

Reviewer's report on Ph.D. thesis

Targeting insulin storage forms in pancreatic β -cell secretory granules

by MSc. Seiya Asai

The submitted Ph.D. Thesis is based on the four articles published in impacted scientific journals and co-authored by Mr. Seiya Asai MSc. He is the first author of two of these manuscripts, with two additional secondary co-authorships. In addition, unpublished results are also included as a part of the thesis. In this way, the candidate has fully complied with the requirements for acceptance of a Ph.D. Thesis.

The thesis itself is dedicated to the research in the field of insulin biology, insulin secretion by pancreatic beta cells, and insulin-like growth factor (IGF-1). It has a common structure, consisting of Introduction, Research Aims, Results, Discussion, Conclusion and References sections, with the inclusion of published papers in the appendices (182 pages in total).

The introductory section provides a brief overview of topics related to the theme of the Thesis, in particular pancreatic beta cells, insulin, insulin secretagogues and methods for the determination of insulin concentration in biological samples, and the bone protein osteocalcin.

Research aims include clearly defined goals for each publication and research objectives.

The results section is divided into six parts related to the 4 published articles and 2 unpublished projects. Each part is subdivided into Background, Methods, Results and discussion, Conclusion sections. It also includes additional information about the candidate's contribution to each topic. The design of the experiments, research techniques and achieved results are presented in a comprehensive form. Results sections are supplemented with well-prepared figures and graphs.

The Discussion and Conclusion sections recapitulate the major findings of the thesis, present them in an understandable manner, and relate them to current knowledge in specific research areas. Overall, the thesis is well-written and the language is clear and comprehensive. The structure and content of the text make it easy to read and understand the aims of the thesis and the results achieved.

Within the scope of the Ph.D. thesis, the candidate has presented the results achieved in the development and evaluation of a new assay for the determination of insulin concentration in biological samples. This assay was used in subsequent research to determine the effect of various peptides and compounds on insulin secretion by the rodent pancreatic beta cells and established beta cell lines. Further, his research has achieved an important and original result by demonstrating the presence of a crystalline form of insulin in the secretory granules of native beta cells. In addition, the results devoted to the production and characterization of IGF-1 dimers are also presented. Finally, unpublished results related to the bone protein osteocalcin and tryptophan metabolites, and their effects on beta cells and insulin secretion expand complex research conducted by the candidate.

Questions to the candidate:

- 1) How was the normalization of cell amount/number for rat pancreatic islet, INS-1E, and BRIN-BD11 cell lines conducted for the analysis of total insulin content in the study “The efficiency of insulin production and its content in insulin expressing model β -cells correlate with their Zn²⁺ levels” (3.2)?
- 2) Is there any information about the biological activity of the crystallized form of insulin? Can it be re-solubilized or is it eventually degraded by the cellular proteolytic systems?
- 3) Can you explain the molecular mechanisms behind the dopamine-restricted insulin secretion?
- 4) How do you explain discrepancies in the binding affinities of various lots of IGF-1 dimer 2 in the study “Recombinant Insulin-Like Growth Factor 1 Dimers: Receptor Binding Affinities and Activation Abilities” (3.6.)?

Conclusion:

In general, this is a valuable Ph.D. thesis in which the candidate has demonstrated excellent theoretical and practical skills and the ability to produce high-quality and independent scientific work. The text of the thesis is well-written and comprehensive, with only rare typographical errors and unclear sentences. It contains actual and valuable findings that advance our knowledge about insulin and beta cell biology. Therefore, I fully recommend the candidate's thesis for acceptance and awarding of the Ph.D. degree to Mr. Seiya Asai MSc.

In Prague, September 15, 2023

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