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MELATONIN AND THE THYROID GLAND - AN OVERVIEW

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SUMMARY

In this review, published data on the relationship occurring between melatonin - the main pineal hormone - and the thyroid gland, are briefly summarized. Numerous experimental data suggest an inhibitory influence of melatonin on thyroid growth and secretion. These effects have been presented in different experimental models *in vivo* and *in vitro*, the former including short-term and/or chronic melatonin administration to various animal species, in pinealectomized animals, also following light restriction which is known to increase the activity of the pineal. On the other hand, stimulatory effects of thyroid hormones on the pineal growth and function have been demonstrated. Oxidative stress is involved in physiological and pathological processes in the thyroid gland. Recent results of experimental studies suggest that melatonin can protect against oxidative damage in the thyroid. A confirmation of the mutual relationship between the pineal gland and the thyroid is - in clinical studies, in humans - rather difficult, as both human beings and animal species used in experimental studies, live far away from their natural/original habitats. It makes almost impossible to compare the results of studies on the pineal-thyroid interrelationship, obtained in particular experiments in different animal species.

Key words: Cell proliferation; Growth processes; Melatonin; Pineal gland; Thyroid gland; Thyroid hormones.

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is the main secretory product of the pineal gland. The molecule performs several functions in living organisms. It is known for its role in seasonal reproductive physiology, circadian rhythmicity and sleep processes and for its ability to reduce the "jet lag" symptoms in humans (1). Additionally, melatonin has been shown to modulate immune functions, growth processes, oxidative processes and, to some extent, the complex process of cancerogenesis (2-10).

The existing relationship between the pineal and the thyroid gland has been evidenced by results of numerous experimental studies. Several questions, however, have still remained unanswered, namely: 1) to what extent is the relationship in question direct? 2) are there any intermediate substances or factors involved in this regulation? 3) are there any cells in the body capable of producing both thyroid hormones and melatonin? 4) is there a local (paracrine?) regulation of thyroid hormone secretion by melatonin in the thyroid gland? 5) does melatonin participate in the regulation of the peripheral metabolism of thyroid hormones (T_4-monodeiodination)? 6) does melatonin regulate the activity of type II T_4-5'-monodeiodinase in pinealocytes? 7) does melatonin control the expression of certain thyroid gland-related genes, i.e, NIS, TPO, thyroglobulin, pendrin, TSH, TSH receptor (TSHR), etc.? 8) is T_3 involved in the regulation of the expression level of genes, encoding for key enzymes, which participate in melatonin synthesis (hydroxy-indole-O-methyltransferase - HIOMT; N-acetyltransferase - NAT)?

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Numerous evidence has been gathered that, beside the pineal gland, other organs, tissues or cells (11) are sites of melatonin production, with exclusively high amounts found in the bone marrow (12, 13) and in the gut (14). The presence of this hormone has been observed in APUD cells of airway epithelium, along the border between the cortex and medulla of adrenals, beneath the hepatic capsule, in kidney cortex, paraganglia, gall bladder, ovary, endometrium, placenta, and the inner ear (15). Colocalizations of melatonin and calcitonin in thyroid C-cells, melatonin and histamine in mast cells, melatonin, somatostatin and beta-endorphins in natural killer cells, and melatonin and prostaglandin F_2 in thymic reticulo-epithelial cells have been observed (16, 17). No studies have yet been performed to reveal a possible presence of melatonin in thyroid follicular cells.

As far as the regulatory mechanisms between melatonin and thyroid hormones are concerned, typical endocrine (due to the fact that melatonin is a hormone), paracrine (due to the presence of melatonin in thyroid C cells), and - possibly - autocrine (if melatonin is present in thyroid follicular cells) regulation should be considered.

The inhibitory influence of melatonin on growth processes in the thyroid gland

Numerous data, from our and other laboratories, indicate a suppressive effect of melatonin on thyroid growth processes. Already in the sixties of XX century, it has been shown that removal of the pineal gland, being the main source of melatonin, resulted in an increased thyroid weight in rats (18) and mice (19). Later on, the inhibitory effects of melatonin on thyroid growth processes were confirmed, using different indices of growth processes and different experimental models.

