RADIOIMMUNOASSAY FOR CHICKEN CALCITONIN

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1. Introduction

Calcitonin is the hypocalcemic, hypophosphatemic hormone produced by the thyroid in mammals and the ultimobranchial gland of submammalian vertebrates. Recent studies have resulted in the isolation, detailed structural analysis [1, 2], and synthesis of salmon and several mammalian calcitonins and, in turn, the development of sensitive radioimmunoassays for the hormone in these species [3].

Uncertainty regarding the physiological importance of calcitonin in mammals has prompted us to begin studies on the secretion and function of the hormone in submammalian species such as birds. Although in all species studied the hormone consists of a 32 amino acid polypeptide [4], considerable variation in amino acid sequence and, as a result, in antigenic determinants, has necessitated the development of separate immunoassays for each species of calcitonin except for the phylogenetically very similar porcine, ovine and bovine species. No purified calcitonins from any of the avian species are currently available to permit development directly of an avian calcitonin assay. However, we have found it possible to develop a radioimmunoassay for chicken calcitonin which employs antisera to synthetic salmon calcitonin. The assay has the requisite sensitivity for application to measurements of calcitonin in both plasma and ultimobranchial glands of chicks during the period of embryogenesis and after hatching.

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2. Methods

High affinity antisera were readily produced within 30–40 days in rabbits by immunization with purified synthetic salmon calcitonin (2700 MRC U/mg, available from Armour Pharmaceutical Company) using the method of immunization described by Vaitukaitis et al. [5]. Two of the three antisera produced against salmon calcitonin gave identical slopes of displacement of labelled salmon calcitonin by chick ultimobranchial extracts and synthetic salmon calcitonin. The most sensitive of the antisera for the chicken hormone was selected for further studies. Several antisera made to both human and to porcine calcitonin were found, incidentally, not to react with chicken calcitonin, suggesting that a closer structural similarity exists between avian and salmon calcitonins than between avian and mammalian calcitonins.

^{125}I-labelled salmon calcitonin was prepared by the chloramine-T oxidation method [6] and purified using powdered silica and gel filtration, as described previously [7]. Synthetic salmon calcitonin served as the assay standard. Typical assay mixtures contained sample antiserum in a final dilution of 1:40 000 and ^{125}I-labelled salmon calcitonin (200–400 Ci/g) sufficient to provide a counting rate of 10 000 cpm and diluted in buffer containing 0.02 M phosphate (pH 7.0), 0.001 M EDTA, 0.005% merthiolate, and 15% human plasma, in a final volume of 0.5 ml. Incubations were carried out at 4°C for 5 days. However, to enhance sensitivity, ^{125}I-labelled calcitonin was added after 3 days of incubation, and the incubation was then continued for an additional 2 days. Antibody-bound and free calcitonin were separated by precipita-

tion of bound ^{125}I-labelled calcitonin in 66% dioxane [8]. Radioactivity in the bound and free fractions was measured by a gamma spectrometer and the bound to free ratio of ^{125}I radioactivity was calculated using standard methods [8].

Chick ultimobranchial calcitonin was prepared by homogenization of freshly dissected ultimobranchial glands in 10–20 vol of 8 M urea –0.2 N HCl at 4°C. The extracts were clarified by centrifugation and were immunoassayed in multiple dilutions.

Blood was collected by venipuncture of allantoic or umbilical veins of chick embryos up to 19 days and, from 20 days on, by direct cardiac puncture. The blood was placed in heparinized tubes and plasma was separated immediately by centrifugation. Plasma was immunoassayed in multiple dilutions.

To assess the non-specific (non-immune) binding of ^{125}I-labelled calcitonin in the radioimmunoassay, duplicate aliquots of chick plasma or of ultimobranchial extract were incubated in each assay without addition of antiserum to the incubation mixture. Appropriate corrections were then made for the non-specific binding as described previously [8].

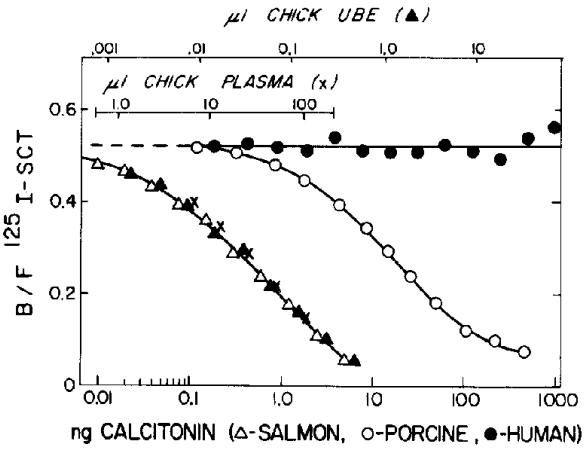

Fig. 1. Radioimmunoassay for chicken calcitonin; competitive displacement curves. Graph depicts relative displacement of ^{125}I-labelled salmon calcitonin (^{125}I-SCT) from binding to antiserum by increasing amounts of: (X—X—X) chicken plasma; (▲—▲—▲) chick ultimobranchial extract (UBE); (△—△—△) salmon calcitonin; (●—●—●) human calcitonin; (○—○—○) porcine calcitonin. Ordinate scale is expressed as bound to free ratio of ^{125}I radioactivity (B/F). Note parallel displacement curves produced by salmon calcitonin, chick plasma and ultimobranchial extract.

