Appendix
Short communication

Effects of vanadate on glycolytic enzymes and malic enzyme in insulin-dependent and -independent tissues of diabetic rats

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The effects of sodium orthovanadate (0.6 mg/ml in drinking water) on hexokinase isozymes, pyruvate kinase and malic enzyme in liver and kidney of control and alloxan diabetic rats were studied and compared. Vanadate treatment of diabetic rats normalized hyperglycemia and almost completely restored the differentially altered enzyme profile of liver (a tissue that underutilizes glucose in diabetic) and kidney (a tissue that overutilizes glucose during diabetes). Vanadate therapy, however, could not restore the depressed plasma insulin level of diabetic rats. The study clearly indicates that vanadate can effectively normalize many metabolic abnormalities even at a low insulin level in both insulin-dependent and -independent tissues of diabetic rats.

Vanadate; Alloxan diabetes; Insulin mimetic effects; Liver; Kidney

1. Introduction

Recent reports on vanadium, a dietary trace element, suggest that it can act as a novel and potent insulin mimetic. In vitro and in vivo studies, vanadate increases glucose transport and metabolism in skeletal muscle, liver and adipose tissue (Heylinger et al., 1985; Pugazhenthi and Khandelwal, 1990; Shechter, 1990). Recently it has been reported from our laboratory that vanadate administration prevents renal hypertrophy and restores polyol pathway activity in diabetic rat kidney (Saxena et al., 1992). However, the mechanism of the insulin mimetic action of vanadate is still not clear.

During diabetes, the liver and kidney are severely but differently affected as the liver is an insulin-dependent tissue whereas the kidney is an insulin-independent tissue which does not require insulin for glucose transport and which overutilizes glucose during persistent hyperglycemia (Soczewinski et al., 1979, 1985). Glucose overutilization is of critical importance in diabetes as exemplified by the glycosylation of proteins, cataract formation, renal hypertrophy, etc. (Brownlee and Cerami, 1981).

The present study investigates the antidiabeticogenic effects of vanadate on hexokinase isozymes, pyruvate kinase (glycolytic regulatory enzymes) and malic enzyme (an NADPH generating lipoic acid enzyme) in the liver and kidney of alloxan diabetic rats. The study is of critical importance in view of the different alterations in insulin requirement and in glucose uptake, utilization and flux into different metabolic pathways in the two selected tissues and their responsiveness to vanadate even at a lowered insulin-level.

2. Materials and methods

Male Wistar rats (220–240 g) were made diabetic by a single subcutaneous (s.c.) injection of alloxan monohydrate (200 mg/kg body weight; Sigma Co., USA). Treated rats were given 2 IU of protamine-zinc insulin for the next 7 days. After insulin withdrawal, the rats were divided into four groups: control, vanadate-treated control, diabetic and vanadate-treated diabetic rats. The vanadate-treated rats were given sodium orthovanadate (Sigma Co., USA) in the drinking water at a dose of 0.6 mg/ml. This concentration has been found to be optimal for exerting insulin mimetic effects (Heylinger et al., 1985; Saxena et al., 1992). NaCl (5 mg/ml) was also added in the vanadate-supplemented water to reduce the toxicity of vanadate (Heylinger et al., 1985). All groups of rats were maintained for 3 weeks and were fed ad libitum.
### TABLE 1

**General parameters, plasma insulin and vanadium levels of different experimental groups.**

Values are means ± S.E.M. of five observations. Statistical analysis was done with Student's t-test. P values are shown as: * P < 0.001, and † P < 0.05 vs. control. Abbreviations used: C. control; C + V, vanadate-treated control; D. diabetic; D + V, vanadate-treated diabetic rats; KW. kidney weight; LW, liver weight; ND, not detectable.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>C + V</th>
<th>D</th>
<th>D + V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>259 ± 8</td>
<td>247 ± 7</td>
<td>197 ± 5 *</td>
<td>205 ± 5 *</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.73 ± 0.05</td>
<td>1.68 ± 0.07</td>
<td>2.25 ± 0.04 *</td>
<td>1.63 ± 0.05 *</td>
</tr>
<tr>
<td>KW/100 g body weight</td>
<td>0.67 ± 0.03</td>
<td>0.68 ± 0.04</td>
<td>1.14 ± 0.04 *</td>
<td>0.79 ± 0.02 *</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>10.58 ± 0.43</td>
<td>10.30 ± 0.29</td>
<td>7.78 ± 0.24 *</td>
<td>7.94 ± 0.24 *</td>
</tr>
<tr>
<td>LW/100 g body weight</td>
<td>4.05 ± 0.11</td>
<td>3.90 ± 0.12</td>
<td>3.76 ± 0.18</td>
<td>3.91 ± 0.22</td>
</tr>
<tr>
<td>Blood glucose (mM)</td>
<td>5.7 ± 0.3</td>
<td>5.5 ± 0.4</td>
<td>25.7 ± 0.9 *</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>Fluid intake (ml)</td>
<td>38 ± 3</td>
<td>34 ± 3</td>
<td>203 ± 9 *</td>
<td>33 ± 4</td>
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<tr>
<td>Vanadium intake (mg/kg per day)</td>
<td>1.63 ± 0.17</td>
<td>9.6 ± 0.8 *</td>
<td>†</td>
<td>5.4 ± 0.9 *</td>
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<tr>
<td>Plasma insulin (µU/ml)</td>
<td>ND</td>
<td>3.04 ± 0.75</td>
<td>ND</td>
<td>3.35 ± 0.84</td>
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<td>Vanadium level (µ mol/100 g wet weight)</td>
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<td>ND</td>
<td>ND</td>
<td>3.09 ± 0.71</td>
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<tr>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>13.09 ± 2.31</td>
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</table>

Rats were killed by cervical dislocation. Liver and kidney homogenates (1:5) were prepared in 0.25 M sucrose, 0.02 M triethanolamine pH 7.4, containing 0.12 mM dithiothreitol. Mitochondrial and cytosolic fractions were prepared by differential centrifugation of the homogenate (Sochor et al., 1985). Hexokinase isozymes were estimated with glucose-6-phosphate dehydrogenase by the method of Giuranna and McLean.

### TABLE 2

**Effect of vanadate on the activities of hexokinase isozymes, pyruvate kinase and malic enzyme in rat kidney and liver of different experimental groups.**

Enzyme activities are expressed as total units/100 g body weight in all cases. One unit of enzyme activity is defined as 1 µmol of NADH/NADP oxidised/reduced per min at 25°C. Statistical analysis was done with Student's t-test. Values are means ± S.E.M. of five separate experiments. P values are shown as: * P < 0.001, † P < 0.005, ‡ P < 0.01 and † † P < 0.05 vs. control. Abbreviations used: C. control; C + V, vanadate-treated control; D. diabetic and D + V, vanadate-treated diabetic rats.

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<tr>
<th>Parameter</th>
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<th>D</th>
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<tr>
<td>Hexokinase</td>
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<td>Cytosolic</td>
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</tr>
<tr>
<td>Total</td>
<td>0.64 ± 0.04</td>
<td>0.59 ± 0.03</td>
<td>0.88 ± 0.06 *</td>
<td>138</td>
<td>0.68 ± 0.03</td>
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<tr>
<td>Type I</td>
<td>0.41 ± 0.02</td>
<td>0.40 ± 0.03</td>
<td>0.58 ± 0.05 *</td>
<td>141</td>
<td>0.44 ± 0.02</td>
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<tr>
<td>Type II</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.30 ± 0.02 *</td>
<td>130</td>
<td>0.24 ± 0.01</td>
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<td>Mitochondrial</td>
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<tr>
<td>Total</td>
<td>0.47 ± 0.04</td>
<td>0.40 ± 0.01 †</td>
<td>0.58 ± 0.04 *</td>
<td>123</td>
<td>0.49 ± 0.03</td>
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<td>Pyruvate kinase</td>
<td>8.13 ± 0.75</td>
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<td>13.10 ± 1.27 *</td>
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<td>9.14 ± 0.60</td>
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<td>Malic enzyme</td>
<td>1.08 ± 0.13</td>
<td>1.01 ± 0.09</td>
<td>1.15 ± 0.09</td>
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<td>1.17 ± 0.08</td>
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<td><strong>Liver</strong></td>
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<td>Hexokinase</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9.74 ± 0.47</td>
<td>9.18 ± 0.62</td>
<td>2.84 ± 0.12 *</td>
<td>29</td>
<td>9.49 ± 0.62</td>
</tr>
<tr>
<td>Type I</td>
<td>1.44 ± 0.10</td>
<td>1.47 ± 0.15</td>
<td>0.96 ± 0.07</td>
<td>67</td>
<td>1.39 ± 0.14</td>
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<tr>
<td>Type II</td>
<td>1.32 ± 0.04</td>
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<td>0.46 ± 0.04 *</td>
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<td>1.26 ± 0.11</td>
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<tr>
<td>Type IV</td>
<td>6.98 ± 0.34</td>
<td>6.58 ± 0.47</td>
<td>1.39 ± 0.15 *</td>
<td>20</td>
<td>6.84 ± 0.58</td>
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<tr>
<td>Pyruvate kinase</td>
<td>106 ± 7</td>
<td>94 ± 5</td>
<td>68 ± 6 *</td>
<td>64</td>
<td>94 ± 6 *</td>
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<tr>
<td>Malic enzyme</td>
<td>4.50 ± 0.43</td>
<td>4.19 ± 0.31</td>
<td>2.52 ± 0.21 *</td>
<td>56</td>
<td>4.24 ± 0.21</td>
</tr>
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</table>
Malic enzyme was assayed by the method of Wood et al. (1969). Pyruvate kinase and blood glucose were measured spectrophotometrically as described in Bergmeyer (1974).

