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Original Article

Enigmatic role of vagal network as a construction worker on thyroid gland creation by inspiration from neurohistology studies

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Abstract

Aim: Thyroid functions regulate higher brain centers and olfactory informations are wireless universal stimulators for thyroid hardware and software development by way of vagal complex management. We wonder if thyroid architecture and functions are regulated by vagal nerve.

Material and Methods: The animals were divided to three groups according to their T4 hormone levels which 1.89±0.10 µg/dl values was accepted as normal (G0; n=12); 2.13±0.19 µg/dl values was accepted as hyperfunctioned (GI; n=7) and 1.61±0.09 µg/dl values was accepted as hypofunctioned (GII; n=5). All of the thyroid glands, thyroidal branches of vagal nerves and brainstem section at the level of motor nucleus of vagal nerves sections were stained with haematoxylin-eosin, GFAP and tunnel dye for histological examination. All microscopical Stereological evaluations were done to understand how vagal morphology effects thyroid tissue architectures and T4 levels. All of the thyroid glands, thyroidal branches of vagal nerves and vagal motor nucleus evaluated stereologically to recognise whether some histomorphological variations thyroid follicles and volumes per cubic centimeter compared with thyroid hormone levels statistically.

Results: The mean neuron density of vagal nucleus (NDVN), axon density of thyroidal branches of vagal nerves (TBVN), TFV, and T_4 levels were measured as G0;GI and GII consequtively: (312±91)x10^6/mm^3, 19.543±2.321/mm^2, 1.89±0.10 µg/dl and (296±72)x10^6µm^3/cm^3 in G0; (387±93)x10^6/mm^3, 22.576±2.864/mm^2, 2.13±0.19 µg/dl and (331±65) x10^6µm^3/cm^3 in GI and (245±56)x10^6/mm3, 12.432±1.234mm^2, 2.13±0.19 µg/dl and (231±45)x10^6µm^3/cm^3 in GII.

Conclusion: It seems that decrased hormone levels due to ineffective/unadequate thyroid gland development results from inadequately developing vagal complex architecture.

Keywords: Vagal network; thyroid innervation; thyroid

INTRODUCTION

Thyroid is the largest, oldest and important endocrine gland (1). Functional unit consists of basal parafollicular cells that synthesize T3 and T4 which effects on cellulary growth and architectural development to induce metabolic cascades of embrios. Parasympathetic innervation is sustained by inferior laryngeal nerve and it's section result in, decreased hormon secretion with increased TSH level (2). Degeneration of the vagal circuitry (3) could cause thyroid gland atrophy. Smell and taste problems rely on thyroid gland disorders with hormone unresponsiveness (4). Thyroid hormones regulate olfactory epithelium and maturation (5). Subarachnoid hemorrhage mat induce hypopituitarism (6). Loss of smell cause cellulary degeneration in temporal cortex, insula, hippocampus and thyroid gland (7,8). If vagal nuclear degeneration induced thyroid gland degradation is responsible for diminished hormone secretion due to induced by olfactory bulbectomy (OBX) in contrary to common belief that anosmia is a result from hypothyroidism; we easily postulated that normal vagal network is required for a normal thyroid gland hardware and software.

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MATERIAL and METHODS

Animal Selection And Study Groups

A total of 24 rats were studied according to the study protocol and permissions were reviewed and approved by the Ethics Commitee for Ataturk University. The animals were placed in individual metal cages at room temperature with 12 h of light per day dark light periods and under control of veterinary supervision. Animals feeded with standard laboratory diets. The animals were classified as three groups according to their T4 hormone levels which 1.89±0.10 µg/dl values was accepted as normal (n=12); 2.13±0.19 µg/dl values was accepted as hyperfunctioned (n=7) and 1.61±0.09 µg/dl values was accepted as hypofunctioned (n=5). All of the thyroid glands, thyroidal branches of vagal nerves and brainstem section at the level of of vagal motor nucleus and were stained with haematoxylin-eosin, GFAP and tunnel dye for histological examination. All microscopical Stereological evaluations were done to understand how vagal morphology effects thyroid tissue architectures and T4 levels. All of the thyroid glands, thyroidal branches of vagal nerves and brainstem section at the level of motor nucleus of vagal nerves were evaluated stereologically to recognise whether histomorphological variations hormone levels, thyroid follicles volumes per cubic centimeter.

