Effect of Graded Levels of Thyrocalcitonin upon Thyroid Secretion Rate, Endocrine Glands, Liver, Kidney and Spleen Weights of Female Rats[1] (36596)

SAMARENDRA N. BAKSI,[2] AND RALPH R. ANDERSON

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

Thyrocalcitonin (TCT) is a hypocalcemic and hypophosphatemic polypeptide hormone synthesized in and secreted by the mammalian thyroid parafollicular or "C" cells, which are embryologically derived from the ultimobranchial body (1, 2). Reports indicate that thyroxine, the hormone secreted by the follicular cells of the thyroid, and TCT have an interrelationship (3–6). We found that the thyroid secretion rate (TSR) and the thyroidal content of TCT in mature female rats have an inverse relationship (7), while Yasumura *et al.* (4) reported a significant depletion of thyroidal TCT following the administration of L-thyroxine. An investigation by Aldred *et al.* (8), concerning the chronic injection of TCT in graded doses into dogs and rabbits, revealed no changes in various tissues both grossly and microscopically.

The present study was undertaken to investigate the possible influence of graded levels of TCT given daily for at least 14 days upon TSR in rats. The chronic effect of exogenously administered TCT on the weights of endocrine glands, liver, kidneys and spleen was determined because such observations have not been reported previously in the rat. Studies published to date on the long-term effect of TCT have been concerned primarily with bone. In the event that TCT is used as a therapeutic agent the present findings will be helpful.

Materials and Methods. Fifty female rats of the Sprague-Dawley-Rolfsmeyer strain born on the same day were purchased from Dan Rolfsmeyer Co., Madison, WI, when they were 30 days old. They were kept at a room temperature of 26 ± 1° with uniform lighting conditions and were fed Purina laboratory pellets and tap water *ad libitum*. The rats were allowed 7 days time in the animal room for acclimatization after shipment. TSR was determined by the method described previously by Grosvenor and Turner (9) as modified slightly by Dryden *et al.* (10).

Twenty-eight days after the TSR was determined initially, the rats were divided into 5 groups of 10 animals each so that each group had a mean TSR equal to any other group. Group I served as the normal intact control group, while group II was injected intraperitoneally twice daily with 0.5 ml/100 g body weight of a carrier vehicle composed of 0.1 *N* HCl, 0.9% NaCl, and 0.1% albumin. Groups III, IV and V received 30, 60 or 90 mU TCT/100 g body weight twice daily by intraperitoneal injection in the carrier vehicle described. The TCT was purified porcine calcitonin (Armour Pharmaceutical Co., Kankakee, IL, Lot No. 423-079). Animals were treated with TCT for 14 days prior to the initiation of and during the TSR determination, which continued for an average of 10 additional days.

When TSR estimations were completed, the rats were killed by an overdose of ether and the following glands and organs were removed and weighed: ovaries, adrenals, pituitary, thyroid, uterus, liver, kidneys and spleen. The balance was sensitive to 0.01 mg.

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[2] Present address: The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

TABLE I. Effect of Graded Levels of Thyrocalcitonin (TCT) upon Thyroid Secretion Rate (TSR) in Intact Female Rats.

Treatment	Group	No. of rats	Before treatment	After treatment		
			Body wt (g) mean ± SE[a]	Body wt (g) mean ± SE	Mean TSR (μg/100 g body wt) mean ± SE	Percentage change (compared to normal)
Normal intact (no treatment)	I	10	240 ± 3.7	264 ± 3.3[b]	1.05 ± 0.03	—
Vehicle control (acid saline)	II	10	262 ± 4.1	274 ± 4.9[b]	1.06 ± 0.04	—
TCT (MRC mU/100 g body wt) twice daily						
30	III	10	251 ± 5.5	255 ± 4.1	0.78 ± 0.03[c]	—34.5
60	IV	10	255 ± 4.5	262 ± 5.3	0.97 ± 0.05	— 8.2
90	V	10	265 ± 6.7	284 ± 4.6[b]	0.89 ± 0.05[c]	—19.1

[a] SE = standard error.
[b] Significantly different ($p < .05$) by Student's t test from the before treatment body wt.
[c] Significantly different ($p < .01$) by Student's t test from normal intact and vehicle control groups.

Group means for TSR and endocrine and organ weights were tested for significant differences by Student's t test.

Results. Administration of 30 or 90 MRC mU of TCT/100 g body weight twice daily to rats for at least 14 days caused a significant decrease in TSR ($p < .01$), but the decrease in TSR in the group treated with 60 MRC mU of TCT was not significant when compared to either normal intact or vehicle treated groups, which were statistically similar (Table I). Body weight in normal intact and vehicle-treated groups increased significantly during the period comparable to treatment and TSR determination ($p < .01$). It also increased significantly ($p < .05$) during the treatment period in the group receiving 90 mU TCT but not in the groups receiving 30 or 60 mU.

