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Changes in Response to Calcitonin Following Prolonged Administration to Intact Rats (38163)

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Calcitonin (CT) is used in the treatment of a number of bone disorders, including Paget's disease and osteoporosis (1–3). Patients receiving long-term CT therapy normally have intact parathyroid glands, and a compensatory hyperparathyroidism has been reported as a result of the prolonged exposure to CT (3). Similar findings have been described in experimental animals (4–6). We have investigated the effects of daily CT administration for up to 90 days in rats with intact parathyroid glands, to determine the extent to which a compensatory hyperparathyroidism abolishes the response to CT.

Methods. The study was divided into two parts, to study the effects of CT on growing and mature animals. In the first part, 24 weanling male Long–Evans hooded rats received daily ip injections of CT[1] or vehicle for 90 days, at a dose level of 100 mU/day for the first 45 days and 200 mU/day for the remaining 45 days. Additional rats of the same age, and not previously exposed to CT received a single ip injection of 200 mU CT on the 90th day. All animals were sacrificed under ether anesthesia 2 hr after the last injection. Plasma was analyzed for calcium (7), phosphorus (8), and acid and alkaline phosphatase activities (9). The right humerus was ashed and analyzed for calcium (7) and phosphorus (8).

In the second part of the study, 80 mature (15-wk-old) male rats received daily ip injections of 500 mU CT or vehicle for up to 42 days. Animals were sacrificed at day 0 and after 2, 7, 21, and 42 days; in all cases the last injection was given 2 hr before sacrifice. Plasma and bone analyses were performed as in the first part of the study.

Results and discussion. The final body weight of rats receiving CT for 90 days was almost 10% higher than that of vehicle-injected rats (significance level $P < 0.02$; Table I). The dry weight of the humerus was also significantly higher ($P < 0.05$) in CT-treated animals, although the ash content and the composition of the mineral phase did not differ significantly from control values (Table I).

The final plasma calcium levels of rats receiving CT for 90 days were not significantly lower than in the vehicle-injected group, while plasma phosphorus was significantly depressed ($P < 0.005$) (Table II). By contrast, rats of the same age exposed to only a single injection of CT on the 90th day showed a significant decrease in both clacium ($P < 0.001$) and phosphorus levels ($P < 0.02$). No effect of the long-term CT treatment was observed on plasma phosphatase activities (Table II).

When larger doses of CT (500 mU/day) were administered for 6 wk to mature rats, no significant differences from control values were apparent in body weight (control group: 386.4 ± 19.1 g; CT group: 416 ± 12.5 g) or in dry weight of the humerus (control group: 279 ± 8.5 mg; CT group: 302.7 ± 6.9 mg). However, the same trend, although not significant, occurred toward an increased body weight (7.8% above control values) and bone mass (7.4% above control values) as was seen in the younger animals receiving CT for 90 days.

[1] Supported by Grant MT-320 of the Medical Research Council of Canada.

[2] Postdoctoral Fellow, Medical Research Council of Canada.

[3] Pure natural salmon calcitonin, supplied by Armour Pharmaceutical Company (Lot No. AL1025). The calcitonin preparation was dissolved in 0.1 *M* acetate buffer, pH 4.3, plus 0.1% gelatin, immediately before injection.

TABLE I. Effect of Daily Calcitonin Injections[a] for 90 Days on Body Weight and Bone Composition of Intact Rats

	Vehicle	Calcitonin
n	12	10
Initial body wt (g)	42.5 ± 1.1[b]	41.4 ± 1.0
Final body wt (g)	351.8 ± 8.3	384.9 ± 10.0**
Humerus		
Dry fat free wt (mg)	230.5 ± 5.2	245.7 ± 4*
% Ash	65.9 ± 0.3	65.2 ± 0.3
Ca content[c]	39.1 ± 0.1	39.4 ± 0.6
P content[c]	18.7 ± 0.1	18.6 ± 0.2
Ca: P molar ratio	1.62 ± 0.02	1.64 ± 0.01

[a] Natural salmon calcitonin, dissolved in 0.1 *M* acetate buffer, pH 4.3, + 0.1% gelatin. Dose levels: 100 mU/day for first 45 days, 200 mU/day for final 45 days. Final injection was given 2 hr before sacrifice.

