Urea as an Agonist in Inner Medullary Collecting Duct Cells

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Introduction
Cells of the inner medullary collecting duct are exposed to varying urea concentrations that can result in an osmolarity that ranges from100 mOsm/L to 1200 mOsm/L. The effects of high urea concentration were examined in this study.

Hypothesis
Physiologically relevant concentrations of urea alter the cell signaling pathways in renal epithelial cells.

Aims
We exposed murine inner medullary collecting duct (mIMCD3) cells to urea concentrations that ranged from zero to 300mM. The exposures lasted either one, three, or six hours. Cells were also exposed to other substances such as vasopressin, which is seen on the basolateral side. The presence or absence of several kinases, heat shock proteins, osmotic shock proteins, and cell surface proteins was investigated. These included EGR1, ERK, HSP70, HSP90, SAPK/JNK, and UT-A1.

Methods
mIMCD3 cells aliquoted into wells were subjected to urea and other substrates for various lengths of time. The cells were then lysed and protein extracted. Using gel electrophoresis and western blot techniques, the cell protein was separated and blotted on a membrane. The membrane was then probed for various proteins of interest. A chemiluminescent approach was used to visualize the membrane

Results
The presence of several proteins is not only dependent on the concentration of urea but also the length of exposure. EGR1 is expressed when exposed to 200mM urea for three or six hours. Only the 300mM six hour sample showed signs of EGR1. Maximum Phospho-SAPK/JNK signal intensity was seen at 200mM for the both three and six hour time points. Similar signal strength was observed in the three hour 300mM sample. Phospho-ERK was upregulated at the time points of one and three hours, for both the 200mM and 300mM samples.

Conclusions
Urea activates cell signaling pathways in mIMCD3 cells. This is a concentration and time dependent relationship. The receptor for the initiation of this signaling pathway is yet to be elucidated and is an area of ongoing research.

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