Significant increases in weights of adrenal glands and liver were noted in all TCT treated groups when compared to normal intact or vehicle treated controls ($p < .05$). There were also significant ($p < .05$) increases in the weights of kidneys in groups treated with 60 or 90 mU TCT but not in the groups receiving 30 mU (Table II). TCT had no significant effect upon thyroid, pituitary, ovary, uterus or spleen weights over the dose range or time period used in this study.

Discussion. The relationship between TCT and thyroxine is uncertain although many investigators have reported interesting findings which suggest that one exists (3–6). Yasumura *et al.* (4) reported a significant reduction in thyroidal content of TCT after thyroxine injection in rats, but thyrotropin caused a significant reduction in only 1 or 2 experiments. They concluded that thyroxine increased the recycling of skeletal calcium by accelerating bone resportion and thereby influencing TCT secretion indirectly. Our finding (7) that the thyroidal content of TCT is inversely related to TSR in rats is consistent with this concept. In the present study TCT-treated rats had lower TSR than control animals. Injection of TCT twice daily in 0.1% serum albumin presumably causes a transient hypocalcemia lasting for 4 to 6 hr in rats (11). The parathyroid hormone (PTH) is probably secreted in response to hypocalcemia. The time required for PTH to act upon bone to cause hypercalcium is believed to be 4 to 6 hr (12). Therefore, it is reasonable to expect that TCT injection twice daily results in hypocalcemia over a

TABLE II. Effect of Graded Levels of Thyrocalcitonin (TCT) upon Endocrine Glands, Liver, Kidney and Spleen Weight in Intact Female Rats.

Item	Normal control (no treatment)	Vehicle control (acid saline)	TCT (MRC mU/100 g body wt) twice daily		
			30	60	90
No. of rats	10	10	10	10	10
Wt					
Body (g)	264 ± 3.3[a]	274 ± 4.9	255 ± 4.1	262 ± 5.3	284 ± 4.6
Ovary (mg)	86.5 ± 1.6	87.5 ± 1.9	87.9 ± 2.2	85.9 ± 1.8	89.0 ± 3.2
Adrenal (mg)	74.2 ± 1.4	73.2 ± 1.6	85.9 ± 1.3[b]	85.2 ± 2.6[b]	86.4 ± 3.3[b]
Pituitary (mg)	15.3 ± 0.4	15.4 ± 0.3	14.4 ± 0.6	14.5 ± 0.6	15.2 ± 0.4
Thyroid (mg)	14.6 ± 0.4	15.4 ± 0.3	14.0 ± 0.5	14.9 ± 0.3	15.2 ± 0.5
Uterus (mg)	790 ±69	770 ±63	610 ±50	760 ±56	870 ±54
Liver (g)	11.5 ± 0.3	12.0 ± 0.3	15.2 ± 0.5[b]	15.7 ± 0.3[b]	16.6 ± 0.3[b]
Kidney (g)	2.08 ± 0.92	1.92 ± 0.62	2.29 ± 0.15	2.59 ± 0.13[b]	1.61 ± 0.35[b]
Spleen (g)	1.56 ± 0.14	1.57 ± 0.13	1.37 ± 0.14	1.51 ± 0.12	1.61 ± 0.15

[a] Mean ± standard error.

[b] Significantly different from control by Student's t test ($p < .05$).

period of 7 to 8 hr in each day. During hypocalcemia animals undergo general depression of most physiological processes including cardiac function (13). The hypocalcemia probably causes a reduction in TSH secretion, thyroxine secretion, and the rate of utilization of thyroxine by peripheral tissues, resulting in a reduced TSR.

There was a significant increase in body weight ($p < .05$) in the control groups and the group treated with 90 mU TCT but not in the groups receiving 30 or 60 mU TCT. Aldred *et al.* (8) found no change in body weight after 7 days of TCT treatment in rats. Nevertheless, Copp, Low and Belanger (14) reported that in growing cockerels prolonged administration of TCT caused faster growth and earlier maturity compared to the controls. TCT has also been shown to increase bone formation, decrease bone resorption, or both (15–18). On the other hand, Helmer-Sorensen, Hindberg and Bank-Mikkelsen (19) reported that chronic TCT injection had no conclusive effect on bone in rats.

