Chapter 6

INTERACTIONS OF THE AUTONOMIC NERVOUS SYSTEM AND THE PITUITARY-ADRENOCORTICAL AXIS IN THE REGULATION OF BLOOD SUGAR LEVEL

The metabolic regulation of the body is maintained in general by two main systems, the autonomic nervous system and the endocrine system; these two in turn are functionally interdependent, each one affecting the activity of the other, either by stimulation or inhibition. Amongst the multiple functions that these systems govern, the regulation of the blood glucose level is of utmost importance for the existence of an animal. Glucose being the main metabolic fuel of the brain, it is essential that its concentration in the blood is maintained at an optimum. Various endocrine glands with their products and in conjunction with the autonomic nervous system act to keep the glucose concentration within the physiological range. The glands involved include the anterior pituitary, the pancreas and the adrenal. These glands with their secretions, viz. adrenocorticotropic hormone, growth hormone, insulin glucagon, catecholamines and glucocorticoids, regulate the blood glucose level. Of these many hormone systems, in the present study, an effort has been made to elucidate the secretory patterns of the hypothalamo-pituitary-adrenocortical axis in various states of manipulations of the autonomic nervous system and endocrine glands.

Kleitman and Holzwarth (1985a) have demonstrated adrenergic nerves in the rat adrenal cortex, and Kesse et al. (1988) have shown specifically both pre and post ganglionic sympathetic fibers to innervate structures within the cortex, especially the zona glomerulosa. Greene (1959) has shown a possible branch of the vagus to innervate the adrenal gland after it merges with the celiac plexus. The hypothalamo-pituitary-adrenocortical axis and autonomic nervous systems interact in complex ways to maintain homeostasis: 1) exposure
to stressors often increases hypothalamo-pituitary-adrenocortical and sympathoneural outflows concurrently; 2) central administration of corticotrophin releasing factor (CRF) evokes large increases in plasma levels of adrenocorticotropic hormone (ACTH) and catecholamines (CA); 3) ACTH (probably via adrenal corticosteroids) increases activities of dopamine-β-hydroxylase and PNMT, enhancing the capacity to synthesize norepinephrine (NE) and convert NE to epinephrine (E); 4) steroids generally inhibit extraneuronal uptake of CA and 5) they augment β-adrenoceptor-mediated processes (Collins et al., 1988; Szemeredi et al., 1989). Conversely, catecholaminergic pathways in the brain contribute to ACTH release (Al-Damluji, 1988) and β-adrenoceptor agonists increase (Axelrod and Reisine, 1984) or decrease (Eisenhofer et al., 1987) pituitary ACTH secretion. Thus the manifold interactions of the sympathoneural system and the pituitary adrenocortical system are found to manifest in response to stress and other physiological situations.

Glucocorticoids exert a wide variety of physiological effects, including inhibition of inflammatory responses and have wide therapeutic applications. Most tissues in the body appear to possess glucocorticoid receptors (Cake and Litwak, 1975). For a number of these tissues, glucocorticoid are necessary for the full expression of a hormone-induced cellular response. These steroids 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). They interact with several aspects of the sympathoadrenal system, such as enhancing the actions of CA by increasing the synthesis and affinity of the β-adrenoreceptor (Harrison et al., 1968; Davies et al., 1981; Malbon and Hadcock, 1988). They also enhance the synthesis of CA in various tissues, including lungs, heart and skeletal muscle (Kennedy and Ziegler, 1990; 1991; Kennedy et al., 1993). They also affect gluconeogenesis by increasing the supply of gluconeogenic precursors (Goldstein et al., 1995) by causing protein degradation (Bolander et al., 1981) and lipolysis (DeBodo and Altzuler, 1958).

The adenohypophysial hormone ACTH is required for maintenance and functioning of adrenal cortex. It stimulates the growth of the adrenal gland in vivo. An elevated concentration of ACTH causes enlargement of the gland and increases the synthesis and content of DNA in adrenal cortex (Liddle et al., 1962; Masui and Garren, 1970). A key role in transduction of ACTH signal leading to differentiation of the adrenocortical cells is played by cAMP (Arola et al., 1993).
It is now well recognized that the adenohypophyseal secretion of ACTH is regulated in a complex manner, which besides corticotropin releasing factor may involve epinephrine also (Axelrod and Reisine, 1984). CRF action can be modulated by a direct stimulatory action of peripheral E mediated by β-adrenoreceptors (Axelrod and Reisine, 1984). Insulin hypoglycemia has been proposed to activate ACTH release via a direct effect of peripheral catecholamines on anterior pituitary through β-adrenergic receptors (Mezey et al., 1984).

