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

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Abstract: Autonomic nervous system together with endocrine glands plays in an intricate manner a significant role in the regulation of glucose homoeostasis. The two divisions of autonomic nervous system have opposing actions while several hormones have similar antagonistic actions. The interplay of all these neural and endocrine regulatory mechanisms and the balance of the actions of them finally fine tune the glycaemic level during various physiological states. Many times the action one autonomic system or a hormone depends upon the presence or absence of the opposing factor. The present study presents an account of the effect of autonomic nervous system and adrenal ablations on the corticosterone and corticotrophin (ACTH).

Vagotomy (VGX) showed an increase in the corticosterone level whereas ACTH concentration in the serum decreased. In the adrenalectomized rats, a steep decline in corticosterone concentration in serum was accompanied with an increased ACTH level. In the rats which were adrenalectomized and vagotomized together, a similar trend was observed. Chemical sympathectomy (CSX) reduced the corticosterone concentration slightly but the ACTH level increased significantly. When sympathectomy was combined with vagotomy (CSX+VGX) an increase in both corticosterone and ACTH levels was observed. Chemical sympathectomy and adrenalectomy (CSX+ADX) also produced a maximum decrease in corticosterone level and a maximum level of ACTH. Vagotomy (VGX) alone or together with CSX or ADX produced hyperglycaemia while chemical sympathectomy alone or together with adrenalectomy produced hypoglycaemia.

In conclusion it could be stated that in rats in which vagotomy was performed singly and in combination with sympathectomy, glucocorticoid secretion increased along with increased corticosterone. Chemical sympathectomy alone or together with adrenalectomy reduced the corticosterone concentration in blood together with hypoglycaemic condition. ACTH concentration by and large was more when corticosterone secretion was decreased.

Key words: Chemical sympathectomy, Vagotomy, Guanethidine, Corticosterone, ACTH, Blood sugar

INTRODUCTION

There are several functions that are regulated by the autonomic nervous system and endocrine system either by activation or inhibition of various reactions or by acting against one another or through synergism. 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 brain, it is essential that its concentration in blood is maintained at an optimum level. Various endocrine secretions in conjunction with autonomic nervous system act to keep the glucose concentration within the physiological range. Anterior pituitary, thyroid, pancreas and adrenal are involved in the glucoregulatory mechanisms. Functions of each of these endocrine glands are in turn are regulated, coordinated or orchestrated by nerves system.

Kleitman and Holzwarth (1985a) have demonstrated the presence of adrenergic nerves in rat adrenal and Kesse et al. (1988) have shown innervation by both pre- and post ganglionic sympathetic nerve fibres 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 to complex ways to maintain homeostasis: 1) exposure to stressors often increases hypothalamo-pituitary-adrenocortical and sympathoneural outflows concurrently; 2) central administration of corticotrophin releasing hormone or factor (CRH or CRF) evokes large increases
in plasma levels of adrenocorticotropic hormone (ACTH) and catecholamines (CA) and 3) ACTH (probably via adrenal corticosteroids) increases activities of dopamine –α-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 α-adrenoreceptor-mediated processes (Collins et al., 1988; Szemeredi et al., 1989). Conversely, catecholaminergic pathways in brain contribute to ACTH release (Al-Damluji, 1988) and α-adrenoreceptor 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.

It is now well recognized that the adrenohypophysal secretion of ACTH is regulated in a complex manner, which besides corticotrophin 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 α-adrenoreceptors (Axelrod and Reisine, 1984). Insulin hypoglycaemia has been proposed to activate ACTH release via a direct effect of peripheral catecholamines on anterior pituitary through α-adrenergic receptors (Mezey et al., 1983).

The adrenohypophysal 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).

Glucocorticoids exert a wide variety of physiological effects, which include 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, glucocorticoids 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 intact with several aspects of the sympathoadrenal system, such as enhancing the actions of the CA by increasing the synthesis and affinity of the α-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 Zeigler, 1990; 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).

Parasympathetic denervation caused a decreased insulin secretion (Pilo and Yadav, 2003) and how this is affecting adrenal gland secretion directly or through hypothalamic–pituitary axis that release adrenocorticotropic hormone (ACTH) can be assessed by carrying out vagotomy. Sympathectomy would provide an opportunity for evaluating the contribution of this autonomic division in controlling the release of adrenocorticoids. 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 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). Adrenalectomy removes both cortical and medullary hormones.

Estimations of glucocorticoid and ACTH were carried out in vagotomized, sympathectomized and adrenalectomized rats as well as combinations of these surgical and chemical ablations to understand the interactions of sympathetic and parasympathetic nervous system with adrenal functions either directly or through hypothalamus.

**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.

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Surgical Procedures

I. Vagotomy (VGX): The rats were subjected to bilateral subdiaphragmatic vagotomy. Under ether anaesthesia, 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 is 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 29 days.

Control Chemical Sympathectomy (CSS): 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.

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 treatment 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 and Glucose estimation: After respective surgery or drug treatment, the overnight fasted animals were given mild anaesthesia and sacrificed. Blood was collected from the jugular vein. A portion of the samples was used for Glucose estimation by glucose oxidase method and expressed as mg glucose / 100 mg blood, and the rest 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 was estimated by Enzyme Immuno Assay by using an EIA kit from Biomerica (CA, USA), and the concentration was expressed as ìg/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.