[b] Mean ± SEM.

[c] Expressed as percentage of ash weight.

Significantly greater than control (vehicle injected) values (Student's *t* test): * $P<0.05$; ** $P<0.02$.

Both plasma calcium and phosphate concentrations in the mature rats were decreased in response to CT, and the effect appeared undiminished throughout the entire 6-wk period (Fig. 1). By contrast, CT produced only a transient decrease in plasma acid phosphatase activity, and the effect was abolished within 21 days (Table III). Plasma alkaline phosphatase activity was not influenced by CT at any time period.

Thus a preferential loss of response to CT was observed, rather than a total abolition of the action of the hormone. The depression of plasma acid phosphatase activity was the most short-lived effect, followed by the loss of the hypocalcemic response. No diminution in the hypophosphatemic or growth-promoting action of CT was observed in either the young or mature animals.

The loss of hypocalcemic response can be explained by an enhanced secretion of endogenous PTH, since plasma calcium concentration appears to be the stimulus evoking PTH secretion (10). The compensatory action of PTH may be mediated via an increased renal reabsorption of calcium (11) to balance the

TABLE II. Effect of Calcitonin Administration on Plasma Calcium, Phosphorus and Phosphatase Levels

	Vehicle	CT (90 days)[a]	CT (single injection)[b]
Calcium (mg/100 ml)	10.1 ± 0.1[d]	9.8 ± 0.2	9.3 ± 0.2***
Phosphorus (mg/100 ml)	6.1 ± 0.2	5.1 ± 0.2**	5.3 ± 0.1*
Acid phosphatase[c]	0.027 ± 0.002	0.024 ± 0.001	—
Alkaline phosphatase[c]	0.172 ± 0.012	0.181 ± 0.012	—

[a] Natural salmon calcitonin, 100 mU/day for 45 days, 200 mU/day for final 45 days. Last injection was given 2 hr before sacrifice.

[b] Single injection of 200 mU salmon calcitonin 2 hr before sacrifice.

[c] Enzyme activity expressed as μmoles *p*-nitrophenol liberated/min/ml plasma.

[d] Mean ± SEM for 10–12 observations in all cases.

Significantly lower than control (vehicle injected) value (Student's *t* test): * $P<0.01$; ** $P<0.005$; *** $P<0.001$.

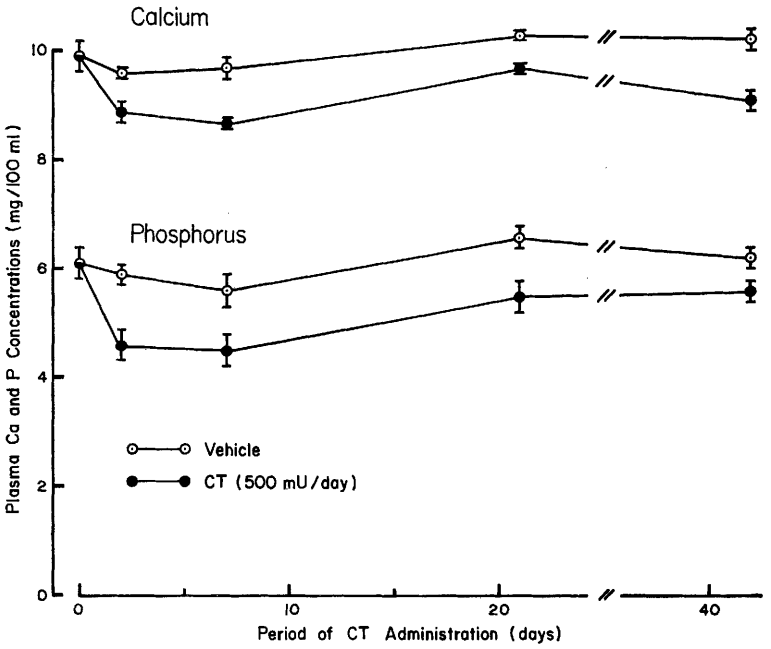

FIG. 1. Response of plasma calcium and phosphorus to daily calcitonin administration. Intact rats weighing approximately 340 g received 500 mU/day pure salmon calcitonin for up to 42 days. Final CT injection was given 2 hr before sacrifice. Each point represents the mean ± SEM of 8–10 observations.

