Chapter 5

SYMPATHOADRENAL MODERATION OF PROTEIN METABOLISM, TRANSAMINASES AND TRANSPORT ENZYMES IN RAT KIDNEY

It is generally accepted that in all forms of diabetes, an imbalance exists between the availability of insulin from endogenous sources (B-cell secretion) and the amount required by target tissues to maintain normal balance between disposal and mobilization of carbohydrate, fat and protein. Maintenance of such a balance of metabolic processes requires equalization of the supply of and demand for insulin and other hormones. In maturity onset diabetes, the demand for insulin is increased in the face of diminished (but not exhausted) capacity for B cell secretion. Treatment is thus directed at reducing the demand for insulin by dietary management involving a hypocaloric intake. In circumstances in which these measures fail, stimulation of endogenous insulin secretion may be helpful. If there is an absolute deficiency of insulin, as in juvenile onset diabetes, which will not be improved by such stimulating agents, nor substantially mitigated by dietary management, insulin therapy is the mainstay of management. However, even under optimal conditions, complete normalization of blood glucose levels by conventional administration of exogenous insulin, may never be achieved. Under such conditions, glycemic control may be attained by measures involving reduction of glucose production in the body.

Implication of the sympathetic nervous system and its associative hormones in the pathology of diabetes has led to the hope that suppression of these may be of help in improving the diabetic control. An increased concentration of glucagon, catecholamines, glucocorticoids or growth hormone, relative to insulin is believed to change metabolic processes toward catabolism and away from anabolism. By that it is meant that breakdown
of macromolecules such as glycogen, triglycerides, and protein is accelerated to provide a source of fuels, and the biosynthesis of macromolecules from energy yielding precursor components is reduced.

Glucagon is essential for metabolic homeostasis. Its secretion is responsive to insulin induced hypoglycemia (Unger et al., 1962) and to infusion or ingestion of large loads of amino acids (Assan et al., 1967). Insulin enhances cellular amino acid uptake and inhibits the overall rate of protein breakdown (Lundholm et al., 1981). Insulin administration lowers plasma amino acid concentrations in normal individuals (Schauder et al., 1983). It has been suggested by Unger et al. (1969) that the relative levels of insulin and glucagon are important in fasting and after amino acid challenge. In fasting they have demonstrated elevated plasma levels of glucagon in the presence of lowered insulin levels and have suggested a synergistic effect on glucose production.

Circulating catecholamines clearly influence pancreatic secretion, stimulating glucagon secretion via β-adrenergic receptors (Iverson, 1973) and inhibiting insulin secretion via β-adrenergic receptors (Porte et al., 1966). Flakoll et al. (1994) have reviewed the works of various groups suggesting that hyperglucagonemia is one of the hallmarks exhibited in patients with uncontrolled diabetes, a state often associated with elevated catabolism, loss of tissue protein stores, stimulated hepatic gluconeogenesis, enhanced amino acid oxidation and escalated urea synthesis. Together, these factors are thought to lead to an increased nitrogen loss.

Both sympathetic innervation to the kidney and the hormones could possibly affect the renal protein metabolism. Therefore in the present study, chemical sympathectomy has been performed individually and in combinations with adrenalectomy and vagotomy, to find out derangements in protein metabolism in the kidney, and also to know if any changes occur in transport enzymes in overall movement of materials across the membranes of parenchymatous and tubular cells of the kidney during manipulations of the neural and endocrine systems.

MATERIAL AND METHODS
Male albino rats (Rattus norvegicus albinus) of Charles Foster strain weighing around 150-
200 gm were used for the study. The animals were acclimatized at standard laboratory conditions with food and water *ad libitum*. They were divided into three groups and subjected to different surgical operations and drug treatments.

