Abstract

The main function of insulin as a hormone is to control glucose homeostasis in the blood. This control takes place at different levels in different parts of the body. The complexity of the regulation of blood glucose levels is manifested through the insulin receptor (IR) and its two isoforms, IR-A and IR-B. IR-B is responsible for metabolic effects and the distribution in adipocytes, muscles and hepatic cells, whereas IR-A has, above all, mitogenic effects with lymphocytes, spleen, brain and cancer cells.

Today‘s treatment of diabetes patients is focused on the use of insulin analogues, insulin replacemensts with a different IR-A and IR-B binding affinity. Today‘s patients use two different types of analogues, called *fast-acting* and *basal*, with a focus on the combination of these two types throughout the day.

In 2011, prof. Belfiore from the Universtiry of Catanzzaro published a scientific article about the close relation between diabetes and some cancer types. This article triggered extensive debates about the impact of insulin or insulin analogues on the mitogenic isoform IR-A of the insulin receptor, and IR-A isoform on the insulin-like growth factor type 1. In the same year, two scientific groups from a Danish company Novo Nordisk, published the first hints at receptor isoform-selective insulin analogues.

The main goal of this theses is to focus on the synthesis of new receptor isoform-selective insulin analogues, testing their binding affinity towards both isoforms of the insulin receptor and to determined their abbility to activate signalisation paths in these two isoforms of the receptor. The target was to determine the role od C-terminus of the B chain of insulin on receptor isoform selectivity. In positions B25-B30 (wich are crucial for the binding affinity) aminoacids were changed to proline, eventually hydroxyproline, aiming at a change of the conformation of peptide bonds and have an impact on the receptor binding affinity. It is generally known from literature the positive effect of an amidation of the C-terminus of the B-chain, so we tried testing the combination of these two modifications.

Here we present 4 insulin analogues with B25-B30 modifications, two of which with a hydroxyproline change, and two of which with a C-terminus B-chain amidation. These analogues were tested for their binding affinities towards IR-A and IR-B. Three of these have some selectivity towards different types of IR. Another study was to compare the extent of IR signalisation by these analogues and the isoform-receptor´s response to these analogues. We found out that the extent of the overal IR signalization and the metabolic path more or less correspond to their binding affinities.

The study of B25-B30 positions can help to better understand how the C-terminus of the B-chain interact with the respective insulin receptor isoforms. This information can be an important clue to design new receptor-selective insulin analogues.

Key words: insulin, insulin analogue, C-terminus of B chain, insulin receptor, interaction of human insulin with its receptor, receptor-selective insulin analogues, insulin receptor isoforms, enzymatic semisynthesis, binding affinities studies, study of the signalisation response.