Several endocrine glands are involved in maintaining relatively constant states (homeostasis) internal fluid environment of the body. They generally operate through nervous system in affecting quick responses to a large variety of exteroceptive and interoceptive stimuli. In order to do so, they must be subjected to complex control systems which regulate their rate of output of secretions. Neural signals can contribute to the regulation of blood glucose level, both by having direct effects on tissues that produce or consume glucose and through the control of hormonal secretion (Wada et al., 1995).

Hormonal control of glucose flux occurs at three levels. The first involves regulation of substrate supply. The second level deals with the very significant but relatively slow adaptive changes in enzyme activity through the regulation of protein synthesis or degradation. The third level is concerned with the minute-to-minute regulation of gluconeogenesis. The hormones such as glucagon, insulin and catecholamines play the most significant role in regulation of the glucostatic pathway.

Insulin has broad influences within body and acts directly or indirectly to affect many kinds of biochemical processes (Smith and Davis, 1983). An increase in blood glucose is the main signal for insulin secretion by pancreatic beta cells (Taylor and Agius, 1988; Unger, 1991; McGarry, 1992). Glucose transport and metabolism in the beta cells are necessary for both prestored insulin release and insulin synthesis de novo (Meglasson and Matschinsky, 1986; Unger, 1991). Thus, an overall effect of the hormone is to facilitate the uptake and
utilization of glucose by the cells and to prevent the excessive breakdown of glycogen (Pisters et al., 1991).

The pancreatic hormones have well established roles to play in the regulation of metabolic homeostasis, which for many years was thought about entirely in terms of interactions between pancreatic endocrine cells and circulating substrates. Today, however it is known that several non-pancreatic hormones and neural factors also contribute heavily to the control mechanisms governing the activity of endocrine pancreas (Gerich et al., 1976; Smith and Porte, 1976; Smith et al., 1979; Woods et al., 1980; Miller, 1981). Hormone producing cells of the islets are adequately supplied with autonomic nerve endings. Neural transmitters are released from nerve terminals of the ANS on the islet cells and acetylcholine and norepinephrine stimulates insulin and glucagon release via cholinergic and β-adrenergic receptors, respectively. Thus, the release of insulin and glucagon is through a coordinated mechanism. There are many different afferent and efferent signals integrated within the central nervous system that are neurally mediated or mediated via substrates (eg. glucose) or hormones (eg. insulin), which ultimately modulate hormonal secretion (Rohner-Jeanrenaud, 1983).

Previous investigations have shown that the chemical sympathectomy induced by guanethidine sulphate have been exclusively morphological (Heath and Burnstock, 1977) and largely concerned with its influence on the cell body rather than the axon (Zochodne et al., 1988). The physiological consequences of chronic guanethidine sulphate administration on somatic sympathetic and parasympathetic conduction have received only limited attention. Two possibilities require consideration. First, whether the effect of drug alters the control of hormonal secretion and second, does the depletion of somatic peripheral nerve norepinephrine content influence physiological functions? To understand the mechanism underlying the interaction between the parasympatho and sympathoadrenal system on insulin profile the present study has been carried out. Insulin titre was measured after performing subdiaphragmatic vagotomy, bilateral adrenalectomy and chemical sympathectomy.

MATERIAL AND METHODS

Adult male albino rats of Charles Foster strain weighing around 120-150 gms were taken as the experimental model for the present study. Rats maintained in standard laboratory
conditions with *ad libitum* food and water were divided into twelve groups with five animals in each group. Various surgical operations and drug treatments of respective groups were executed in the similar manner as discussed in chapter materials and methods. Post-operation care were taken and animals were maintained in stress free environment.

GROUP I  VAGOTOMY (VGX)
GROUP II  SHAM VAGOTOMY (VGS)
GROUP III ADRENALECTOMY (ADX)
GROUP IV  SHAM ADRENALECTOMY (ADS)
GROUP V  VAGOTOMY + ADRENALECTOMY (VGX + ADX)
GROUP VI  SHAM VAGOTOMY + SHAM ADRENALECTOMY (VGS + ADS)
GROUP VII CHEMICAL SYMPATHECTOMY (CSX)
GROUP VIII CONTROL CHEMICAL SYMPATHECTOMY (CSS)
GROUP IX CHEMICAL SYMPATHECTOMY + VAGOTOMY (CSX + VGX)
GROUP X  CONTROL CHEMICAL SYMPATHECTOMY + SHAM VAGOTOMY (CSX + VGS)
GROUP XI CHEMICAL SYMPATHECTOMY + ADRENALECTOMY (CSX + ADX)
GROUP XII CONTROL CHEMICAL SYMPATHECTOMY + SHAM ADRENALECTOMY (CSS + ADS)

After respective treatments, overnight fasted animals were sacrificed under mild anaesthesia. Blood was collected immediately by puncturing the jugular vein. All care were taken to avoid hemolysis and other contaminations. Blood was centrifuged in cold centrifuge at 2000rpm at -10°C for 20 minutes and serum collected was stored in frozen condition prior to the analysis.