I  CHEMICAL SYMPATHECTOMY (CSX)
   CONTROL CHEMICAL SYMPATHECTOMY (CSS)

II CHEMICAL SYMPATHECTOMY + VAGOTOMY (CSX + VGX)
   CONTROL CHEMICAL SYMPATHECTOMY + SHAM VAGOTOMY (CSS + VGS)

III CHEMICAL SYMPATHECTOMY + ADRENALECTOMY (CSX + ADX)
   CONTROL CHEMICAL SYMPATHECTOMY + SHAM ADRENALECTOMY (CSS + ADS)

Overnight fasted animals of both experimental and control groups were sacrificed after the completion of respective treatments. The kidney was excised, blotted free of blood and used for preparing various homogenates to carry out the following estimations:

Protein was estimated by the method of Lowry *et al.* (1951) and expressed as mg/100 mg wet tissue. Acid phosphatase and alkaline phosphatase were estimated by the method of Linhardt and Walter (1963), and the activity was estimated as μ moles PNP released / mg protein/ 30 min. Aspartate and alanine transaminases were estimated by the method of Bergmeyer and Bernt (1963) and expressed as Karmen units/ mg protein/ min. Na*-K*-ATPase was estimated by the method of Post and Sen (1967) and expressed as μmoles P released/mg protein / 15 min.

Statistical analysis:
Data were analyzed by employing Student's *t*-test to determine the significance of the data. A level of *P* < 0.05 was considered to be significant.

RESULTS (Tables 5.1, 5.2; Figures 5.1 to 5.4)
In the present study, animals of all the three groups showed an increase in the total protein content of the kidney. The magnitude of the increase was however highest in CSX + ADX being 56 % (*p* < 0.001), followed by CSX (30 %) and lastly CSX + VGX (26 %).
Table 5.1 Protein, transaminases and transport enzymes in rat kidney after Chemical Sympathectomy singly and in combination with Vagotomy and Adrenalectomy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chemical Sympathectomy</th>
<th>Chemical Sympathectomy + Vagotomy</th>
<th>Chemical Sympathectomy + Adrenalectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Experimental</td>
<td>Sham</td>
</tr>
<tr>
<td>Protein (mg/100 mg Wet Tissue)</td>
<td>21.78(\pm 1.26)</td>
<td>28.22(\pm 1.66)</td>
<td>24.92</td>
</tr>
<tr>
<td>Aspartate Aminotransferase (Karmen units/mg Protein/Min.)</td>
<td>102.80</td>
<td>80.00*** (\pm 5.53)</td>
<td>114.00</td>
</tr>
<tr>
<td>Alanine Aminotransferase (Karmen units/mg Protein/Min.)</td>
<td>25.20</td>
<td>13.00*** (\pm 2.13)</td>
<td>22.00</td>
</tr>
<tr>
<td>Acid Phosphatase ((\mu)M PNP released/mg Protein/30 Min.)</td>
<td>1.22</td>
<td>0.92** (\pm 0.09)</td>
<td>1.25</td>
</tr>
<tr>
<td>Alkaline Phosphatase ((\mu)M PNP released/mg Protein/30 Min.)</td>
<td>1.60</td>
<td>1.98** (\pm 0.11)</td>
<td>1.60</td>
</tr>
<tr>
<td>Na⁺-K⁺-ATPase ((\mu)g P released/mg Protein/10 Min.)</td>
<td>6.73</td>
<td>5.91** (\pm 0.24)</td>
<td>6.21</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± SEM of 6 experiments; * p< 0.05; ** p< 0.02; *** p< 0.01; **** p< 0.001.
Table 5.2  Percentage change (compared to controls) in protein, transaminases and transport enzymes in the kidney of rats subjected to chemical sympathectomy singly and in combination with vagotomy and adrenalectomy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sympathectomy</th>
<th>Sympathectomy + Vagotomy</th>
<th>Sympathectomy + Adrenalectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/100 mg Wet Tissue)</td>
<td>30° *** ↑</td>
<td>26 ** ↑</td>
<td>56 **** ↑</td>
</tr>
<tr>
<td>Aspartate Aminotransferase (Karmen units/mg Protein/ Min.)</td>
<td>22 *** ↓</td>
<td>16 ** ↓</td>
<td>28 **** ↓</td>
</tr>
<tr>
<td>Alanine Aminotransferase (Karmen units/mg Protein/ Min.)</td>
<td>48 *** ↓</td>
<td>39 ** ↓</td>
<td>59 **** ↓</td>
</tr>
<tr>
<td>Acid Phosphatase (µM PNP released/mg Protein/30 Min.)</td>
<td>25 ** ↓</td>
<td>11 * ↓</td>
<td>26 *** ↓</td>
</tr>
<tr>
<td>Alkaline Phosphatase (µM PNP released/mg Protein/30 Min.)</td>
<td>24 ** ↑</td>
<td>13 * ↓</td>
<td>30 ** ↑</td>
</tr>
<tr>
<td>Na⁺-K⁺-ATPase (µg P released/mg Protein/10 Min.)</td>
<td>12 ** ↓</td>
<td>19 * ↓</td>
<td>41 *** ↓</td>
</tr>
</tbody>
</table>