Plasma insulin was measured with a RIA kit supplied by BARC, India. Samples for vanadium measurement were prepared according to a standard protocol (Stroops et al., 1985) and vanadium was measured by inductively coupled plasma-atomic emission spectrometer (Perkin Elmer Plasma 40 model).

3. Results

General parameters, plasma insulin and vanadium levels are shown in table 1. Vanadate therapy of diabetic rats normalized the 4-fold elevated blood glucose level within 4 days and levels remained normal until the day of the experiment. However, an increase in the blood glucose level was observed when the vanadate treatment was withdrawn from diabetic rats. The increased water consumption of diabetic rats also came back to normal values, and vanadate-treated diabetic rats did not show signs of dehydration. Diabetic rats were characterized by an increase in kidney weight (30%) and a significant decrease in liver weight (26%). Vanadate reduced the increased kidney weight by 82%, when expressed per 100 g body weight but had no effect on the decreased liver weight of diabetic rats. The body weight of vanadate-treated diabetic rats remained almost the same as that of diabetic rats.

The changes in enzyme activities are depicted in table 2 and are expressed as total units/100 g body weight, a parameter relating biochemical activity to the functional requirement of the whole animal, which makes comparisons possible (Sochor et al., 1979). In liver, type I and type II isozymes of hexokinase were significantly decreased and glucokinase was almost completely depleted during diabetes. It was observed that the considerably reduced activities of different isozymic forms of hexokinase (type I, type II and glucokinase), pyruvate kinase and malic enzyme in the liver of diabetic rats were almost completely restored by vanadate treatment. In the kidney, which overutilizes glucose in persistent hyperglycemia during diabetes, enzyme activities were found to increase except for malic enzyme, which remained unchanged. Vanadate therapy restored the elevated enzyme activities in the kidney of diabetic rats to almost normal values. However, vanadate treatment did not normalize the 74% depressed plasma insulin level of diabetic rats (table 1).

In vanadate-treated control rats, no significant effect of vanadate was observed, except for a 41% decrease in plasma insulin level. Long-term therapy with vanadate caused a slight accumulation of vanadium in the tissues of treated rats (table 1). In control and diabetic rats vanadium levels were below the detection limit (0.4 µmol/100 g wet weight).

4. Discussion

Recent studies have clearly indicated that vanadate has insulin-mimetic properties (Geczyling et al., 1985; Pugazhenthi and Khandelwal, 1990; Saxena et al., 1992). During diabetes, in contrast to the general pattern of tissue loss, the kidney increases in weight (Sochor et al., 1979). The liver requires insulin for glucose uptake, glucose phosphorylation and the entry of glucose-6-phosphate into metabolic pathways. The kidney, being an insulin-independent tissue, does not require insulin for the same functions. During diabetes, in the absence of insulin, the enzyme levels are altered differently in the liver and kidney. While in the liver the enzyme activities are depressed, in kidney the enzyme activities are enhanced. The present work clearly demonstrates that vanadate, even in the absence of insulin, exerts antidiabeticogenic effects on both insulin-dependent and -independent tissues, namely the liver and kidney, respectively. The distribution and different isozymic forms of hexokinase were markedly elevated as the total capacity of the kidney for phosphorylation seems to keep pace with kidney growth in diabetes. Vanadate therapy in the dose applied completely normalized the altered different hexokinase isozyme profiles in the liver and kidney of treated diabetic rats. Normalization of hexokinase by vanadate is of critical importance as the enzyme regulates glucose entry in the tissue. Forms glucose-6-phosphate and also regulates glucose entry into alternative metabolic routes. Upon activation during diabetes, these alternative metabolic routes may cause glycogen accumulation, thickening of renal basement membrane, cataract formation and other effects.

The restoration of pyruvate kinase by vanadate in the liver and kidney suggests a probable role of vanadate in the channelling of pyruvate and glucose into the tricarboxylic acid cycle. Malic enzyme, which is an important supplier of NADPH for lipogenesis, remained unchanged in the kidney but was significantly decreased in the liver during diabetes. A marked increase in the hepatic level of malic enzyme was observed in vanadate-treated diabetic rats, suggesting that vanadate therapy may also influence the hydrogen shuttle systems and transhydrogenase reactions of lipogenic pathways.

In control rats, vanadate did not have a significant effect except for causing a decrease in plasma insulin level, which may be due to increased tissue responsiveness to insulin, as was also suggested by Pugazhenthi and Khandelwal (1990).
The present study clearly shows that vanadate has potent antidiabetogenic effects and at the dose administered it restored the differently altered metabolic pathways even under conditions of depressed insulin secretion, both in insulin-dependent and -independent tissues. The mechanism of its insulin-mimetic properties remains unclear. Further research is required to explore its other insulin-like properties and antidiabetogenic effects, which will provide more information about its mechanism of action.

Acknowledgements

Senior research fellowships from UGC (India) are gratefully acknowledged by A.K.S. and P.S. The authors also like to thank Dr. C.R. Jagga for the help in vanadium measurement.

References

EFFECT OF VANADATE ADMINISTRATION ON POLYOL PATHWAY IN DIABETIC RAT KIDNEY

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SUMMARY

Effect of oral administration of sodium orthovanadate for three weeks on polyol pathway in renal cortex and medulla was studied in control and alloxan diabetic rats. An enhancement in aldose reductase in cortex and medulla and sorbitol dehydrogenase in cortex was observed in alloxan diabetic rats. Despite depressed insulin secretion, vanadate treatment to diabetic rats counteracted hyperglycemia, normalized elevated enzyme activities and glucose level, prevented medullary sorbitol accumulation and markedly checked increase in kidney weight. These results show that vanadate causes marked improvement in renal hypertrophy and has an antidiabetogenic effect on polyol pathway in diabetic kidney.

INTRODUCTION

Recent studies have been focussed to reveal the biological significance of vanadium, a nutritional trace element (1). Though vanadium has not been identified with a specific physiological role, it is required for normal growth, development and also for survival of mammalian cells in culture (2). It is known to inhibit ion channel ATPases and phosphotyrosine phosphatases (3). Since the demonstration that vanadate has insulin mimetic effects, interest in its properties and effects on biochemical parameters has increased (4,5). In in vitro studies, vanadate has been shown to increase glucose transport and metabolism in skeletal muscles (6) and adipocytes (7). Oral administration of vanadate to streptozotocin diabetic rats normalizes blood glucose level (8), restores hepatic and skeletal muscle glycogen levels (9,10) and is also shown to normalize glycogen synthase (11) and glycolytic

To whom correspondence should be addressed.
enzymes (12). Insulin mimetic effects of Vanadium are also reported in partially pancreatectomized rats (10) and in genetically obese insulin resistant ob/ob mice (13). Though vanadate stimulates tyrosine kinase activity (14), recent reports indicate that vanadate elicits insulin like effects at a post receptor level (15).

In diabetes, the kidney is severely affected resulting in renal hypertrophy and other complications (16,17). In the present investigation, the in vivo effects of vanadate for the restoration of altered polyol pathway, hypertrophy and other parameters in the kidney of alloxan induced diabetic rat have been studied and a possible antidiabetogenic role of vanadate on diabetic kidney has been suggested.

MATERIALS AND METHODS

Materials: Sodium orthovanadate (Na$_3$VO$_4$), Alloxan monohydrate, enzymes, coenzymes, substrates and other chemicals were purchased from Sigma Chemical Co. (St. Louis, M.O.). Insulin RIA kit was obtained from BARC, India.

Treatment of animals: Albino male rats of Wistar strain weighing between 220-240g were starved for 24 hours, and were made diabetic by a single subcutaneous injection of alloxan monohydrate (200mg/kg body weight) dissolved in 0.15M sodium acetate buffer, pH 4.5. The alloxan treated rats were given intraperitoneal injections of 2 IU protamine zinc insulin daily for the next 7 days to reduce the mortality rate (18). After the insulin withdrawal rats were divided into four groups; Control (C), vanadate treated control (C+V), diabetic (D) and vanadate treated diabetic rats (D+V). Vanadate (0.6 mg/ml) was given in drinking water with NaCl (5 mg/ml; prepared fresh each day) to treated control and diabetic rats and was continued for 3 weeks. NaCl was added to reduce vanadate toxicity (8). As the rats do not like the taste of vanadate supplemented water because of its high pH and bitter taste, so to avoid initial aversion its pH was adjusted to 7 with citric acid. Same concentration of citric acid (neutralised with NaOH) was given in drinking water of untreated rats (C). The vanadate intake was approximately 74 and 92 mg/kg/day for C+V and D+V rats respectively. The severity of diabetes was monitored by testing urinary glucose daily by glucose detection strips.

