Volume : 3 | Issue : 11 | November 2014 • ISSN No 2277 - 8179

Research Paper

EFFECTS OF CHRONIC HYPERTENSION CAUSED BY REPEATED EPISODES OF AIR JET STRESS ON THE PLASMA LEVELS OF TSH,T3 AND T4 IN WISTAR RATS

Biology

KEYWORDS : hypertension; air jet stress; thyroid hormones; rats

Nessaibia Issam* Laboratoire de Neuro-endocrinologie Appliquée, Département de Biologie, Université Badji Mokhtar, BP 12, 23000, Annaba, Algeria. * Corresponding author

Chouba Ibtissem[a] Laboratoire de Neuro-endocrinologie Appliquée, Département de Biologie, Université Badji Mokhtar, BP 12, 23000, Annaba, Algeria.

Tahraoui Abdelkrim[a] Laboratoire de Neuro-endocrinologie Appliquée, Département de Biologie, Université Badji Mokhtar, BP 12, 23000, Annaba, Algeria.

ABSTRACT

Disruptions of hypothalamic-pituitary-thyroid (HPT) axis in response to chronic crises of hypertension is relatively less known mainly regarding the defense mechanisms of the body facing a stressful external aggression and the repercussions caused by neuroendocrine mediation related to this axis. This study deals experimentally with a social deficit model on Wistar rats. This state of hypertension is caused by using chronic exposure to 1 bar 2 hours / day of air jet stress. The results show a significant increase in the TSH plasma and T3, T4 symptomatic of hyperthyroidism. This may be directly caused by the direct impact of hypertension on the activity of the para-ventricular hypothalamic nucleus (PVN) and the production of TRH, as well as a probable imbalance of immune rate Th1 / Th2 towards a Th2 hormonal profile triggered by the stress and consequently, the production of anti-TSH.

1. Introduction

In mammals, the hypothalamic–pituitary–thyroid HPT axis is controlled by neurons located within the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) that synthesize and release thyrotropin-releasing-hormone (TRH) into the median eminence. TRH stimulates the release of thyroid-stimulating hormone (TSH) from the anterior pituitary, andthen TSH travels through the peripheral vasculature to stimulate thyroid hormone (TH) release (both T3 and T4) from the thyroid gland[1].

Several Studies have revealed that the thyroid hormones exert various effects on cardiovascular function[2]. Differing states of disease verify these influences[3]. Heart rate, stroke volume and contractile force, which are results of imbalance in enzymatic function and alterations in protein synthesis[4], decrease in hypothyroidism[5]. In hyperthyroidim, T3 dilates résistance artérioles, reducing systemic vasculair resistance by 300-1000 dynes-sec-cm-5.The decline in systemic vasculair resistance stimulates renin release and sodium reabsorption, resulting in an expansion of blood volume by 5.5% and an increase in venous return to the heart [6].Other potential cardiovasculair consequences of hyperthyroidism include arterial arrhythmais[7], pulmonary hypertension [8], heart failure[9-10]. This evidence shows a significant association between thyroid hormones and cardiac function.

On the contrary, few are the studies that focus on the reverse impact that provoke cardiovascular disorders, including chronic hypertension, on the function of the thyroid. In this research, we consider a third element which is the environmental factor as one of the leading causes of hypertensive variations if exposed a long time to a stressor.

Humans and animals are constantly faced with various stressors in their lives, which can be both physiological and psychological[11]. Many studies demonstrate that stress of physiological or psychological origin results in considerable changes, either directly or indirectly, in serum level of many hormones including pituitary, adrenal and thyroid hormones [12].

Although Graves' disease has a well-known familial tendency, heredity does not explaineverything[13,14,15]. A study of twins, who are not always concordant for the disease, suggeststhat genetic factors explain 79% of the incidence of the disease, while environmental factors are responsible for 21% of the cases[16]. Stressful events is able to disrupt the normal regulation of neuroendocrine axes, and although most research in this field has mainly pointed to the classic stress hormones: glucocorticoids and catech- olamines[17], increasing evidences indicate that serum thyroid hormones (TH): T4, thyroxine and T3, triiodothyronine levels are also changed in response to chronic stress [18,19].