It is well known that light decreases the activity of the pineal gland; suppression of the thyroid growth was found in male mice under conditions of light restriction (20). Melatonin, administered to mice in late-afternoon s.c injections for 10 days, inhibited the basal and thyrotropin (TSH)-stimulated mitotic activity of the thyroid follicular cells; a similar effect was observed in thyroid organ culture (21). The indoleamine prevented the pinealectomy-induced increase of mitotic indices in the rat thyroid gland (22). The effect of short-term s.c. administration of melatonin (5 days) on ^{3}H-thymidine incorporation into DNA of rat thyroid lobes, transferred, after collecting, into incubation *in vitro*, was dose-dependent; melatonin, in dose of 25 µg/daily, effectively reduced ^{3}H-thymidine incorporation, when used in dose of 50 µg/daily - melatonin produced no effect, however, the indoleamine, applied in the highest dose - 100 µg/daily, brought about an increase of ^{3}H-thymidine uptake (23). However, under *in vitro* conditions, melatonin reduced ^{3}H-thymidine incorporation into DNA of rat thyroid lobes only when used in the concentration of 10^{-9} M (24).

The following observation is of special attention : melatonin, when released from s.c. pellets, prevented the inhibitory effect of late-afternoon melatonin injections on growth processes in rat thyroid (25, 26). Those results suggest a counter-antithyroid action of melatonin released from pellets on the growth-inhibiting response of the gland, following melatonin injection. This would suggest that melatonin is able to regulate its own action.

Melatonin and, to a lesser extent, another indoleamine - 5-methoxytryptamine, decreased the mean nuclear volume of thyroid follicular cells in Syrian hamsters (27). Melatonin and its precursor - N-acetylserotonin (NAS), administered to male rats, decreased the mitotic activity in the thyroid gland (28). The inhibitory effect of short photoperiod on the thyroid growth processes was shown in mice (20) and in Indian palm squirrels *Funambulus pennanti* (29). It is to be stressed that the pineal gland involvement in the photoperiodic response of the thyroid cannot be excluded, since parallel changes of melatonin concentrations were observed, following a short photoperiod exposure (29). Moreover, much experimental evidence derived either from our (21, 30) or from other (31,

32) laboratories, speak in favour of direct melatonin influence on thyroid follicular cells. This hypothesis is further confirmed by the finding that the pituitary is not necessary to demonstrate the increase in thyroid weight after pinealectomy in mice (19, 33).

The activities of the following enzymes, treated as indices of growth processes, have been measured at our laboratory: thymidine kinase, thymidine phosphorylase and adenosine kinase. Additionally, we have examined the effect of indoleamines on cyclic AMP generation in rat thyroids *in vitro*. Thymidine kinase (TK: thymidine 5'-phosphotransferase, EC 2.7.1.21) is an enzyme responsible for catalyzing the phosphorylation of thymidine, functioning as a part of the pyrimidine salvage pathway involved in DNA synthesis and being closely correlated with ^{3}H-thymidine incorporation and mitosis. Adenosine kinase (AK; EC 2.7.1.20) is an enzyme which catalyses the phosphorylation of adenosine (Ado) and deoxyadenosine (dAdo) to adenosine monophosphate (AMP) and deoxyadenosine monophosphate (dAMP), respectively. Adenosine kinase functions as a part of the purine metabolic pathway involved in DNA synthesis and is the key enzyme regulating the Ado content. Thymidine phosphorylase (dThdPase, EC 2.4.2.4) is an enzyme catalyzing the reversible phosphorolysis of thymidine, deoxyuridine and their analogues to the respective bases and to 2-deoxyribose-1-phosphate. This enzyme has been proved to be identical with the platelet-derived endothelial cell growth factor (PD-ECGF), which is involved in the process of angiogenesis.

Melatonin and NAS have been shown to decrease the concentration of cyclic AMP (34) and to reduce the activity of TK (35) in rat thyroid lobes incubated *in vitro*. Whereas melatonin under conditions *in vitro*, decreased TK activity in thyroids collected from older rats (35), the indoleamine, added to the incubation medium containing thyroids collected from much younger - intact, sham-operated and hemithyroidectomized animals - increased TK activity (36). Thus, may be in young animals, melatonin serves as a hormone helping organs in growth and differentiation, whereas in adult animals the indoleamine prevents enhanced growth processes.