Preparation of extracts and enzyme determination: Kidneys were excised rapidly following decapitation and separated into cortex and medulla. Cortex and medulla were homogenized separately in 0.25M sucrose, 0.02M Triethanolamine buffer pH 7.4, containing 0.12mM dithiothreitol using a Potter Elvehjem homogenizer fitted
with a teflon plunger. The supernatant fraction was isolated by centrifugation at 105,000g for 45 minutes and dialysed against the same buffer for 2 hr at 3°C. Sorbitol dehydrogenase (EC 1.1.1.14) was assayed as described (19). Aldose reductase (EC 1.1.1.21) was estimated by the method of Kinoshita et al. (20) and Pottinger (21). One unit of enzyme activity was defined as one micromole of NADH or NADPH oxidised per min at 25°C.

**Metabolite determination:** The neutralized perchloric acid extracts of cortex and medulla were prepared (22) for glucose and sorbitol determination. Glucose was estimated as described by Bergmeyer (23). Sorbitol was measured by a modified enzymatic method in which sorbitol dehydrogenase was added to convert sorbitol to fructose. NADH produced thereby was measured fluorometrically (24) using a Shimadzu RF-540 Spectrofluorophotometer.

**Vanadium and Insulin determination:** For vanadium estimation, kidney and plasma samples were prepared by the method of Stroop et al. (25). The concentration of vanadium was then analysed by inductively coupled plasma- atomic emission spectrometer (Perkin Elmer Plasma 40 model). The plasma insulin levels were quantitated by Radioimmunoassay kit supplied by BARC, India.

**RESULTS**

General parameters of all the four groups of rats are summerized in Table I. Alloxan diabetes caused a 4-fold increase in blood glucose level which was restored to normal values after 3-4 days of vanadate treatment, this was continued till the animals were used for the experiment. No weight gain was observed in D+V rats as earlier reported in STZ rats (8). The weight loss in D+V rats was found to be almost equal to the diabetic rats. Weight gain was slower in C+V rats than in controls. D+V rats drank as much water as C+V and showed no signs of dehydration. Alloxan induced diabetes caused a significant (about 32%) increase in kidney weight. This increase was about 82% when expressed per 100g body weight. It was observed that vanadate treatment significantly prevented increase in kidney growth in D+V rats. Kidney weight of D+V rats completely reversed to normal values and 70% reversed when expressed per 100g body weight.

The enzyme activities and metabolite levels in renal cortical and medullary regions of various study groups are shown in Table II. The polyol pathway comprises of two enzymes, aldose
Table 1  General parameters, vanadium levels in plasma and kidney and plasma insulin level of different experimental groups.

<table>
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<th>CONTROL</th>
<th>CONTROL + VANADATE</th>
<th>DIABETIC</th>
<th>DIABETIC + VANADATE</th>
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<tr>
<td>Body weight (g)</td>
<td>265 ± 5</td>
<td>244 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>193 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>200 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Kidney weight (g)</td>
<td>1.74 ± 0.03</td>
<td>1.67 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.29 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Kidney weight/100 g Body weight</td>
<td>0.66 ± 0.02</td>
<td>0.69 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86 ± 0.01</td>
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<td>Blood Glucose (mg/dl)</td>
<td>105 ± 4</td>
<td>92 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>436 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109 ± 5</td>
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<td>Fluid Intake (ml/day)</td>
<td>36 ± 3</td>
<td>30 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>210 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Vanadium intake (mg/kg/day)</td>
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<td>Plasma (pg/g wet wt.)</td>
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<td>Kidney (pg/g wet wt.)</td>
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<td>7.20 ± 1.32&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Plasma insulin (µU/ml)</td>
<td>15.90 ± 1.65</td>
<td>8.90 ± 0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.11 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.81</td>
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Values are mean ± SEM of 6 to 8 observations. Fisher's P values are shown as <sup>a</sup>P<0.001, <sup>b</sup>P<0.01, <sup>c</sup>P<0.025, <sup>d</sup>P<0.05 vs. control.

Conditions are described in Materials and Methods. Vanadium intake was measured as 1000x[average fluid intake per day (ml)xconc. of vanadium (mg/ml)/body wt. (g)].
Table II  Enzyme activities and metabolite levels of polyol pathway in renal medullary and cortical regions of different experimental groups.

<table>
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</tr>
<tr>
<td><em>Sorbitol dehydrogenase</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>6.91 ± 0.12</td>
<td>6.34 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.35 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.71 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>medulla</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td><strong>Aldose reductase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>medulla</td>
<td>0.44 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.62 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td><strong>Metabolites (μ mol/g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sorbitol</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>0.22 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.26 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>medulla</td>
<td>6.40 ± 0.54</td>
<td>6.28 ± 0.41</td>
<td>11.50 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.36 ± 0.45</td>
</tr>
<tr>
<td><em>Glucose</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>2.56 ± 0.13</td>
<td>2.46 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.26 ± 1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.04 ± 0.28</td>
</tr>
<tr>
<td>medulla</td>
<td>0.32 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td>21.30 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ± 0.04</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of six separate experiments. Fisher's P values are shown as

<sup>a</sup><sub>P<0.001</sub>,  <sup>b</sup><sub>P<0.005</sub>,  <sup>c</sup><sub>P<0.01</sub>,  <sup>d</sup><sub>P<0.025</sub> vs. control.

Conditions are described and units are defined in Materials and Methods.
reductase (AR), a regulatory enzyme that oxidises glucose to sorbitol and sorbitol dehydrogenase (SDH) which converts it into fructose. The AR activity was found to be about 2 fold higher in the renal medullary region than in cortex. In diabetic rats 30% and 41% increase above the control values was found in AR level in renal cortex and medulla respectively. The SDH level was found to be very low in medulla and quite high in the cortex. Diabetes caused 35% increase in cortical SDH activity but no change was seen in the medulla. Oral administration of vanadate to diabetic rats caused a complete reversal of AR level to almost control values in both cortex and medulla respectively (100% and 91% respectively). SDH activity was also found to be completely restored in vanadate treated diabetic rats.

Sorbitol formation was much higher in medulla where it is accumulated [30 fold higher than in cortex in contrast to glucose which is 8 fold higher in cortex] (26). Sorbitol level was unaltered in cortex but almost doubled in medulla of diabetic rats. Vanadate administration to diabetic rats significantly lowered the elevated sorbitol content (85% reversed) in renal medullary region and also caused marked decline in glucose level which was elevated by 9 and 67 fold in cortex and medulla respectively during diabetes.

Plasma insulin level of diabetic rats decreased by 74% and it could not be restored by vanadate treatment (Table I). Vanadate treatment to controls did not cause any significant effect in enzyme and metabolite levels but a 44% fall in plasma insulin level was however observed. Vanadium levels were found to be 1.88 and 7.2 μg/g of wet tissue in plasma and kidney of D+V rats which was almost equal to that in C+V rats. Vanadium levels of control and diabetic in plasma and kidney was below the detection limit (detection limit: 0.2 μg/g wet wt.) (Table I).

DISCUSSION

Studies of several investigators have amply suggested that vanadium compounds mimic some of the insulin effects. While previous studies involved effects of vanadate on liver, adipose
tissue and skeletal muscle (8-15), no such study has been conducted on alloxan diabetic kidney. In the present study the responsiveness of renal medullary and cortical regions for the antidiabetogenic in vivo effects of orthovanadate has been shown. Though normoglycemia is achieved in alloxan diabetic rats within 3-4 days of vanadate treatment initiation, hyperglycemia recurs within 48 hours of its withdrawal. This recurring may be due to the progressive destruction of \( \beta \) cells to a critical value before the initiation of vanadate treatment and thus the long term islet protective role of vanadate is lost (27). The dose of vanadate (0.6 mg/ml) in the present experiment was chosen as it is reported to be non-toxic for liver and kidney function (11,12) though only 70% rats responded to it in our experiments.

Vanadate treatment to diabetic rats restores the raised enzyme activities of polyol pathway to almost near control values (Table II). A near complete reversal was observed in AR and SDH in both cortical and medullary regions. In medulla of control animals, sorbitol accumulation was much higher than that in cortex. This may be due to the high AR and low SDH levels in medulla as reported earlier (26). In medulla, sorbitol which is nonpermeable to cells may act as one of the several osmolytes (28). During diabetes, an increased AR and tissue glucose levels may account for a further 2 fold increase of sorbitol in medulla as reported here (Table II). Thus, like in diabetic lens (29), renal sorbitol accumulation may lead to increase in osmotic pressure and cell disruption, thereby causing kidney dysfunction at a later stage. Oral administration of vanadate to diabetic rats not only normalizes the elevated enzyme levels but the sorbitol and glucose content are also effectively restored. As the polyol pathway enzymes are activated by high glucose, their reversal may partially be due to the normalization of blood and renal glucose level by vanadate.