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

RESULTS

In vagotomized animals corticosterone (CORT) level was found to increase by 27% (1.53±0.067 to 1.94±0.103, p<0.01) while a 21% decrease in ACTH level (50.58±2.32 to 39.77±2.81, p<0.02) was observed.
in these animals. In the animals in which adrenalectomy was performed, CORT decreased very significantly by 39% (1.49±0.063 to 0.91±0.039, p< 0.001), while a 32% increase was noted in ACTH concentration (49.86±2.32 to 65.86±3.85 p<0.01). Similarly, in animals with combined vagotomy and adrenalectomy, CORT level decreased markedly by 41% (1.57 ± 0.067 to 0.93±0.05; p<0.001). Conversely, ACTH increased by 26% in these animals. The chemically sympathectomized rats showed a 32% reduction in CORT level but ACTH concentration showed a 39% increase. When chemical sympathectomy and vagotomy was performed together, the CORT level and ACTH level increased by 15% and 24 % respectively. In the animals with chemical sympathectomy and adrenalectomy together, the CORT concentration was found to decrease sharply by 43%, whereas ACTH concentration showed a reciprocal hike in the level by 49% over that of the control animals.

**DISCUSSION**

Subdiaphragmatic vagotomy in rats removes a major part of parasympathetic innervation especially in visceral region. Vagus nerves of parasympathetic division contain both the afferent and efferent fibres and hence the ablation of the nerve eliminates information flow to CNS as well as the motor influence on innervating visceral organs. Vagus nerve conducts several information such as those regarding digestive tract status and activities and glucostatic information. Such information is used for homeostatic regulations within the body and also for regulating hunger and satiety feelings.

Vagotomy caused an increase in corticosterone level while ACTH showed a decrease. Vagotomy caused an increase in glycaemic level (Sule et al., 1996, 1997) while insulin level too registered a lowered value (Pilo and Yadav, 2003). Stimuli such as insulin induced hypoglycaemia (Fisher and Brown, 1980) can evoke significant secretion of E, which may also facilitate ACTH release via β-adrenergic receptors (Tilders et al., 1982). Conditions such as hyperglycaemia and hypoinsulinemia found in vagotomy were also accompanied by an increased corticosterone level in plasma. Glucocorticoid levels are known to increase during diabetes (Walker et al., 1989). ACTH level, on the other hand, decreased in level, understandably due to feed back inhibition by increased corticosterone.

Adrenalectomy as expected reduced the corticosterone concentration in the plasma and an immediate response was the elevation of ACTH release as an attempt to compensate. The removal of endogenous glucocorticoids by adrenalectomy, pituitary ACTH synthesis and secretion increase (Dallman et al., 1972) as do the levels of CRF concentration in hypophyseal portal blood (Plotsky and Sawchenko, 1987). This increase in ACTH was reversed by subsequent treatment with synthetic glucocorticoid dexamethosone or corticosterone (Levin et al., 1988; Dallman et al., 1985). Adrenalectomy when combined with vagotomy (ADX+VGX) the corticos-
terone level was decreased with a concomitant increase in ACTH. In this experiment vagotomy has not affected the increased the glucocorticoid release.

In the chemically sympathectomized rats corticosterone level was lower than the control values but understandably not as markedly reduced as in the ADX rats as the adrenal gland was still intact. However, since the sympathetic stimulus needed for the release of adrenal (both cortical and medullary) hormones was absent the steroid hormone level was found to be low. Adrenergic stimulation of cortical secretion was already reported (Shima et al., 1984; Vinson et al., 1994). 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; Bornstein and Ehrhart-Bornstein, 1992; 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 propanolol suggesting the involvement of α-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 (Kventnansky 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, 1985b), a result that is compatible to what obtained in the present study. The ACTH in CSX rats was lower than the control indicating that adrenergic activation is more pronounced than the activation by pituitary through ACTH.

In chemically sympathectomized rats with simultaneous vagotomy, both corticosterone and ACTH concentrations were seen to rise very moderately. The removal of vagal tone could be stimulatory for the release of Corticosterone form the adrenal cortex. This increase in corticosterone is in spite of the high glycaemic level found in CSX+VGX rats. It is not clear whether the hyperglycaemic level was the result of high level of cortisol or the other way round.

In the CSX+ADX rats the serum corticosterone level was observed to decrease significantly. In these rats the NE and E from both sympathetic nerve endings and adrenal medulla was curtailed by guanethidine treatment and adrenalectomy. The decrease in corticosterone level was maximum in these rats. Like wise the increase in ACTH concentration was also maximum. These rats also showed maximum reduction in glycaemic level. Grino and Oliver (1992) have demonstrated that catecholamines, acting through α-adrenergic receptors have a stimulatory effect on hypothalamic-pituitary-adrenal axis during insulin induced hypoglycaemic rats.

The result indicates that the glycaemic control is the result of interplay of parasympathetic and sympathetic autonomic nervous systems and hormones from pancreas, adrenal, thyroid and pituitary. The levels of the activity of the nervous system and concentration of various hormones could affects the blood sugar level while the changes in glycaemic levels could influence the activities of nervous system and release of hormones.

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