AUTONOMIC NERVOUS SYSTEM AND GROWTH HORMONE SECRETION AND THEIR COMBINED EFFECT ON BLOOD SUGAR LEVEL IN RATS

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Abstract: Growth hormone in addition to its growth promoting function has many other important biological activities. It exerts its influence on the glucose metabolism through its anti insulin and glucose releasing activities. Growth hormone mobilizes glucose from the carbohydrate and noncarbohydrate sources in the body. Its effect on protein metabolism is anabolic in nature. In the present investigation, GH profiles were estimated in various conditions of neural and endocrine manoeuvres by eliminating the different components of the autonomic nervous system, alone and in different combinations.

Vagotomized (VGX) rats manifested an increased GH level, as also did the animals with combinations of vagotomy with sympathectomy (CSX+VGX) and adrenalectomy (VGX+ADX). However, the latter two conditions did not show a very significant increase. CSX rats manifested a decrease in the level of GH in the serum. Rats with ADX and CSX+ADX showed a very pronounced decrease in GH levels.

The increase in GH levels in groups where vagotomy was performed (VGX, VGX+ADX, CSX+VGX) indicates that vagal denervation seems to have a major effect on the status of this hormone. The growth hormone increase in these animals also coincides with hyperglycaemia. The decrease observed in CSX, ADX and CSX+ADX conditions could be because of the removal of catecholamines and glucocorticoids which have a stimulatory effect on the secretion of this hormone. These animals showed a marked decrease in blood glucose level. It is therefore concluded that rats with CSX+ADX combination are able to restrain the rise in GH level more efficiently than either CSX or ADX alone. The growth hormone level is also correlated with glucose level.

Key words: Vagotomy, Adrenalectomy, Sympathectomy, Growth hormone, Glucoregulation.

INTRODUCTION

Studies of the mechanisms involved in the regulation of secretion and the functions of growth hormone (GH) are difficult as GH, unlike other pituitary tropic hormones, does not elicit specific activation in a single target peripheral gland or organ. However, recent development of sensitive and specific immunoassays for GH has allowed studies of the hormone in varying physiological states.

Pituitary GH secretion is under the control of the hypothalamus, where several neurogenic, hormonal and metabolic signals are integrated to modulate the secretion of two hypothalamic peptides, growth hormone releasing hormone (GHRH), a GH releasing hormone and somatostatin (SS), a GH release inhibiting hormone. GHRH stimulates the secretion of GH from the anterior pituitary. Topographical analysis of the neural regulation of GH release carried out by electrical stimulation of certain structures in the central nervous system (Bernardis and Frohman, 1971; Martin et al., 1975) revealed that the ventromedial nucleus of the hypothalamus (VMH), an area sensitive to
hypoglycaemia and causing hunger during hypoglycaemic state and the arcuate nucleus (ARC) are important structures in enhancing GH release. The GH release is inhibited by somatotrophin release inhibiting factor (SRIF). In the lateral hypothalamic area (LHA), scattered immunoreactive SRIF cells are known to be present (Makara et al., 1983). The stimulation of LHA would excite the SRIF fibres directly and this would result in the release of SRIF into the hypophysial portal blood which in turn suppresses GH release induced by GRF. The mechanism by which hypoglycaemia induces a substantial release of pituitary GH has not been elucidated. Abrams et al. (1964) have demonstrated in male squirrel monkeys that chronic lesions in the anterior ventral hypothalamus significantly reduce the GH response to hypoglycaemia. It is tempting to speculate that hypoglycaemia increases GH secretion by stimulating glucose receptors in the CNS, which in turn trigger the release of GHRH.

Growth hormone, in addition to having body growth promoting action, has insulin-like activities which can result in transient hypoglycaemia, promotes increased glucose and amino acid transport and metabolism in tissues (Knobil et al., 1961; Honeyman and Goodman, 1980) on one hand, and on the other hand bring about chronic and prolonged anti-insulin activities that can result in hyperglycaemia, hyperinsulinemia, increased lipolysis (Goodman and Schwartz, 1974) and decreased glucose metabolism (Davidson, 1987).