Parasympathetic denervation would have direct and indirect influence through the insulin level, on the adrenal gland secretion. By carrying out vagotomy, this effect can be achieved, as cholinergic denervation caused decreased insulin secretion (Yadav, 1997). Sympathectomy would provide a method of evaluating the contribution of the sympathoneural system in the regulation of the CORT-ACTH negative feedback loop. Guanethidine induces a species specific adrenergic sympathectomy (Jensen-Holm, 1967; Burnstock et al., 1971; Eranko and Eranko, 1971), destroying adrenergic neurons and leaving cholinergic systems completely intact (Jensen-Holm and Juul, 1971; Angeletti et al., 1972). Adrenal medulla is spared despite uptake of the drug (Johnson and Manning, 1984) and guanethidine does not cross the blood brain barrier (BBB), sparing central noradrenergic neurons (Furst, 1967). It destroys peripheral sympathetic nerves, when administered chronically to newborn rats in high doses (Mills et al., 1986). Plasma CA levels are known to be lowered following guanethidine treatment (Lo et al., 1991). Since adrenal is at the center of this glucocorticoid-ACTH loop, adrenalectomy would allow to observe the direct effects of any neuroendocrine manipulation on this pathway.

As the stimulation of adrenocortical steroidogenesis is the most important action of ACTH, the estimation of both ACTH and corticosteroid level is necessary to assess pituitary adrenocorticotropic function. Vagotomy, adrenalectomy, chemical sympathectomy and various combinations of these maneuvers have been carried out in the present study in order to investigate the interactions between the autonomic nervous system and the ACTH-corticosterone association. The present study has been designed to observe the effects of insulinopenia due to vagotomy and adrenal hormone loss by adrenalectomy, to test the effects of chronic sympathectomy on adrenocortical activity and to determine to what extent activation of sympathetic adrenal innervation participates in the regulation of corticotrophin-adrenocortical hormone system.
MATERIAL AND METHODS

Male albino rats (*Rattus norvegicus albinus*) of Charles Foster strain, weighing between 150-200 gm were used for the study. The animals were acclimatized for one week under standard laboratory conditions (12:12 L:D) and were handled regularly before the experiments. They were divided into six groups and were subjected to the following treatments and surgeries:

I  VAGOTOMY (VGX)
   SHAM VAGOTOMY (VGS)

II ADRENALECTOMY (ADX)
    SHAM ADRENALECTOMY (ADS)

III VAGOTOMY + ADRENALECTOMY (VGX + ADX)
    SHAM VAGOTOMY + SHAM ADRENALECTOMY (VGS + ADS)

IV CHEMICAL SYMPATHECTOMY (CSX)
    CONTROL CHEMICAL SYMPATHECTOMY (CSS)

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

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

After respective surgery or drug treatment, the overnight fasted animals were given mild anesthesia and sacrificed. Blood was collected from the jugular vein, was allowed to clot for an hour and then centrifuged at 3-4° C to obtain clear serum which was used for estimating the hormones corticosterone and ACTH.

Corticosterone was estimated by Enzyme Immuno Assay by using an EIA kit from Biomerica (CA, USA), and the concentration was expressed as μg/dl. ACTH was estimated by Radio Immuno Assay by using RIA kit from Diagnostics Systems Laboratories Inc. (TX, USA), and the concentration was expressed as pg/ml. The procedures for the assays have been described in detail in Material and Methods.
Table 6.1  Serum corticosterone and Adrenocorticotrophic Hormone (ACTH) levels in rats subjected to autonomic and adrenal manipulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone (µg/dl)</th>
<th>ACTH (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Experimental</td>
</tr>
<tr>
<td>Vagotomay</td>
<td>1.53*± 0.067</td>
<td>1.94***± 0.103</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>1.49± 0.063</td>
<td>0.91****± 0.039</td>
</tr>
<tr>
<td>Vagotomy + Adrenalectomy</td>
<td>1.57± 0.067</td>
<td>0.93****± 0.05</td>
</tr>
<tr>
<td>Chemical Sympathectomy</td>
<td>1.51± 0.064</td>
<td>1.02***± 0.074</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Vagotomy</td>
<td>1.45± 0.059</td>
<td>1.66*± 0.067</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Adrenalectomy</td>
<td>1.52± 0.065</td>
<td>0.87****± 0.052</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 corticosterone and Adrenocorticotrophic Hormone (ACTH) levels in rats subjected to vagotomy, adrenalectomy and chemical sympathectomy singly and in combinations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone</th>
<th>ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagotomy</td>
<td>27° *** ↑</td>
<td>21 ** ↓</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>39 **** ↓</td>
<td>32 *** ↑</td>
</tr>
<tr>
<td>Vagotomy + Adrenalectomy</td>
<td>41 **** ↓</td>
<td>26 ** ↑</td>
</tr>
<tr>
<td>Sympathectomy</td>
<td>32 *** ↓</td>
<td>39 *** ↑</td>
</tr>
<tr>
<td>Sympathectomy + Vagotomy</td>
<td>15 * ↑</td>
<td>24 * ↑</td>
</tr>
<tr>
<td>Sympathectomy + Adrenalectomy</td>
<td>43 **** ↓</td>
<td>49 **** ↑</td>
</tr>
</tbody>
</table>