In another study, increased dThdPase activity in the remaining thyroid lobe was found following hemithyroidectomy. Melatonin, applied *in vitro*, decreased the dThdPase activity in thyroid lobes collected from intact animals, sham-operated animals and hemithyroidectomized rats (37). The results suggest an involvement of melatonin in the regulation of thyroid growth, probably by inhibition of the process of angiogenesis.

Concerning AK, a decreased activity of the enzyme was found in the remaining thyroid lobe, following hemithyroidectomy; melatonin, used *in vitro*, increased AK activity in thyroid lobes collected from intact and sham-operated rats, but it did not change AK activity in the remaining thyroid lobes after hemithyroidectomy (36). The results suggest a certain role of AK in the regulation of (patho)physiological processes in the thyroid gland after hemithyroidectomy.

Karyometry is the method used to assess the activity rate - mainly - of secretion but also of growth processes in various tissues and organs. An increased volume of cell nuclei may either result from enhanced DNA synthesis or emerge from a stimulated functional activity (increased protein synthesis). At our laboratory, we examined the influence of melatonin and TSH on karyometric parameters of rat thyrocytes. We found that a short-photoperiod exposure, associated with a stimulation of the pineal gland, resulted in a decrease of the mean volume of thyrocyte nuclei in male gerbils (38). Additionally, we observed that melatonin, administered in late-afternoon injections, decreased the mean nuclear volume of thyrocytes in male Syrian hamsters (27), and, when used *in vitro*, the indole significantly decreased the mean nuclear volume and the nuclear intersection area of thyrocytes (39).

The protective effects of melatonin against cancer is a subject of an intensive research (2-4,40). Because of the potential role of ionizing radiation in the pathogenesis of thyroid cancer, the studies on protective effects of melatonin against radiation-induced oxidative stress and cancer of the thyroid gland seem to be of special value. However, the published data concerning this issue, are rather scarce. It has been found that histoenzymological changes in rat thyroid gland, caused by an exposure to γ-radiation (8 Gy), were partially reversed by pretreatment with melatonin (41). In another study, when using morphometric parameters, melatonin was shown to decrease the height of thyroid follicular cells and the nuclear volume of the cells from rats exposed to 8 Gy-radiation (42). The potential protective effect of melatonin against thyroid cancer will unquestionably be a subject of future studies.

The influence of melatonin on thyroid secretion

Most studies, related to the effects of melatonin on thyroid function, revealed that this influence is an inhibitory one. Late afternoon s.c. injections of melatonin decreased circulating thyroid hormone concentrations in adult Syrian hamsters of both sexes (melatonin - 25 μg/daily) (43) and in male Wistar rats (melatonin - 50 μg/daily) (44).

An increase in serum thyroxine (T_4) concentration was found 10 weeks after pinealectomy performed in male Wistar rats, that process being prevented by melatonin administration, the concentrations of triiodothyronine (T_3) remaining unchanged in the pinealectomized rats (45).

However, an opposite effect was observed when melatonin was chronically released from s.c. pellets, implanted to male Wistar rats; the indoleamine increased both T_3 and T_4 levels after 10 days and also, however to a lesser degree, after 10 weeks; this effect may be called the "prothyroid" action of melatonin (44). On the other hand, the joint effect of late-afternoon melatonin injections and melatonin-implants caused no changes in thyroid hormone concentrations (44). Thus, this could be another piece of evidence, beside that observed in relation to growth processes, that melatonin is able to regulate its own action.

Not only may chronic melatonin availability but also a short-term treatment with the hormone result in a "prothyroid" action under certain conditions. Unexpectedly, when melatonin was injected in a dose of 25 μg/daily to rats for 5 consecutive days in the late light phase, it increased serum T_3 concentration, revealing a slight tendency towards rising serum T_4 (46). In the same study, also a 5-days treatment with NAS resulted in a "prothyroid" effect, concerning thyroid secretory processes.

Petterborg and Rudeen (47) have demonstrated that chronic afternoon melatonin administration in female hamsters results in a loss of estrous cyclicity, a significant gain in body weight and reduction of T_4 levels and T_3 uptake.