Another point of interest which emerged from this study is that the vanadate administration successfully prevents the renal hypertrophy measured as the weight gain in kidney (Table I). In
diabetes, there is a marked increase in kidney growth characterized by hypertrophy and hyperplasia (16) in contrast with the general pattern of tissue loss. Here polyol pathway plays a significant role as aldose reductase activates the pentose phosphate pathway by reoxidising the NADPH produced by it which leads to the formation of nucleotide precursors and activation of other biosynthetic mechanisms that finally cause increase in kidney growth (16,17). Earlier studies have shown that insulin treatment for 3 weeks prevents renal hypertrophy in diabetes (30). Vanadate administration to diabetic rats almost reverses the increase in kidney growth to normal values and a 87% reversal was found when kidney weight was expressed per 100g body weight that may be due to loss in body weight.

Despite the lower insulin level in D+V rats (Table I), vanadate is able to act as an antidiabetogenic agent. This suggests that vanadate action may be independent of insulin action. In vanadate treated control rats, a significant decrease in plasma insulin level was observed. Similar findings were also reported by earlier workers (8,9) who suggested that this decrease may be due to an increased responsiveness to insulin. The vanadium level of kidney is much higher than the plasma level that may be due to its greater accumulation in kidney during its administration. From this study, it can be concluded that oral administration of vanadate markedly improves the diabetic state and has a profound antidiabetogenic effect on polyol pathway accompanying renal hypertrophy.

The mechanism by which vanadium compounds exert insulin mimetic effects is not yet very clear. Tamura et al (14) demonstrated that vanadate can stimulate the tyrosine kinase activity which may account for its insulin like effects. Recently, it has been suggested that the effects of vanadate are due the oxovanadium compound which involves a superoxide anion (31). However, further investigations are required to elucidate its mechanism of action at the cellular and molecular level. It would be of interest to study the effect of vanadate on diabetic lens where similar situation causes cataract formation.
Acknowledgements:

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REFERENCES

IMPAIRED ANTIOXIDANT STATUS IN DIABETIC RAT LIVER

EFFECT OF VANADATE

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Abstract—In vivo effects of vanadate on the antioxidant status of control and alloxan diabetic rats liver were examined. The increased oxidative stress during diabetes caused a decline in the activities of glutathione peroxidase (GPx), catalase (CAT), CuZn superoxide dismutase (CuZn-SOD) and Mn-superoxide dismutase (Mn-SOD) in the liver. Reduced glutathione (GSH) was also depleted, but the level of oxidized glutathione and glutathione reductase activity remained unchanged in the livers of diabetic rats. Vanadate treatment of diabetic rats (0.6 mg/mL in drinking water) resulted in almost complete restoration of GPx and Mn-SOD but caused only a partial restoration of CuZn-SOD. However, CAT and GSH were found to be lowered further in vanadate-treated diabetic rats as compared to untreated diabetic rat. Similar decreases in CAT and GSH levels were also observed in the vanadate-treated controls. These results suggest that vanadate, an insulin-mimetic agent, effectively normalized hyperglycemia, but unlike insulin, could not completely restore the altered endogenous defence mechanisms in diabetic liver.

The biological role of vanadate has not been firmly established [1, 2]. Recent studies, have shown that vanadate is a potent insulin-mimetic agent and promotes glucose uptake [3, 4], glucose oxidation [5] and activation of glycogen synthase in rat liver [6] and adipocytes [7]. Oral administration of vanadate to alloxan and streptozotocin diabetic rats restores elevated blood glucose level and several carbohydrate-metabolizing enzymes to control values [6, 8, 9]. Recently, we have shown that vanadate treatment normalizes glycolytic enzymes and malic enzyme in both insulin-dependent and -independent tissues [9] and prevents renal hypertrophy in diabetic rats [10]. However, the mechanism for its insulin-like actions is still not clear. Evidence is accumulating which suggests that toxic reactive oxygen-derived free radicals (superoxide, peroxide and hydroxyl radicals) play a crucial role in diabetes [11-14]. Thus, the tissue antioxidant status seems to emerge as an important factor in the etiology of diabetic complications.

Wohsieb and Godin [14] demonstrated previously that insulin treatment of diabetic rats resulted in almost complete reversal of all the foregoing alterations in tissue antioxidant status. Hence, an antioxidant role was presumed for vanadate, in view of its insulin-mimetic properties of correcting various carbohydrate-metabolizing enzymes. The present study was, therefore, specifically aimed to examine whether, like insulin, vanadate treatment can normalize the disturbed antioxidant status in experimental diabetes.

MATERIALS AND METHODS

Materials. Sodium orthovanadate, alloxan monohydrate, enzymes, coenzymes and substrates were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). All the other chemicals were standard analytical grade laboratory reagents.

Treatment of animals. Male Wistar rats (200-220 g; age 10-12 weeks) were starved for 24 hr and diabetes was induced by a single subcutaneous injection of alloxan monohydrate (200 mg/kg body weight) dissolved in 0.15 M sodium acetate buffer, pH 4.5. Another group of rats received a corresponding volume of sodium acetate buffer and served as controls. The alloxan-induced diabetic rats were injected i.p. with 2 IU of protamine-zinc insulin for the next 7 days. Insulin was then withdrawn and both groups of rats were randomly divided into four groups: control, vanadate-treated control, diabetic alterations in antioxidant enzyme activities and tissue GSH concentrations have been reported in diabetes [11, 14].

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† Abbreviations: SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione.
and vanadate-treated diabetic rats. Sodium orthovanadate (0.6 mg/mL) was given to the treated rats in drinking water which also contained 80 mM NaCl. As described earlier [8, 9], NaCl was included to reduce the toxicity of vanadate. The untreated control and diabetic rats received drinking water supplemented with 80 mM NaCl. All animals were maintained for 3 weeks and had access to water and food ad lib. At the end of the treatment period, blood was collected by eye vein in heparin and rats were killed by cervical dislocation.

**Determination of GSH and oxidized glutathione (GSSG).** Livers were rapidly excised, blotted dry and immediately homogenized in ice-cold 2 M perchloric acid, 4 mM EDTA using a Potter-Elvehjem homogenizer fitted with a teflon plunger. Homogenates were then centrifuged at 1000 g for 10 min. The clear extracts were neutralized with 2 N KOH, and GSH and GSSG were measured enzymatically as described by Griffith [15] on the same day. For GSH estimation, the reaction mixture contained 0.21 mM NADPH, 0.6 mM 5,5'-dithiobis-(2-nitrobenzoic acid) and 0.5 U glutathione reductase in 125 mM sodium phosphate buffer (pH 7.5), and 6.3 mM EDTA. The rate of 5,5'-dithiobis-(2-nitrobenzoic acid) reduction was continuously followed at 412 nm and GSH content was determined by comparing the rate observed using a standard curve generated with a known amount of GSH. For GSSG, GSH was derivatized by adding 2-vinyl pyridine (2 μL/mL extract) and was kept for 1 hr at 25°. The GSSG content was then measured directly as described for GSH.

**Enzyme assays.** For enzyme assays, livers were homogenized in 10 mM potassium phosphate buffer pH 7.4, supplemented with 30 mM KCl. Homogenates were then centrifuged at 1000 g for 10 min. For SOD measurement, the supernatant was treated with Triton X-100 (1% final concentration) and left for 30 min on ice to solubilize both CuZn-SOD and Mn-SOD. The SOD activity was analysed by the method of Marklund and Marklund [16] based on the ability of the enzyme to inhibit autoxidation of pyrogallol and expressed as units of SOD per gram tissue (one unit is the amount of enzyme that causes half-maximal inhibition of pyrogallol autoxidation).

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Control</th>
<th>Control + vanadate</th>
<th>Diabetic</th>
<th>Diabetic + vanadate</th>
</tr>
</thead>
<tbody>
<tr>
<td>263 ± 9</td>
<td>250 ± 8</td>
<td>203 ± 6*</td>
<td>211 ± 7*</td>
<td></td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>10.4 ± 0.7</td>
<td>10.2 ± 0.5</td>
<td>7.9 ± 0.4*</td>
<td>7.8 ± 0.4*</td>
</tr>
<tr>
<td>Protein content (mg/g wet weight)</td>
<td>159 ± 5</td>
<td>163 ± 6</td>
<td>161 ± 7</td>
<td>156 ± 5</td>
</tr>
<tr>
<td>Fluid intake (mL)</td>
<td>35 ± 4</td>
<td>33 ± 4</td>
<td>190 ± 8*</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Vanadium intake (mg/kg/day)</td>
<td>—</td>
<td>79 ± 10</td>
<td>—</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>Blood glucose (mg/100 mL)</td>
<td>106 ± 6</td>
<td>100 ± 7</td>
<td>415 ± 17*</td>
<td>104 ± 7</td>
</tr>
<tr>
<td>Plasma insulin (μU/mL)</td>
<td>16.8 ± 1.9</td>
<td>10.9 ± 0.9*</td>
<td>4.5 ± 0.8*</td>
<td>5.5 ± 0.4*</td>
</tr>
</tbody>
</table>

**Table 1 General parameters of the four experimental groups**

The treated control and diabetic rats received sodium orthovanadate (0.6 mg/mL)-supplemented water. Values are means ± SE of six rats. Significance of difference was assessed by Student’s t-test. P values are shown as *P < 0.001, †P < 0.01 and ‡P < 0.05 vs control.