° Values corrected to nearest whole number; * p< 0.05; ** p< 0.02; *** p< 0.01; **** p< 0.001.
Figure 6.1 Levels of serum corticosterone and adrenocorticotropin (ACTH) in rats subjected to vagotomy (A) and adrenalectomy (B).

** p < 0.02; *** p < 0.01; **** p < 0.001.
Figure 6.2 Levels of serum corticosterone and adrenocorticotropin (ACTH) in rats subjected to vagotomy and adrenalectomy together (A), and chemical sympathectomy (B).

** p < 0.02; *** p < 0.01; **** p < 0.001.

** p < 0.02; *** p < 0.01; **** p < 0.001.
Figure 6.3 Levels of serum corticosterone and adrenocorticotropin (ACTH) in rats subjected to chemical sympathectomy together with vagotomy (A), and adrenalectomy (B).

* p < 0.05; **** p < 0.001.
Figure 6.4 Percentage change in serum corticosterone (A) and adrenocorticotropin levels (B) in rats subjected to vagotony (VGX), adrenalectomy (ADX), sympathectomy (CSX), and their combinations.
Statistical Analysis:
Data are expressed as means ± standard error of the mean. Analysis was done by Student's t-test and the level of statistical significance was considered to be $p < 0.05$.

RESULTS (Tables 6.1, 6.2; Figures 6.1 to 6.4)
In vagotomized animals corticosterone (CORT) level was found to increase by 27% ($1.53 \pm 0.067$ to $1.94 \pm 0.103$, $p < 0.01$) while a 21% decrease in ACTH con. ($50.58 \pm 2.32$ to $39.77 \pm 2.81$, $p < 0.02$) was observed in these animals. In the animals operated for adrenalectomy, CORT decreased very significantly by 39% ($1.49 \pm 0.063$ to $0.91 \pm 0.039$, $p < 0.001$), while a 32% increase was noted in ACTH concentration ($49.86 \pm 2.32$ to $65.86$, $p < 0.01$). Similarly, in animals with combined vagotomy and adrenalectomy, CORT level decreased markedly, being 41% ($1.57 \pm 0.067$ to $0.93 \pm 0.05$, $p < 0.001$). Conversely, ACTH increased by 26% ($49.46 \pm 2.26$ to $62.48$, $p < 0.02$). The chemically sympathectomized animals showed a 32% reduction in CORT level ($1.51 \pm 0.064$ to $1.02 \pm 0.074$, $p < 0.01$) and a 39% increased ACTH ($47.85 \pm 2.24$ to $66.53 \pm 3.44$, $P < 0.01$) concentration. In the animals with chemical sympathectomy and vagotomy, both CORT ($1.45 \pm 0.059$ to $1.66 \pm 0.067$, $p < 0.05$) and ACTH levels ($49.54 \pm 2.89$ to $61.65 \pm 3.37$, $p < 0.05$) increased, respectively by 15% and 24%. In the animals with chemical sympathectomy and adrenalectomy together, the CORT concentration was found to decrease sharply by 43% ($1.52 \pm 0.065$ to $0.87 \pm 0.052$, $p < 0.001$), whereas ACTH concentration showed a reciprocal hike of 49% ($51.37 \pm 2.57$ to $76.69 \pm 4.09$, $p < 0.001$).

DISCUSSION
In the present study, some of the mechanisms involved the interactions of the autonomic nervous system and the pituitary-adrenocortical axis, were investigated.