°Values corrected to nearest whole number; * p< 0.05; ** p< 0.02; *** p< 0.01; **** p< 0.001
Figure 5.1 Level of Protein and activity of Acid Phosphatase in kidney of rats subjected to chemical sympathectomy (CSX) singly and in combination with vagotomy (VGX) and adrenalectomy (ADX)

(A) Level of Protein

(B) Activity of Acid Phosphatase

* p < 0.05; ** p < 0.02; *** p < 0.01; **** p < 0.001.
Figure 5.2 Activities of Aspartate and Alanine Aminotransferase in kidney of rats subjected to chemical sympathectomy (CSX) singly in combination with vagotomy (VGX) and adrenalectomy (ADX)

(A)

(B)

** p < 0.02; *** p < 0.01; **** p < 0.001.
Figure 5.3 Activities of Alkaline Phosphatase and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in kidney of rats subjected to chemical sympathectomy (CSX) singly and in combination with vagotomy (VGX) and adrenalectomy (ADX)

\[ \text{Alkaline Phosphatase} \]

\[ \mu \text{M PNP released/mg Protein/30 Min.} \]

\[ \text{Na}^+\text{-K}^+\text{-ATPase} \]

\[ \mu \text{g P released/mg Protein/10 Min.} \]

\[ * p < 0.05; ** p < 0.02; *** p < 0.01. \]
Figure 5.4 Percentage change in renal protein content and the activities of transport enzymes and transaminases after sympathectomy alone (A) and in combination with vagotomy (B) and adrenalectomy (C).
Both aspartate and alanine transaminases showed reduced activities in all the groups, the activities being the most significantly reduced in the kidney of the CSX + ADX rats, respectively 28 % and 56 % (p < 0.001). CSX rats also showed a significant reduction of 22 % and 48 %, followed by CSX + VGX (16 % and 39 %).

Acid phosphatase activity was reduced in all the groups, the degree of reduction being the highest of 26 % in the CSX + ADX, (p < 0.01). CSX rats showed a 25 % decrease. In CSX + ADX rats, there was only a small decline of 11 % in the activity.

Alkaline phosphatase activity was seen to rise by 24 % in CSX and and by 30 % in CSX + ADX, whereas it decreased in CSX + VGX rats (13 %).

Na⁺-K⁺-ATPase activity declined in all the three groups, but the magnitude of the decrease was higher in CSX + ADX (p < 0.01) being 41 %. In CSX and CSX + VGX this decrease was not as significant.

DISCUSSION
In the present set of experiment, the animals with chemical sympathectomy (CSX), and the animals with combined chemical sympathectomy and adrenalectomy (CSX + ADX), manifested a metabolic profile which leads to the inference that there is reduced protein metabolism after both the experimental designs manipulating the neuroendocrine system. As reported in chapter 3, chemically sympathectomized rats showed hypoglycemia, which is more significant or pronounced in the animals with chemical sympathectomy and adrenalectomy in combination. Glycogenolytic rate was found to be low, whereas, synthesis and deposition of glycogen increases. Gluconeogenesis slowed down, fat catabolism showing a reduction as evident from increased lipid content of the tissue.