By and large, GH has anti-insulin like activity. Hypoglycaemia has been found to be a potent stimulus for the release of growth hormone (Roth et al., 1963a). Prolonged fasting also raises the growth hormone level, and administration of glucose may result in a decrease of the hormone level in plasma. Roth et al. (1963b) have observed that GH release is in response to low glucose concentration in plasma, fasting and muscular exercise.

It is generally believed that among the above mentioned effects, the principal physiological action of GH is the anti-insulin activity, which results in hyperglycaemia (Davidson, 1987). Therefore, as one of the glucose counter-regulatory hormones, GH has been believed to influence the manifestation of diabetes mellitus (Schaper, 1990). Perhaps the most prominent action of GH in this respect could be inducing insulin resistance in tissues (Press et al., 1984). Increased GH and catecholamine levels are noticed in Insulin Dependent Diabetic Mellitus (IDDM) during periods of poor metabolic regulation (Hayford et al., 1980). Even glucocorticoids have been observed to influence GH release (Wehrenberg et al., 1990).

In an attempt to find a comprehensive picture of regulation of glucose by nerves and hormones a series of studies have been initiated. In this paper the GH concentration was studied in rats subjected to parasympathectomy (vagotomy), chemical sympathectomy and adrenalectomy and combinations of these.

**MATERIALS 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 (12L : 12D) and were divided into various groups as in table A.

**Surgical procedures**

**I. Vagotomy (VGX):** The rats were subjected to bilateral subdiaphragmatic vagotomy. Under ether anesthesia, 3 cm midventral incision was made directly posterior to the xiphisternum. A piece of each vagus, ventral and dorsal to oesophagus was snipped off. Appropriate post operational care was taken. The animals were considered to be vagotomized only after the observation that at 48 hrs the stomach was distended with partially digested food.

**Sham vagotomy (VGS):** In these animals, sham operations were performed with vagi being only lifted at the subdiaphragmatic level and then allowed to drop.
back intact in their normal positions.

II. Adrenalectomy (ADX): The animals of this group were subjected to bilateral adrenalectomy. They were anaesthetized with ether. A 2cm dorso-lateral incision was made on one side, posterior to the rib cage. The kidney was located and the adrenal on its dorsal side was separated from the tissue surrounding it and was gently lifted out. The process was repeated on the other side. The animals were supplemented with 0.9% saline.

Sham adrenalectomy (ADS): Animals were sham operated by lifting the adrenal and allowing it to fall back in its place intact.

III. Vagotomy + adrenalectomy (VGX+ADX): The animals were subjected to both vagotomy and adrenalectomy. Subdiaphragmatic bilateral vagotomy was executed through a midventral incision as in the case of Group I animals. Bilateral adrenalectomy was done through two dorsolateral incisions as in the case with Group II rats. Both were performed in the same animals. The animals were supplemented with 0.9% saline.

Sham vagotomy + sham adrenalectomy (VGS+ADS): The animals of this group were sham operated. Both the ventral and dorsal vagi were lifted and kept back without sectioning them. The adrenals were also like wise lifted and released back in their normal positions.

IV. Chemical sympathectomy (CSX): Chemical sympathectomy was induced by injecting guanethidine sulphate (1-(2-guanidinoethyl)octa hydroazine) from Sigma Chemical Co., USA. The drug powder was dissolved in 0.9% physiological saline (pH 10.2). The dose of 50mg/kg body weight was administered intraperitoneally daily over a period of 28 days.

Control chemical sympathectomy (CSS): Control animals received 0.9% saline for 28 days, administered daily intraperitoneally.

V. Chemical sympathectomy + vagotomy (CSX+VGX): The animals of this group were subjected to chemical sympathectomy by administering guanethidine sulphate (Sigma Chem. Co., USA), as in Group IV, for 28 days. Before 48 hrs of completion of treatment of guanethidine, the animals were subjected to subdiaphragmatic vagotomy through a midventral incision.

Control chemical sympathectomy + sham vagotomy (CSS+VGS): These control animals were injected with 0.9% saline i.p. for 28 days. Forty eight hours before the completion of the treatment, a sham operation for vagotomy was executed through a midventral incision, by lifting both the vagi and releasing them back to their original positions without sectioning.

