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C-peptide induces ribosomal protein S6 phosphorylation in the pancreatic β-cell line, INS-1E.

A. M. Janabi[1,2], S. J. Ennion[3], G. B. Willars[1]. [1]Molecular and Cell Biology, University of Leicester, Leicester, UNITED KINGDOM, [2]Faculty of Pharmacy, University of Kufa, Najaf Governorate, IRAQ, [3]Leicester Medical School, University of Leicester, Leicester, UNITED KINGDOM.

Introduction: Proinsulin connecting peptide (C-peptide) joins A- and B-chains of proinsulin and coordinates folding during synthesis. C-peptide has been considered an inert by-product of insulin biosynthesis but more recently, physiological roles and beneficial effects against chronic complications of long-term diabetes have been suggested (1, 2). A major limitation is that the putative C-peptide receptor has not been identified convincingly. Little is known about C-peptide signalling in pancreatic β-cells with studies focussing on antioxidant effects. To extend understanding of C-peptide signalling in these cells, we explored effects on activation of ribosomal S6 (rpS6), a key regulator of translation and ultimately cell metabolism, size and proliferation.

Method: The pancreatic β-cell line, INS-1E, was cultured in monolayers under standard conditions and serum-starved (16 h) before use. Cells were challenged with ligands in EBSS-based buffer at 37 ^{o}C and activation of signalling pathway proteins determined by immunoblotting of phosphorylated forms using standard techniques.

Results: C-peptide (10 min) evoked concentration-dependent increases in phosphorylated rpS6 at Ser240/244 and Ser235/235 (pEC_{50} values:9.56$\pm$0.32, 9.72$\pm$0.16 respectively; data are mean$\pm$SEM, n$\geq$3). Furthermore, levels of both Ser240/244 and Ser235/236 phospho-rpS6 were significantly ($p<0.05$, Bonferroni's test) elevated by 10 min challenge with either C-peptide (10 nM; 1.93$\pm$0.09 and 2.53$\pm$0.31 fold over basal respectively) or insulin (100 nM; 2.99$\pm$0.06 and 4.10$\pm$0.59), although scrambled C-peptide (10 nM) had no significant effect (1.16$\pm$0.12 and 1.17$\pm$0.34). Key pathways leading to phosphorylation of rpS6 involve Ras/Raf/MEK/ERK/RSK or PI3K/Akt/TSC/Rheb/mTORC1/S6K. Phospho-ERK1/2 was examined to explore the former and phospho-Akt Ser473 and phospho-p70S6K Thr389 the latter. Challenge of INS-1E with C-peptide (5 min) evoked concentration-dependent increases in phospho-ERK1/2 (pEC_{50} 9.98$\pm$0.62). C-peptide (10 nM, 10 min) also increased both phospho-Akt (Ser473) and phospho-p70S6K (Thr389) ($p<0.05$, unpaired t-test). Moreover, C-peptide-induced rpS6 phosphorylation (1.80$\pm$0.15 fold over basal) was sensitive to inhibition of PI3K (LY-294002, 20 μM), MEK1/2 (UO126, 20 μM), PKC (RO318220, 10 μM) and mTOR (rapamycin, 100 nM) but was insensitive to PKA inhibition (KT5720, 1 μM). Activation of rpS6 by insulin (100 nM; 2.44$\pm$0.09 fold over basal) was similarly sensitive to the inhibitors except the MEK1/2 inhibitor.

Conclusions: Thus, in the pancreatic β-cell line, INS-1E, C-peptide evoked phosphorylation of rpS6 at two clustered phospho-sites key to activation (Ser235/236, Ser240/244). Pharmacological inhibition of signalling intermediates suggested upstream signalling pathways include those involving ERK1/2 and Akt.

References: 1) Luppi P *et al*. (2011). *Pediatric Diabetes* **12**: 276-292. 2) Yosten GL *et al.* (2014). *Am J Physiol Endocrinol Metab* **307**: E955-E968.