Protein content in the kidney of the animals of all the three groups increased, the rise being the most significant in the CSX + ADX animals, and CSX rats showing an increase of a lesser degree, followed by the CSX + VGX rats. During euglycemia, insulin is known to decrease plasma amino acid concentration, by inhibiting protein breakdown, and by increasing intracellular amino acid transport (Abumrad et al., 1982; Flakoll et al., 1989). Chronic physiologic hyperglucagonemia has been associated with a decrease in insulin mediated
glucose disposal by peripheral tissues (Del Prato et al., 1987). Hyperaminoacidemia is a well
known glucagon secretagogue (Unger, 1971). Glucagon has been shown not only to induce
glycogenolysis in liver, but also to be capable of augmenting gluconeogenesis (Ercan et al.,
1995), in opposition to the effect of insulin in diminishing gluconeogenesis. Relative
hyperglucagonemia combined with increased availability of glucogenic amino acids may result
in a relative resistance to the suppressive effects of insulin on hepatic glucose production.
The catecholamines would also exert a protein catabolic effect. Also, the insulin antagonistic
effect of E has been amply demonstrated in both insulin suppression of HGP and stimulation
of peripheral glucose uptake (Deibert and DeFronzo, 1980; Rizza et al., 1980; Lager et al.,
1986). Thus the lack of protein breakdown in all the three conditions could be because of the
absence of the catabolic influence of the catecholamines, glucagon and glucocorticoids, which
have been removed by the experimental maneuver of the respective system.

In the experimental groups with CSX and CSX + ADX, there could be a supplementary rise in the parasympathetic tone resulting in an increased insulin secretion as reported by Yadav (1997). Also, even if there is a regular / normal insulin secretory rate, it would have a more pronounced effect on the metabolic procedure, because of the want of the counteracting sympathetic system. Therefore, the lesser content of protein in the kidney of rats with CSX + VGX as compared to the CSX and CSX + ADX groups could be because of the absence of the influence of insulin due to vagotomy.

The increased protein content of the tissue was also explainable from the activities of the enzymes involved in amino acid conversion to glucose precursors, namely the transaminases. Aspartate aminotransferase showed reduced activity in the kidney in all the three groups, being most significantly reduced in the CSX + ADX group, followed by CSX rats and with yet lesser degree of reduction in the CSX + VGX group. The activity of this enzyme is regulated by glucocorticoids, insulin and cAMP in kidney and liver (Pave-Preux et al., 1990). Insulin which is known to prevent the effect of glucocorticoids on AST, decreased the AST mRNA transcription (Pave-Preux et al., 1990). In all the three groups, the kidney showed a decreased activity of this enzyme. Probably the elimination of the hormones that enhance the activity of this enzyme justifies the reduced rate of aspartate transamination. Davis et al. (1935) have reported increased plasma levels of epinephrine, norepinephrine, corticosteroids and glucagon in response to hypoglycemia. Increased rates
of gluconeogenesis usually occur when there are low circulating levels of insulin. In response to fasting, when the insulin level falls, an increased gluconeogenic rate is observed (Wasserman et al., 1989). Reduced insulin level after CSX + VGX (Yadav, 1997) could be the reason for a lesser degree of reduction in the activity of aspartate transaminase in this group. Contrarily, it has been demonstrated in vitro, that insulin represses the activity of PEPCK, a key gluconeogenic enzyme (Exton, 1972) indicating that hyperinsulinemia usually inhibits gluconeogenesis. In the present study, a decrease of its activity in the CSX and CSX + ADX rats could be because of increased insulin levels in these conditions (Yadav, 1997), in addition to the lowered catecholamines and corticosteroids (Chapter 6).

The activity of the other transaminase, the alanine transaminase was seen to decrease in all the three groups. The trend of the decrease in the activity in different groups was similar to that of the AST, being the most significant in CSX + ADX, followed by CSX and CSX + VGX. Alanine is the pre-eminent gluconeogenic amino acid (Rodwell, 1985), 30% alanine carbon entering the circulation as a result of direct release from tissue protein (Roberts et al., 1982; Darmaun et al., 1988). This alanine can then be transported to liver and kidney for production of glucose (Chochinov et al., 1978; Chiasson et al., 1979). It is well established that glucocorticoid hormones regulate the hepatic gluconeogenic pathway and that amino acid transport is a rate-determining step for the pathway (Exton, 1972). Glucocorticoids also amplify the multifocal effects of glucagon on the pathway (Exton, 1972). Quinlan et al. (1982) have shown that glucocorticoid administration permits the enhanced alanine uptake activity brought about by starvation as seen in non-adrenalectomized rats. ADX abolishes increased transport activity of L-alanine, whereas glucocorticoid administration restores it. Decreased uptake of alanine and hence less transamination occurs, as can be seen in the animals with CSX + ADX, which register decreased transamination from alanine showing decreased ALT activity. Abolishment of the sympathetic system in the other two treatments, would result in decreased glucagon and CA secretion, and also deficient glucocorticoid release, because of the loss of the sympathetic control of the adrenal cortex; and therefore reduced ALT activity.

