000-1758-30

MASTER

THYROID SECRETION RATE AND THYROCALCITONIN

CONTENT OF THE THYROID IN FEMALE RATS^{1,2,3,4.}

Samarendra N. Baksi and Ralph R. Anderson

**Animal Science Research Center and Department
of Dairy Husbandry, University of Missouri
Columbia, Missouri 65201**

THYROID SECRETION RATE AND THYROCALCITONIN.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

1. Contribution from Mo. Agr. Exp. Sta. Journal Series No. 6197
Approved by the Director. This investigation was supported in part by a research grant from U.S. Atomic Energy Commission, Contract No. AT (11-1) C00-1758-30.
 2. Part of this investigation appeared as an abstract (Proc. 5th. Midwest Conf. of the Thyroid, Univ. Mo. Columbia, 1969.pp.90).
 3. Taken in part from the thesis submitted by S.N. Baksi for the requirement of the Ph.D. degree at the University of Missouri, Columbia, June, 1971.
 4. We are indebted to Dr. J.A. Parsons, Division of Biological Standards, National Institute for Medical Research, Mill Hill, London, N.W. 7, for the generous donation of Thyroid Calcitonin "B" preparation. Thanks are also extended to Drs. E.E. Pickett and S.R. Koirtyohann of the Spectroscopic Laboratory, Department of Agr. Chemistry, University of Missouri, Columbia for the help in calcium determinations.
-

Thyrocalcitonin (TCT), a hypocalcemic and hypophosphatemic polypeptide hormone secreted by the parafollicular or "C" cells of the mammalian thyroid (1), plays an important role in calcium homeostasis (2,3,). The main action of TCT was attributed to be on bone where it inhibits resorption and thereby decreases serum calcium level (4,5,6,7). It was suggested that TCT also helps in bone accretion (8,9). Thyroxine, the hormone secreted by the follicular cells of the thyroid gland, has an important role in regulating metabolic rate. Thyroxine reportedly increases the rate of bone formation and resorption and raises the levels of plasma and urinary calcium (10,11,12). The action of parathyroid hormone and thyroxine on calcium metabolism appears somewhat similar. Adams and Jowsey (12) suggested that in vivo thyroxine stimulates the metabolic activity of the bone cells directly. However, Gabbiani et al. (13) reported a somewhat opposite effect of thyroxine to parathyroid hormone in which they showed in rats thyroxine pretreatment prevents the morphologic lesions of kidney, heart, aorta and bone induced by parathyroid extract overdoses. The exact mechanism of action and role of thyroxine on bone is largely obscure. The anatomical proximity of the parafollicular cells to the follicular cells and the assumption that thyroxine may stimulate bone resorption, a process which TCT reportedly inhibits, led us to speculate a relationship between the functional ability of the thyroid gland to elaborate thyroxine and thyrocalcitonin. The present study was undertaken to investigate the possible relationship between thyroid hormone secretion rate (TSR) and the thyroidal content of TCT in mature female rats.

Materials and Methods:

a. Experimental Animals: Forty mature female rats of the Sprague-Dawley-Rolfsmeyer strain weighing 150-317 g were kept at a room temperature of $26 \pm 1^{\circ}\text{C}$ with uniform lighting conditions and fed Purina laboratory pellets and tap water ad libitum. All rats were ear marked for identification.

b. Estimation of TSR: TSR of all the rats was determined by the method described previously by Grosvenor and Turner (14) and slightly modified by Dryden et al. (15). All forty rats were injected i.p. with 10 uCi carrier free ^{131}I (Nuclear Consultant Corp., St. Louis, Mo.) in 0.2 ml distilled water. Forty-eight hours were allowed for the uptake of ^{131}I by the thyroid gland and for urinary excretion of excess isotope. External thyroid counts were taken at this time and at 48-hour intervals thereafter, by first anesthetizing each animal with ether, then placing it on a lead plate with its thyroid region over a scintillation probe. Measurements of thyroïdal radioactivity were made with a scintillation counter, Nuclear-Chicago (N.C.) Model DS5, connected to a pulse height analyzer (N.C. Model 1810) which in turn was connected to a rate meter (N.C. Model 1620A). Conventional corrections were made for radioactive decay and background. The rats were injected subcutaneously with 0.25 ug L-thyroxine (L-T_4) for two consecutive days commencing 2 days after the injection of ^{131}I . The thyroxine dose was increased at 0.25 ug/100 g bw increments every two days thereafter to the end of TSR estimation. All rats were injected s/c with 0.4 mg/100 g bw 1-methyl 1-2 mercaptoimidazole (methimazole), a goitrogen, to prevent recycling of metabolized radioiodine every day