Serum insulin level was estimated after respective treatments using tube ELISA kit from Boeringer Mannheim, Germany. Insulin concentration (µU/ml) of the samples were determined by reading off against the respective absorbance value of the calibration curve obtained from the standards.

**Statistical Analysis**

All the results are expressed as mean ± SE. And, statistical comparisons of means were made using Student's paired 't' test p value <0.05 was considered as statistically significant.
Table 6.1 Serum insulin level in rats subjected to parasympathetic and sympatho-adrenal manipulation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin (μU/ml serum)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Merchad</td>
<td>Experimental</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>21.39±1.26</td>
<td>16.55±0.65***</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>21.33±1.13</td>
<td>27.65±2.06*</td>
</tr>
<tr>
<td>Vagotomy + Adrenalectomy</td>
<td>21.06±1.09</td>
<td>16.44±0.75***</td>
</tr>
<tr>
<td>Chemical Sympathectomy</td>
<td>19.69±0.72</td>
<td>24.49±1.09***</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Adrenalectomy</td>
<td>20.38±1.15</td>
<td>31.93±1.93****</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Vagotomy</td>
<td>21.09±1.28</td>
<td>16.92±0.61**</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 6.2  Percentage change (compared to controls) in serum Insulin level in rats subjected to autonomic and adrenal manipulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagotomcy</td>
<td>23&lt;sup&gt;**&lt;/sup&gt; *** ↓</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>30&lt;sup&gt;*&lt;/sup&gt; ↑</td>
</tr>
<tr>
<td>Vagotomcy + Adrenalectomy</td>
<td>22 *** ↓</td>
</tr>
<tr>
<td>Sympathectomy</td>
<td>24 *** ↑</td>
</tr>
<tr>
<td>Sympathectomy + Adrenalectomy</td>
<td>57 **** ↑</td>
</tr>
<tr>
<td>Sympathectomy + Vagotomcy</td>
<td>20 ** ↓</td>
</tr>
</tbody>
</table>

<sup>®</sup> Values corrected to nearest whole number; <sup>*</sup> p< 0.05; <sup>**</sup> p< 0.02; <sup>***</sup> p< 0.01; <sup>****</sup> p< 0.001.
Figure 6.1 Serum insulin level in rats subjected to parasympathetic and sympathoadrenal manipulation.

* p < 0.05; ** p < 0.02; *** p < 0.01; **** p < 0.001.
Figure 6.2 Percentage change in serum insulin level in rats subjected to vagotomy (VGX), adrenalectomy (ADX), sympathectomy (CSX) and their combinations.
RESULTS

Table 6.1, 6.2 and Figure 6.1, 6.2 illustrates the average plasma insulin concentrations and percentage change measured in control and experimental groups of rats in response to various treatments (VGX, ADX, VGX + ADX, CSX, CSX + ADX and CSX + VGX, respectively).

Serum insulin level decreased significantly ($p<0.01$) in vagotomized rats after 48hrs. Similarly, 22% decline in insulin level was noticed in rats subjected to vagotomy and adrenalectomy in combination (Table 6.2). In these animals, in response to suppressed parasympathetic tone and deprived cortical and medullary hormones insulin level decreased to $16.44 \pm 0.75$ from $21.06 \pm 1.09$ of control animals (Table 6.1). But, the reverse was the condition in adrenalectomized rats. In these rats serum insulin level increased by 30% compared to sham operated rats (Figure 6.2).

On the contrary, suppression of sympathetic tone by injecting guanethidine sulphate resulted a significant increase in insulin concentration ($p<0.01$). This increase in insulin level was more evident in CSX + ADX rats ($p<0.001$; Figure 6.1). But, rats treated for chemical sympathectomy and vagotomy together showed a significant decrease in serum insulin concentration from $21.09 \pm 1.28$ to $16.92 \pm 0.61$ ($p<0.02$).