Samples for CAT were prepared by incubating the supernatant with ethanol (10 μL/mL) for 30 min in ice and then Triton X-100 was added to a final concentration of 1%. CAT assay was performed by the method of Aebi [17] and the activity was expressed as IU/g tissue × 10^3. In the 1% Triton X-100-treated samples GPx and glutathione reductase (GR) (EC 1.6.4.2) were measured by the method of Lawrence and Burk [18] and Erden and Bor [19], respectively. One unit of GPx and GR activity was defined as one micromole of NADPH oxidized per minute at 25°. All measurements were carried out using a Beckman DU-68 spectrophotometer.

**Blood glucose, plasma insulin and protein determination.** Blood glucose was measured by an enzyme coupled assay using glucose-6-phosphate dehydrogenase and hexokinase as described [20]. The plasma insulin levels were quantitated by radioimmunoassay kit supplied by BARC, India. Protein content was estimated in the liver extracts by the method of Bradford [21].

**RESULTS**

The general parameters as observed in the four experimental groups are shown in Table 1. Diabetes was characterized by hyperglycemia (4-fold increase) and hypoinsulinaemia (73% decrease). Vanadate administration (0.6 mg/mL), within 3–4 days, normalized the elevated blood glucose level but was unable to restore the decreased plasma insulin level of diabetic rats. If the vanadate treatment was withdrawn from the diabetic rats, hyperglycemia was found to recur within 72 hr. Vanadate treatment reversed the increased water intake of diabetic rats to normal values with no signs of dehydration. The body weight of treated diabetic rats remained almost equal to that of untreated diabetic rats. Vanadate treatment caused a 35% decrease in plasma insulin level of control rats that could be due to an increased tissue responsiveness for insulin, as suggested earlier [10, 22]. There was no change in the total protein content/g liver after treatment as compared to the control values.

**Table 2** depicts the antioxidant status of liver in various experimental groups. Measurement of free radical-scavenging enzymes in diabetic rats showed a generalized decrease in the activities of GPx, CAT,
1. "certain pathological states like diabetes, the increased oxidative stress during diabetes (involving increased \( O_2 \) and \( H_2O_2 \) production) is controlled to some extent only. Paradoxically, we found that, instead of restoration, the decreased levels of CAT and GSH in diabetic rats had declined further in vanadate-treated diabetic rats. This observation was not expected from an insulin-mimetic agent like vanadate. A similar pattern of decrease in CAT and GSH was seen in vanadate-treated controls. However, the present results are contradictory to those of Sekar et al. [25] who observed the restoration of both CAT and GSH in vanadate-treated diabetic rats, which could be due to the low dose of orthovanadate (0.3 mg/mL) used in their study. Degani et al. [26] showed by electron paramagnetic resonance studies that externally applied vanadate (\( V^{V,V}_V,V_0^O_4 \) is reduced.

CuZn-SOD and Mn-SOD as also reported earlier [14]. Oral administration of vanadate to diabetic rats almost completely normalized the 30% depressed GSH levels. In diabetic rats, a 28% decline in the Mn-SOD was observed that returned to normal value following vanadate administration. However, the treatment only partially restored the 40% decreased antioxidant state CuZn-SOD level.

In the diabetic rat liver, a 29% and 31% decrease in CAT and GSH levels was observed, respectively. Vanadate treatment of diabetic rats was, however, found to be totally ineffective in the restoration of the CAT and GSH level. On the other hand, the depressed CAT and GSH levels in the diabetic rats were lowered further in the vanadate-treated diabetic rats. A similar pattern was observed in the treated control rats where CAT and GSH levels were decreased from the control values (19% and 24%, respectively). No effect of vanadate was seen on the GR activity which remained unaltered during diabetes. GSSG levels were also found to be unchanged in the diabetic and treated rats.

**DISCUSSION**

Vanadium is now considered an essential nutritional trace element and has therapeutic value in pharmacological doses, but is toxic in excess and causes stimulation of \( H_2O_2 \) production and lipid peroxidation [2]. Our studies, along with those of others, have demonstrated that vanadate mimics many of the actions of insulin and its oral administration improves the altered glucose homoeostasis and other metabolic disorders of the diabetic state [4–10].

There is now ample evidence to suggest that, in certain pathological states like diabetes, the increased production and ineffective scavenging of toxic reactive oxygen species may play a crucial role in determining tissue injury [11–14]. Diabetes causes a depression in overall liver antioxidant status making it more vulnerable to oxygen radical attack which may cause oxidative damage to membranes and alterations in subcellular organelle structural and functional integrity [14]. The decreased levels of GPx, CAT, CuZn-SOD and Mn-SOD in the diabetic state may be due to inactivation caused by reactive oxygen species [23]. This decrease in antioxidant enzymes may also be due to the emaciation observed in the diabetic rats [14]. In diabetes, non-enzymatic glycation due to persistent hyperglycemia may also inactivate the antioxidant enzymes as shown previously with SOD by Arai et al. [24]. Insulin treatment of diabetic rats was shown previously to normalize all the foregoing alterations in the antioxidant status [14].

In view of the various insulin-mimetic effects of vanadate, an attempt was made to study the efficacy of vanadate treatment in controlling the altered antioxidant status of the liver of diabetic rats. The dose (0.6 mg/mL) of sodium orthovanadate \( (Na_3V_0_4) \) was carefully chosen since it had been established previously as optimal for normalizing hyperglycemia and for exerting insulin-mimetic effects in diabetic rats [8–10]. GPx has a key role in enzymatic defence systems and acts on peroxides \( (H_2O_2 \) lipid or organic peroxides) to remove them. In the present investigation, it was observed that vanadate therapy could effectively normalize the GPx and Mn-SOD level but it only partially restored the level of CuZn-SOD. This suggests that the increased oxidative stress during diabetes (involving increased \( O_2 \) and \( H_2O_2 \) production) is controlled to some extent only. Paradoxically, we found that, instead of restoration, the decreased levels of CAT and GSH in diabetic rats had declined further in vanadate-treated diabetic rats. This observation was not expected from an insulin-mimetic agent like vanadate. A similar pattern of decrease in CAT and GSH was seen in vanadate-treated controls. However, the present results are contradictory to those of Sekar et al. [25] who observed the restoration of both CAT and GSH in vanadate-treated diabetic rats, which could be due to the low dose of orthovanadate (0.3 mg/mL) used in their study. Degani et al. [26] showed by electron paramagnetic resonance studies that externally applied vanadate \( (V^{V,V}_V,V_0^O_4) \) is reduced.

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**Table 2. GPx, GR, CAT, CuZn-SOD and Mn-SOD activities and GSH and GSSG levels in the livers of different experimental groups**

<table>
<thead>
<tr>
<th>Enzyme activities (U/g)</th>
<th>Control</th>
<th>Control + vanadate</th>
<th>Diabetic</th>
<th>Diabetic + vanadate</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (6)</td>
<td>22.1 ± 1.6</td>
<td>20.1 ± 1.8</td>
<td>15.4 ± 1.5§</td>
<td>21.8 ± 1.9</td>
</tr>
<tr>
<td>GR (6)</td>
<td>6.8 ± 0.2</td>
<td>6.0 ± 0.28</td>
<td>6.6 ± 0.2</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>CAT* (6)</td>
<td>34.0 ± 2.2</td>
<td>27.4 ± 2.8</td>
<td>24.0 ± 1.74</td>
<td>20.9 ± 2.14</td>
</tr>
<tr>
<td>CuZn-SOD (8)</td>
<td>2238 ± 276</td>
<td>2077 ± 270</td>
<td>1333 ± 174§</td>
<td>1682 ± 184</td>
</tr>
<tr>
<td>Mn-SOD (8)</td>
<td>225 ± 22</td>
<td>213 ± 17</td>
<td>162 ± 188</td>
<td>220 ± 17</td>
</tr>
<tr>
<td>Metabolite levels (( \mu mol/g ))</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GSH (6)</td>
<td>5.5 ± 0.4</td>
<td>4.2 ± 0.5§</td>
<td>3.8 ± 0.48</td>
<td>3.0 ± 0.3§</td>
</tr>
<tr>
<td>GSSG (6)</td>
<td>0.18 ± 0.03</td>
<td>0.16 ± 0.02</td>
<td>0.19 ± 0.03</td>
<td>0.17 ± 0.03</td>
</tr>
</tbody>
</table>

The treated control and diabetic rats received vanadate-supplemented water. Values are means ± SE. Number of animals (N) is given in parenthesis.

*Activity is expressed in U/g x 10⁶.

Significance of difference was assessed by Student's t-test. P values are shown as **P < 0.001, §P < 0.01 and #P < 0.05** vs control.