Olfactory functions, feding behavior and body weigths were reported. After four weeks follows up, they were decapitaded humanely given general anesthesia. Their brains, vagal complexes and thyroid glands evaluated with routin and immunohistochemical methods.

Anatomical Finding

All animals sacrified general anesthesia. Before decapitation, intracardiac formaline was injected and brains-spinal cord-thyroid glands were extracted. Macroscobical examination was done under operation microscope. We noticed that thyroid gland volume was more prominent in thyroid hormone values upper levels and vs/vs. Vagal branches have more diameter (260±73µm) than the others. In brainstem examinations shown that brain morphologia and cranial nerve architectures were normal limits in normothyroidic animals; but brain volumes and macroarchitecture found as underdeveloped limits in hypothyroidic and have less diameter owned vagal nerves.

Histological Procedures

5 µm sections were done for thyroid glands were at the distances of 30 µm. Each 30th-31st sample sections were used for stereological way to thyroid follicles volumes calculation by fractionator method (9). Neural materials and thyroid tissue sections stained with haematoxylin-eosin (H&E), and tunel methods.

Stereological-Algebraic Analysis Methods

Used stereological methods for vagal nuclei and thyroid follicles are clearly described our former studies (7,9,10). The first sampled sections pair was choosen at random from a starting point within the first 30-section interval. Because, thyroid follicles volumes and numbers profoundly described by Aydin et al. (9) there is no need new explanation in that article to avoid from article volume expantion. Because follicles volume and densities did not show normal distribution we use Kolmogorov-Smirnov and Shapiro-Wilk test ($p<0.05$) as same as the Aydin N, et al. Methods (7). Before score datas, Mann-Whitney-U test was applied for the groups. To compare for pair wise comparisons of independent samples Kruskal-Wallis test used. For multiple comparisons by dividing 0.05 by six with Bonferroni correction, the p value used which p values were accepted significant under ≤ 0.0098 at the level of 0.05 confidence interval 95%.

Thyroid follicles volumes was calculated at the following formula. The x,y and z are half of the ellipsoid axex in x, y, and z apsis. (9):

$$\sum_1^n \mathbf{FV} = \sum_{f=1}^{n} \mathrm{n}[\![\tfrac{4}{3}\pi(\tfrac{x+y+z}{3})^3]\!] *$$

And the TFV was estimated as:

$$\mathbf{TFV} = \sum_{\mathbf{N=1}}^{\mathbf{N=N}} \mathbf{NxV}\,\boldsymbol{n}$$

RESULTS

Figure 1 shown as vagal nucleus just under the fourth ventricle vagal nucleus, neuron estimation method with cubic milimeter of vagal nuclei. Histologic appearance of thyroid gland with vagal nerve, thyroidal arter. Vagal nerve axon estimation method via divided of vagal nerve section to quadrangulary region as four equal part.

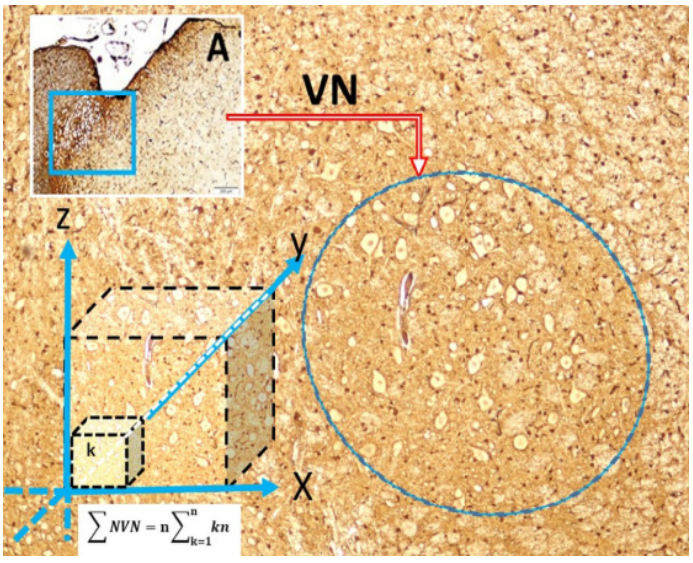

Figure 1. Histological appearance of vagal nucleus just under the fourth ventricle (LM, GFAP, x4/A) vagal nucleus (VN) inside in blue circle (LM, GFAP, x10/Base), neuron estimation method with cubic milimeter of vagal nuclei via serial number equation formula