In the first set, in vagotomized rats, a major part of the parasympathetic innervation was curtailed by means of sectioning the vagi subdiaphragmatically. The corticosterone concentration in the serum of these rats showed an increase. The concentration of its governing hormone action ACTH showed a decrease. Vagotomy represents a true pathologic situation of insulinopenia. Insulin level has been shown to decrease in VGX animals (Wasserman et al., 1989a; Yadav, 1997) Loss of insulin after vagotomy, leads to a decreased response of ACTH. This could be through the negative feedback loop due to increased
corticosterone level after vagotomy. Cortisol is known to increase during diabetes (Walker et al., 1989). In this situation it could be acting with the elevated levels of catecholamines and glucagon to elevate the plasma glucose level. In this experimental condition, the acute hyperglycemia which was observed (Chapter 2) could have been facilitated by this rise in the corticosterone. The metabolic profile of the kidney which tilts towards catabolism (Chapters 2, 3), might be a contribution of this accelerated production and release of the glucocorticoid into circulation after vagotomy.

The serum corticosterone level was observed to decrease in ADX animals, whereas the ACTH concentration increased. Glucocorticoids are important inhibitory regulators of the hypothalamo-pituitary-adrenocortical system. It is known that the corticotroph itself is a site at which glucocorticoids act to inhibit synthesis and secretion (Sayers and Portanova, 1974). The fall of the plasma corticosterone concentration abolishes the normal suppressive effect of corticosterone on the release of ACTH. ACTH secretion becomes excessive and can be detected easily in peripheral blood. In the absence of the usual target organ, there is a lack of the normal secretory response. After the removal of endogenous glucocorticoids by ADX, pituitary ACTH synthesis and secretion increase (Dallman et al., 1972), as do the levels of CRF mRNA in the paraventricular nucleus of the hypothalamus (Jingami et al., 1986) and immunoreactive CRF concentrations in hypophysial portal plasma (Plotsky and Sawchenko, 1987). These increases were reversed by subsequent treatment with synthetic glucocorticoid dexamethasone, or corticosterone (Levin et al., 1988). It has been discussed in detail by Dallman et al. (1985), that in the intact rat removal of corticosteroids by adrenalectomy (ADX) results in marked increases in transcription of ACTH gene and the synthesis and secretion of ACTH, and that these effects of ADX are reversed or prevented by treatment with glucocorticoids. Adrenalectomy resulted in a hypoglycemic state and the renal metabolism was depressed (Chapters 2, 3). Persistence of insulin because intact vagal discharge and lack of the glucocorticoids, could be accounted for this.

In the VGX + ADX rats, a marked decrease was observed in the corticosterone level, whereas, the ACTH concentration increased. Ablation of adrenal resulted in the loss of the corticosteroids, and subsequently, a rise in the ACTH secretion which could have been due
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to the removal of the inhibitory influence of the glucocorticoid. This state of the
adrenocortical secretion would help produce the marginal hyperglycemia and the partially
accelerated renal metabolism (Chapters 2, 3).

In the CSX animals, the serum CORT level was lower (p < 0.05) than the control
value but understandably not as markedly reduced as in the ADX animals. In the
sympathectomized animals, the adrenal gland is present. However, since the sympathetic
system is absent, the neural stimulus required for the secretion of the adrenocortical steroids
is not available. In addition to the splanchnic innervation, the adrenal cortex of adult rats
receives autonomic input from the medulla (Vinson et al., 1994). The occurrence of β-
receptors on the adrenal cortical cells has been reported (Shima et al., 1984). Moreover, it
has been proposed that the sympatho-adrenal system has a role to play in local paracrine
regulation of the adrenal cortex (Bornstein et al., 1990; Charlton, 1990). Epinephrine has
been shown to stimulate the release of cortisol and aldosterone in a cAMP dependent manner
(Walker et al., 1988). The effect can be blocked by propranolol suggesting the involvement
of β-receptors (Kawamura et al., 1984). Butler et al. (1994) have shown an 85% decrease in
plasma NE levels in sympathectomized rats than in saline treated controls. Other attempts
at CSX have yielded similar results. Guanethidine treatment reduced plasma NE levels by
70% in stressed Sprague-Dawley rats (Kvetnansky et al., 1979) and by more than 80% in
normotensive SD rats (Lo et al., 1991). Chronic guanethidine treatment has been
demonstrated to affect adrenocortical activity and reduce corticosterone secretion in adult
rats (Kleitman and Holzwarth, 1985), a result that is compatible to what obtained in the
present study.