This work aims to study the interactions between these three elements: the environment, hypertension and thyroid hormones to determine the influences of hypertension provokedin a prolonged way on the activity of the TSH axis by sending anair jet; a simple method to induce stressful hypertensive episodes in animals [28].

2. Materials and methods

2.1. Animals and housing

20 adult male Wistar rats were obtained from Pasteur Institute (Algiers, Algeria) and individually housed in trans- parent cages in a temperature-controlled facility room (23± 1 °C). Rats were maintained on a 12-h/12-h light cycle (lights on at 07:30 a.m.) with access to standard rodents chow and tap water ad libitum. The experimental protocol was carried out according to the NIH revised Guidelines for the Care and Use of Laboratory Animals (No. 80-23, 1996).

2.2. Study protocol

In this laboratory experimental study, male Wistar rats were randomly divided to control group (T), and groups exposed to 2 h/day of 10 rats in each group.The subjects were artificially exposed to constant air pressure of 1 bar using a compressor equipped with a gauge in the rat cage through a side port. At the end of 4 weeks, the rats were sacrificed by decapitation under mild anesthetic diethyl ether and blood samples were collected into the glass tubes without anticoagulant and were allowed to clot. It was centrifuged to obtain serum and stored at –20 ^{0}C until the assay. Serum TSH hormone levels were measured using TSH IRMA kit and total T3–total T4 hormonelevels were measured using RIA kit (Diagnostic Products Corporation, LA, USA). All decapitations occurred at 13:00 h to minimize variations caused by diurnal rhythm [29].

2.3. Data analysis

The obtained results were statistically analyzed by using the student t-test according to the method of Snedecor and Cochran [20], $P \leq 0.05$ considered significant while $P \leq 0.01$ highly significant.

Volume : 3 | Issue : 11 | November 2014 • ISSN No 2277 - 8179

3. Results

The data are indicated as mean ± SEM. P values are expressed in comparison with control group. The results indicated that serum TSH (fig 1 A) and T3, T4 (fig 1B and C)levels were significantly increased in rats exposed to air je stress 2 h/day compared with control animals.

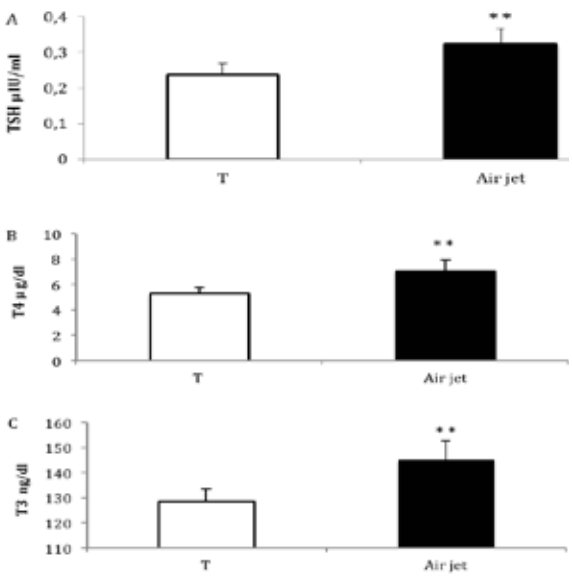

Figure 1. Serum concentrations of TSH and T3, T4 in control and rats exposed to air jet stress. The results are expressed as the mean ± SEM. * *P< 0.01 T vs. Air jet.

4. Discussion

Functionally, the short-term benefits and advantages of a stress-induced decrease in thyroid hormone levels may reflect a conservation of energy and resources in an unpredictable environment[21]. However, stress-induced hormonal changes that are initially adaptive can become inappropriate or excessive and may lead to stress-related pathologies and disease [22, 23].

