

**P020** Evaluation of the role of insulin-stimulated kinases in heart PFK-2 activation

V. Mouton, D. Vertommen, D.R. Alessi, L. Hue and M. H. Rider  
*Hormone and Metabolic Research Unit, University of Louvain Medical School, and Institute of Cellular and Molecular Pathology, Avenue Hippocrate, 75, B-1200 Brussels, Belgium.*

Insulin stimulates heart glycolysis via 6-phosphofructo-2 kinase (PFK-2) activation and by stimulating glucose transport. A PFK-2 kinase called WISK (wortmannin-sensitive and insulin-stimulated protein-kinase) was partially purified from perfused rat hearts and from HeLa cells. The preparation phosphorylated and activated recombinant bovine heart PFK-2 and was shown to contain PKC $\zeta$  by immunoblot analysis. Recombinant PKC $\zeta$  phosphorylated and activated PFK-2 *in vitro* and the phosphorylation sites for WISK and PKC $\zeta$  were identical, namely Ser 466 and Thr 475 as identified by mass spectrometry. In parallel we studied SGK3, as this protein-kinase has similar properties to WISK. In rat hearts treated with insulin, SGK3 was activated, as assessed by its activation loop Thr 320 phosphorylation. Moreover, SGK3 activated PFK-2 *in vitro* by phosphorylating Ser 466 and Ser 483 the same sites phosphorylated by PKB. Transfection of CHO-IR cells with siRNA indicated that PKB participates in heart PFK-2 activation by insulin.