together with L-T₄. The dose of L-T₄ which prevented the release of thyroid stimulating hormone (TSH) from the anterior pituitary, thereby preventing the release of thyroïdal ¹³¹I output in each rat (95-100 per cent of the previous does), was estimated as its TSR. TSR values reported herein are the thyroxine equivalents of the animal's endogenous secretion of both L-T₄ and triiodothyronine (L-T₃).

c. Biological Assay of TCT: After the TSR estimation, a period of two weeks duration was given each rat in order for the pituitary-thyroid axis to normalize and to eliminate any possible carry-over effects of methimazole. The rats were sacrificed and their thyroid glands were dissected out under a magnifying glass and the parathyroid glands were carefully removed. Homogenate from each thyroid was made separately in a glass homogenizer under ice using 6.67 ml of normal saline which has been adjusted to pH 4.6 with 0.1 N hydrochloric acid. One percent crystalline bovine albumin was added to each thyroid homogenate in a separate glass vial. The contents were quickly frozen at -20°C for bioassay later. All bioassays were conducted within three months using five thyroid homogenates for each assay. Rat thyroid homogenates in acid saline kept frozen have been reported to be potent for at least six months (16). Male rats weighing 115 g (110-120 g), 36 days old obtained from the Holtzman Co., Madison, Wis., were used for bioassay (17). All rats in each assay were born on the same day and randomly assigned to treatment groups. The diet was withdrawn for 17-20 hr prior to use but distilled water was supplied ad libitum. Five frozen thyroid homogenates (in 6.67 ml acid saline) were used in one day. The samples were thawed at room temperature. Two different dose levels with a threefold increment between the low and high dose were used in each assay. The high dose

was made up of 5 ml of the original homogenate whereas the low dose was prepared by diluting the remaining 1.67 to 5 ml with the same diluent. Similarly standard TCT (MRC Thyroid Calcitonin Standard B) was dissolved in acid saline in two different doses, a low dose of 18 mU and a high dose of 54 mU per ml. A single group of five rats was used for an acid saline control in all the eight assays. Each rat received 1 ml of the sample, standard and control vehicle. In one assay 65 rats were used with 5 animals per dose level. All 65 rats were injected sc in the back of the neck within 1 hr period (18). Blood samples of 3 ml were collected from each rat 1 hr after the injection by decapitating the animals using a heparin rinsed glass funnel for each rat. The plasma was separated by centrifugation at room temperature after overnight storage in the refrigerator and 0.3 ml of each sample was measured in each vial and kept frozen pending calcium analysis within two weeks.

d. Calcium Analysis: A volume of 0.3 ml plasma from each rat for each dose was diluted with 1040 ppm lanthanum in 0.06 N HCl (7.2 ml) making a 25-fold dilution and analyzed on a Perkin-Elmer 290 Atomic Absorption Spectrophotometer (19).

e. Statistical Analysis: The standard statistical design of the assay was the 20 dose factorial method (20) and the potencies were calculated accordingly. The index of precision, lambda (λ), was also estimated for the assay. The regression and correlation coefficient (r) between TSR and thyroidal TCT content was also calculated (21).

Results:

The TSR of the forty rats were estimated to be 1.10 ug L-T₃ per 100 g bw (range 0.50 to 2.0), whereas the TCT content was 395 MRC mU/gland (range 248 to 772 as shown in Table 1. The

average body weight of the rats was 232 g (range 150-317). The negative correlation coefficient ($r = -0.384$) between TSR and TCT content per gland was significant ($P < 0.05$ at 38 d.f.). The slope b was -107.66 and intercept was 512.53. These observations suggest that with higher TSR, TCT content of the gland decreases as shown in Figure 1. The index of precision, lambda (λ), of all the eight TCT bioassays was calculated to be 0.20.

