

**P064** PAS kinase is regulated by glucose independently of cellular glucose metabolism

**F.Semplici and G.A. Rutter**

*Imperial College London, UK*

Background. Studies in cultured pancreatic beta cells have shown that PASK (Per-Arrnt-Sim (PAS) Kinase), previously implicated in the control of insulin gene expression by glucose, is acutely stimulated by high glucose concentrations. In order to investigate whether the increase of PASK activity is dependent on the metabolism of glucose we measured PASK activity after treatment with the nonmetabolized glucose analogue 3-O-(methyl-3H)-D-glucose (3-MG), which can enter the cell but cannot be phosphorylated by glucokinase, or with the nonmetabolized glucose analogue 2-deoxyglucose (2-DG), which is converted only to 2-deoxyglucose-6-phosphate.

Methods. HEK293 cells, MIN6 and INS1 (832/13) beta cells were infected with a human PASK-expressing adenovirus. 24 h post infection cells were cultured for 16 h in 3 mmol/l glucose, then incubated in modified Krebs–Ringer medium for 1 h in, respectively, 30 mmol/l or 15 mmol/ glucose, 3-MG or 2-DG. The activity of immuno-precipitated PASK was assessed by SAMS peptide assay.

Results. A similar 0.5-fold increase in PASK activity in response to high glucose or other stimuli was observed in clonal beta and in HEK293 cells.

Conclusions. These data suggest that the regulation of PASK activity by glucose is unlikely to be due to changes in glucose metabolism *per se* but may be directly exerted by a derived organic compound, binding to the PAS domain.