DOPAMINE RECEPTOR SIGNALLING IN PANCREATIC β-CELLS

Marco Carli[1], Stefano Aringhieri[1], Shivakumar Kolachalam[1], Francesca Vaglini[1], Michela Novelli[1], Marco Scarselli[1]
[1]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa, Pisa - Italy

Introduction: The main signal for β-cells insulin release is glucose, but the modulation of glucose-stimulated insulin secretion (GSIS) is further complicated by many nervous and endocrine stimuli converging on the β-cell. Among the best characterized are glucagon-like peptide 1, acetylcholine, noradrenaline and adrenaline, all of them acting on their cognate G-protein coupled receptor (GPCR). Generally, the activation of the associated G_s and G_q protein enhances insulin secretion, while G_i inhibit GSIS. Dopamine (DA) has a physiological role in regulating insulin release, however its action in β-cells is less studied than in other systems. Besides expressing D_1-D_5receptors, rodent and human islet tissues express enzymes to produce and store DA, such as aromatic L-amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), vesicular monoamine transporter 2(VMAT2), dopamine transporter (DAT) and large amino acid transporter (LAT). DA is locally produced by β-cells and acts on D_2/D_3receptors to activate a negative feedback loop which reduces insulin secretion. The aim of this project is to investigate the signalling pathways involved in the fine tuning of insulin secretion downstream to D_2/D_3receptors and analyse how dopamine agonists and antagonists influence the physiology of insulin release.

Materials and methods: We analysed the expression of dopaminergic proteins in INS-1E 832/13cells using western blot, immunocytochemistry and immunofluorescence. For $D_1$and D_2receptors, we performed real time-PCR. Western blot was also used to assess the activation of ERK and Akt downstream D_2/D_3receptors. Insulin release was measured with a radioimmunoassay. β-arrestin knock down was performed by electroporation with a siRNA mix provided by Dharmacon.

Results: INS-1E express DAT and TH, as previously reported for other β-cell lines. The D_2receptor expression is high, while D_1receptor was not detectable by rt-PCR. DA and l-DOPA reduced insulin release after 60-90' incubation. Dopamine agonists, such as ropinirole and quinpirole, significantly reduced GSIS and the pre-incubation with haloperidol, a D_2/D_3antagonist, partially restored insulin release. The action of quinpirole on $D_2$was further investigated in order to follow the activation of specific pathways downstream to the receptor, i.e. the G protein-dependent and/or β-arrestin-dependent mechanisms. Short stimulation of D_2receptor increased the activation of ERK, while longer stimulation determined a dephosphorylation of Akt. The role of the Gi or β-arrestin on kinases activity and insulin secretion is under investigation by using pertussis toxin and β-arrestin knock-down.

Discussion and conclusions: INS-1E β-cells respond to DA and are equipped with a complete dopaminergic apparatus. DA and dopamine agonists inhibit the release of insulin activating a negative feedback loop mediated by D_2/D_3receptors. The downstream pathway responsible for this effect is probably G_i-mediated, however the role of β-arrestin will be further investigated. The precence of dopaminergic receptors on β-cells makes them a target for dopamine agonists and antagonists, and it is partially responsible of the metabolic side effects induced by dopaminergic drugs. Moreover, the action of dopamine agonists on β-cells could be exploited for the treatment of diabetes, as already happens for bromocriptine.