

## BETAINE TRANSPORT IN MOUSE HEPATOCYTES AND EFFECT OF HYPERTONIC STRESS

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Betaine is an osmolyte and methyl donor in the kidney and liver, respectively. In the kidney medulla, it is used to balance extracellular hypertonic stress. It enters medullary cells via a sodium-dependent transport protein, known as the betaine/GABA transport system (BGT1), which is upregulated in response to hypertonic stress (Kempson, 1998). It was recently shown that BGT1 is more abundant in the liver but its function is unknown (Zhou, et al., 2012). In the present study, hepatocytes were used to investigate betaine transport. These cells were obtained from anesthetized mice by collagenase perfusion of the liver through the vena cava. Isolated cells were plated in plastic 24-well plates for 1-4 days in Williams E medium (10% fetal bovine serum and antibiotics). Betaine uptake was measured by an assay in which hepatocytes were incubated for various time intervals in buffered isotonic solution containing 0.1 mM [ $^{14}\text{C}$ ]betaine and in the presence and absence of 100 mM  $\text{Na}^+$ . After the specified time interval, the cells were washed in ice-cold saline solution to stop further uptake, followed by incubation in Triton detergent. Intracellular [ $^{14}\text{C}$ ]betaine was measured by scintillation counting and expressed in pmol betaine/mg cell protein. Betaine uptake was increased in the presence of extracellular  $\text{Na}^+$  compared to replacement of  $\text{Na}^+$  with N-methyl-D-glucamine, proving that the uptake of betaine into liver-hepatocytes is a  $\text{Na}^+$ -dependent process. Healthy hepatocytes plated for 24 hours showed BGT1 protein present in the plasma membrane, as detected by immunohistochemical staining. Cells plated for 4 days showed a drastic change in appearance suggesting hepatocytes changed morphology to adapt to a new environment. Overnight incubation of hepatocytes in hypertonic growth medium (400 mOsm) upregulated  $\text{Na}^+$ -dependent betaine transport, suggesting a normal response to hypertonic stress. Further studies are needed determine if BGT1 is the primary pathway of betaine uptake by hepatocytes.

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