Discussion:

The role of thyroxine on calcium metabolism and its relationship with parathyroid hormone (PTH) and TCT has been investigated by many authors (22, 23, 24). The findings however are somewhat contradictory in different animals. Gittes and Irvin (25) reported that thyroxine has an additive effect with that of TCT in rats in combating induced hypercalcemia. Gabbiani et al. (13) also reported that pretreatment with thyroxine inhibits the calcification of the kidney, heart and aorta as well as osteitis fibroosa produced by parathyroid extract overdose in normal, adrenalectomized, parathyroidectomized and thyroparathyroidectomized rats. These authors suggested that the prevention of morphologic lesions is probably mediated through the antihypercalcemic effect of thyroxine on blood calcium. Krane et al. (11) previously showed that the thyroid gland plays a role in determining the rate of clearance of calcium from circulation. Logan et al. (26) observed an increase in urinary calcium and the development of a negative calcium balance in puppies given thyroid extract for less than a week. However, there was no change in serum calcium level. These effects of thyroid hormone were abolished by prior parathyroidectomy. Adams and Jowsey (12) reported that thyroxine-induced hyperthyroidism causes an increase in bone

turnover, an increase in the serum calcium and a decrease in the serum calcium and a decrease in the plasma protein concentrations in parathyroid-intact and parathyroidectomized dogs. They suggested that thyroxine probably stimulates the metabolic activity of the bone cells in vivo and is responsible for the increased bone resorption. In our present study the endogenous thyroxine secretion rate was found to be inversely related to the TCT content of the thyroid gland in rats $r = -0.384$, $P < 0.05$ with 38 d.f.). This finding is in agreement with the idea that increased thyroxine level stimulates the metabolic activity of bone cells with increased bone resorption. The low TCT content in rats with higher TSR probably reflects the idea that calcium compartment sizes and rates of flow to and from these compartments are increased in hyperthyroidism (11). These studies indirectly support our view that increased metabolic state brings about increased exchange in calcium pool and hence need of TCT to keep the blood calcium level within normal range. It was also suggested that PTH induced acceleration of the rate of bone resorption is inhibited by TCT (27). Yasumura et al. (28) reported a significant depletion of thyroidal TCT which followed the administration of L-thyroxine. This is in agreement with our conclusion that higher secretion rate of thyroxine in vivo also depletes thyroidal TCT. It was also reported thyroidal content of TCT and TCT secretion rate are inversely related (16). Since less TCT in thyroid indicated higher secretion rate of TCT, it is quite probable the secretion rate of TCT is directly related to TSR.

The inverse relationship between thyroidal content of TCT and thyroxine secretion rate in rats reported in this study is in

agreement with the above assumption. The view of Yasumura et al. (28) that the action of thyroxine is probably due to an increased recycling of skeletal calcium secondary to the ability of thyroid hormone to accelerate bone resorption, rather than a direct effect of thyroid hormone on the secretion of TCT, is a feasible explanation of our observations also. The exact mechanism by which thyroxine affects a probably turnover of circulating calcium is not clear however.

TABLE 1.

Mean Thyroid Secretion Rate, Thyrocalcitonin Content of the Thyroid and Body Weight in Normal Mature Female Rats.

No. of Rats	Mean Body Weight $\pm$ SE* (gm)	Mean TSR ug/100 g bw/day $\pm$ SE	Mean TCT Content MRC mU/gland $\pm$ SE
40	232.9 $\pm$ 8.1	1.10 $\pm$ 0.05	395 $\pm$ 13

*SE = Standard Error of Mean

Figure 1. Relation of Thyroxine Secretion Rate (μ g L-T₄/100 g bw/day and Thyrocalcitonin Content of Thyroid gland (MRC mU/gland) in mature female Sprague -Dawley Rolfsmeyer rats.