Other details are given in Materials and Methods.
intracellularly to vanadyl (VIV, VO2) and binds endogenously with GSH to form vanadyl-GSH complexes which may produce its insulin-like effects. Thus, the decrease observed in the level of GSH in treated control and diabetic rats may be due to its consumption in the formation of such vanadyl-GSH complexes. The downward trend in CAT level seen in treated control and diabetic rats as compared to their counterparts cannot be explained at this stage. Vanadate may produce some other form of free radical or factor leading to site-specific inactivation of CAT. It is also possible that vanadate administration in the dose applied has some other role in the biological system independent of its insulin-mimetic effects. This is likely as vanadium is a transition element with variable oxidation states (+3, +4, +5, +7) and theoretically should influence the tissue redox state and, in turn, the antioxidant enzymes.

In conclusion, the present investigation indicates that vanadate therapy is only partially effective in controlling the impaired antioxidative system of diabetic rats and is accompanied by some adverse effects as, unlike insulin, it fails to normalize CAT and GSH levels which are of crucial importance for the host defence. On the basis of the foregoing study it is suggested that vanadate treatment accompanied by some known antioxidant (e.g. flavonoids [27]), to improve the concomitant defence system disturbances, may produce better therapeutic results. More incisive studies are warranted to explore other insulin-like effects of vanadate that may also elucidate the mechanism of vanadate action at the molecular and cellular level.

Acknowledgements—A.K.S. and P.S. gratefully acknowledge senior research fellowships from UGC, India.

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Insulin-Like Effects of Vanadate on Renal Hypertrophy and Polyol Pathway in Diabetic Rats


School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

ABSTRACT

Vanadate has been shown to be a potent insulin mimetic agent. Present investigation examines and compares the effect of vanadate and insulin on renal hypertrophy, disturbed polyol pathway in the whole kidney (an insulin-independent tissue) and other parameters of the diabetic rats. Vanadate administration caused stable normoglycemia and like insulin, effectively normalized the altered polyol pathway and prevented increased kidney growth in diabetic rats. Insulin injections to diabetic rats, however, could not completely normalize hyperglycemia and frequent variations in blood glucose levels were observed.

INTRODUCTION

A new interest in vanadate has emerged since the discovery that vanadium compounds possess potent insulin-mimetic properties (13). Vanadate ions have been shown to produce insulin-like effects by stimulating glucose transport and metabolism in various tissues (3,18). In non-diabetic rats, oral vanadate has no effect on plasma glucose levels but in diabetic rats, vanadate administration is shown to markedly decrease the elevated blood glucose levels and to restore many glucose metabolizing enzymes (9,10). However, the mechanism of action for its insulin-like effects remains unclear. These effects may be due to vanadate's ability to enhance
phosphorylation of insulin receptor by stimulating tyrosine kinase activity (16) or due to direct vanadate esterification of certain tyrosine residues (19). Recent studies have shown mechanisms distal to insulin receptor accounting for its insulinomimetic properties (5).

During diabetes mellitus, the clinical expression of absolute or relative insulin deficiency, most of the organs are adversely affected giving rise to pathological states like renal hypertrophy, cataract and peripheral vascular diseases (17). Kidney, an insulin independent tissue, is severely affected in diabetes and in contrast to general pattern of tissue loss, it undergoes a net increase in weight resulting in renal hypertrophy which is found to be closely linked with a highly activated polyol pathway (10,12). Activation of polyol pathway, comprising of two enzymes aldose reductase (AR) and sorbitol dehydrogenase (SDH), in turn activates other pathways like pentose phosphate pathway and the biosynthetic mechanisms leading to increased growth of kidney. Sorbitol, an intermediate product of polyol pathway, accumulates in diabetes and is suspected to be a major factor in osmotic damage and tubular nephropathy (2,11,12).

In the present study, insulin injections and sodium orthovanadate in drinking water were given to diabetic rats and their effects on polyol pathway in whole kidney, renal hypertrophy and other important metabolic parameters were studied and compared. A possible therapeutic role of vanadate as a potent substitute of insulin has been suggested.

MATERIALS AND METHODS

Male rats (210-230 g) of Wistar strain were made diabetic by a single subcutaneous injection of alloxan monohydrate (200 mg/kg body weight; Sigma Co., St Louis) as previously described (10,11). After 7 days, rats were divided into 4 groups (i) control (ii) untreated diabetic rats (iii) insulin treated diabetic rats (D+I) given 4 IU of protamine zinc insulin daily and (iv) vanadate treated diabetic rats (D+V) given 0.6 mg/ml sodium orthovanadate (Sigma Co.) in drinking water also containing 80 mM NaCl. All groups of rats were maintained for 3 weeks and were fed ad lib.

Rats were killed by cervical dislocation. Kidney
was rapidly excised, decapsulated and the whole kidneys were homogenized for the enzyme assays and metabolite measurements separately as described (12). Sorbitol dehydrogenase and aldose reductase activities were assayed by the method of Gerlach and Hiby (4) and Kinoshita et al. (6) respectively. One unit of enzyme activity was defined as one umol of NADH or NADPH oxidized/min at 25°C. Glucose and sorbitol content were measured in the neutralized perchloric acid extracts as described earlier (1,8). Vanadium samples were prepared by the method of Stroops et al (14) and were analysed by inductively coupled plasma-atomic emission spectrometer.

RESULTS AND DISCUSSION

Recent studies have clearly indicated that vanadium compounds mimic many of the actions of insulin (9,10,13). Alloxan induced diabetes was characterized by elevated blood glucose level, increased water intake and a marked decline in body weight (Table 1).

Administration of vanadate to diabetic rats normalized hyperglycemia within 4 days, in the dose applied (Fig. 1) and also reversed the increased water

<table>
<thead>
<tr>
<th>TABLE 1. General parameters of 4 experimental groups.</th>
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<tr>
<td>Body weight (g)</td>
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<tr>
<td>Kidney weight (g)</td>
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<tr>
<td>Fluid intake (ml/day)</td>
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<tr>
<td>Blood glucose (mM)</td>
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</table>

Values are mean ± SD of five animals. Significance of difference was assessed with the student's t test. P values are shown as <sup>a</sup>p<0.001, <sup>b</sup>p<0.01 and <sup>c</sup>p<0.05 vs. control. Abbreviations: C, control; D, diabetic; D+I, insulin injected (4 IU insulin daily, i.p.) diabetic and D+V, vanadate treated diabetic rats.
consumption to normal values (Table 1). This stable normoglycemia was observed in the vanadate treated diabetic rats as long as the treatment was continued. Vanadate withdrawal from the diabetic rats again resulted, within 72 hours, in the reappearance of diabetic signs namely increased blood glucose level and increased fluid intake. Insulin injections to diabetic rats lowered the elevated blood glucose levels to 66% but frequent variations were observed in the blood glucose levels (Fig. 1) and also it could not reverse the increased fluid intake. Vanadate treatment, like insulin therapy, effectively regressed renal hypertrophy, measured as weight gain in kidney. The long term treatment of vanadate caused a slight accumulation of vanadium in plasma and kidney (38±14 and 141±40 μmol/kg wet weight, respectively) of the vanadate treated diabetic rats. The vanadium levels were not detectable in control, untreated diabetic and insulin treated diabetic animals.

Diabetes resulted in highly enhanced polyol pathway activity in kidney as observed by an increased levels of AR, SDH and sorbitol content (27%, 37% and 68% increase, respectively) (Fig. 2 & Fig. 3). Glucose
IMG. 2. Percent change in the activities of AR and SDH in the whole kidney of different groups of rats. Enzyme activities of control rats: AR, 0.22±0.03 and SDH, 6.5±0.67 U/g.

FIG. 3. Percent change in sorbitol and glucose content in the whole kidney of different groups of rats. Metabolite levels of control rats: sorbitol, 0.31±0.05 and glucose, 2.4±0.41 µmol/g.
concentration was also significantly elevated, up to 8 fold, from the normal value in the diabetic kidney (Fig. 3).

As kidney is an insulin-independent tissue, it does not require insulin for glucose uptake and glucose phosphorylation. Thus, in persistent hyperglycemia, during diabetes, increased glucose flux in kidney activates the polyol pathway which plays an important role in the development of renal hypertrophy. This enhanced polyol pathway activates pentose phosphate pathway which supplies ribose-5-phosphate used for nucleotide and nucleic acid synthesis and NADPH for reductive biosynthesis that finally cause increase in kidney growth.

Vanadate therapy proved to be very effective and completely normalized the increased aldose reductase, sorbitol dehydrogenase (Fig. 2) and metabolite levels namely sorbitol and glucose (Fig. 3) in the kidney of diabetic rats. Insulin injections to diabetic rats also caused a significant reversal of enhanced enzyme activities and metabolite levels to the normal values. This antidiabetogenic reversal effects of vanadate seem to be contributed primarily because of its glucose lowering capacity leading to stable normoglycemia. Earlier reports demonstrated that vanadate has the ability to restore the expression of a glucose transporter (15), thereby regulating the glucose homeostasis. As insulin injections to diabetic animals could not lead to stable normoglycemia, thus due to the variations in the blood glucose level, a steady control of glucose flux in metabolic pathways cannot be expected. This could explain the inability of insulin therapy to completely reverse the pathways activated by increased glucose flux.

