

Abstract

Insulin and insulin-like growth factors (IGF-1 and -2) together with their receptors take part in a complex system, which affects both basal metabolism of carbohydrates, lipids and proteins as well as cell growth, proliferation, differentiation and apoptosis. Defects in action of insulin or IGFs can lead to serious diseases such as diabetes or cancer. Both of these disorders represent nowadays one of the biggest health threats to the world's population. Insulin and IGFs induce different biological effects through their cognate receptors; two isoforms of the insulin receptor (IR-A and IR-B) and the receptor for IGF-1 (IGF-1R). These receptors bind insulin and IGFs with different affinities and induce different but partially overlapping signalling events leading towards metabolic (especially insulin) or mitogenic responses (IGFs and insulin). To understand the mechanism of action of insulin and IGFs it is important to specify which structural domains of these hormones are responsible for binding to the receptors and exerting specific effects.

One region that is missing in insulin is the D-domain of IGF-1 and -2. For this reason, we decided to prepare insulin analogues with the A-chain extended by either the whole D-domain of IGF-1 or IGF-2, or by fragments of the IGF-1 D-domain in order to define the impact of these domains on binding specificity to the IR-A and IR-B receptors, and on the ability to activate specific signalling pathways. We chose the synthetic model of insulin because of an easier synthesis of its molecule than that of IGF.

Four insulin analogues were prepared: A²¹-T-P-A-K-S-E²⁷-insulin, representing insulin with the A-chain extended by the D-domain of IGF-2; A²¹-P-L-K-P-A-K-S-A²⁹-insulin which is insulin with the A-chain extended by the D-domain of IGF-1; A²¹-P-L-K²⁴-insulin with the A-chain of insulin extended by the initial four amino acids of the D-domain of IGF-1; and A²¹-P-L²³-insulin with A-chain extended by the initial three amino acids from the D-domain of IGF-1.

Binding affinities of the first three analogues to the IR-A and IR-B were determined. The impacts of analogues on the activation of IR-A, IR-B and IGF-1R and on the receptor-induced phosphorylation of specific intracellular proteins (Akt and Erk) were also tested. The results obtained in this study helped to understand better the role of D-domains of IGF-1 and -2. These little pieces of knowledge could contribute to the design of new molecules of insulin/IGF with more selective effects. (In Czech)