The use of air jet is a simple method to induce hypertension in rats because this technique simulates a collapse of its external environment which triggers a typical adaptive stress response including increased heart rate and arterial tension linked to the action of stress hormones.Apart from this psychological stressor aspect, the pressure difference caused bysending constantly theair leads the body to trigger an internal pressure to compensate this pressure difference [24-29].Chronic application of this stress mode has a completely original way about the effect of stress on the thyroid axis,because this interaction remains specific and depending on the type of stressor present in the environment, the exposure period, intensity and predictability, as well as the biological fluid, the time of hormonal sampling and ultimately the animal strains used [30-34].

For example, the levels of peripheral TH have been found to be decreased (Hohtari et al., 1987) [35] increased (Opstad, 1994) [36] or unchanged (Baumgartner et al., 1988) [37] after physical or acute psychological stress in humans. Controversial results were also observed in experimental models of stress. Therefore, immobilization stress has been shown to both increase (Turakulov et al., 1994) [38] and decrease (Cizza et al., 1996) [39] T4 and T3 levels. Exposure to foot shock stress generated a stress reaction that has also been associated with increases or decreases on T3 (triiodothyronine) or T4 (thyroxine) levels (Josko, 1996; Pollard et al., 1979) [32,40]. Regarding different stressors paradigms, while Servatius et al. (2000) have described decreased thyroid hormones after either one or three sessions of inescapable tail shock [41],found no changes in plasma TH levels after 14 days of cold swim stress [42]. [30,31]demonstrated a TH levels increase in plasma of rats after 2 min of immobilization stress. Curiously, TH sharply decreased later on. In chronic mild stress (CMS), increased serum TH levels were found in two different rat strains, with no parallel increase in the TSH, thyroid-stimulating hormone [43]. Changes in TSH levels also depend on the kind of stressful stimuli. While immobilization stress has been shown to increase serum TSH [39] other stressors, such as lipopolysaccharide (LPS) injection [44] or foot-shock [45] been shown to reduce TSH values.

Concerning the work of our stressor chosen to conduct this experiment, it appears that rats exposed chronically 2h/day to the jet air (4 weeks) develop an overproduction of TSH. This is probably due to hypertension triggeringduring the episodes of air jet stress, which triggers an abnormal neuroendocrine response including elevated circulating levels of thyrotropin(TSH) [46, 47]. The underlying cause for the inappropriate secretion of TSH in hypertensive rat is presently unknown. A defect involving thyrotropin-releasing hormone (THR) synthesizing neurons located in the paraventricular nucleus (PVN) of the hypothalamus could be the primary cause of this abnormality. It is well established that paraventricular TRH neurons which are under thyroid hormone regulation[48,49] stimulate pituitary TSH synthesis and secretion [50-51]. Additionally, the PVN plays a key role in the development of hypertension [52]. Since the PVN is directly innervated by noradrenergic fibers from brain stem structures involved in relaying cardiovascular information [53-54] and also projects to neurons of the intermediolateral cell column of the spinal cord, the origin of preganglionic sympathetic neurons [55], it might be expected that peptidergic hypophysiotropic neurons located in the PVN are affected by an increased sympathetic tone in hypertensive rats. It has been proposed that excessive sympathetic nervous system activity may be involved in the onset of hypertension[56]. Thus, an increased noradrenergic input into the PVN could cause hypertension and at the same time chronically activate neurons encoding for TRH leading to hypersecretion of TSH.

The higher levels of TSH explain the systematic increase of thyroid hormones T3 and T4 in the hypertensive group compared with the control one.However, negative feedback regulation should curb the increased hypothalamic tone and normalize elevated TSH secretion from the pituitary gland. Since the development of hypertension air jet group is associated with a chronic hypersecretion of TSH[57], we hypothesized that negative feedback regulation at the pituitary and/or hypothalamic level may be impaired in these animals.

