EFFECT OF VANADATE TREATMENT ON INSULIN RECEPTOR BINDING IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Studies from numerous laboratories in the past 20 years have elucidated the principal features of insulin action at the molecular level (22). Binding of insulin to its heterotetrameric receptor activates its intracellular tyrosine kinase activity (42). The balance between tyrosine phosphorylation and dephosphorylation appears to be critical in the regulation of the earliest steps in insulin action (38,39).

A new turning point occurred in 1985, when Heyliger, McNell and coworkers demonstrated that oral administration of vanadate to streptozotocin (STZ) induced diabetic rats, a representative model of type-1-diabetes, lower their high levels of blood glucose to normal levels (109). Unlike insulin, which is not absorbed orally (125), vanadate has the physical attributes of being a low molecular weight substance and a phosphate analogue, enabling it to permeate plasma membranes and the intestinal wall with relative ease. Perfused, isolated liver is a suitable system that connects between the relatively simpler in vitro (i.e isolated cells) systems and the more sophisticated and complex intact animal models. Several in vitro (141,234,235,236 ) and in vivo (141,234,235,236 ) studies have shown that vanadate can produce insulin like effects.
The interest in vanadate was greatly augmented by the findings that vanadate \((V_2O_5)\) and vanadyl sulfate \((VOSO_4)\) could mimic insulin to stimulate glucose uptake and metabolism in insulin target tissues \textit{in vitro} \((112,110,111)\).

The exact mechanism of action by which vanadate produces its insulin-like effects is not very clear. Vanadate has been shown to enhance glucose transport \((237)\) and glycogen synthesis \((238,239,240,241)\) in adipocytes by stimulating insulin receptor kinase \((242)\). Gherzl et al \((248)\) have shown that autophosphorylation and tyrosine kinase activity of the isolated insulin receptors can be stimulated by vanadate \textit{in vitro}. It has been also shown that vanadate could act at the post insulin receptor level \((242,243)\) without having any effect on insulin receptors.

Hence this study was taken up to investigate the effect of sodium ortho vanadate on insulin secretion and insulin receptor binding in diabetic animals.

\textbf{Experimental design}

\textbf{Animals:} Two months old male \textit{wistar} rats were made diabetic by a single Intraperitoneal injection of Streptozotocin \((65\text{mg/kg body weight})\) after 24hr fasting. \textit{STZ} was dissolved in \(100\text{mM citrate buffer (pH 4.5)}\) containing \(150\text{mM NaCl}\) and the control rats were injected with this buffer alone. One week later, the rats were divided in to three groups of six rats each 1) control, 2) diabetic untreated and 3) vanadate treated
diabetic rats. Vanadate treatment to the third group of rats was initiated at a concentration of 0.1mg/ml in drinking water and increased up to 0.5mg/ml over a period of one week and continued for five more weeks. At the end of the treatment period, all the three groups of rats were killed in fed condition. Their livers were removed, frozen in liquid Nitrogen, pulverised and stored at -80°C. Plasma samples were prepared and levels of insulin were determined by using BARC RIA-Klt and glucose was measured by the method of O-toluidine.

**Purification of Insulin receptor**

Insulin receptors from rat livers were isolated and purified by the method described by kruszynska et al (193) and Boni-Schnetzler (194).

**Insulin Binding**

Insulin receptor binding activity was measured according the procedure of Subbalah and Ramji (259).

**RESULTS**

The changes in body weight and the biochemical parameters of the different groups of rats are given in table-9.

There was a significant loss in body weight in the untreated diabetic rats as compared to non-diabetic rats (P<0.001); whereas, the vanadate treated diabetic rats gained weight (P<0.01) compared to the untreated diabetic rats. There was significant increase in plasma glucose.
Table-9: Changes in Body weight, plasma glucose, insulin and glycosylated Hb(HbA₁) levels in controls, diabetics and vanadate treated diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Body weight change (g/week)</th>
<th>Plasma glucose (mg/dl)</th>
<th>Plasma insulin (p moles/L)</th>
<th>Glycosylated haemoglobin (HbA₁%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (n=6)</td>
<td>+25.5±1.2</td>
<td>67.56±9.25</td>
<td>262.35±30.52</td>
<td>7.2±0.69</td>
</tr>
<tr>
<td>Diabetic rats (n=6)</td>
<td>-18.5±0.82*</td>
<td>280.68±26.23**</td>
<td>75.26±6.85**</td>
<td>13.65±0.95**</td>
</tr>
<tr>
<td>Vanadate treated diabetics rats (n=6)</td>
<td>+23.24±0.75*</td>
<td>105.25±28.36**</td>
<td>92.26±5.86</td>
<td>8.72±1.02*</td>
</tr>
</tbody>
</table>

** P<0.001  *P<0.01 compared with their respective controls
level in the streptozotocin induced diabetic rats compared to that in control rats; whereas, in the vanadate treated diabetic rats the blood glucose level was significantly decreased (P<0.001).

The plasma insulin levels in diabetic untreated rats were decreased significantly compared to those of control rats (P<0.001).

However, in the vanadate treated diabetic rats the plasma insulin level was not improved. The levels of Glycated haemoglobin (HbA1c) in the diabetic rats were significantly higher (P<0.001) than those in control rats. The vanadate treatment decreased the level of glycosylated haemoglobin significantly to near control levels.