Table 1
Ultimobranchial (UB) and plasma calcitonin in embryo and newborn chicks.

Age of embryo (days)	Chick weight (grams)†	UB weight (mg)*	UB calcitonin (ng)** (per mg gland wt.)	Plasma calcitonin (ng/ml)**
12	3.6 ± 0.1 (12)	0.04	3.1 ± 0.6	–
14	8.3 ± 0.3 (8)	0.18	6.2 ± 0.2	< 0.6
16	13.3 ± 0.4 (5)	0.33	4.7 ± 0.3	
18	21.6 ± 0.4 (5)	0.42	20.0 ± 0.6	0.8
19	–	–	–	1.3 ± 0.5
20	29.8 ± 0.6 (5)	0.42	99 ± 6	3.4 ± 1.1
21 (Hatch)	–	–	–	6.9 ± 0.4
1	–	–	–	4.1 ± 1.7
2	41.4 ± 2.5 (5)	0.49 ± .05	805 ± 17	< 0.6
4	35.7 ± 1.4 (5)	0.43 ± .04	1370 ± 20	< 0.6

† Mean ± SE. Parentheses indicate number of embryos or chicks used at each age.
* Wet weight per gland except for 2 and 4 days after hatching, where glands were weighed individually and recorded as mean ± SE, weights are determined from the weight of pooled glands.
** ng In salmon equivalent units, determined by radioimmunoassay. [Concentration of standard salmon calcitonin was determined by amino acid analysis]. Values represent mean ± SE of multiple assay determinations.

3. Results

The immunoassay for chicken calcitonin, which utilizes an antiserum produced by immunization with salmon calcitonin and ^{125}I-labelled salmon calcitonin, detects as little as 50 pg of salmon calcitonin (fig. 1). The calcitonin in chick plasma or in an extract of chicken ultimobranchial gland gives a completely parallel curve of displacement to the salmon calcitonin standard, which reflects a high degree of immunochemical similarity between the chicken and salmon calcitonins in this assay system. Human and porcine calcitonins, two representative mammalian calcitonins, either do not cross-react at all (human) or very poorly (porcine) in the immunoassay. The assay is sufficiently sensitive to measure the amount of calcitonin contained in 1/12 000 of an ultimobranchial gland from a 4-day old chick (see table 1). No calcitonin was detected in extracts of chick thyroid, parathyroid or liver.

Measurements of calcitonin in chick ultimobranchial glands were made during embryonic development (table 1). A relatively constant amount of calcitonin per gland weight was observed during the phase of rapid ultimobranchial growth, from days 12 to 16. However, from day 16 through the hatching period, a striking exponential rise was found in the content of calcitonin in the ultimobranchial; the increase in calcitonin was more than 200-fold during this period. In addition, calcitonin in the plasma of the chick was found to rise sharply at the time of hatching and then to fall below the limit of assay detection by two days after the hatch.

4. Discussion

These studies demonstrate that antisera produced by immunization of rabbits with synthetic salmon calcitonin can be used as the basis for a sensitive radioimmunoassay for chicken calcitonin in both ultimobranchial tissue and blood. The identical curves of displacement seen between chicken and salmon calcitonins in the immunoassay indicates that immunological similarities and thus, similarities in amino acid sequence, must exist between portions of the chicken and salmon calcitonin molecules. Such immunological similarities are not seen between chicken and the human and porcine calcitonins and leads one to conclude that the structure of the chicken hormone is considerably different from that of the mammalian hormones. These observations are consistent with the evolutionary relationships between the calcitonins of known structure [9].

Our results thus far have shown a striking increase in the calcitonin content of the ultimobranchial glands of chicken in the few days just before and after hatching, as well as a brisk but transient neonatal phase of calcitonin secretion. These results are consistent with previous evidence based on morphological [10] and bioassay [11] studies. This rapid rate of accumulation of calcitonin stores is similar to the pattern of accumulation of insulin that has been observed in rat embryo pancreas [12]. Although the physiological significance of our observations has yet to be determined, the rapid burst of synthesis and secretion of calcitonin in the few days immediately preceding and following hatching is intriguing with regard to possible physiological actions of calcitonin at this critical phase in the metabolism of the chick. It appears that the late embryo and newborn period in the chick might be a promising phase of development in this species to further evaluate the physiological role of calcitonin through systematic studies of the factors controlling secretion of the hormone.

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