Furthermore, numerous studies have indicated a connection between stress and autoimmunity and that stress may trigger or worsen autoimmune disease, also demonstrated that, psychological and physiological stressors induce various immunological changes [58-61]. Exposed to stress as the air jet stress led to overproduction of the thyroid hormones T3, T4 and it is the most commonly caused of the development of Grave's disease, an autoimmune disease in which antibodies are produced which stimulate the thyroid to secrete excessive quantities of thyroid hormones [62].Stress hormones resulting from chronic exposure to air jet acting on antigen- presenting immune cells, may influence the differentiation of bipotential helper T-cells away from a Th1 phenotype towards a Th2 phenotype.

These results of suppression of cellular immunity and in potentiating of humo- ralimmunity. Thyroid autoimmunity is clinically expressed as Hashimoto's thyroiditis or as Graves' disease [63,64]. The different phenotypic expressions of thyroid autoimmunity are largely dependent on the balance of Th1 versus Th2 immune response. Predominance of Th2-mediated immune response may induce antigen specific B lymphocytes to produce anti-TSH receptor (TSHr)

antibodies causing Graves' disease [65,66].

Finally, this work has demonstrated the impact of chronic hypertension induced by air jet stress on the likelihood of developing hyperthyroid problems expressed by increasinglevels of TSH, T3 and T4 plasma in the hypertensive rat.