Wright *et al.* (48) were the first authors to have succeeded in showing an influence of melatonin on the amphibian thyroid gland. They found that melatonin directly antagonized the action of T_4 in promoting regression of tadpole tail tips *in vitro* (48). The *in vitro* secretion of T_4 from prometamorphic *Rana catesbeiana* tadpoles was significantly inhibited by melatonin in concentration of 10 mg/ml, and - especially - by that indoleamine in a higher concentration - 100 mg/ml, also in response to TSH; a complete suppression of the thyroid response to TSH was observed (49). In another *in vitro* experiment, thyroids from larval *Rana catesbeiana* or adult *Rana pipiens* were incubated in control or melatonin (0.01 to 100 mg/ml) media; melatonin directly inhibited T_4 secretion by thyroids from both tadpoles and frogs at all concentrations of melatonin used and at both prometamorphic and climax tadpole stages (50).

Melatonin administration to turtles decreased plasma T_4 levels, which was accompanied by reduced thyroid weight, reduced follicular epithelial cell height and a decreased activity of thyroid peroxidase (51). Effects of continuous light (CL; 24 L : 0 D), continuous darkness (CD; 24 D : 0 L) and pinealectomy (Px) were tested on the pineal-thyroid-gonadal axis of a tropical seasonally bred rodent, *Funambulus pennanti*, during its sexually active reproductive phase (February-March). CL had no effect on pineal, thyroid and ovarian functions, as demonstrated by thyroid and ovarian weight, plasma levels of T_4 and estradiol (E_2). However, CD reduced significantly thyroid and ovarian weight and plasma T_4 and E_2 concentrations (52). Pinealectomy resulted in stimulation of thyroid and ovarian functions under normal photoperiod, and under CL, Px significantly increased thyroid and ovarian weights with no observable changes in their hormonal levels. Under CD condition, Px prevented the reduction in thyroid and ovarian weights but the hormone levels were increased (52). These results suggest that CD may inhibit thyroid and ovarian functions by the stimulation of melatonin secretion (52). Furthermore, melatonin injection reduced T_4 concentration in control rats, and T_3 concentration in rats with transplanted anterior pituitary (53). The influence of melatonin on thyroid hormone secretion could either be direct or indirect. It has recently been shown that injections of melatonin caused a decrease in both blood TSH and thyroid hormone concentrations in rats (54). On the other hand, it has been shown that melatonin stimulates, whereas pinealectomy decreases TSH accumulation in the unique thyroid hormone-immunoreactive cells in rat pars tuberalis (55).

The influence of melatonin on the activities of monodeiodinases - enzymes participating in thyroid hormone metabolism in peripheral tissues - was also studied. It was previously shown that melatonin, released from s.c. pellets for 15 days, enhanced type II thyroxine 5'-monodeiodinase in brown adipose tissue of Syrian hamsters, without changing of serum thyroid hormone concentrations (56). Similarly, activation of cerebrocortical type II 5'-deiodinase activity in Syrian hamsters, kept under short photoperiod or subjected to the indoleamine liberated from s.c. pellets, was observed (57). It has been found in the more recent studies that treatment with melatonin results in an increased activity of type I 5'-monodeiodinase in the liver and kidney and of type II 5'-monodeiodinase in adipose tissue of newborn rabbits, the changes being accompanied by increased concentrations of serum T_3 and reverse T_3, and - unexpectedly - by increased concentrations of serum T_4; according to the authors' interpretation, the rise in T_4 concentration was probably due to the stimulatory effect of melatonin on the secretory activity of the thyroid gland (58). Moreover, melatonin supplementation in hyperthyroid rats was found to suppress secretion of thyroid hormones and of testosterone (59).

Melatonin as antioxidant and the thyroid gland

Several thyroid disorders are accompanied by enhanced oxidative stress. In an experimental model of hyperthyroidism, a 2-week-treatment with L-T_4 (100 µg/kg b. w. for 14 days) resulted, expectedly, in an increased concentration of free fractions of both thyroid hormones; a co-treatment with melatonin (5 mg/kg b. w. for 7 days) completely prevented the increase in the concentration of free T_3, being the most active form of thyroid hormones (60).

In another study, L-T_4-treatment resulted in an increased level of Schiff's bases, malondialdehyde and conjugated dienes concentrations - all being parameters of oxidative stress - in lung, brain and kidney homogenates; these changes were reversed by melatonin (61, 62).

