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 CytPTK 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 metal oxide.

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 tyrosine phosphatases. Most of the vanadate-sensitive PTPases 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

<table>
<thead>
<tr>
<th></th>
<th>CytPTK</th>
<th>InsRTK</th>
</tr>
</thead>
<tbody>
<tr>
<td>estimated molecular-weight</td>
<td>53 kDa</td>
<td>350-400 kDa</td>
</tr>
<tr>
<td>requirements for bivalent anion</td>
<td>Co^{2+}</td>
<td>Mn^{2+}</td>
</tr>
<tr>
<td>good exogenous substrate</td>
<td>Polyglu4Tyr</td>
<td>Polyglu4Tyr</td>
</tr>
<tr>
<td>sensitivity to N-ethylmaleimide</td>
<td>No effect</td>
<td>Inactivation</td>
</tr>
<tr>
<td>inhibition by staurosporine (IC_{50})</td>
<td>2 nM</td>
<td>8 nM</td>
</tr>
</tbody>
</table>
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 potentiates its insulinomimetic efficacy, 4-6 fold. This is valid both in vivo and in vitro (Goldwaser et al., 1999). The therapeutic 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 undergo slow reactivation under physiological conditions both in vivo and in vitro.

Selected Publications


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