In this study, we found significant increases in adrenal gland and liver weights of all treated groups and in kidney weights of groups treated with 60 or 90 mU TCT. However, Aldred *et al.* (8) reported no significant changes in these organs when purified TCT was injected twice daily for 7 days at 10 U/kg/day in 0.9% saline or 5 U/kg/day in 16% gelatin. DeLuise, Martin and Melick (20, 21) have shown that the liver is the main site for accumulation and degradation of TCT injected into rats, while the kidney plays a minor role. Saba and Cunningham (22) recently reported that clinical hypocalcemia may cause sufficient stress in ewes to result in a rise in plasma adrenal steroid levels. It is possible that chronic injection of TCT caused a nonspecific stress reaction, with a concomitant increase in weight of the adrenal glands.

Summary. Fifty mature female rats were divided into 5 groups of 10 animals each on the basis of equal thyroxine secretion rate (TSR). Twenty-eight days after the TSR was determined initially, groups III, IV and V were treated with 30, 60 and 90 MRC mU of thyrocalcitonin (TCT)/100 g body weight twice daily intraperitoneally. Groups I and II acted as normal and vehicle treated groups, respectively. The treatment was started 14 days prior to the start of the TSR estimation and continued until the TSR of the animals was estimated. The animals were then sacrificed and the following glands and organs were removed and weighed: ovaries, adrenals, pituitary, thyroid, uterus, liver, kidneys and spleen. Administration of 30 and 90 mU

TCT/100 g body weight twice daily for at least 14 days caused a significant ($p < .01$) decrease in TSR, but the 60 mU dose of TCT did not. Significant ($p < .05$) increases in weights of the adrenals and liver were noticed in all the treated groups, whereas kidney weight changes were significant only in 60 and 90 mU groups. Exogenous TCT injection has a depressing effect on TSR. The increase in adrenal, liver and kidney weights might be due to nonspecific stress.

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1. Pearse, A. G. E., Proc. Roy. Soc. Ser. B **164**, 478 (1966).

2. Copp, D. H., Cockroft, D. W., and Kueh, Y., Science **158**, 924 (1967).

3. Gittes, R. F., and Irvin, G. L., Endocrinology **79**, 1033 (1966).

4. Yasumura, S., Burk, M., Chausmer, A., Mittleman, R., and Wallach, S., Endocrinology **81**, 256 (1967).

5. Cherny, S. N., Chausmer, A. B., Bellavia, J. V., and Wallach, S., Endocrinology **86**, 1337 (1970).

6. Jowsey, J., and Detenbeck, L. C., Endocrinology **85**, 87 (1969).

7. Baksi, S. N., PhD thesis, Univ. of Missouri, Columbia, MO, 1971.

8. Aldred, J. P., Clements, G. R., Schlueter, R. J., Bastian, J. W., Dailey, J. P. and Wazeter, F. X., *in* "Calcitonin: Proceedings of the Symposium on Thyrocalcitonin and "C" cells" (S. Taylor, ed.). Heinmann, London (1968).

9. Grosvenor, C. E., and Turner, C. W., Proc. Soc. Exp. Biol. Med. **99**, 517 (1958).

10. Dryden, G. L., Bauman, T. R., Conaway, C. H., and Anderson, R. R., Gen. Comp. Endocrinol. **12**, 536 (1969).

11. Schlueter, R. J., Aldred, J. P., and Fisher, J. D., Acta Endocrinol. **58**, 268 (1968).

12. Munson, P. L., *in* "The Parathyroids" (R. O. Greep and R. V. Talmage, ed.). Thomas, Springfield, IL (1961).

13. West, T. C., Fed. Proc. **27**, 120 (1968).

14. Copp, D. H., Low, B. S., and Belanger, L. F., 52nd Meet. Endocrine Soc. (Abstr. No. 173). St. Louis, MO (1970).

15. Foster, G. V., Doyle, F. H., Bordier, P. H., and Matrajt, H., Lancet **2**, 1428 (1966).

16. Wase, A. W., Solewski, J., Rickes, E., and Seidenberg, J., Nature (London) **214**, 388 (1967).

17. Matrajt, H., Bordier, P. H., Tun-Chot, S., Hioco, D., Foster, G. V., and Doyle, F. H. *in* "Calcitonin: Proceedings of the Symposium on Thyrocalcitonin and "C" Cells" (S. Taylor, ed.). Heinmann, London (1968).

18. Fujito, T., Orimo, H., Ohata, M., and Yoshikawa, M., Endocrinol. Jap. **15**, 8 (1968).

19. Helmer-Sorensen, O., Hindberg, I, and Bank-Mikkelsen, O., Acta Endocrinol. **68**, 203 (1971).

20. DeLuise, M., Martin, T. J., and Melick, R. A., J. Endocrinol. **48**, 181 (1970).

21. DeLuise, M., Martin, T. J., and Melick, R. A., J. Endocrinol. **48**, 173 (1970).

22. Saba, N., and Cunningham, N. F., Res. Vet. Sci. **12**, 483 (1971).

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