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Alternative pathways to manifest the metabolic bioeffects of insulin

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Objectives of Research

A highly investigated field for several decades now relates to insulin-dependent signal transmission networks. A key question having clinical significance as well is whether insulin effects can be manifested via *alternative (insulin-independent) signaling pathways.* Our long-term studies on the insulinomimetic actions of vanadium, revealed that the metabolic effects of insulin can be fully manifested through insulin-receptor independent pathways. The identification and characterization of the 'key players' in this backup system is the main objective of my research.

Recent Findings

Vanadium salts that mimic virtually all the metabolic effects of insulin, have no or minor effect in activating the insulin-receptor tyrosine kinase (InsRTK) in intact cellular systems). This vanadate bypassing receptor activation was fully validated, using specific cell permeable blockers of InsRTK. Endogenous tyrosine-phosphorylation, however is a prerequisite condition for manifesting the metabolic effects of insulin. We therefore searched for additional vanadium-activatable non-receptor protein tyrosine kinase. Such a protein has been identified in the cytosolic fraction of rat adipocytes (CytPTK). The relevant feature of CytPTK, and the basic differences between CtyPTK and the insulin-receptor are summarized in Table I.

CytPTK is activated several fold by vanadium salts and participate in several of the insulin-like effects manifested by this metalooxide.

Establishment of a cell-free system. The lack of a cell-free experimental system for studying insulin-dependent signaling pathways was the predominant factor in slowing down this field of research. Recently, however, we have managed to establish such a cell-free system for the investigation of vanadium-dependent mechanism(s) of action. This experimental system allowed us to determine that CytPTK activation is preceded and dependent on inhibiting vanadium-sensitive protein phosphotyrosine phosphatases. Most of the vanadate-sensitive PTPases are intrinsic plasma membrane proteins. The more relevant PTPases to manifest the bioresponses of insulin are now identified (in preparation). An additional non-receptor protein-tyrosine kinase that is activated by vanadte was identified as well (Elberg et al., 1997).

Sensitization: Vanadium therapy, also sensitizes, peripheral diabetic tissues, to respond to insulin. This, sensitizing mechanism, was recently elucidated. It is due to vanadium-evoked restoration of glucose-6-phosphate levels in diabetic tissues, in situ (Sekar et al., 1998, and Sun et al., submitted manuscript).

Therapeutic considerations: Any manipulations to elevate, the insulin-like efficacy of vanadium salts, without increasing its toxicity is of clinical interest to the future care of diabetes in human. The ligand L-glutamic-acid(γ)monohydroxamate, chelates vanadium, decreases its toxicity and potentiate its insulinomimetic efficacy, 4-6 fold. This is valid both in vivo and in vitro (Goldwaser et al., 1999). The therapeutical implication of these findings are currently evaluated (Patent WO97-IL265).

In summary, vanadium mimics insulin through a backup system, involving the inhibition of PTPases, and the activation of nonreceptor protein tyrosine kinases. It sensitizes diabetic tissues to respond to insulin, by restoring glucose-6-phosphate levels in target tissues in situ. Its therapeutic value can be considerably synergized by chelating vanadium with certain derivatives of amino acids.

Table I. Comparison between CytPTK and InsRTK

CytPTK	CytPTK	InsRTK
estimated molecular-weight	53 kDa	350-400kDa
requirement for bivalent anion	Co^{2+}	Mn^{2+}
good exogenous substrate	$Polyglu_4Tyr$	$Polyglu_4Tyr$
sensitivity to N-ethylmaleimide	No effect	Inactivation
inhibition by staurosporine ($I.C_{50}$)	2 nM	8 mM

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