REFERENCE

1. Zoeller, R.T., Tan, S.W., Tyl, R.W. (2007), General background on the hypothalamic– pituitary–thyroid (HPT) axis. Crit. Rev. Toxicol. 37, 11–53. | | 2. Williams, G.H and Braunwald, R. (1980), Endocrine and nutritional disorders and heart disease. Braunwald E (ed). Heart disease: A textbook of cardiovascular medicine. Philadelphia: W.B.Saunders, 825-53. | | 3. Woeber, KA. (1992), Thyrotoxicosis and the heart.N Engl J Med, 327, 94-8. | | 4. Philipson, K.D., and Edelman, I.S. (1977), Thyroid hormone control of Na+- K+- ATPase and K+- dependent phosphatase in rat heart. Am J Physiol, 232, 96-201. | | 5. Johnson, P.N., Freedberg, A.S., Marshall, J.M. (1973), Action of thyroid hormone on the transmembrane potentials from sinoatrial node cells and atrial muscle cells in isolated atria of rabbits. Cardiology,58, 273-89. | | 6. Resnick, L.M., and Laragh J.H. (1982), Plasma reninactivity in syndromes of thyroid hormone excess and deficiency. Life Sci, 30, 585-586. | | 7. Sawin , C.T., Geller, A., Wolf, P.A., et al. (1994), lowserumthyrotropin concentrations as risk factor for atrial fibrillation in olderpersons. N Engl J Med, 331,1249-1252. | | 8. Marvisi, M., Brianti, M., Marani, G., et al. (2002), Hyperthyroidism and pulmonary hypertension. Respir Med, 96, 215-220. | | 9. Klein, I., and Ojamaa, K. (2001),Thyroidhromone and the cardiovascular system. N Engl J Med, 344, 501-509. | | 10. Wocher, K.A.(1992),Thyrotoxicosis and the heart.NEngl J Med, 327, 94-98. | | 11. Turakulov, IaKh., and Burikhanov, R.B. (1993), Role of norepinephrine in the regulation of thyroid gland functional activity in rabbits. ProblEndokrinol (Mosk), 39 (4), 45-8. | | 12. Aguilera, G., Kiss, A., Sunar-Akbasak B. (1995), Hyperreninemichypoaldosteronism after chronic stress in the rat. J Clin Invest, 96 (3), 1512-9. | | 13. Andrey, V. (2010), Endoplasmic reticulum stress in proteinuric kidney disease –Department of Medicine MCGill University Health centre.Montreal Quebec, Cananda, 77,187-193. | | 14. Kidd, P. M. (2007), Glutathione: systemic protectant against oxidative and free radical damage. Altern. Med Rev, 2, 155 – 176. | | 15. Mari, G., Morganti, B., Merlo, C. (2009), Administration of sulpiride or domperidone for advancing the fist ovulation in deep an estrous mares.J. Nestrous Mares Theriogenology, 71, 959–965. | | | 16. Blotta, M. H., Dekruyff, R.H., Umetsu, D.T., (2007), Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in DC4+ lymphocytes. J. Immunol, 158, 5589 – 5595. | | 17. McEwen, B.S., (2000). Allostasis and allostatic load: implications for neuro psychopharmacology. Neuropsychopharmacology, 22, 108-124. | | 18. Cremaschi, G.A., Gorelik, G., Klecha, A.J., Lysionek, A.E., Genaro, A.M., (2000). Chronic stress influences the immune system through the thyroid axis. Life Sci, 67, 3171-3179. | | 19. Silberman, D.M., Wald, M., Genaro, A.M., (2002). Effects of chronic mild stress on lymphocyte proliferative response: participation of serum thyroid hormones and corticosterone. Int. Immunopharmacol , 2, 487- 497. | | 20. Snedecor, G.W., Cochran, W.G., (1980), Statistical Method.United State University Press, Lowa, London , 59-60. | | 21. Engel, G., and Schmale, A. (1972), Conservation-withdrawal: a primary regulatory process for organismic homeostatsis. CIBA Found. Symp ,8, 57–75. | | 22. McEwen, B., Wingfield, J., 2003. The concept of allostatis in biology and biomedicine.Horm. Behav. 43, 2–15. | | 23. Davis, J.D., Tremont, G., 2007. Neuropsychiatric aspects of hypothyroidism and treatment reversibility. Minerva Endocrinol 32, 49–65. | | 24. Lundin, S., Ricksten, S.E., Thorén, P., (1984). Interaction between "mental stress" and baroreceptor reflexes concerning effects on heart rate, mean arterial pressure and renal sympathetic activity in conscious spontaneously hypertensive rats. ActaPhysiolScand,120, 273-281. | | 25. Lundin, S., Ricksten, S.E., Thorén, P. (1983), Interaction between mental stress and baroreceptor control of heart rate and sympathetic activity in conscious spontaneously hypertensive (SHR) and normotensive (WKY) rats. J Hypertens-Suppl,1, 