Using isolated perfused pig adrenals with preserved nerve supply, it has been shown
that splanchnic nerve activation causing stimulatory effect may be mediated by chromaffin
cells in a paracrine manner, since perfusion of the adrenals with CA also provoked a
significant release of corticosteroids (Bornstein and Ehrhart-Bornstein, 1992). The ACTH
concentration in the CSX animals is higher than the control value and this increase is as
significant as the concentration in the ADX animals. The reason for this could be that the
removal of the sympathetic system leaves only the ACTH in effect which in turn is secreted
excessively. Several studies have supported a stimulatory effect of E on the hypothalamic-
pituitary-adrenal axis (Tilders et al., 1982). Spinedi et al. (1988) also support a stimulatory
role of E on central CRF levels and ACTH release. Besides, stimuli such as insulin induced hypoglycemia (Fisher and Brown, 1980) can evoke significant secretion of E, which may also facilitate ACTH release via β-adrenergic receptors (Tilders et al., 1982). In the present situation, sympathectomy resulting in an elevated insulin secretion (Yadav, 1997) might have facilitated the rise in ACTH secretion. The reduced corticosterone in the animals of this set might also be one of the factors accountable for the hypoglycemia and the decreased renal metabolism (Chapters 4, 5).

In chemically sympathectomized rats with a simultaneous vagotomy, corticosterone concentration was seen to rise. Simultaneously, the ACTH concentration also increased. In this experimental setup, the ACTH concentration increased, in response to chemical sympathectomy. Though there is chemical sympathectomy in CSX + VGX rats, corticosterone was seen to rise, which seems to be contrary to the results of sympathectomy. However, this increase could be due to the increase in the ACTH concentration. In the CSX + VGX condition, the only control existing over the adrenal secretion would be of the ACTH, and hence the rise in the concentration of corticosterone and ACTH in the serum of the CSX + VGX rats. This rise in the corticosterone would aid the marginal hyperglycemic condition of the rats of this set and the partially elevated renal metabolic rate (Chapters 3, 4).

In the CSX + ADX animals, the serum corticosterone level was observed to decrease significantly. Here, peripheral sympathetic nerves were chemically ablated by guanethidine and total plasma catecholamine supply was curtailed by the removal of the adrenal glands were removed. The possible contribution of neuronal and humoral CA could then be investigated. The inhibition of the sympathetic system and the removal of the adrenal itself led to a decreased corticosterone level. The ACTH concentration showed a significant rise. The only governing factor in the system that remained would be ACTH. Low corticosterone levels induce a negative feedback and therefore a rise in the ACTH concentration is only expected. In sympathectomized rats with adrenalectomy, the NE levels were found to be lower than in ADX rats (Butler et al. 1994). Mezey et al., (1984) have reported that insulin injection to adult rats with pituitary stalk transection or with lesion of the median eminence resulted in increased ACTH secretion that was blocked by prior treatment with propranolol, suggesting that hypoglycemia stimulates ACTH release by a mechanism in which CA of peripheral origin act directly on the anterior pituitary. Grino and Oliver (1992) have
demonstrated that CA, acting through $\beta_2$-adrenergic receptors have a stimulatory effect on HPA axis during insulin induced hypoglycemic rats. Adrenal sensitivity to ACTH and the resulting corticosterone production in the adult rat is regulated by ACTH secretion and under stress conditions it could also be influenced by the level of splanchnic activity to the adrenal gland (Dallman et al., 1985). The paradoxical results obtained in the present situation of CSX and CSX + ADX showing ACTH rise could be because, brain catecholamines are not affected by guanethidine as it does not cross the blood brain barrier (Furst, 1967).

It is evident that the adaptive mechanisms at work in the anterior lobe with manipulations of the autonomic nervous system can be complex, involving changes in biosynthesis, content and releasability of ACTH and corticosteroids. These changes may permit the gland to meet physiological requirement in response to changes in the ambient internal milieu. In general, rats in which vagotomy was carried out singly, and in combination with chemical sympathectomy, glucocorticoid secretion was found to increase. This condition is akin to the insulinopenic condition comparable to diabetes and the pituitary-adrenal profile in that situation. In all the conditions in which the adrenal gland has been removed, a significant decrease was noted in the glucocorticoid secretion; the decrease being equally significant in ADX alone, and in combination, and also more significant than CSX alone. Therefore manipulation of secretion of the adrenal gland alone could be competent enough to check the hypercortisolism often found in diabetes.