The electrophoretogram of sucrose density gradient purified normal rat insulin receptor is shown in Fig. 4. Four protein bands were observed corresponding to the α subunit of insulin receptor (MW 116 KDa), β subunit (MW 95 KDa), whole receptor (MW 400 KDa) and αβ subunit (MW 200 KDa).

125I-Insulin binding to Insulin Receptor

The binding of 125I-Insulin to the purified hepatic insulin receptors from the control, diabetic and vanadate treated diabetic rats is shown in Fig.5. The 125I-Insulin binding increased significantly (P<0.001) in diabetic rats as compared with that in controls, while in the vanadate treated diabetic rats the insulin binding was decreased but not to the control level.
Figure-4: SDS-PAGE of sucrose density gradient purified insulin receptor
Lane 1. Whole receptor
Lane 2. Nonreducing conditions
Lane 3. Reducing conditions
Lane 4. Marker proteins
FIGURE 4
Figure-5: Insulin binding to WGA purified insulin receptors of liver from control (●–), diabetic (●–) and vanadate treated (●↑) rats.
Figure-5
DISCUSSION

In the present study, sodium ortho vanadate, treatment resulted in a reduction in fasting plasma glucose and HbA1c, without changes in plasma insulin levels. In association with the insulin-mimetic effects, vanadium compounds have been shown to normalize hyperphagia associated with experimental diabetes (234). This normalization of food intake has led repeatedly to the issue of the effects of dietary restriction on glycaemic responses after vanadate administration. In 1994, Malabu et al (249) claimed that the decreases in plasma glucose levels observed after administration of vanadate were entirely attributed to a reduction in food intake. In the present investigation, vanadate was administered daily to streptozotocin induced diabetic rats for five weeks. There was significant increase of body weight in vanadate treated diabetic rats when compared with diabetic untreated rats. Our results are in agreement with that of Yuen et al (250).

Although the exact cellular mechanism(s) and/or mediators involved in vanadate action remain elusive, it appears that the final action of vanadate may be mediated by a synergy between several post-receptor events in the insulin-signalling cascade. Recognition of the insulin molecule by its receptor is a complex molecular event and is closely linked to signal transmission.

Recent evidences have revealed that the insulin receptor is a tyrosine-specific protein kinase activated by insulin binding (251-253). Several studies have shown that the tyrosine kinase activity of insulin
receptor is essential for mediating the biological effects of insulin (148). Previous studies on the effect of vanadate on insulin receptor kinase had yielded varying results (138, 242, 243, 244). Several in vitro studies had demonstrated that vanadate can activate the tyrosine kinase activity of the insulin receptor (242, 243). However, some investigators had shown that vanadate can exert insulin-like effects without stimulating the receptor kinase suggesting that this agent might be acting at the post receptor level (138, 244). In the present study, it was shown that vanadate decreases insulin binding though not to the control level. But there is an indication that vanadate may have an independent insulin signal transduction.

Two models of the insulin receptor interactions have been suggested. One proposes that the binding of insulin leads to negative cooperativity i.e. binding of one ligand molecule causes a decrease in the receptor's affinity for subsequent molecules (254, 255). The other model hypothesizes the existence of multiple binding sites, with either low or high affinity for insulin (256, 257).

In our study, we observed that there was an increase in insulin binding in diabetic rats when compared to normal rats, while in vanadate treated diabetic rats, there was a decrease in insulin binding but not to the level of control rats. These results clearly indicate that insulin - deficient diabetic rats show increased insulin binding in liver membranes as shown by other studies (258, 259). Increased insulin binding was observed in other tissues like adipocytes (260, 261), and skeletal muscle
(262), attributable to increased receptor affinity (263). As there is significant insulin resistance in the target tissues of these animals, the increased receptor binding evidently cannot generate normal transmembrane signals that mediate insulin's metabolic effects. To address the paradox between increased binding and defective insulin action in hypoinsulinaemic diabetes, several studies have explained a possible role for receptor tyrosine kinase activity. Thus, further studies are warranted to define the exact role of vanadate in clinical diabetes.

Though some investigators did not find any effect of vanadate at the receptor level they did observe the insulin-mimetic action of vanadate (138,244,267). Peripheral glucose utilization and hepatic glucose production in diabetic rats were normalized by vanadate with the insulin receptor phosphorylation remaining unaltered (267). Vanadate increased glycogen synthesis in mouse diaphragm but did not activate insulin receptor kinase (138). Further, it has been demonstrated that some of the effects of insulin might not involve its receptor kinase. However, one cannot rule out the possibility of vanadate acting at the insulin receptor level.

Several studies have shown that sodium ortho vanadate mimics insulin action (122), and produces insulin like effects in vitro (112, 137, 138 242, 243, 244, 266). Vanadium in this form has been suggested to cause diarrhoea when fed to animals (113). However, no such observations were made in this study and also in other studies (122, 143,238,245,264). It has been reported that vanadate administration
to diabetic animals normalized the abnormalities associated with insulin deficiency and insulin resistance (143,238,245,246,247,265).

However, the exact mechanism of action of vanadate still remains unclear. Since vanadate mimics insulin action in diverse metabolic pathways, it seems probable that its site of action could be at a proximal level in the cascade of insulin-induced signal transduction.