DISCUSSION

In the present study, involvement of autonomic nervous system and adrenal hormones in the maintenance of homeostasis through their effect on insulin secretion has been highlighted. In response to fasting and prolonged exercise, when the insulin level falls, an increased gluconeogenic rate is observed (Wasserman et al., 1989; Davis et al., 1995). Similarly, an acute selective insulin deficiency following vagotomy bring about a prompt hyperglycaemia by increasing hepatic glucose production and decreasing glucose clearance by the target tissues (Cherrington et al., 1978). The hyperglycaemic condition caused by decreased circulating insulin levels also results in significant increase in glucagon level (Rao et al., 1995), which could activate glucose mobilizing mechanisms. Even, studies by Marette and Bukowiecki, (1991) has proposed that noradrenaline in the absence of insulin stimulates both glucose transport and utilization and that in addition, it increases insulin sensitivity. An acute increase in corticosterone (Sule, 1997) in conjunction with simultaneous increase in glucagon or epinephrine can have additive or synergistic effects on carbohydrate metabolism (Goldstein...
et al., 1995). This has confirmed the presence of vagal-insulin axis and suggest that the secretion of insulin can be influenced by vagus nerves. In other words, vagal stimulation will be promptly responded by B cells to release insulin.

Hypoglycaemia is a severe complication of insulin dependent diabetes mellitus (IDDM), especially in those treated with intensive insulin therapy (Cryer et al., 1989; Orskov et al., 1991; Gardner et al., 1993). Hypoglycaemia is associated with impaired glucagon release (Gardner et al., 1993; Orskov et al., 1991) and hepatic glucose production (Hetenyi et al., 1989; Orskov et al., 1991) and also excess insulin release. In vivo and in vitro observations by Exton (1972) has pointed out that hyperinsulinaemia usually inhibits gluconeogenesis. One exception to this general rule occurs during prolonged hypoglycaemia (Caprio et al., 1988; Frizzell et al., 1988; Lecavalier et al., 1989).

The stimulation of insulin secretion by the B cells is partly achieved by blood substrates (glucose, amino acids etc.) (Woods and Porte, 1974). Insulin secretion is also modulated (i.e. stimulated or inhibited) by hormones arising from other cell types of the islets of Langerhans and by several other hormones of a gastrointestinal origin (Rohner-Jeanrenaud et al., 1983). A central nervous system controlled neural regulatory system finally intervenes to tune the output of endocrine pancreatic hormones i.e. the parasympathetic and sympathetic systems (Woods and Porte, 1974).

Apart from the above discussed interplay between vagus and insulin secretion and their control on glycaemic state, even adrenal gland have a multifactorial glucoregulatory mechanisms through its cortical and medullary hormones directly, through their effect on the target tissues and indirectly through their influence on the hormonal secretion of other endocrine glands. In response to stress and diabetic condition, plasma epinephrine and cortisol levels increases (Christensen, 1974; Walker et al., 1989). Studies by Shamoon et al. (1980) have demonstrated that acute hypercortisolemia and hypercatecholaminemia decreased glucose clearance by decreasing insulin level and leading to an increased plasma glucose level.

However, catecholamines are known to cause a significant reduction in plasma insulin levels (Porte and Woods, 1990) And glucocorticoids are known to induce hepatic insulin resistance when administered in vivo (Issekutz and Allen, 1972) and have been known to
cause insulin resistance at both the receptor and postreceptor levels in adipocytes and fibroblasts \textit{in vitro} (Carter-Su and Okamoto, 1985; Nelson and Murray, 1987). But, bilateral adrenalectomy nullifies the respective effects of glucocorticoids and catecholamine on insulin. Enhanced insulin concentration in these rats (ADX rats) initiates a cascade of events culminating in activation of glucose uptake.

The individuals treated for both vagotomy and adrenalectomy together have low insulin and adrenal cortical hormone concentration (Sule, 1997). Catecholamine reduction following adrenalectomy rescind its suppressive impact on insulin, which in turn could be the cause of reduced insulin level. Moreover, subdued parasympathetic tone along with adrenalectomy also enhances the reduction of insulin level. The undisturbed sympathetic tone manifests its effect on homeostatic flux, thereby resulting marginal hyperglycaemia (chapter 2). It has been shown that prolonged hyperglycaemia can induce altered sensitivity to pancreatic A and B cells to glucose in healthy humans (De Fronzo \textit{et al.}, 1985).