In the rats with CSX, CSX + VGX and CSX + ADX, acid phosphatase showed a diminished activity, indicating that there is reduced proteolysis in all the three conditions, and supporting the increased protein content of the tissue. Intracellular protein breakdown is
believed to play a major role in regulating cytoplasmic protein content (Hutson and Mortimore, 1982) and the supply of amino acid for gluconeogenesis and other metabolic pathways (Schworer and Mortimore, 1979). It is under moment to moment control by amino acid (Schworer et al., 1981), insulin (Mortimore and Mondon, 1970; Schworer et al., 1981) and glucagon (Schworer and Mortimore, 1979). Autophagy and proteolysis are enhanced by amino acid or insulin lack and reversed when these agents are replaced (Mortimore and Schworer, 1980). It has been demonstrated that glucagon induces proteolytic and autophagic responses which are equivalent to those produced by stringent amino acid deprivation (Schworer and Mortimore, 1979). Inhibition of glucagon secretion following sympathectomy could reverse this response, resulting in a reduced activity of this enzyme.

Alkaline phosphatase, the enzyme associated with carbohydrate metabolism, by means of its capacity of aiding glucose uptake from renal tubules, was seen to increase in the animals with CSX and CSX + ADX. This change in the activity of the enzyme led to the inference that, there was an increased uptake of glucose in the kidney tubules. Capaldo et al. (1992) have reported E to antagonize insulin induced glucose uptake. Studies on isolated cells have shown that E inhibits insulin induced translocation of glucose transporters from the intracellular pool to the cell membrane (Kashiwagi, 1983; Smith et al., 1984). In the animals with CSX + VGX, a reduction is seen in the activity of this enzyme, indicating a decrease in, glucose uptake, a justification to the slight hyperglycemia observed in the animals of this group.

The efferent renal nerves are capable of directly influencing renal tubular sodium reabsorption independent of changes in systemic or renal hemodynamics or hormones such as mineralocorticoids, angiotensin II and prostaglandins. Increases and decreases in efferent renal sympathetic nerve activity are associated with parallel changes in renal tubular sodium excretion. These neural influences are mediated by α-adrenoreceptors located on PCT and possibly DCT as well (DiBona and Sawin, 1982). In CSX, CSX + VGX and CSX + ADX rats, the Na⁺-K⁺-ATPase activity was observed to be reduced, the degree of the reduction being less in CSX + VGX. This indicates reduced Na⁺ flux. Beach et al. (1987) have shown that NE increases Na⁺-K⁺-ATPase and Na⁺ flux in rabbit proximal tubules. After chemical sympathectomy by guanethidine, NE elimination would be resulting in the reverse or opposite effect. Gill and Bartter (1966) demonstrated that normal subjects in whom autonomic
insufficiency had been produced by guanethidine administration were unable to lower daily urinary sodium excretion sufficiently to avoid negative external sodium balance in response to a reduction in dietary sodium intake despite decreases in GFR. Besides, in the CSX + ADX rats, there is an additional deficiency of aldosterone, which increases the biosynthesis of mRNA encoding for Na⁺-K⁺-ATPase in the A₉ renal cells (Verrey et al., 1987). The reason for a smaller reduction in the CSX + VGX rat kidney could be the effect produced from vagotomy.

The findings throw significant light on the multihormonal regulatory system which controls these processes. Decrease in protein breakdown in the animals of two groups CSX and CSX + ADX, suggests that this decrease would be in large measure due to insulin influence on these pathways unopposed by the catecholamines, and glucocorticoids. In the CSX + VGX animals, although there is a lack of insulin, the effect of the absence of the protein mobilizing hormones is not overridden, and the slight hyperglycemia which has been the consequence of this treatment, is not due to gluconeogenesis through protein derived precursors.