Oral administration of insulin is ineffective and insulin injections, due to large variability in insulin absorption among diabetics cause frequent variations in blood glucose levels that is supposed to have links with various cardiovascular diseases and shorten the life expectancy of diabetic patients (7). Thus, availability of orally administered potent insulin-mimetic agents like vanadate could be of immense importance for better therapeutic intervention in the treatment of diabetes. However, further research is required to explore its other insulin like effects that may also give insight into the mechanisms involved.
ACKNOWLEDGEMENTS

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REFERENCES

INSULIN LIKE EFFECTS OF LITHIUM AND VANADATE ON THE ALTERED ANTIOXIDANT STATUS OF DIABETIC RATS

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Abstract

Lithium is widely used for treatment of behavioral disorders and has been shown to possess insulin-mimetic properties. The present study examines the in vivo effects of lithium alone, as well as in combination with vanadate (a potent insulin-mimetic agent), on the altered antioxidant status in the liver and kidney of diabetic rats. The elevated blood glucose levels in diabetic rats were about 50% restored by oral administration of lithium (0.3 mg/ml) and were completely normalized following vanadate addition (0.05 mg/ml) to lithium. Lithium therapy effectively normalized the decreased activities of catalase (CAT) and glutathione peroxidase (GSH-PX) but could not restore the lowered superoxide dismutase (SOD) in the liver of diabetic rats; while in kidney, the treatment proved to be ineffective. Inclusion of vanadate produced synergistic effect and caused partial restoration of the altered CAT, GSH-PX and CuZn-SOD levels in diabetic kidney and the depressed SOD activity in diabetic liver. These results suggest that lithium therapy may prove effective in improving the impaired antioxidant status during diabetes and vanadate supplementation at a low dose potentiates the effectiveness of lithium action.

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Introduction

Earlier studies have shown the intracellular deficiency of trace elements in diabetes (Failla and Kiser, 1981). Recently, much interest has been focused on the insulin-mimetic properties of several trace elements following various in vitro and in vivo studies (Shechter and Karlish, 1980; Bosch et al., 1986; Rossetti et al., 1990). Particularly, lithium and vanadate have been shown to stimulate glucose uptake and glycogen synthase activity in hepatocytes, adipocytes, and skeletal muscle (Bosch et al., 1986; Bigornia and Bihler, 1985; Shechter and Karlish, 1980). Rossetti et al. (1989, 1990) have recently demonstrated that oral administration of lithium alone, as well as with vanadate, to 90% partially pancreatectomized rats, improves glucose tolerance, normalizes insulin mediated glucose uptake and stimulates muscle and liver glycogen synthesis. However, insulin-mimetic actions of lithium need further characterization.

Recent studies have implicated the toxic reactive oxygen centered free radicals in the development of diabetes (Oberley, 1988). Isolated pancreatic cells, when exposed to the diabetogenic agent alloxan, were shown to sustain damage preventable by free radical scavengers like superoxide dismutase and catalase (Granquist et al., 1979). The ability of alloxan to generate oxygen radicals has been suggested as a primary determinant of its diabetogenic properties (Oberley, 1988). Diabetic complications like ischemic heart disease (McCord, 1985) and renal injury (Paller et al., 1984) have also been suggested to involve free radical-related processes such as the disturbed antioxidative system and oxidative damage to membranes. In diabetes, alterations in the endogenous free radical scavanging defense mechanisms comprising of enzymes; SOD (EC 1.15.1.1.), CAT (EC 1.11.1.6), GSH-PX (EC 1.11.1.9), and glutathione reductase (GSSG-R; EC 1.6.4.2); and nonenzymatic scavengers like reduced glutathione (GSH) may lead to ineffective scavanging of reactive oxygen species resulting in oxidative damage (Loven et al., 1986; Wohaiib and Godin, 1987; Mclennan et al., 1991). This altered antioxidant status, during diabetes, has been shown to be restored by insulin treatment (Loven et al., 1986; Wohaiib and Godin, 1987), thereby, indicating the possibility of an antioxidant role for lithium, an insulin-mimetic trace element. To examine this question, we investigated the effects of oral administration of lithium on the disturbed levels of antioxidant enzymes and GSH in both insulin-dependent and
-independent tissues, namely liver and kidney, respectively of the alloxan-diabetic rats. Vanadate, previously shown by us (Saxena et al., 1992a, 1992b) and others (Heylinger et al., 1985; Pugazhenthi and Khandelwal, 1990) to be a novel and potent insulin-mimetic trace element, was also supplemented with lithium to investigate any synergistic effect.

Materials and Methods

Treatment of animals

Male rats of the Wistar strain (200-250 g) were randomly divided into control and experimental groups. The experimental group was starved for 24 hours and then made diabetic with a single subcutaneous injection of alloxan monohydrate (200 mg/kg body wt.; Sigma chemical, St Louis) dissolved in 0.15 M sodium acetate buffer, pH 4.5. To reduce the mortality rate, the alloxan injected rats were given 2 IU of protamine zinc insulin i.p. daily for the next 7 days (Saxena et al., 1992a, 1992b). After insulin withdrawal, the diabetic rats were divided into 3 groups: (i) untreated diabetic rats (ii) diabetic rats treated with lithium and (iii) diabetic rats treated with lithium and vanadate.

Lithium carbonate (0.3 mg/ml; Sigma) and sodium orthovanadate (0.05 mg/ml; Sigma) were given in drinking water for 16 days. All groups of rats were fed ad lib. Blood was collected by eye vein for glucose and plasma insulin measurement.

Determination of GSH

Animals were killed by cervical dislocation, liver and kidney were excised rapidly and homogenized in 2 M perchloric acid, and 4 mM EDTA. Extracts were neutralized with 2 N KOH and GSH levels were measured enzymatically (Griffith, 1984).

Enzyme activity determination

Tissues were homogenized in 10 vol of 10 mM potassium phosphate buffer, pH 7.4 containing 30 mM KCl and centrifuged at 1000 g for 30 min to remove nucleus and cell debris. For SOD assay, the supernatant was treated with Triton X-100 (1% final concentration; Sigma) for 30 min in ice to solubilize both CuZn-SOD and Mn-SOD. SOD activity was then measured spectrophotometrically (Marklund and Marklund, 1974) and
expressed as units of SOD per gram tissue (one unit is the amount of enzyme that causes half maximal inhibition of pyrogallol autoxidation). Mn-SOD was measured by using KCN (10 mM final concentration) to inhibit CuZn-SOD. CAT activity was assayed in the ethanol (10 μl/ml) and Triton X-100 (1% final concentration) treated samples by the method of Aebi (1974) and expressed as IU/g x 10^1 (one unit was defined as mol of H_2O_2 decomposed/min/g). GSH-PX and GSSG-R were assayed in the Triton X-100 treated samples by the method of Lawrence and Burk (1976) and Erden and Bor (1984), respectively. One unit of GSH-PX and GSSG-R activity was defined as one umol of NADPH oxidised per min at 25°C.

Blood glucose was measured by a coupled enzymatic method (Bergmeyer et al., 1974). The plasma insulin levels were measured with a RIA kit supplied by BARC, India. Results are expressed as mean values ± SEM. Statistical analysis was performed with the Student's t test.

Results

General parameters of all the four groups of rats are summarized in Table I. The decreased body weight and liver weight as observed in alloxan-diabetic rats remained unchanged with lithium or with combined lithium and vanadate treatment. However, only a small restoration of the increased kidney weight in diabetic rats was noticed by a combined lithium and vanadate treatment, whereas no change was observed in diabetic rats treated with lithium alone.

Blood glucose levels were observed to be 4 fold elevated in diabetic rats during the experimental period. While treatment with lithium only partially restored (about 50%) hyperglycemia to normal level, vanadate supplementation in a low dose to lithium, however, proved to be more effective and within 4 days completely normalized the elevated blood glucose level that remained at normal value till the day of experiment (Fig I). Diabetes induced hypoinsulinemia (about 70% decrease) remained unchanged by either of the treatments.

Changes in hepatic enzyme and GSH levels

In liver, the activities of CAT, GSH-PX, CuZn-SOD, Mn-SOD and GSH were relatively higher than in kidney (Table II). Diabetes caused a generalized decrease in the
Figure 1: Effect of lithium alone and combined lithium and vanadate treatment on the blood glucose levels of diabetic rats. Control (○—○), untreated diabetic (△—△), lithium treated diabetic (■—■) and lithium and vanadate treated diabetic rats (■—■). Values are mean ± SEM of five rats.

activities of all the enzymes and GSH content as also reported earlier (Loven et al., 1986; Wohaieb and Godin, 1987). GSSG-R activity, however, remained unaltered during diabetes. Treatment of diabetic rats with lithium alone and with vanadate supplemented lithium completely normalized the decreased CAT and GSH-PX activities, however, lithium alone could not restore the decreased CuZn-SOD and Mn-SOD levels. Interestingly, the addition of vanadate to lithium caused a synergistic effect as shown by the augmentation of CuZn-SOD and Mn-SOD (Table II). No significant change was observed in the GSSG-R activity in lithium as well as combined lithium and vanadate treated diabetic rats. The decreased GSH levels, during diabetes, could not be brought to control levels by either of the treatments.

