Modulation of mRNA levels of liver arginase by insulin and vanadate in experimental diabetes

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This work was carried out to study the modulation of arginase expression in experimental diabetes. Here, we have demonstrated that liver arginase activity and mRNA levels increased significantly in diabetic condition. Insulin treatment reverses the increased activity and mRNA levels nearly to the control values. The reversal effects of vanadate are found to be similar to that of insulin and this observation further reiterates the insulin-like effects of vanadate. ELISA and slot blot assay observations are consistent with biochemical measurements of enzyme activity. These results show an increase in arginase activity and mRNA levels in diabetes and decrease in treated animals may be due to the transcriptional regulation of arginase gene.

Arginase (L-arginine ureohydrolase, E.C. 3.5.3.1), one of the urea cycle enzymes, catalyzes the hydrolysis of arginine to urea and ornithine. Mammalian arginase exists in multiple forms (A1, A2, A3 and A4), and A1 (the cytosolic) is the main form (>95%) in rat liver. The gene of this enzyme seems to be regulated in response to nutritional conditions and showed an upregulation during starvation, high protein diet and diabetes. The mRNA level of arginase increases in response to glucocorticoids and glucagon which appears to be a consequence of transcriptional activation. A characteristic feature of arginase induction is that it is caused in a "secondary manner". The "primary" response to glucocorticoids is brought about by binding of the receptor complex to regulatory sequences of target genes and the "secondary response is mediated by trans acting factors synthesized de novo through primary response."

Vanadate mimics most of the insulin actions and elicits insulin-like effects at the post receptor level by inhibiting the phosphotyrosine phosphatases activity, though its functions in cellular regulation is still not fully understood. Insulin acts at the receptor level and initiates a process of phosphorylation and dephosphorylation of protein factors responsible for signal transduction. Administration of insulin is effective in reversing most of the changes found in diabetes. Similarly, oral administration of sodium orthovanadate to streptozotocin or to alloxan induced diabetic rats was also found to restore the activity of the enzymes of gluconeogenesis, glycolysis, glycogen synthesis and polyol pathway, thereby resulting in the reversal of the diabetic hyperglycaemic condition. The present study was undertaken to investigate the effects of insulin and vanadate on the arginase expression during experimental diabetes.

Materials and Methods

Treatment of animals and preparation of extracts
Female albino Wistar rats, weighing between 180-200 g, were made diabetic using alloxan monohydrate as described earlier from our laboratory. Two units of protamine zinc insulin was injected to the alloxan treated animals for one week. This procedure was found to decrease the mortality rate of the animals. After the insulin withdrawal, rats were divided into four groups; control (C), diabetic (D), insulin treated diabetic (D+I) and vanadate treated diabetic (D+V) rats as described by Saxena et al. Vanadate (0.6 mg/ml) was given in the drinking water with NaCl (5 mg/ml, prepared fresh each day) to diabetic rats for three weeks. At the end of the treatment, blood was collected from the tail vein and the rats were killed by cervical dislocation. Liver was excised rapidly and homogenised in 0.25M sucrose, 0.2M triethanolamine buffer (pH 7.4) containing 0.12 mM DTT using a Potter Elvehjem homogenizer fitted with a teflon plunger. Cytosolic fraction was prepared by differential centrifugation of the homogenate as described in our earlier paper.

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Enzyme assay

Hepatic cytosolic fractions were activated in the presence of 0.01 M MnCl$_2$ at 55°C for 15 min. Activated samples (0.5 ml) were added in the reaction mixture (0.46 M arginine, pH 9.5 and 1.25 M glycine buffer, pH 9.5) and incubated for 30 min at 25°C. Reaction was stopped by adding 2 ml of 1 M perchloric acid. Samples were centrifuged and the supernatant was used for the estimation of urea$^{14}$. One enzyme unit is defined as one micromole of ammonia formed per min per gram fresh weight of liver at 25°C. In each group at least 6-8 separate animals were used.

Vanadium and insulin determination

Plasma samples were prepared by the method of Stroop et al.$^{15}$ and the vanadium concentration was analyzed using an inductively coupled plasma-atomic emission spectrometer (Perkin Elmer plasma 40 model) as described in earlier papers and are expressed as mg/100 gm body weight$^{13}$. The plasma insulin levels were quantified by using RIA kit supplied by Bhabha Atomic Research Centre, India.

Blood glucose and protein estimations

Blood glucose was measured by an enzyme coupled assay system using glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and hexokinase (E.C. 2.7.1.1)$^{16}$, protein was estimated according to the method of Lowry et al.$^{17}$

ELISA

ELISA was performed as described by Sambrook et al.$^{18}$ Antibodies against human liver arginase were given by Prof Mori, Japan. Secondary antibodies conjugated with horse radish peroxidase were used. O-phenylene diamine (OPD) was used as a substrate at a concentration of 17 mg/ml in sodium acetate buffer. Pale yellow colour of the product was measured at 492 nm on an ELISA reader.

RNA slot blot analysis

Total RNA from rat liver was prepared by the guanidium thiocyanate-phenol-chloroform extraction procedure$^{19}$. The slot blot manifold was used to transfer the RNA on nylon membrane. Transferred RNA on nylon membrane was immobilized by UV-crosslinking. To detect the mRNA transcripts specific to arginase, labelled cDNA of rat liver arginase was used as probe for hybridization$^{18}$. After hybridization, nylon membrane was washed, dried and exposed to detect the signals derived from hybridized probe on the X-ray film.