Under physiological conditions, numerous free radicals and reactive species are produced in the thyroid gland (63). However, the oxidative reactions undergoing in the thyroid gland, could be - under certain pathological conditions - the source of oxidative damage. Iron, which is present in the thyroid peroxidase (TPO) - the key enzyme catalyzing thyroid hormone synthesis - and H_2O_2, which is essential for TPO activity, constitute substrates for Fenton reaction. Thus, using ferrous iron (Fe^{2+}) and H_2O_2, we have recently induced oxidative damage to lipids in

homogenates of porcine thyroid; Fenton reaction-induced lipid peroxidation [measured by the level of malondialdehyde + 4-hydroxyalkenals (MDA+4-HDA)] was prevented by melatonin in a concentration-dependent manner (64). Thus, we have shown for the first time that, under experimental conditions, it is possible to induce lipid peroxidation in the thyroid by using substrates of Fenton reaction and that this process could be prevented by melatonin.

Thyroid hormone-stimulation of pineal function or growth processes

The stimulatory effect of the thyroid hormones on the pineal gland is supported by many morphological, biochemical and clinical findings. Peschke (65-67) reported that T_4 significantly increased the surface area of cross sections of nuclei in rat pinealocytes *in vivo*. Thyroidectomy and/or methylthiouracil (an antithyroid drug) treatment caused a significant decrease of the surface area in question. Also the results of our studies speak in favour of pineal growth stimulation by thyroid hormones; thyroid hormones increased the mean nuclear volume of pinealocytes in organ culture, as well as slightly increased the mean mitotic activity rate of pinealocytes (68). In turn, Milcou *et al.* (69) found a significantly increased amount of DNA in rat pineals, following the administration of T_4 to *culture medium."Our hypothesis was further supported by the observation that* T_4 *and* T_3 *increased* melatonin concentration and norepinephrine-stimulated NAS content in cultured rat pineals (70).

The synthesis and release of melatonin were studied in pineal explants from 14-day-old (young) and 60-day-old ("maturing") male Long-Evans rats, in either the absence or the presence of T_3 being at or close to physiological levels and under light and dark conditions (71). Under light conditions, T_3 increased melatonin content in pineals and in medium of pineal cultures, the gland being collected from either young or maturing animals. Under dark conditions, T_3 decreased melatonin levels in the pineals of either age, but it did not affect melatonin levels in the medium (71). Since it is known from another study that 14-day-old rat pineal glands do not yet have a complete sympathetic innervation system, it seems evident that T_3 can modulate directly the pineal synthesis and the release of melatonin, while not depending upon a mature sympathetic innervation (71). Light - in the studied conditions - was permissive for the stimulatory action of T_3 on pineal synthesis and release of melatonin *in vitro* (71). The results confirm the pineal-thyroid feedback hypothesis, proposed originally by us (72-74).

With agreement with above observations is the finding that treatment with methimazole (resulted in hypothyroidism) caused a decrease in NAT activity in the Harderian gland of the male Syrian hamsters (75). In studies *in vivo,* treatment with T_4 resulted in an increased nocturnal peak of melatonin in rats (76).

Pineal-thyroid relationship in humans

The clinical data on the pineal-thyroid relationship are rather scarce. Whereas no changes were observed by some authors (77) in melatonin levels in either hypothyroidism or hyperthyroidism of human subjects, others investigators have found an increase in nocturnal melatonin concentrations in hypothyroid patients (78). Patients with hypothyroidism were found to have higher peak serum melatonin values, total nocturnal melatonin secretion and urinary excretion of melonin than normal individuals (78). Neither of those values differed significantly from normal in patients with thyrotoxicosis or obesity. Although thyrotoxic patients released normal amounts of melatonin during the night, their melatonin secretion peaks were phase-advanced (melatonin secretion peak appearing at 1.1 ± 0.5 h in thyrotoxic, and at 3.4 ± 0.5 h in normal participants). No such phasal shifts were seen in patients with obesity or hypothyroidism (78). Those findings imply that both hypothyroid and thyrotoxic patients have disturbed pineal function, which is not the case in patients with obesity (78). A decreased nocturnal melatonin blood concentration was observed in patients with recurrent non-toxic nodular goitre when compared to respective values in controls (79).