68-70. | | 26. Lundin, S., and Thorén, P. (1982), Renal function and sympathetic activity during mental stress in normotensive and spontaneously hypertensive rats. ActaPhysiolScand,115, 115-124. | | 27. Koepke, J.P., DiBona,G.F., (1985), Central beta-adrenergic receptors mediate renal nerve activity during. Hypertension ,(3 Pt 1), 350-6. | | 28. Julien, C.,Cerutti, C., Kandza, P., Barres C., Su D., Vincent, M., Sassard, J., (1988), Cardiovascular response to emotional stress and spontaneous blood pressure variability in genetically hypertensive rats of the Lyon strain. Clin ExpPharmacolPhysiol, 15, 533-538. | | 29. Kalsbeek, A., Fliers, E., Franke, A., Wortel, J., Buijs, R.(2000), Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. Endocrinology, 141, 3832–3841. | | 30. Langer, P., Foldes, O., Kvetnansky, R., Culman, J.,Torda, T., El Daher, F., (1983a), Pituitary-thyroid function during acute immobilization stress in rats. Exp. Clin. Endocrinol, 82, 51-60. | | 31. Langer, P., Vigas, M., Kvetnanský, R., Földes, O., Culman, J.(1983b), Immediateincrease of thyroid hormone release during acute stress in rats: effect of biogenic amines rather than that of TSH? Acta Endocrinol, 104 (4), 443-449. | | 32. Osko, J. 1996. Liberation of thyrotropin, thyroxine and triiodothyronine in the | controllable and uncontrollable stress and after administration of naloxone in | rats. J. Physiol. Pharmacol. 47 (2), 303-310. | | 33. Kioukia-Fougia, N., Christofidis, I., Strantzalis, N. (1999), Physicochemical conditions affecting the formation/stability of serum complexes and the determination of prostate-specific antigen (PSA). Anticancer Res, 19 (4B), 3315-3320. | | 34. Kirkegaard, C., Faber, J., (1998), The role of thyroid hormones in depression. Eur. J. Endocrinol, 138, 1-9. | | 35. Hohtari, H., Pakarinen, A., Kauppila, A.(1987), Serum concentrations of thyrotropin, thyroxine, triiodothyronine and thyroxine binding globulin in female endur-ance runners and joggers. ActaEndocrinol, 114, 41-46. | | 36.Opstad, P.K. (1994), Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. Eur. J. Endocrinol.,131, 56-66. | | 37. Baumgartner, A., Graf, K.J., Kurten, I., Meinhold, H. (1988), The hypothalamic-pituitary-thyroid axis in psychiatric patients and healthy subjects: parts 1e4. Part 4: TRH tests, thyroxine, trimdothyronine, and reverse triiodo- thyronine determinations in medical students during a major examination. Psychiatry Res, 24, 316-332. | | 38. Turakulov, Y., Burikhanov, R., Pakitdinov, P., Myslitskaya, A. (1994), Influence of immobilization stress on the levels of thyroid hormones. Neurosci. Behav. Physiol, 24, 462-464. | | 39. Cizza, G., Brady, L., Escpales, M., Blackman, M., Gold, P., Chrousus, G. (1996), Age and gender influence basal and stress-modulated hypothalamic–pituitary–thyroidal function in Fisher 344/N rats. Neuroendocrinology, 64, 440–448. | | 40. Pollard, J., Basett, J.R., Cairncross, K.D. (1979), Plasma thyroid hormone and gluco- corticosteroid concentrations in the male rat following prolonged exposure to stress. Aust. J. Biol. Sci, 32, 237-242. | | 41. Servatius, R., Natelson, B., Moldow, R., Pogach, L., Brennan, F., Ottenweller, J. (2000), Persistent neuroendocrine changes in multiple hormonal axes after a single or repeated stressor exposures. Stress, 3, 263–274. | | 42. Kioukia-Fougia, N., Antoniou, K., Bekris, S., Liapi, C., Christofidis, I., Papadopoulou-Daifoti, Z.(2002), The effects of stress exposure on the hypothalamic-pituitary- adrenal axis, thymus, thyroid hormones and glucose levels. Prog. Neuro- psychopharmacol. Biol. Psychiatry, 26 (5), 823-830. | | 43. Kioukia, N., Bekris, S., Antoniou, K., Papadopoulou-Daifoti, Z., Christofidis, I.(2000), Effects of chronic mild stress (CMS) on thyroid hormone function in two rat strains. Psychoneuroendocrinology, 25 (3), 247-257. | | 44. Kondo, K., Harbuz, M., Levy, A., Lightman, S.