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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. Another key question having clinical significance 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; Shisheva and Shechter, 1993). The relevant feature of CytPTK, and the basic differences between CtyPTK as well as the insulin-receptor are summarized in the Table. CytPTK is activated several fold by vanadium salts and participates 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 PYPases are intrinsic plasma membrane proteins. The more relevant PTPases to manifest the bioresponses of insulin have now been identified (in preparation). An additional non-receptor protein-tyrosine kinase that is activated by vanadate was previously identified (Elberg et al., 1997).

Sensitization: Vanadium therapy also sensitizes peripheral diabetic tissues to respond to insulin. This sensitizing mechanism was recently elucidated and found to be due to vanadium-evoked

Table. Comparison between CytPTK and InsRTK

	CytPTK	InsRTK
estimated molecular-weight	53 kDa	350-400 kDa
requirements 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 ∝M

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(g)monohydroxamate, chelates vanadium, decreases its toxicity and potentiates 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 these tissues in situ. Its therapeutic value can be considerably synergized by chelating vanadium with certain derivatives of amino acids.

Other Projects

A technology to elongate life-time of proteins *in vivo* has been developed. FMS moieties are covalently linked to proteins to form an inactive prodrug that undergoes slow reactivation under physiological conditions both *in vivo* and *in vitro*.

Selected Publications

Elberg, G., et al. (1997) A vanadate-activatable membranal non-receptor protein tyrosine kinase in rat adipocytes. Diabetes, 46, 1684-1690.

Sekar, N., Li, J., He, Z.B., and Shechter, Y. (1997) A novel assay for evaluating glycogenolysis in rat adipocytes and inability of insulin to antagonize glycogenolysis. Biochemistry (in press).

Li, J., Elberg, G., Sekar, N., He, Z.B., and Shechter, Y. (1997) Antilipolytic actions of vanadate and insulin in rat adipocytes mediated by distinctly different mechanisms. Endocrinology, 138, 2274-2279.

Sekar, N., Qian, S., and Shechter, Y. (1998) Vanadate elevates the lipogenic capacity of adipose tissue of fasted rats: mechanism of action.Endocrinology 139, 2514-2518.

Goldwaser, I., Li, J., Gershonov, E., Armoni, M., Karnieli, E., Fridkin, M., and Shechter, Y. (1999) L-glutamic acid γ-monohydroxamate: A potentiator of vanadium-evoked glucose metabolism *in vitro* and *in vivo*. J. Biol. Chem. 274, 26616-26624.

Goldwaser, I., Qian, S., Gershonov, E., Fridkin, M., and Shechter, Y. (2000) Organic vanadium chelators potentiate vanadium-evoked glucose metabolism *In vitro* and *In vivo*: Establishing criteria for optimal chelators. Mol. Pharmacol. 58, 738-746.

Shechter, Y., Patt, L.C., Schreiber, G., and Fridkin, M. (2001) Prolonging the half-life of human interferon-α-2 in circulation: Design, preparation and analysis of FMS_7-IFN-α2. Proc. Natl. Acad. Sci. USA 98, 1212-1217.

Shechter, Y., I. Goldwaser, I., Lavon, I., Gershonov, E., Mester, B., Mironchik, M., Patt, L., and Fridkin, M. (2001) A new approach for prolonging the half-life of peptides, proteins and low molecular-weight drugs *in vivo*. Drugs of the Future 26, 669-676.

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