Apart from the parasympathetic influence, the sympathetic system also have a remarkable effect on insulin flux. More recently, the importance of adrenergic mechanisms to whole body glucose economy has been thoroughly appreciated in a human model with prolonged hypoglycaemia (DeFeo, \textit{et al.}, 1991a; Fanelli, \textit{et al.}, 1992). Combined alpha and beta adrenergic blockade accentuated the severity of insulin hypoglycaemia and this occurred as a consequence of two mechanisms: i) inhibition of the rebound increase in hepatic glucose production that normally occurs despite sustained hyperinsulinaemia (Sacca \textit{et al.}, 1979b) and ii) potentiation of insulin action on tissue glucose uptake.

Guanethidine sulphate induced chemical sympathectomy resulted in an increased serum insulin concentration over four weeks period of time. This shows that, in these animals the glucoregulatory mechanisms are steered by the parasympathetic component of the ANS. Acetylcholine, the parasympathetic transmitter causes the release of insulin from B cells \textit{in vivo} and \textit{in vitro} (Iversen, 1973; Campfield and Smith, 1983; Berthoud, 1984). In adult rat, high doses of insulin causing profound hypoglycaemia (Lautt, 1980b) are capable of activating peripheral pathways involving release of adrenal epinephrine, which may then act at the pituitary level, through $\beta_2$ adrenergic receptors as reported by Mezey \textit{et al.} (1984). But
adrenergic blockade by guanethidine hinders the activation of peripheral pathways, thereby, hypoglycaemia in these rats could be the manifestation of high serum insulin level.

Extensive evidence are there that show that epinephrine counteracts both insulin suppression of hepatic glucose production and stimulation of peripheral glucose uptake (Rizza et al., 1980a; Lager et al., 1986). Glucocorticoids impair the stimulation by insulin of whole-body glucose uptake and oxidation, and of non-oxidative glucose disposal in vivo (Nosadini et al., 1983; Baron et al., 1987; McMahon et al., 1988). However, the response to insulin is less dependent on the presence of NE (Gaillet et al., 1991).

Hypoglycaemic condition in individuals treated for chemical sympathectomy and adrenalectomy together (chapter 4) could be due to complete depletion of cortical and medullary hormones and suppressed sympathetic tone. Lack of adrenal hormones following adrenalectomy along with chemical sympathectomy nullifies the counterregulatory effect of catecholamines and cortical hormones in maintaining the glucose homeostasis. In these rats, the glycaemic control is mainly under the influence undisturbed persisting parasympathetic tone. This is revealed by the present observation of significant increase in serum insulin level in CSX + ADX group of rats and in turn a drastic hypoglycaemia. Insulin may counteract the actions of other glucose mobilizing hormones by its ability to decrease the cAMP concentration (Martin, 1985). It also antagonizes the gluconeogenic and glycolytic effects of glucagon and other agents (Ito et al., 1995) and thus could be able to manifest low blood sugar level.

Rats subjected to suppressed sympathetic and parasympathetic tone (CSX + VGX) have direct neural inhibitory influence on pancreatic hormone secretion. The insulin level in these rat has decreased prominently but the corticosterone level has increased (Sule, 1997), which simultaneously could be the reason for marginal hyperglycaemia (chapter 4). The mild hyperglycaemic response to surgical trauma in rats is controlled by a dual control system whereby the presence of either intact adrenals or hepatic sympathetic nerves will allow a normal response to occur. In these rats, the glycaemic control is mainly through the adrenal hormones. The hyperglycaemic effects of the catecholamine involve both stimulation of hepatic glucose production and limitation of gluconeogenesis Studies by Lautt and Cote (1977) have also revealed the importance of adrenal catecholamine in resulting hyperglycaemic state.
in response to surgery under full anesthesia. It has been illustrated in rats treated for chemical sympathectomy and vagotomy together that the hyperglycaemic state developed due to the suppression of parasympathetic tone in vagotomized rats can be corrected to some extent, by performing chemical sympathectomy (Chapter 4). This could be due to lowered insulin concentration.

It can be concluded that substantial neurally stimulated insulin release emphasizes the important role of the nervous system in the control of metabolism. Also, the insulin releasing capacity of acetylcholine (Burr et al., 1976; Holst et al., 1981) or vagal stimulation (Bloom and Edward, 1981) depends on the plasma glucose concentration. Thus, parasympatho-pancreatic and sympatho-pancreatic interaction plays an important role in the regulating glucostatic metabolic pathways. In addition to this, present study demonstrates the complex mechanism regulating insulin's interaction with the autonomic-adrenomedullary counterregulatory mechanisms.