OP 02 News on the insulin secretion front

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What makes beta cells 1st responders, and are they temporally consistent?

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Background and aims: Calcium (Ca^{2+}) uptake drives glucose-stimulated insulin secretion from the pancreatic β-cells. Functional subpopulations of β-cells disproportionally control the oscillatory phase of Ca^{2+} uptake, which is disrupted with ageing and in diabetes. Less is known about β-cells which impact the 1st phase of Ca^{2+} uptake, disrupted in early diabetes. Here we determine whether "1st-responder" cells that lead the 1st phase of Ca^{2+} uptake are the same as "hub" cells that coordinate oscillatory Ca^{2+} (2nd) phase. We study what makes β-cell a 1st responder, and whether 1st responders are a transient state or a distinct temporally stable subpopulation.
Materials and methods: We used MIP-CreER GCaMP6s mouse model which expresses Ca^{2+}-sensitive GFP specifically in β-cells. We performed simultaneous recording of Ca^{2+} dynamics and gap junction permeability in individual islets. We stimulated islets with glucose, Katp channel blocker glibenclamide, and KCl. Based on Ca^{2+} dynamics we defined the 10% of cells responding to the glucose stimulation sooner than the rest of the islet as "1st responders", and the 10% of cells responding slower as "last responders". We tested their temporal consistency over 1, 24, and 48 hours. We used laser ablation to remove specific cells from the islet. We performed computational modelling of the islet electrophysiology.
Results: We found that Ca^{2+} wave coordination of the 1st responders was not greater than the islet-average, and hence they are not overlapping with highly-coordinated "hub" cells. In fact, according to our gap junction permeability data, 1st responders had lower than average electrical coupling (p=0.0157). Furthermore, our computational model showed lower electrical coupling conductance in both 1st and last responders (p=0.0447, p=0.0279). This may be explained by our finding that 1st responders are located at the islet's periphery (at 0.8 ± 0.1 of the islet's radius). We found 1st responders to be consistent under glibenclamide stimulation: cells which respond first to the glucose remained in the 15th percentile of the time response distribution when stimulated with glibenclamide (SEM 10%). This is consistent with our computational results: 1st responders had lower Katp conductance (hence higher membrane depolarization probability) (p=0.0086). Glucose elevations with 1h period showed that 1st responders remained consistent: with reaction time within the 25% of the reaction time distribution for all cells. With an elevation period of 24 hours, their reaction time shifted to the second quartile of the distribution, and with 48 hours to the median. Unlike 1st responders, last responder cells were not consistent at any time interval. Ablation of the 1st responders dis-coordinated, but did not disrupt, the Ca^{2+} response of the islet. A different cell took over the role of the 1st responder post-ablation. This new 1st responder was a cell which originally, pre-ablation, was within a leading 7th percentile of the time response distribution (SEM 3%).
Conclusion: In conclusion, 1st responders are distinct from "hub" cell subpopulation, have higher membrane depolarization probability and are less strongly coupled to other cells. After the laser ablation of a 1st responder, new 1st responder taking on it's role comes from a pool of original leading cells. While initially consistent over a short 1h period of time, 1st responders may be losing temporal consistency over longer time periods.
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Beta-arrestin 2 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. F-actin 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.
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Pancreatic beta cell-selective deletion of the mitofusins 1 and 2 (Mfn1 and Mfn2) impairs glucose-stimulated insulin secretion in vitro and in vivo

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