Calcitonin in tissues of thyroidectomized monkey[1]

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Summary. Immunochemical studies indicate that immunoreactive calcitonin (iCT) is present in many tissues of monkey following thyroidectomy (thx) (e.g. liver, thymus, lung). Extrathyroidal iCT may play a role in calcium metabolism.

Previously, we found immunoreactive calcitonin (iCT) in serum and/or urine of thyroidectomized man[2]. These results suggested that iCT synthesis is not confined to the thyroid gland. Subsequently, in an autopsy study of 23 patients, iCT was found in many extrathyroidal tissues[3]. Since these latter patients had intact thyroid glands, it appeared likely that some of the tissue iCT was being extracted from receptor sites; therefore, a control study was undertaken in monkeys.

Materials and methods. 5 young-adult male rhesus monkeys had an initial, 1-min i.v. infusion of pentagastrin (0.5 µg/kg) plus calcium gluconate (4 mg calcium/kg), and serum and urine iCT was determined by radioimmunoassay as described previously[4,5]. 3 animals then had a total thx, sparing the parathyroid glands. The completeness of thyroidectomy was verified by low serum thyroxine values and radio-technetium scans. The monkeys were maintained on thyroxine and sacrificed after 2 months. Serum, urine, thyroid gland, and 11 nonthyroidal tissues were assayed in all 5 monkeys. Tissues were boiled, defatted, and iCT was extracted with a mixture of CH_3CN and 1 M NH_4OH (1:1), and, subsequently, with butanol: $CH_3COOH:H_2O$ (12:3:5)[3]. Lyophilized extracts were reconstituted, boiled, and gel filtered (BioGel P-2) before radioimmunoassay. Data was obtained using 2 antisera having differing region specificities for calcitonin: Ab-IIIb (midportion) and Ab-IV (carboxyl terminal)[6]. Incubation damage was negligible and recovery was 65%.

Results. All tissues studied contained iCT (table). In the intact monkey, the level of iCT (Ab-IIIb) in all nonthyroidal tissues, excluding the liver, was 420±120 (mean±SD) pg/g wet weight, which is at least 21-fold that found in serum (<20 pg/ml). Comparable values were obtained with Ab-IV. 2 months after thx, the tissues had similar amounts of iCT (mean of nonthyroidal tissues excluding liver, Ab-IIIb: 570±190 pg/g wet weight) (figure 1). The liver of intact animals, which contained 21±12 ng/g wet weight of hormone, decreased by 41-fold after thx. The size (3×110 cm superfine G-75 Sephadex column) and charge (isoelectric focusing) characteristics for monkey (intact) liver iCT are nearly identical to those found for iCT of human thyroid, liver and serum fraction IV[3,5,6]. Similarly, liver iCT from thx monkeys contained principally fraction IV, which corresponds to calcitonin monomer[6]. Thyroid and lung extracts for intact monkeys also contained principally fraction IV although the thyroid contained a significant amount of fraction IIIA (iCT-dimer?). Although the thyroidal iCT concentration of

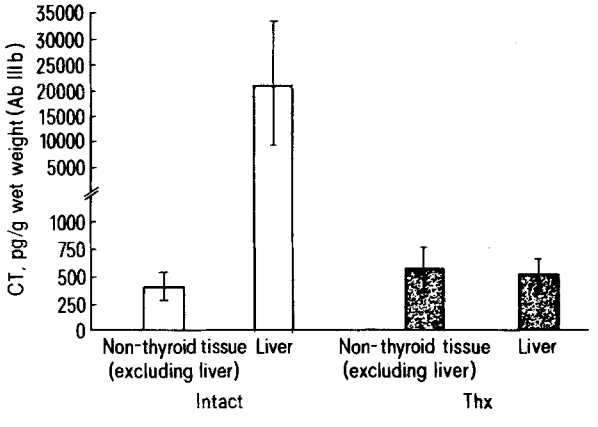

Fig. 1. Immunoreactive calcitonin in nonthyroidal tissue of intact monkeys, and animals 2 months after thyroidectomy.

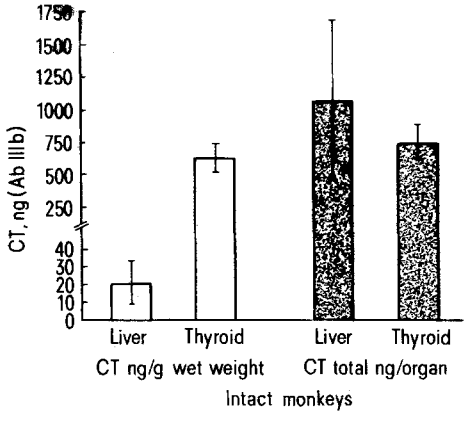

Fig. 2. Immunoreactive calcitonin in the liver and thyroid of intact monkeys.