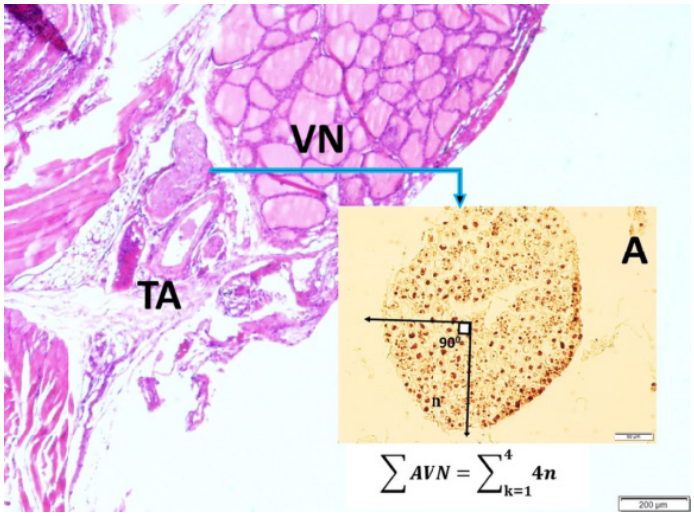

Figure 2. Histologic appearance of thyroid gland with vagal nerve (VN), thyroidal arter (TA) (LM, H&E, x4/Base). Vagal nerve axon estimation method via divided of vagal nerve section to quadrangulary region as four equal part. The mean axon number of one part (n) multiplied with number four and total numbers of thyroidal branch of vagal nerve axons estimated (LM, S100, x20/A). Our formula located at the right bottom

The axon number estimation methods summerized in Figure 2 legend. Histological appearance of thyroid gland, follicles and external carotid arter, vagal fibers in thyroid gland and thyroid gland artery (Figure3). Thyroid follicles volume estimation method is summerized in Figure 4 legend.

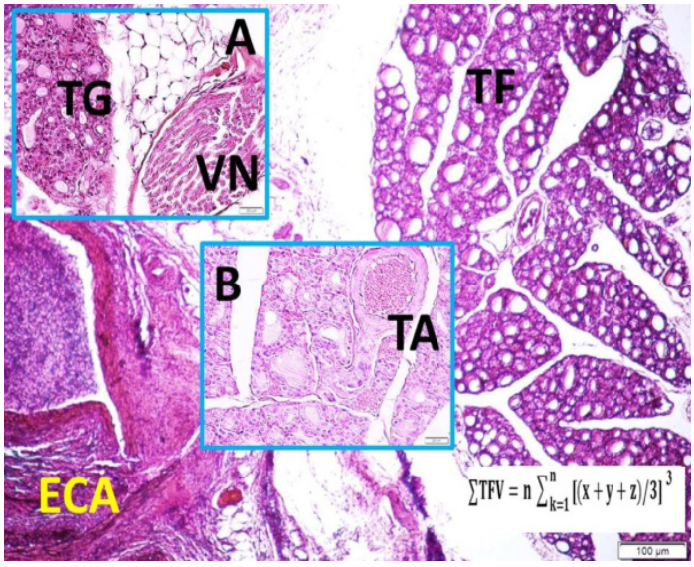

Figure 3. Histological appearance of thyroid gland, follicles (TF) and external carotid ater just near the thyroid gland (ECA) (LM,H&E,10/Base); vagal nerves (VN) of thyroid gland (TG) (LM,H&E,x40/A); and thyroid gland artery (TA) is seen in a normal rat (LM,H&E,x40/B)

The mean neuron density of vagal nucleus (NDVN), axon density of thyroidal branches of vagal nerves (TBVN), T_4 levels and TFV were measured as G0;GI and GII consequtively: (312±91)x10⁶/mm³, 19.543±2.321/mm², 1.89±0.10 µg/dl and (296±72) x10⁶µm³/cm³ in G0; (387±93)x10⁶/mm³, 22.576±2.864/mm², 2.13±0.19 µg/dl and (331±65)x10⁶µm³/cm³ in GI and (245±56)x10⁶/mm³, 12.432±1.234mm², 2.13±0.19 µg/dl and (231±45) x10⁶µm³/cm³ in GII. Results were summarised in Table 1.