VI. Chemical sympathectomy + adrenalectomy (CSX+ADX): In this group, chemically sympathectomized animals were subjected to bilateral adrenalectomy. The animals were given guanethidine sulphate (Sigma Chem. Co., USA) as in Group IV. 28 days after the treatment of guanethidine the animals were adrenalectomized following the procedure described for Group II.

Control chemical sympathectomy + sham adrenalectomy (CSS+ADS): The animals of this group were controls, being administered only the vehicle for 28 days. On 28th day, sham adrenalectomy was performed by lifting the adrenals and keeping them back in their original positions.

Hormone assay: After the respective treatment, the overnight fasted animals were given mild anaesthesia and sacrificed. Blood was collected by puncturing the jugular vein and allowed to clot for an hour. It was then centrifuged at 3-4°C to obtain clear serum which was used for estimating Growth Hormone. GH was estimated by Double Antibody Radio Immuno Assay using a kit from Diagnostic Products Corporation (CA, USA). The concentration of GH in the serum is expressed as ng/ml.

Statistical analysis: Data were analyzed by Student’s ‘t’ test and the level of significance was considered to be P<0.05.

RESULTS

GH profiles of the animals with various surgeries and drug treatments showed considerable variability. Vagotomized (VGX) rats manifested an increased concentration of GH, the hike being 37% (108.20±5.32 to 148±6.01, P<0.001). Adrenalectomized (ADX) rats showed a 29% decline in GH level in the serum (111.80±6.51 to 73.20±4.03, P<0.001). In the rats with vagotomy and adrenalectomy together (VGX+ADX), a small increase of 18 % was observed (107.60±5.16 to 127.20±6.53, P<0.05). Animals with chemical sympathectomy (CSX) showed a 21% reduction in the level
of GH (115.80±5.76 to 91.20±4.58, P<0.01), the animals with combined treatment for chemical sympathectomy and vagotomy (CSX+VGX) showed a 21% rise in the GH level (109.40±5.11 to 132.80±6.13, P<0.02), whereas, the animals with combined treatment for chemical sympathectomy and adrenalectomy (CSX+ADX) registered a marked reduction of 35% in the concentration of GH in the serum (107.40±5.21 to 76.00±3.41, P<0.001).

**DISCUSSION**

The most significant observation in these experiments was that vagotomy alone or in combination with adrenalectomy or chemical sympathectomy caused an increased GH release whereas chemical sympathectomy, adrenalectomy or chemical sympathectomy together with adrenalectomy produced a decrease in GH titre in the plasma. Absence of vagal tone is a potent stimulus for GH release probably due to elevated glucose level prevalent in such animals (Yadav et al., 1997). It is known that GH secretion is affected by metabolic and nutritional perturbations (Frohman et al., 1992), glucose level in plasma being the foremost factor. The diabetogenic and insulin-antagonistic properties of GH are well documented. Chronically elevated GH level in humans or animals can lead to glucose intolerance, insulin resistance and diabetes mellitus (Gerich, 1984; Press et al., 1984). Physiological concentrations of exogenous GH have also been shown to antagonize insulin action in humans (Bratusch-Marrain et al., 1982; Rizza et al., 1982; Sherwin et al., 1983; Fowelin et al., 1993) whereas chronic GH deficiency is typically associated with hypersensitivity to insulin (Altszuler, 1974). In diabetic animals, hypophysectomy leads to decreased hyperglycaemia and glucosuria (Scow, 1963; Altszuler, 1974) and increased glucose utilization in adipose tissue and liver (Goodman and MacDonald, 1969). Endogenous GH might contribute to hyperglycaemia and insulin resistance in diabetes by suppressing tissue responses to the low ambient levels of endogenous insulin and / or by insulin independent mechanisms. Serum glucose concentration showed a 10% decrease in diabetic rats treated with ArGH (Tatro and Schwartz, 1987). In the present situation in rats where vagotomy caused a lowering of insulin level (Pilo and Yadav, 2004) and a simultaneous hyperglycaemia (Yadav et al., 1997), GH was also observed to increase which in turn produced hyperglycaemia. Isaacs et al. (1987) opined that insulin can decrease rat GH synthesis and secretion and rat GH mRNA synthesis. Thus it could be assumed that removal of vagal tone and resultant reduction in insulin secretion could produce an elevated GH level in vagotomized rats. When vagotomy is combined with chemical sympathectomy or adrenalectomy only a very