Immunoreactive calcitonin content of tissues of intact monkeys, and animals 2 months after thyroidectomy (mean±SD)

Tissue	Ab-IIIb (pg/g wet weight)		Ab-IV (pg/g wet weight)	
	Intact	Thx	Intact	Thx
Thyroid	532,000±143,000	-	396,000±83,000	-
Liver	21,210±12,100	512±227	12,290±9350	478±161
Thymus	1840±71	2360±1080	1640±370	1780±910
Lung	509±284	1400±1870	596±438	982±1116
Submaxillary	490±35	292±13	842±27	836±127
Parotid	468±424	178±9	759±554	490±13
Duodenum	272±70	179±66	751±110	490±115
Kidney	168±14	245±109	241±2	285±58
Stomach	166±48	143±130	420±139	335±125
Jejunum	167±71	48±16	463±80	415±134
Skeletal muscle	134±78	128±38	266±35	308±115
Hypothalamus	29±17	723±1253	48±11	457±791

the 5 monkeys (620± 110 ng/g wet weight, Ab-IIIb) greatly exceeded that in liver of intact animals, when the total mass of the organs involved is considered, there is more iCT in the liver than in the thyroid (figure 2). Basal serum iCT was undetectable (< 20 pg/ml) in both thx and intact monkeys. Pentagastrin-calcium infusion resulted in an increase in thx monkey serum iCT values (Ab-IIIb) which was less than for intact animals (peak of 45± 50 pg/ml vs 104± 104 pg/ml). The basal urine iCT of intact and thx monkeys was detectable after boiling 5 min, concentration (lyophilization of 300 ml), extraction with NH_4OH and acetonitrile, and chromatography[5] (intact, Ab-IIIb - 97 pg/mg creatinine, thx, Ab-IIIb - 6 pg/mg creatinine).

Discussion. We have found that iCT is not confined to the thyroid gland. Undoubtedly, some of the iCT (such as that in the liver, muscle, or kidney) is receptor-bound hormone. The liver iCT decreased strikingly post thx. However, all tissues studied continued to have significant amounts of iCT 2 months after thx. Calcitonin is synthesized by C cells, which are believed to arise from the neural crest and migrate to the last branchial pouch and, subsequently, in mammals, to reside in the thyroid gland. These cells are part of a diffuse system of so-called APUD cells (staining characteristics: amine content and/or amine precursor uptake and decarboxylation)[7]. It seems likely that extrathyroidal iCT is the result of iCT secretion by these cells. In this regard, we have recently reported finding iCT in the bronchial K cells (Kultchitzky, enterochromaffin, argyrophil or Feyrter cells) of man[8]. Thus, the iCT content in any specific tissue of the thx animals could be the result of extrathyroidal secretion within that tissue, or receptor iCT which emanated from another extrathyroidal tissue, such as lung. Experiments implying a relative unimportance of iCT in the control of calcium metabolism are based upon the assumption that the thx animal is calcitonin-free; it would appear that such assumptions require critical re-examination.

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Pituitary response to gonadotropin-releasing hormone in diabetic male rats[1]

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Summary. The rise in serum luteinizing hormone concentration after treatment with gonadotropin-releasing hormone was less in diabetic castrated male rats than control castrates. In intact male rats, gonadotropin-releasing hormone treatment resulted in higher serum luteinizing hormone concentrations in diabetic than in control rats.

Diabetes mellitus seriously impairs reproductive function in male rats. Following experimental induction of diabetes, animals characteristically display reduced sexual behaviour[2], low fertility[3], reduced accessory sex gland weight[3-6], reduced numbers of interstitial cells in the testes[3], degenerative changes, in seminiferous tubules[7-9] and low serum levels of testosterone[3,5]. At least some of these changes could be related to reduced serum levels of luteinizing hormone (LH)[3,5].

Since glucose metabolism in anterior pituitary tissue is insulin-dependent[10], it is possible that the pituitary glands of insulin deficient rats are unable to respond adequately to gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus. The following experiments were conducted to test this hypothesis.

Materials and methods. Male Sprague-Dawley rats from our own colony were made diabetic at 3 months of age by an injection of streptozotocin (7 mg/100 g b.wt) into a tail vein. The induction and persistence of diabetes was confirmed by observations of glycosuria, polyuria and polydipsia.

In the 1st experiment, castrated rats were used. The testes were removed on the day before streptozotocin was injected. After 21 days, control and diabetic animals were lightly anesthetized with ether, a blood sample was obtained by heart puncture and GnRH (40 ng/100 g b.wt, in saline) was injected into a tail vein. Rats were decapitated 10 min after the injection and trunk blood was collected.

In a 2nd experiment, intact control and diabetic male rats were used on 3 occasions beginning 2 months after induction of diabetes. On each occasion rats were anesthetized with ether and injected via a tail vein with 500 ng GnRH/100 g b.wt. Rats were again anesthetized and a blood sample was obtained from the orbital sinus (first 2 occasions) or heart 10 min after GnRH injection.

In each experiment the sera obtained from the clotted blood samples were frozen and subsequently assayed[11] for LH concentration. The data were subjected to analysis of variance.

Table 1. Effect of diabetes on body weight and response to GnRH in castrated male rats (11 rats/group)

Group	Body weight (g)**	Serum LH (ng/ml) Before GnRH*	After GnRH*	ΔLH (ng/ml)*
Control	350± 19	728± 61	2507± 415	1775± 443
Diabetic	492± 10	522± 50	1402± 73	880± 64

* $p<0.05$; ** $p<0.01$; values are mean± SEM.

Results and discussion. The 1st experiment was designed to test the responsiveness of the pituitary to GnRH in the absence of the inhibitory effect of gonadal steroids. The data (table 1) indicate that diabetic rats had lower ($p < 0.05$) serum levels of LH than control rats both before and after treatment with GnRH. The reduced level of LH