Results and Discussion

Experimental diabetes is characterized by a fourfold increase in blood glucose level and hypoinsulinemia. About 30% decrease in the insulin level was observed by the oral administration of sodium orthovanadate (0.6 mg/ml) to control rats with no change in the level of glucose$^{13}$. Vanadate treatment restored the normoglycemia without altering the level of insulin in diabetic animals (Table 1).

From the above observations it may be concluded that vanadate mimics insulin action without inducing the insulin secretion. It has been reported earlier that vanadate inhibits the phosphotyrosine phosphatases and maintains the phosphorylation of insulin receptors for longer time, so that the signal can be transduced into the cell even in the presence of minute quantity of insulin$^{19}$.

Liver is an insulin dependent tissue and insulin is required for glucose transport into the hepatic cells; under diabetic and hypoglycemic conditions, glucose transport in the liver is inhibited$^{20}$. The synthesis of glucose, through gluconeogenesis is increased in a diabetic animal. The deamination of amino acids to produce carbon skeleton, which is subsequently used as an energy source, is catalyzed by several enzymes including aminotransferases and glutamate dehydrogenase. The residual ammonium ions are excreted out in the form of urea, catalyzed by the enzyme arginase. The activity of liver arginase increases (by 210%) in diabetes (Fig. 1) which could
Table 1 — Insulin and vanadium levels in plasma of control, diabetic, diabetic rats treated with insulin and diabetic rats treated with vanadate

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Insulin treated diabetic</th>
<th>Vanadate treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>190 ± 8</td>
<td>145 ± 7a</td>
<td>176 ± 8c</td>
<td>163 ± 9a</td>
</tr>
<tr>
<td>Fluid intake (ml/day/rat)</td>
<td>38 ± 3</td>
<td>203 ± 9a</td>
<td>34 ± 3</td>
<td>33 ± 4b</td>
</tr>
<tr>
<td>Vanadate intake (mg/kg/day)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>98 ± 10</td>
</tr>
<tr>
<td>Soluble protein (mg/gm)</td>
<td>132 ± 6</td>
<td>134 ± 10b</td>
<td>143 ± 7c</td>
<td>139 ± 9c</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>113 ± 9</td>
<td>383 ± 43a</td>
<td>129 ± 7a</td>
<td>118 ± 8b</td>
</tr>
<tr>
<td>Plasma insulin (μU/ml)</td>
<td>14.6 ± 1.54</td>
<td>4.15 ± 0.94a</td>
<td>8.48 ± 0.92b</td>
<td>5.07 ± 0.78b</td>
</tr>
<tr>
<td>Vanadium levels (ng/100gm body wt)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>158 ± 36</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 6 to 8 observations. Fisher's values are shown as *p<0.001, bp<0.005, cp<0.001 vs control. Experimental conditions are described in Materials and Methods section. Vanadium levels were found to be too low to be measured in control and diabetic rat liver.

Fig. 2 — The quantitation of liver arginase in the cytosolic fractions of control (C), diabetic (D), diabetic treated with insulin (I, D+I) and diabetic treated with vanadate (V, D+V) rats using ELISA. [The values are expressed as percentage change in optical density]

Fig. 3 — Slot blot assay of liver arginase mRNA in control (C), diabetic (D), diabetic treated with insulin (D+I) and diabetic treated with vanadate (D+V) rats.

activity in diabetic rats is either due to increased concentration of its cofactor, Mn²⁺ or due to the enzyme protein synthesis²⁴. The results of ELISA and slot blot assay in the present study point to the latter factor. ELISA was performed by using the antibodies against human arginase. These antibodies have been used by earlier workers and have been shown to work

be due to the high rate of protein catabolism³,²¹. Vanadate and insulin treatment were found to restore the increased activity nearly to the control values (Fig. 1) probably by reversing the enzymes of gluconeogenesis and glycolysis³⁰. The reversal effects of vanadate and insulin are almost similar and the observation further emphasizes the insulin-mimetic effects of vanadate. Oral administration of Momordica charantia extracts also showed insulin-mimetic effects and reversed the activity of liver arginase in diabetic rats²²,²³. The increased arginase activity in diabetic rats is either due to increased concentration of its cofactor, Mn²⁺ or due to the enzyme protein synthesis²⁴. The results of ELISA and slot blot assay in the present study point to the latter factor. ELISA was performed by using the antibodies against human arginase. These antibodies have been used by earlier workers and have been shown to work
quite well for the detection of rat liver arginase. Fig. 2 shows the increase in the concentration of arginase protein in the liver of diabetic animals as compared to that in the control. The reversal of enzyme activity in treated animals (D+I and D+V) is also confirmed by these results (Fig. 2). Our results show that the mRNA concentration in control, D+I and D+V rat was almost equal whereas in diabetic animals, mRNA concentration was much higher as compared to C, D+I and D+V (Fig. 3). The data presented here shows that the increased activity of arginase in diabetes can be due to increase in arginase specific transcripts suggesting that the arginase gene may be modulated at transcriptional level.

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References