(1997), Inhibition of the hypothal-amic-pituitary-thyroid axis in response to lipopolysaccharide is independent of changes in circulating corticosteroids. Neuroimmunomodulation,4, 188-194. | | 45. Servatius, R., Natelson, B., Moldow, R., Pogach, L., Brennan, F., Ottenweller, J. (2000), Persistent neuroendocrine changes in multiple hormonal axes after a single or repeated stressor exposures. Stress, 3, 263-274. | | 46. Werner, S.C., Manger, W.M., Radichevich, I., Wolff, M. and Von Estorff, I. Excessive thyrotropin concentrations in the circulation of the spontaneously hypertensive rat (38679) (1975), Proc. Soc. Exp. Biol. Med,148 ,1013-1017. | | 47. Sowers, J.R., Tempel, G., Resch, G. and Colantino, M. (1978), Pituitary response to TRH and LHRH in spontaneously hypertensive rats (40356). Proc. Soc. Exp Biol. Med, 159, 397-399. | | 48. Segerson, T.P., Kauer, J., Wolfe, H.C., Mobtaker, H., Wu, P., Jackson, I.M.D. and Lechan, R.M. (1987), Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus, Science, 238,78-80. | | 49. Dyess, E. M., Sergerson, T. P., Liposits, Z., Paull, W. K., Kaplan, M. M., Wu, P., Jackson, I. M. D. (1988), and Lechan, R.M., Triiodothyronine exerts direct cell-specific regulation of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus, Endocrinology, 123, 2291-2297. | | 50. Szabo, M., Kovathana, N., Gordon, K., and Frohman, L.A. (1978), Effect of passive immunization with an antiserum to thyrotropin (TSH)-releasing hormone on plasma TSH levels in thyroidectomized rats, Endocrinology, 102 ,799-805. | | 51. Taylor, T., Wondisford, F.E., Blaine, T. and Weintraub, B.D. (1990), The paraventricular nucleus of the hypothalamus has a major role in thyroid hormone feedback regulation of thyrotropin synthesis and secretion, Endocrinology, 126, 317-324. | | 52. Ciriello, J., Kline, R.L., Zhang, T. X. and Caverson, M.M. (1984), Lesions of the paraventricular nucleus alter the development of spontaneous hypertension in the rat, Brain Res., 310, 355-359. | | 53. Sawchenko, P. E., and Swanson, L. W. (1982), The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat, Brain. Res. Rev, 4, 275-325. | | 54. Swanson, L.W., and Sawchenko, P.E. (1980),Paraventricular neurons: a site for the integration of neuroendocrine and autonomic mechanisms, Neuroendocrinology, 31, 410-417. | | 55. Henry, J. L., and Calaresu, F. R. (1972), Topography and numeral distribution of neurons of the thoraco-lumbar intermediolateral nucleus of the cat, J. Comp. Neurol., 144, 205-214. | | 56. Qualy, J.M. and Westfall, T.C. (1988), Release of norepinephrine from the paraventricular hypothalamic nucleus of hypertensive rats, Am. J. Physiol, 251 ,993-1003. | | 57. Kojima, A., Kubota, T., Sato, A., Yamada, T., Yamori, Y. and Okamoto, K. (1975), Congenital abnormality of pituitary-thyroid axis in spontaneously hypertensive rats (SHR) and stroke-prone rats (SPR) (39081), Proc. Soc. Exp. Biol. Med, 150, 571-573. | | 58. Murata, Y.T., Shimamura, T., Tagami, et al . (2008), The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. Immunopharmacol, 2, 673 -689. | | 59. Abbas ,A.K., Murphy, W. B. K. M., Sher, A. (2009), Functional diversity of helper T-lymphocytes. Nature, 383, 787 - 795. | | 60. Webster, E.L.,Torpy, D. J., Elenkov, I.J., and Chrousos, G. P. (2009), Corticotropin-releasing hormone and inflammation. Ann. N. Y Acad. Sci, 840, 21 - 23. | | | 61. Panzani, D.I., Zicchino, A., Taras, P. (2011),Sulpiride use of dopamine antagonist sulpiride to advance first ovulation in transitional mares .Theriogenology, 75, 138-143. | | | 62. Rook, G. A. W. (2010), Glucocorticoids and immune function. Bail. Clin. Endocrinol. Metab, 13: 567 – 581. | | 63. Schwartz, K.E. (2002), Autoimmunity,dehydroepiandrosterone (DHEA), and stress. J. Adolesc. Health, 305, 37 - 43. | | 64. Roura – Mir, C. M.,Catalf M., et al. (2010), Single-cell analysis of intrathyroidal lymphocytes shows differential cytokine expression in Hashimoto's and Graves' disease. Eur. J. Immunol, 27, 3290 – 3302. | | 65. Trinchieri, G. (2008),Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen- specific adaptive immunity. Annu. Rev. Immunol, 13, 251 – 276. | | 66. Awerman, L. M. (2010), Chronic Psuchlogical stress alters body weight and blood chemistry in European starlings. Comparative Biochemistry and Physiology, 156,136-142. |