WP1.6. Targerting insulin storage forms in pancreatic secretory granules (Jiráček Group)

Research aims

- To identify the main structural forms of insulin in pancreatic β-cells secretory granules.
- To define the role of different structural forms of insulin in insulin stability, and its release to the blood circulation.
- To develop novel small molecule ligands for the rational steering of insulin release from pancreatic cells and for better, native-like clinical formulations of the storage/injection forms of insulin

Research plan and methodology

(i) *In vitro/silico* work. Insulin interaction with selected endogenous ligands (e.g. dopamine, serotonin and arginine) will be investigated in parallel *in vitro* and *in silico* experiments that will aim to provide models for the *in vivo* environment. We will use molecular dynamics simulations to predict the mode of the binding of ligands to insulin hexamers under defined conditions (Pavel Jungwirth' IOCB Group). In parallel, these small molecule ligands will be co-crystallized with insulin, and the structures of complexes will be determined (A. M. Brzozowski's Group, University of York, UK). The mode of binding of selected ligands with insulin (both stoichiometry and affinity) will be analysed using isothermal titration microcalorimetry, and different spectroscopic techniques.

(ii) *In vivo pancreatic cell work.* The ability of available pancreatic permanent cells lines to produce, store and secrete insulin will be investigated. It is known that some cultured pancreatic cell lines (e.g. insulinoma) are proned to over-produce proinsulin in favour of the mature hormone. Hence, there is a need for the assessment and establishment of cell-lines with real, pancreas-like insulin production characteristics. These experiments will be paralleled by studies on animal pancreatic tissues and primary cells (rat or porcine). We will optimize the protocols for isolation of intact granule fractions from selected cells lines or tissues. The still not fully known chemical/molecular content of insulin secretory granules will be analysed using advanced mass spectrometry techniques by J. Cvačka's IOCB Group (using a new high-resolution mass spectrometer, which is part of the proposed capital investment of this project) and by Carol Robinson's Group collaborating at the University of Oxford, UK, and using different EM microscopy and immunohistochemistry methods.

(iii) *In vivo storage-granules* work. The intact native secretory granule fractions will be subjects of X-rays (single and powder diffraction), and Cryo-Electron microscopy studies to analyse the type of crystalline/amorphous character of stored insulin, and to determine their structures if applicable (collaboration with A. M. Brzozowski's Group, University of York, UK, and Diamond Light Source facility, Didcot, U.K.).

(iv) *In vivo ligands:pancreatic-cell* work. The research in (i-iii) should lead to rational studies of the effects of selected small molecules ligands on insulin structural storage forms and insulin release in model cell lines, primary cell cultures and tissues. The structures of the most interesting ligands will be chemically modified, and optimized to achieve their desired effects on insulin physiology. Here we will extensively use the newly purchased semipreparative HPLC apparatus (Jiráček group) for the purification of synthesized compounds.

The project will be realized in a close collaboration with P. Jungwirth's Group, I. Pichová's Group, J. Konvalinka's Group and J. Cvačka's mass spectrometry department.

Research schedule

2017-2019

- Study of insulin interaction with small ligands in vitro and in silico.

2019-2022

- Investigation of the available permanent pancreatic cell lines, primary pancreatic cells and pancreatic tissues for their ability to produce, store and release insulin.
- Optimization of protocols for the isolation of intact and native insulin secretory granules.
- Mass spectrometry analysis of insulin secretory granule content.

2020-2022

- Structural analysis of insulin structures in isolated insulin secretory granules.

2021-2022

- Study of the effects of selected ligands on insulin structural storage forms and insulin release in model cell lines, primary cell cultures and tissues.
  Chemical derivatization of the most interesting ligands and studying of their effects *in vitro* and *in vivo*.

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	1
2019	1
2020	2
2021	2
2022	2
Total	8

Biochemical Journal
Diabetes
Diabetologia
Journal of Medicinal Chemistry
ACS Chemical Biology
Journal of Biological Chemistry
Biochemistry
PlosOne

Patents and patent applications

	Patents (granted)	International patent applications (filed)
2017	0	0
2018	0	0
2019	0	0
2020	0	0
2021	0	1
2022	1	0
Total	1	1

We expect IP protection in the following areas:
New ligand modulating insulin storage forms.

Cooperation with foreign institutions

- Dr. Andrzej M. Brzozowski from the Structural Biology Laboratory (YSBL) of the University of York in the U.K.,
- Prof. Carol Robinson's team at the University of Oxford in UK