DOCTORAL THESIS

Insulin-potentiating effects of chromium(III): a mechanistic study

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Insulin-Potentiating Effects of Chromium(III): A Mechanistic Study

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ABSTRACT

Chromium, a trace nutrient essential for maintaining a normal carbohydrate and lipid metabolic status, has been shown to exert its effects via its insulin-potentiating activity. A dietary agent known as the glucose tolerance factor (GTF), which alleviates the diabetic-like symptoms, such as glucose intolerance, associated with chromium deficiency, was shown to depend on Cr(III) for its biological activity. However, despite four decades of research, neither the structure of this Cr(III)-containing GTF nor the mechanism by which Cr(III) potentiates the actions of insulin has been elucidated.

In this study, 11 structurally characterized Cr(III) complexes and a synthetic analog of the GTF, referred to as the synthetic GTF, were prepared and their potentiating activities towards insulin-induced lipogenesis, anti-lipolysis, total glucose uptake and glucose transport were measured using isolated rat adipocytes. The Cr(III) complexes studied were chosen in such a way that some of their ligand compositions closely resembled the chemical composition of the GTF while others bore no such resemblance at all. Only the synthetic GTF was shown to display a strong insulin-potentiating effect towards these four insulin-induced activities. Furthermore, in the presence of the synthetic GTF, an increase in insulin responsiveness, but not in insulin sensitivity, was observed in the insulin dose-response curve towards lipogenesis. This observation suggested that the Cr(III) most likely exerts its potentiating effect at a postreceptor-binding event along the insulin signal transduction pathway. This notion was reinforced by an observed lack of effect of these Cr(III) compounds on insulin-receptor binding. Trypsin pretreatment of the adipocytes, which resulted in a significant attenuation of the lipogenesis activity stimulated by both insulin and the synthetic GTF, showed that a structurally intact insulin receptor is a prerequisite for the observed insulin-potentiating effect. Experiments conducted in the presence of specific inhibitors for the insulin receptor protein tyrosine kinase (IR-PTK), cytosolic PTK and protein tyrosine phosphatase (PTPase) activities strongly suggested that the IR-PTK must be involved in this process. A detailed investigation of the effects of the Cr(III) compounds on the IR-PTK activity of partially purified insulin receptor preparation from rat skeletal muscle revealed that the $[Cr(L\text{-}cys\text{-}N,O,S)_2]^-$ complex, at 1 mM concentration, was the only compound which induced a significant enhancement (ca. 2-fold) of the IR-PTK activity over the insulin control. PTK assays conducted in the presence and absence of 1 mM $NaVO_3$, a potent PTPase inhibitor, indicated further that the Cr(III) potentiates insulin actions via a *direct* activation of the IR-PTK activity.

The distinct structure-activity profile obtained in assays using intact cells, such as lipogenesis where only the synthetic GTF was found to be active, versus those using a cell-free system, such as the PTK assay where only the $[Cr(L\text{-}cys\text{-}N,O,S)_2]$ complex was shown to be active, suggested the following: (i) the GTF probably represents a readily absorbable form of Cr(III) which can only potentiate insulin action after being transported into the cell, and (ii) the intracellularly active Cr(III) is likely to contain cysteine as a key ligand.

TABLE OF CONTENTS