The reduction of the transport function mediated by Pgp leads to intracellular accumulation of drugs and toxic compounds resulting in increased tubular cells toxicity
- This could be a further factor of progression of kidney damage linked to proteinuria

Conclusions:

The results of this study show that the albumin induced down-regulation of Pgp can be reversed by MPA and PAR and point out a perspective of limiting the negative effect of albumin on tubular cells