### Table 1: Serum Growth Hormone level in rats subjected to parasympathetic and sympatho-adrenal manipulation. Percentage increase (↑) or decrease (↓) in experimental rats over the sham/control rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Hormone (ng/ml)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagotomy (VGX)</td>
<td>108.20±5.32</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomy (ADX)</td>
<td>107.40±5.21</td>
<td></td>
</tr>
<tr>
<td>Vagotomy + Adrenalectomy (VGX+ADX)</td>
<td>107.60±5.16</td>
<td>37 ↑</td>
</tr>
<tr>
<td>Chemical Sympathectomy (CSX)</td>
<td>115.80±5.76</td>
<td></td>
</tr>
<tr>
<td>Chemical Sympathectomy + Vagotomy (CSX+VGX)</td>
<td>109.40±5.11</td>
<td>21 ↑</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Adrenalectomy (CSX+ADX)</td>
<td>111.80±6.51</td>
<td>35 ↓</td>
</tr>
</tbody>
</table>

* * P < 0.05; ** P < 0.02; *** P < 0.01; **** P < 0.001

### Table 2: Serum glucose in rats subjected to parasympathetic and sympatho-adrenal manipulation. Percentage increase (↑) or decrease (↓) in experimental rats over the sham/control rats. (cited from Pilo and Sule, 2004)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose (mg/dl serum)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagotomy (VGX)</td>
<td>134.68±10.92</td>
<td>62 ↑</td>
</tr>
<tr>
<td>Adrenalectomy (ADX)</td>
<td>129.63±6.64</td>
<td>26 ↓</td>
</tr>
<tr>
<td>Vagotomy + Adrenalectomy (VGX+ADX)</td>
<td>120.68±6.42</td>
<td>22 ↑</td>
</tr>
<tr>
<td>Chemical Sympathectomy (CSX)</td>
<td>123.19±8.20</td>
<td>28 ↑</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Vagotomy (CSX+VGX)</td>
<td>118.03±7.75</td>
<td>24 ↑</td>
</tr>
<tr>
<td>Chemical Sympathectomy + Adrenalectomy (CSX+ADX)</td>
<td>121.02±8.95</td>
<td>44 ↑</td>
</tr>
</tbody>
</table>

* * P < 0.05; ** P < 0.02; *** P < 0.01; **** P < 0.001
moderate increase in glycaemic level as well as GH level in the plasma. Removal of vagal tone has been counteracted by sympathectomy and adrenalectomy thereby the glucose level did not increase very much.

Adrenalectomy alone or together with sympathectomy lowered the GH level in the blood. Removal of corticosteroids especially corticosterone in adrenalectomy (chronic hypoadrenalism) has been shown to be associated with decreased GH responsiveness to pharmacologic stimuli both rats (Wehrenberg et al., 1983) and man (Guistina et al., 1989a; b). Patients with idiopathic ACTH deficiency have impaired GH responses to arginine, L-dopa and insulin induced hypoglycaemia, which are reversible during glucocorticoid replacement therapy (Guistina et al., 1989a; b). The present observation also indicates that the decrease in GH an ADX rats is due to absence of stimulatory effect of adrenal corticosteroids. Bancroft (1981) has reported from studies on pituitary tumour cell line that glucocorticoids are known to stimulate the cellular production of GH. A moderate glucocorticoid excess augments spontaneous pituitary GH secretion in normal humans (Casanueva et al., 1990).

Sympathectomy alone or together with adrenalectomy produced a significant reduction in GH level. In these experimental animals, the absence of catecholamines is expected to be the prominent feature. It is known that β adrenergic agents stimulate the release of GH (Perkins et al., 1983; Krieg et al., 1986; Cronin et al., 1982).

In conclusion it could be stated that sympathetic action and adrenal hormones facilitate the release of GH. Parasympathetic actions on the other hand inhibit the release of GH. The result of increased or decreased GH level is felt on the glucose level in the blood. When GH level is increased, the glucose level is also very high and the low GH level has a corresponding low glucose level.

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REFERENCES