decreased bone resorption, rather than a total abolition of the effect of CT. Sorenson *et al.* (1972) (6) reported that the degree of hypocalcemia in rats was diminished after 24 hr when CT was given at 4-hr intervals, but that a continuing depression of plasma calcium persisted for up to 10 days. In kittens, the hypocalcemia was abolished completely within 5 days when CT was administered every 4 hr (4). The slower loss of effect in the present

TABLE III. Plasma Phosphatase Activities of Intact Rats Receiving Daily Injections of Calcitonin[a]

Days of treatment	Treatment	Acid phosphatase[b]	Alkaline phosphatase
0	—	0.031 ± 0.003	0.197 ± 0.011
2	Vehicle	0.036 ± 0.001	0.144 ± 0.013
	CT	0.024 ± 0.001**	0.231 ± 0.056
7	Vehicle	0.029 ± 0.002	0.219 ± 0.017
	CT	0.023 ± 0.002*	0.204 ± 0.019
21	Vehicle	0.036 ± 0.002	0.215 ± 0.017
	CT	0.036 ± 0.003	0.187 ± 0.015
42	Vehicle	0.033 ± 0.003	0.178 ± 0.008
	CT	0.029 ± 0.002	0.171 ± 0.017

[a] Pure salmon CT, 500 mU per day. Final injection given 2 hr before sacrifice.

[b] Enzyme activity expressed as μmoles *p*-nitrophenol liberated/min/ml plasma. Mean ± SEM for 8–10 observations).

Significantly lower than vehicle-injected value: * $P<0.05$; ** $P<0.001$.

study can be attributed to the lower stimulus to increased parathyroid activity, since the duration of hypocalcemia following the once-daily CT injection was almost certainly only intermittent. Similarly, the restoration of normal plasma acid phosphatase activity, an indicator of bone resorption (12, 13), would be expected when an increased PTH secretion balanced the inhibitory effect of CT on bone resorption. The reason for the difference in the duration of the two effects is not clear.

The hypophosphatemic effect of CT is well known (14), and the persistence of hypophosphatemia following the loss of the hypocalcemic response has been described in kittens receiving CT for up to 10 days (4). Talmage *et al.* (1974) (15) have shown that the hypocalcemic and hypophosphatemic effects of CT may be at least partially independent; the hypophosphatemic response includes extraosseous and extrarenal effects, and involves an efflux of phosphate from plasma. Thus the loss of hypocalcemia without a loss of hypophosphatemia may result from a separation of the two effects of the hormone. The observation would also be consistent with an increased endogenous secretion of PTH, since PTH also lowers plasma phosphate levels, by increasing renal excretion of phosphate (16).

It is not possible to determine from this study whether the growth-promoting action of CT resulted from a direct effect on bone, or from a more generalized anabolic effect. An increase in bone mass in response to CT could occur via increased bone formation (17, 18) or decreased resorption (19, 20). Such a direct effect on bone may also be explained in terms of a secondary hyperparathyroidism. Kalu *et al.* (1970) (21) reported that the prolonged administration of PTH to intact or thyroparathyroidectomized rats exerted a marked anabolic effect on bone, as measured by total calcium and collagen content and by the rate of collagen synthesis. Selye (1932) (22) had earlier provided histological evidence of an anabolic effect of PTH on bone.

However, the increase in bone mass does not account for the total increase in body weight, and a more generalized anabolic effect of CT appears to be a more satisfactory explanation. This growth-promoting effect has also been reported in chickens (23) and in turtles raised on a calcium-deficient diet (24), following prolonged administration of CT. However, in previous studies of shorter duration in rats (5, 25) the effect was not observed, despite an increase in bone density (25).