Summary

The thyroid hormone secretion rates (TSR) of forty Sprague-Dawley-Rolfsmeyer rats, average body weight 233 g (150-317 g), were estimated using radioactive ^{131}I and increasing increments of exogenous L-thyroxine (L-T_4). The mean daily TSR was found to be 1.10 $\mu\text{g L-T}_4/100 \text{ g bw}$ (range 0.50-2.0 μg). The thyroïdal content of thyrocalcitonin (TCT) of these rats was estimated by bioassay using young rats. The average TCT content was found to be 395 MRC mU/gland (range 248-772 mU). It was found that thyroïdal TCT content and TSR have an inverse relationship ($r = -0.384$, $P < 0.05$ with 38 d.f.). It is concluded that higher TSR depletes thyroïdal TCT indirectly due to increased metabolic activity, increased bone resorption and increased recycling of skeletal calcium rather than directly increasing the TCT secretion rate.

1. Pearse, A.G.E. Proc. Roy. Soc. Ser. B 164, 478 (1966).
2. Copp, D.H. J. Endocrinol. 43, 137 (1969).
3. Hirsch, P.F. and Munson, P.L., Physiol. Rev. 49, 548 (1969).
4. Raisz, L.G., Au, W.Y., Friedman, J. and Neiman, I., Am. J. Med. 43, 684 (1967).
5. Hirsch, P.F. Endocrinology 80, 539 (1967).
6. Friedman, J., Raisz, L.G., Science 150, 1465 (1965).
7. Johnston, C.C. Jr., Deiss, W.P., Endocrinology 78, 1139 (1966).
8. Hirsch, P.F., Orimo, H., Sliwowski, A., Federation Proc. 28, 383 (1969).
9. Wase, A.W., Solewski, J., Rickes, E., and Seidenberg, J., Nature 214, 388 (1966).
10. Engfeldt, B., and Hjertquist, S.V., Acta. Endocri. 15, 109, (1954).
11. Krane, S.M., Brownell, G.L., Stanbruy, J.B., and Corrigan, H. J. Clin. Invest. 35, 874 (1956).
12. Adams, P., and Jowsey, J., Endocrinology 81, 735 (1967).
13. Gabbiani, G., Tuchweber, B., and Cote, G., Endocrinology 81, 798 (1967).
14. Grosvenor, C.E. and Turner, C.W., Proc. Soc. Exp. Biol. Med. 99, 517 (1958).
15. Dryden, G.L., Bauman, T.R., Conaway, C.H., and Anderson, R.R., Gen. Comp. Endocrinol. 12, 536 (1969).
16. Frankel, S., and Yasumura, S., Endocrinology 87, 602 (1970).
17. Cooper, C.W., Endocrinology 82, 1015 (1968).
18. Schlueter, R.J., and Caldwell, A.L. Jr., Endocrinology 81, 854 (1967).
19. Perkin-Elmer Corp: Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corp., Norwalk, Conn., 1965.
20. Bliss, C.I. The Statistics of Bioassay. Academic Press, New York 81, 735 (1967).
21. Snedecor, G.W., Cochran, W.G., Statistical Methods., The Iowa State University Press, Ames, 1967.

22. Cherny, S.N., Chausmer, A.B., Bellavia, J.V., and Wallach, S.,
Endocrinology 86, 1337 (1970).
23. Burkhardt, J.M., Jowsey, J., Endocrinology 81, 1053 (1967).
24. Jowsey, J., and Detenbeck, L.C., Endocrinology 85, 87 (1969).
25. Gittes, R.F., and Irvin, G.L. III., Endocrinology 79, 1033 (1966).
26. Logan, M.A., Christen, W.R. and Kirklin, J.W., Am. J. Physiol.
135, 419 (1942).
27. Mittleman, R., Wallach, S., Chausmer, A., Bellavia, J., In
Taylor, S. (ed.) Calcitonin. Proc. Symp. Thyrocalcitonin
and C cells. Heinemann, London, p. 276 (1968).
28. Yasumura, S., Burk, M., Chausmer, A., Mittleman, R., and Wallach,
S., Endocrinology 81, 256 (1967).