ABSTRACT

Modern lifestyle with its lack of exercise and healthy diet often leads to obesity which is accompanied by a decreasing biological effect of insulin and the onset of hyperinsulinemia, and consequently type 2 diabetes. Persistently high levels of insulin stimulate signalling pathways with growth effects; cells thus become more sensitive to mitogenic effects of all growth factors which may even lead to the loss of control over cell proliferation and the rise of various malignancies.

Due to a high degree of structure homology of insulin, IGF-I/II as well as particular IR (existing in “mitogenic” IR-A isoform and “metabolic” IR-B isoform) and IGF-1R, there are a number of cross-interaction among hormones and receptors; nevertheless, the biological response may be different during the binding to a receptor. The determination of the crucial structural regions in insulin and IGF which are responsible for binding to the receptors could lead to the evolution of selective insulin analogues with strengthen metabolic effects, or could lead to the evolution of selective antagonism of IGF which would, in turn, suppress the mitogenic effect.

The highest overlap is between insulin and IGF-II since both hormones are able to bind to the isoform A of an insulin receptor (IR-A) with a high affinity, and to activate it. There are, however, some differences after a binding event because insulin triggers predominantly metabolic pathways and IGF-II mitogenic pathways. Previous studies showed that C domain of IGF participates in the interaction with IR and IGF-1R, and it significantly modulates their affinities. This thesis examines the C domain of IGF-II. We developed several insulin analogues to which C-terminal of B-chain we gradually extended the first four amino acids of IGF-II (SRVSRRSR), exactly S_{B31}^B, SR_{B31-32}^B, SKV_{B31-33}^B, SKVS_{B31-34}^B-insulin. Based on the binding abilities of IGF-II, we assumed that created insulin analogues will have the increased affinity to IR-A and IGF-1R receptors, and decreased to IR-B.

All the analogues had a lower affinity to IR-A compared to human insulin while the rate of a decrease did not correlate with the extension of the chain. SKV_{B31-33}^B-insulin showed, according to our assumption, a lower affinity to IR-B in comparison with its affinity to IR-A. Despite our expectations, S_{B31}^B- and SKVS_{B31-34}^B-insulin, there were a higher affinity to IR-B with respect to the affinities to IR-A while SKVS_{B31-34}^B had an even higher affinity than human insulin (150% of the value of human insulin). Surprisingly, SR_{B31-32}^B-insulin had a very low value of affinity to both IR isoforms (IR-A: 0,09%, IR-B: 0,2% of the values of human insulin). All the analogues (except SR_{B31-31}^B-insulin which was not tested) had a similar binding affinity to IGF 1R to that of insulin (100%); but with the extending B-chain, the affinity increased slightly (104 – 150 % of the value of human insulin). Consequently, the SKV_{B31-33}^B-insulin showed the highest affinity to IGF-1R (150 %).

The next aim of this study was to recombinantly prepare Gly¹IGF-II in E. coli. The protein was prepared in order to introduce the new preparation method of analogues IGF-II and simultaneous creation of a reference substance. The main purpose of the preparation of the protein was to introduce a new method of the preparation of analogues of IGF-II, and to prepare a referent substance at the same time. Gly¹IGF-II was then compared to a commercially available human wild type of IGF-II while the characteristics were comparable in all tested fields. (In Czech)