Summary. The responses of intact rats to prolonged administration of CT, as measured by different parameters, were of variable duration. CT promoted an increase in body weight and bone mass (humerus) of approximately 1% per week above control values, and this effect was undiminished up to 90 days. Similarly the hypophosphatemic response to CT persisted for 90 days, while the hypocalcemic effect was not observed after 42 days. The decrease in plasma acid phosphatase levels was the most transient effect of CT, which was not obtained beyond 7 days. These findings are consistent with a compensatory hyperparathyroidism. The persistence of the hypophosphatemia and growth-stimulating effect may result from a concerted action of CT and PTH, while the disappearance of hypocalcemia and depression of acid phosphatase activity could be the result of antagonistic effects of the hormones.

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1. Bijvoet, O.L.M., Sluys Veer, J.v.d., Wildiers, J. and Smeenk, D., Proc. 2nd Symp. Calcitonin (1969), p. 531 (1970).

2. Woodhouse, N.J.Y., Reiner, M., Kalu, D.N., Galante, L., Leese, B., Foster, G.V., Joplin, G.F. and MacIntyre, I., Proc. 2nd Symp. Calcitonin (1969), p. 504 (1970).

3. Dubé, W.J., Goldsmith, R.S., Arnaud, S.B. and Arnaud, C.D. *in* "Calcium, Parathyroid Hormone and the Calcitonins" (R.V. Talmage and P.L. Munson, eds.), p. 113. Excerpta Medica, Amsterdam (1972).

4. Indech, M. and Jowsey, J., Endocrinology **88**, 1489 (1971).

5. Hindberg, I. and Sorenson, O.H., Acta Endocrinol. **66**, 137 (1971).

6. Sorenson, O.H., Hindberg, I. and Madsen, S.N., Acta Endocrinol. **71**, 313 (1972).

7. Instructional Manual Series 82-360 Jerrell-Ash Company (1964).

8. Fiske, C.H. and Subba Row, Y., J. Biol. Chem. **66**, 375 (1925).

9. Linhardt, K. and Walter, K., *in* "Methods of Enzymatic Analysis" (H.U. Bergmeyer, ed.), p. 783. Academic Press, New York (1965).

10. Patt, H.M. and Luckhardt, A.B., Endocrinology **31**, 384 (1942).

12. Vaes, G., J. Cell Biol. **39**, 676 (1968).

13. Doty, S.B., Schofield, B.H. and Robinson, R.A., *in* "Parathyroid Hormone and Thyrocalcitonin (Calcitonin)" (R.V. Talmage and L.F. Belanger, eds.), p. 169. Excerpta Medica Foundation, Amsterdam (1968).

14. Hirsch, P.F., Voelkel, E.F. and Munson, P.L., Science **146**, 412 (1964).

15. Talmage, R.V., Anderson, J.J.B. and Kennedy, J.W., Endocrinology (in press).

16. Kleeman, C.R., Rockney, R.E. and Maxwell, M.H., J. Clin. Invest. **37**, 907 (1958).

17. Gaillard, P.J., Proc. Kon. Ned. Akad. Wet. **C70**, 309 (1967).

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

19. Friedman, J. and Raisz, L.G., Science **150**, 1465 (1965).

20. Copp, D.H., Ann. Rev. Physiol. **32**, 61 (1970).

21. Kalu, D.N., Doyle, F.H., Pennock, J., Denys-Matrajt, H. and Foster, G.V., Calc. Tiss. Res. **4** (Suppl.) 72 (1972).

22. Selye, H., Endocrinology **16**, 547 (1932).

23. Copp, D.H., Gen. Comp. Endocrinology Suppl. **3**, 441 (1972).

24. Copp, D.H., Belanger, L.F., Dimond, M., Newsome, F., Ng, D. and O'Dor, R.K., *in* "Endocrinology 1971: Proceedings of the Third International Symposium" (S. Taylor, ed.), p. 27. Heinemann Medical Books, London (1972).

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

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