beta-arrestin2 is absolutely required for the potentiation of insulin secretion by GIP

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Background and aims : The scaffold protein beta-arrestin2 (ARRB2) is known to uncouple G protein coupled receptors (GPCRs) from the G protein and to recruit new signaling pathways (such as the ERK1/2, PI3K, FAK…). In non beta cells, ARRB2 interacts with a wide range of GPCRs, but its interaction with the GIP receptor (GIPR) is still unclear. Our aim is to determine if ARRB2 is involved in the signaling of the GIPR in pancreatic beta cells.

Materials and methods : The experiments were carried out in beta cells from five-month-old *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ male mice. cAMP production (CAMPS-EPAC), endogenous PKA (AKAR3) and ERK1/2 (EKAR) activations, [Ca^{2+}] in the cytosol ([Ca^{2+}]$_c$; Fura2-LR) and in the endoplasmic reticulum ([Ca^{2+}]$_{ER}$; D4ER) were assessed by live cell imaging in mouse pancreatic beta cells. EPAC2 (EPAC2-GFP) recruitment beneath the plasma membrane was monitored by total internal reflection fluorescence microscopy. Actin-F depolymerisation was evaluated by phalloidin staining (Alexa Fluor 488-conjugated phalloidin) and the phosphorylation of Focal Adhesion Kinase (FAK) by immunofluorescence.

Results: Insulin secretion from *Arrb2*$^{-/-}$ islets was reduced by 50% compared to *Arrb2*$^{+/+}$ islets in response to GIP (100pM-10nM, $p<0.01$). When ARRB2 (ARRB2-GFP) was re-expressed in *Arrb2*$^{-/-}$ beta cells, insulin secretion in response to GIP was restored to a similar level than in *Arrb2*$^{+/+}$ islets. Surprisingly, upon GIP stimulation (10pM-10nM), the cAMP production, PKA activation and EPAC2 recruitment were similar in *Arrb2*$^{+/+}$and *Arrb2*$^{-/-}$ beta cells. Both [Ca^{2+}]$_c$ and [Ca^{2+}]$_{ER}$ remained comparable. Finally, the activation of ERK1/2 was also similar in *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ beta cells. By contrast, the F-actin depolymerisation induced by 10nM GIP was significantly reduced (~25%, $p<0.01$) in *Arrb2*$^{-/-}$ beta cells. PI3Kγ and FAK have been reported to be involved in F-actin depolymerisation in response to GIP and glucose, respectively, and to be required for optimal insulin secretion. As expected, the PI3Kγ inhibitor (AS604850; 1µmol/l) reduced F-actin depolymerisation (~30%, $p<0.01$) by GIP stimulation in *Arrb2*$^{+/+}$ beta cells, but no additional effect was observed in *Arrb2*$^{-/-}$ beta cells. Moreover, GIP-induced FAK activation was also reduced by 50% in *Arrb2*$^{-/-}$ beta cells.

Conclusion: Our study revealed that ARRB2 is required for the potentiation of insulin secretion by GIP, through F-actin depolymerisation probably via FAK activation and PI3Kγ recruitment, but independently from the canonical cAMP signalling (PKA and EPAC2) and the ERK1/2 pathway. Therefore, any variation in the expression of ARRB2, as observed in diabetic states, should functionally affect the incretin effect produced by GIP.