Changes in renal enzyme and GSH levels

In the kidney, the pattern of changes for CAT and CuZn-SOD were similar to that in the liver and a decrease was observed in their activities during diabetes (Table II). GSH-PX activity was, however, increased whereas no change was found in GSSG-R, Mn-SOD and the GSH levels of the diabetic rats. Lithium administration to diabetic rats
<table>
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<tr>
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<th>C</th>
<th>D</th>
<th>D + L</th>
<th>D + LV</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>258 ± 9</td>
<td>197 ± 5*</td>
<td>206 ± 7**</td>
<td>210 ± 8**</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>10.5 ± 0.8</td>
<td>8.1 ± 0.4***</td>
<td>8.6 ± 0.6</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.68 ± 0.05</td>
<td>2.30 ± 0.05*</td>
<td>2.25 ± 0.06*</td>
<td>1.96 ± 0.05**</td>
</tr>
<tr>
<td>KW/100 g body weight</td>
<td>0.66 ± 0.03</td>
<td>1.15 ± 0.04*</td>
<td>1.10 ± 0.02*</td>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>Blood Glucose (mg/dl)</td>
<td>104 ± 6</td>
<td>405 ± 22*</td>
<td>250 ± 12*</td>
<td>115 ± 8</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>16.1 ± 2.2</td>
<td>4.2 ± 0.7*</td>
<td>3.6 ± 0.5*</td>
<td>3.7 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of five animals. Significance of difference was assessed with student's t test. P values are shown as * p<0.001, ** p<0.01 and *** p<0.05 vs. control. Abbreviations used: C, control; D, diabetic; D+L, lithium treated diabetic; D+LV, lithium and vanadate treated diabetic rats; KW, kidney weight.
TABLE II. Catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase and reduced glutathione levels in rat liver and kidney of different experimental groups.

<table>
<thead>
<tr>
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<th>C</th>
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<th>D + L</th>
<th>D + LV</th>
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</thead>
<tbody>
<tr>
<td>LIVER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>34.2 ± 2.4</td>
<td>24.3 ± 2.4</td>
<td>33.1 ± 2.4</td>
<td>34.8 ± 2.1</td>
</tr>
<tr>
<td>GSH-PX</td>
<td>22.8 ± 2.1</td>
<td>16.5 ± 1.5</td>
<td>21.6 ± 1.9</td>
<td>23.8 ± 1.8</td>
</tr>
<tr>
<td>GSSG-R</td>
<td>6.8 ± 0.3</td>
<td>6.6 ± 0.2</td>
<td>6.3 ± 0.2</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>2173 ± 208</td>
<td>1365 ± 132</td>
<td>1381 ± 140</td>
<td>1835 ± 179</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>225 ± 22</td>
<td>172 ± 17</td>
<td>166 ± 15</td>
<td>199 ± 20</td>
</tr>
<tr>
<td>GSH (μmol/g)</td>
<td>5.2 ± 0.4</td>
<td>3.6 ± 0.4</td>
<td>3.9 ± 0.3</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>KIDNEY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>12.8 ± 1.2</td>
<td>7.5 ± 1.4</td>
<td>7.7 ± 1.2</td>
<td>9.2 ± 1.1</td>
</tr>
<tr>
<td>GSH-PX</td>
<td>6.1 ± 0.6</td>
<td>10.1 ± 0.9</td>
<td>9.8 ± 0.5</td>
<td>8.7 ± 0.5</td>
</tr>
<tr>
<td>GSSG-R</td>
<td>5.3 ± 0.4</td>
<td>5.9 ± 0.3</td>
<td>5.9 ± 0.2</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>CuZn-SOD</td>
<td>1131 ± 135</td>
<td>829 ± 79</td>
<td>811 ± 77</td>
<td>930 ± 81</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>125 ± 12</td>
<td>137 ± 19</td>
<td>135 ± 16</td>
<td>132 ± 14</td>
</tr>
<tr>
<td>GSH (μmol/g)</td>
<td>2.1 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of five separate experiments. Significance of difference was assessed with student's t test. P values are shown as *p<0.01 and **p<0.05 vs. control. Enzyme activities are expressed as units/g wet wt. Activity expressed in U/g x 10^3. Abbreviations: CAT, Catalase; GSH-PX, Glutathione peroxidase; GSSG-R, Glutathione reductase; SOD, Superoxide dismutase; C, Control; D, Diabetic; D+L, Lithium treated diabetic; D+LV, Lithium and vanadate treated diabetic rats.
was found to be ineffective in the restoration of the altered renal enzyme levels. Like in liver, vanadate supplementation to lithium caused synergism and partially restored the altered CAT, GSH-PX and CuZn-SOD activities of the diabetic rats. A group of lithium treated and a combined lithium and vanadate treated control rats were also made and no significant change was, however, observed in enzyme activities, GSH content and other parameters except for a slight decline in plasma insulin levels (16% and 18% decrease respectively) [data not shown].

**Discussion**

Insulin like activity of several trace elements, particularly, lithium and vanadate has been demonstrated in various studies (Shechter and Karlish, 1980; Bosch et al., 1986). Rossetti et al. (1989) for the first time reported that lithium administration normalizes insulin sensitivity in 90% of the pancreatectomized rats. Our reports (Saxena, 1992a, 1992b) along with those of others (Heylinger et al., 1985; Pugazhenthi and Khandelwal, 1990), clearly indicate that vanadate possess insulin-mimetic properties and its oral administration, at a therapeutic dose of 0.6-0.8 mg/ml, completely normalizes the elevated blood glucose levels in diabetic rats. However, in the present study the applied dose of sodium orthovanadate (0.05 mg/ml) supplemented with lithium, is 12 times lower than the minimal effective dose of orthovanadate (0.6 mg/ml) when administered alone to diabetic rats for its insulin like effects. This combination of lithium and vanadate seems to act in a synergistic manner on glucose homeostasis and also allows significant reduction in the effective dose of vanadate.

Present data show that lithium therapy to diabetic rats only partially restored while vanadate supplementation completely normalized the elevated blood glucose concentration despite a decreased plasma insulin level. This suggests that the mechanism of action of these elements may share common steps with that of insulin.

The altered tissue antioxidant status due to diabetes may cause increased production of free radicals leading to oxidative damage and tissue injury (Wohaieb and Godin, 1987; Oberley, 1988). Liver, during diabetes, showed a relatively more severe impairment in antioxidant capacity than the kidney. The decreased activities of CuZn-SOD and CAT in both liver and kidney, GSH-PX and Mn-SOD in liver during diabetes, may be due to the increased production of reactive oxygen radicals that can themselves reduce the activity of these enzymes (Wohaieb and Godin, 1987). An increase in GSH-PX activity in the kidney, where its activity is relatively lower than in the liver, may be a compensatory response to conditions of increased oxidative stress in diabetes. GSH depletion, during diabetes, may be as a result of several factors including
emaciation observed in diabetic animals, disturbances in hepatic $\gamma$-glutamyl cycle or, due to the altered efflux of GSH via the biliary tract (Mclennan et al., 1991).

CAT and GSH-PX are the enzymatic components of the defense system that protect the cells against peroxidative damage. In the present investigation, we found that lithium therapy to diabetic rats almost completely normalized the CAT and GSH-PX activities in the liver, an insulin-dependent tissue but it could not restore CuZn-SOD and Mn-SOD. This suggests that lithium alone has the capability to control the weakened defense system of liver to some extent. However, vanadate supplementation to lithium, though in a low dose, caused a synergistic effect and potentiated lithium action as observed by reversal of CuZn-SOD and Mn-SOD activities towards the normal values in diabetic liver. The diabetic rat kidney shows unresponsiveness to lithium treatment as no improvement was observed in the disturbed free radical scavenging enzyme activities. However, a combined lithium and vanadate treatment to diabetic rats proved effective in the normalization of the differentially altered enzyme levels in kidney to some extent. Although the enzyme GSH-PX is restored, the decreased GSH content in diabetic rat liver as shown by the present data (Table II) and others (Loven et al., 1986; Wohaieb and Godin, 1987) did not return to control values by lithium or by combined lithium and vanadate therapy. This remains unexplained at this stage and may partly be due to emaciation as observed in both diabetic and treated rats. Present results suggest that lithium has an insulin like role in the improvement of the impaired defense system of the diabetic rat liver. This insulin-mimetic action of lithium can be potentiated by the incorporation of a low dose of vanadate.

In conclusion, the present data clearly indicate that trace element therapy, either alone or in combination, may prove effective in the treatment of metabolic disturbances of the diabetic state. However, the mechanism of action of lithium and vanadium for their insulin like actions, remains unclear at this stage. Tamura et al. (1984) demonstrated that vanadate can stimulate the tyrosine kinase activity which may account for its insulin like effects. Recently, it has been suggested that the effects of vanadate are due to oxovanadium compounds which involves a superoxide anion (Ramanadham et al., 1989). Mechanisms distal to the insulin-receptor may also play a role (Green, 1986). Further investigations are required to delineate their biological role at the cellular level that may also help to produce therapeutic beneficial improvements in the treatment of diabetes.

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293