Blood concentrations of melatonin were evaluated in patients with very large non-toxic nodular goitre before and after thyroidectomy; unexpectedly, nocturnal melatonin concentrations

were significantly higher after than before the operation (80). The authors have drawn a conclusion that a very large goitre can possibly compress the superior cervical ganglia, and in consequence indirectly alter melatonin synthesis. Another explanation can be based on the assumption that melatonin may actively be taken up by enlarged thyroid with subsequent decrease in blood concentration of the indoleamine.

Melatonin treatment for 3-6 months in perimenopausal and menopausal women (aged 42-62 years), with initial low level of blood melatonin, resulted in a significant increase in thyroid hormone concentrations (81); thus, melatonin reveals a recovery effect of thyroid function towards a more juvenile pattern of regulation. It is not excluded that the described effect is a direct one because a similar treatment with melatonin in aging patients did not result in any changes in TSH concentration (82).

Concluding remarks

All the above mentioned results, while proving the suppression of thyroid growth and/or thyroid function by the pineal (83), as well as the reports on stimulation of the pineal gland activity and growth processes by the thyroid hormones (68, 70, 76, 84), have prompted us to formulate a hypothesis on the existence of a reciprocal relationship between the thyroid and the pineal gland (73, 74, 83) (Fig. 1).

The main conclusions from the up-to-date results, concerning thyroid-pineal relationship, are as follows: 1) melatonin influence on thyroid growth processes and thyroid hormone synthesis seems to be complex; it should be stressed once again that the evidence of the mutual relationship between the pineal gland and the thyroid is derived, almost exclusively, from studies performed in experimental animals; 2) the confirmation of these relations in clinical studies meets numerous difficulties and pitfalls, resulting, among others, from the fact that, nowadays, human beings, as well as animal species used in experimental studies, live far away from their natural and original habitats; 3) however, still much evidence indicates an undoubtful role of melatonin in physiological and pathological processes of the thyroid gland, providing "green light" for the future use of this indoleamine under certain clinical conditions.

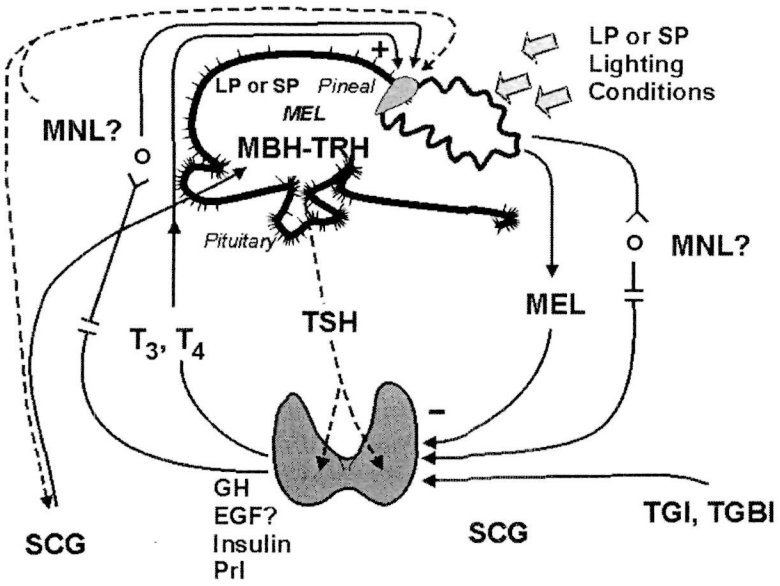

Fig. 1. A general model of possible reciprocal relationships between the pineal gland, the superior cervical ganglia and the thyroid, involved in the control of thyroid growth. EGF - epidermal growth factor, GH - growth hormone, IGFs - insulin-like growth factors, LP - long photoperiod, MBH - medio-basal hypothalamus, MEL - melatonin, MNL - multineuronal link, Prl - prolactin, SCG - superior cervical ganglion, SP - short photoperiod, T_3 - triiodothyronine, T_4 - thyroxine, TGBI - thyroid growth-blocking immunoglobulins, TGI -thyroid growth-stimulating immunoglobulins, TRH - thyrotropin releasing hormone, TSH -thyrotropin, VIP - vasoactive intestinal peptide.

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