International Journal Of Bilogical Sciences

Measurement of Hepatic Protein Fractional Synthetic Rate with Stable Isotope Labeling Technique in Thapsigargin Stressed HepG2 Cells

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Abstarc :

Severe burn-induced liver damage and dysfunction is associated with endoplasmic reticulum (ER) stress. ER stress has been shown to regulate global protein synthesis. In the current study, we induced ER stress *in vitro* and estimated the effect of ER stress on hepatic protein synthesis. The aim was two-fold: (1) to establish an *in vitro* model to isotopically measure hepatic protein synthesis and (2) to evaluate protein fractional synthetic rate (FSR) in response to ER stress. Human hepatocellular carcinoma cells (HepG2) were cultured in medium supplemented with stable isotopes 1,2-$^{13}C_2$-glycine and L-[ring-$^{13}C_6$]phenylalanine. ER stress was induced by exposing the cells to 100 nM of thapsigargin (TG). Cell content was collected from day 0 to 14. Alterations in cytosolic calcium were measured by calcium imaging and ER stress markers were confirmed by Western blotting. The precursor and product enrichments were detected by GC-MS analysis for FSR calculation. We found that the hepatic protein FSR were 0.97±0.02 and 0.99±0.05%/hr calculated from 1,2-$^{13}C_2$-glycine and L-[ring-$^{13}C_6$]phenylalanine, respectively. TG depleted ER calcium stores and induced ER stress by upregulating p-IRE-1 and Bip. FSR dramatically decreased to 0.68±0.03 and 0.60±0.06%/hr in the TG treatment group ($p<0.05$, vs. control). TG-induced ER stress inhibited hepatic protein synthesis. The stable isotope tracer incorporation technique is a useful method for studying the effects of ER stress on hepatic protein synthesis.

Key Word :
Hepatic protein synthesis, Endoplasmic reticulum (ER) stress, calcium, Gas chromatography-mass spectrometry (GC-MS), in vitro

Volume 8, Number 2, - 2012 , ISSN 1545-1003