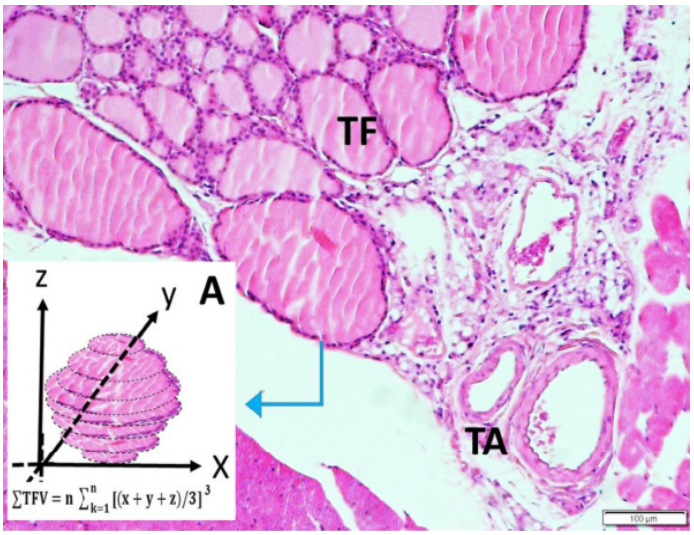

Figure 4. Histological view of thyroid follicles (TF), thyroidal artery (TA) (LM, H&E, x10/Base). To estimate of any throid follicle volume, many consequtive sections of follicles was taken which follicles shape was exhausted. Later, all sections arranged over and over and optained a sypher/ellipsoid/conic or any curved 3D shapes. Many of them resemble ellipsoid shapes, we estimated that follicles volume used the located at the left bottom (A). To estimate total thyroidal follicles volume, one follicles volume multiplied with follicles numbers. Because the most important determinator of thyroid functions are thyroidal follicles architectures which constructed by vagal nerves

Table 1. The mean neuron density of vagal nucleus (NDVN), axon density of thyroidal branches of vagal nerves (TBVN), TFV, and T^4 levels

Numerical results of study	Group 0 (n=12)	Group I (n=7)	Group II (n=5)
NDVN (x10⁶/mm³)	312±91$^{\alpha}$	387±93$^{\beta}$	245±56$^{£}$
TBVN (mm²)	19.543±2.321$^{\alpha}$	22.576±2.864$^{\beta}$	12.432±1.234$^{\gamma}$
TFV (x10⁶µm³/cm³)	296±72$^{\beta}$	331±65$^{\beta}$	321±65$^{\gamma}$
T_4 (µg/dl)	1.89$^{\beta}$	2.13±0.19$^{\alpha}$	1.61±0.09$^{£}$

Group 0: normal, Group I: hyperfunctioned group, Group II: hypofunctioned group, NDVN: neuron density of vagal nucleus, TBVN: axon density of thyroidal branches of vagal nerves, TFV: Thyroid follicles volume
$^{\alpha}$ p < 0.0005 Group 0 vs I, Group I vs II
$^{\beta}$ p < 0.0001 Group 0 vs I, Group I vs II
$^{\gamma}$ p < 0.001 Group 0 vs II
$^{£}$ p< 0.005 Group 0 vs II, Group I vs II

DISCUSSION

Thyroid is the largest and oldest endocrine glands in animals (1). Architecturel unit of the thyroid hormones synthesising basal-parafollicular cells and luminal colloid in follicles. Thyroid hormons provoke growth and development of organs in response to environmental factors. The other content of the thyroid follicular cell reflect changes in hormone synthesis, secretion and absorption (11,12). Active follicles are typically involves more cuboidal epithelium and may have resorption vacuoles and the colloid. Thyroidal cells and volumes are different in all thyroid gland and the larger follicles localised at the periphery. According to physical law, migration of follicles movement vector directed from central to periphery and the most bigger follicless inflated the more earlier. For that reason, fibrotic chances firstly begin on periphery. Ageing process cause volume reduction and calcification.

Reduction of adrenergic and M-cholinergic influences thyroidogenesis and spermatogenesis (13). Thyroid disorders managing drugs may also congenital thyroid gland malformations (14). Blood pH abnormalities cause thyroid gland pathologies in newborn (15). Aydin et al. Reported that blood pH is highly important (16) for life expectancy because %10 changes of pH could result in incompatible with life (17).

Thyroid gland have principal roles on the development of sexual organs and continuations of sexual functions (18) with their autonomic network linked with olfactory nerves (7). Described/nondescribed many neuroendocrinological malfunctions may be develop if that neurothyroidal networks could not be developed in normal cytoarchitectures. Optic and gustatory stimulant starts vagal cephalic-phase reflexes to induce thyroid hormone secretion (19). Vasodilatatory effect of vagal nerves modulate thyroid hormone release by influencing thyroid blood flow with thyroid stimulating hormone (20). The earliest thyroid disfunctions is commonly seen by degradation of olfactory bulb and dorsal motor nucleus of the vagal nerve in neurodegenerative disease (21). The bipolar sensory neurones synaptically in entorhinal cortex, hippocampus, amygdala and autonomic secretomotor centers related to long life span producing vagal network (22). Hypothalamus and insula controlled vagal complex, cervicothorasic dorsal root ganglia, sympathetic/parasympathetic chain (23,24), olfactory network (7) have esstial roles on the construction of thyroid gland.

Parasympathetic system

Postganglionic parasympathetic nerve fibers that arrised from the nervus lingualis (25) and cervical sympathetics (26) have prominent role on salivary ad thyroid glands development. Superior laryngeal nerve produce thyroid blood flow with dilated thyroidal arteries augment thyroid hormone secretion (20) by help of hypothalamus, nodose ganglia of the vagal nerve (27).

Growth and secretory activity thyroid gland controlled by sympathetic and parasympathetic systems (2). Parasympathetic denervation cause thyroid and parathyroid atrophy (28) and decreased circulating T4 levels. Bilateral cervical vagotomy rely on decreased thyroid volume and T4-T3 levels. Vagotomy is characterized by thytoidal cell enlargement, promoted colloid droplets, hypertrophied mitochondria/lysosomes/microvilli and Golgi complex, decreased T4/icreased T3 level. These pathological changes has been attributed to vagal insuficiency (29).

Cholinergic fibers of inferior laryngeal nerve innervate thyroid gland (28) and it's section result in atrophy and decreased hormon levels (2). Vagal network ischemia following subarachnoid hemorrhage is a possible reason for hypopituitarism (6). Olfaction and taste dysfunctions cause subclinical hypothyroidism. FT3 levels is an important mediatore than TSH or FT4 levels with olfactory parameters (30). It is well known that olfactory impulses stimulate vagal nerve connections (31). So, olfactory bulbs removal triggered neurodegeneration in the vagal network result in cellular loss in thyroid gland (32). Although earliest degradation of olfactory bulb and the dorsal motor nucleus of the vagal nerve accused of dementia (21) and neurodegeneration (33), we observed that olfactory nerve ablasion induced vagal complex disruption may be responsible for hardware/software abnormalities of thyroid glands in contrary to common belief.

Sympathetic System

Activation of the central a-adrenergic mechanisms increases the release of thyroid-stimulating hormone (34). The sympathetic superior cervical ganglia (35) is required for hardware/software compartments development of thyroid and parathyroid glands.

Psychiatric Aspects of Olfactory Nerve/Vagal Nerve Interactions

Recent researchs show that on sensorial interactions of olfactory nerve/vagal nerve ınteractions play a major role on the regulation of internal homeostazis to avoid of neurophsyschiatric entrophy induced catastrophes (36). The hypotheses of olfaction loss is commonly seen in Alzheimer's disease (37) may be wrong because hyposmia/anosmia should be considered as a causative agents for that disease and also in Crohn's disease (38). Aydin N et al. shown that olfaction sense and vagal network relations have mor important factor on thyroid gland morphology (7) and all body structures.

CONCLUSION

In summary we profoundly adviced that blood pH regulating glossopharyngeal and vagal nerves injuries can be responsible for many endocrine metabolic abnormalities with their unexplained mechanisms; and even in oogenesis, spermatogenesis, embriogenesis, neonatal period and all lifelong.

Future Insight

Congenital disorders or malformations such disorders what all nervous system else have seen but vagal nerves thoughout that no nerves else has seen. Fort hat reason pH regulating autonomic nervous system should be examined all neurobiological and even neuropsychiatric disorders.

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