Calcitonin-like peptide in the shrimp *Palaemon serratus* (Crustacea, Decapoda) during the intermolt cycle

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Summary. A molecule immunologically related to salmon calcitonin has been detected in the hemolymph of the shrimp *Palaemon serratus*. Its concentration varies inversely with the calcium level during the molt cycle; a maximum (14 ng/ml) is found in the post-molt stage and a minimum (0.5 ng/ml) during the premolt stage.
Key words. Crustacean; calcitonin; radioimmunoassay; calcium; molt.

The calcitonins are single chain polypeptides containing 32 amino acids with a 1–7 disulfide bridge at the amino terminus and proline amide at the carboxyl terminus[1].

The hormone is implicated in the regulation of calcium metabolism. Its major function in mammals is the inhibition of bone resorption[2], while in seawater teleosts calcitonin decreases the influx and increases the efflux of calcium across the gills[3,4]. Thus its major role in both higher and lower vertebrates is the protection of the 'milieu intérieur' (internal environment) from calcium stress.

Recently, calcitonin-like molecules have been detected in an invertebrate[5,6], *Ciona intestinalis* (prochordate). Their physiological role is unknown. To this end, we have searched for the presence of calcitonin-like molecules in decapod crustaceans, as in this class the exoskeleton is calcified. During the molt cycle of these animals variations of calcium concentrations in the hemolymph have been reported[7]. Here we demonstrate the presence of salmon calcitonin-like molecules in the hemolymph of a crustacean: *Palaemon serratus* (Crustacea: Decapoda). The levels in the hemolymph reach a maximum during the premolt stage and this increase is coincident with a fall in calcium concentrations.

Material and methods. Animals. The shrimps used in this study were caught during the summer of 1984 in the bay of Concarneau. They were grouped according to their molt stages. Uropoda were cut, and hemolymph (50–100 µl) collected in microfuge tubes and separated from haematocytes by centrifugation. Approximately 100 animals were used in this study.

The total calcium concentration of hemolymph was estimated by a colorimetric method[8].

A heterologous radioimmunoassay[9] for shrimp calcitonin was used with synthetic salmon calcitonin (sCT) (biological activity 4000 U/mg, batch number 20,051, Sandoz) labeled with 125 I by the chloramine T-method[10]. In brief, the radioimmunoassay was performed with antibodies (final dilution $^1/_{20,000}$), 50 pg labeled hormone and increasing amounts of synthetic sCT or aliquots of shrimp hemolymph incubated in a buffer (0.1 M phosphate, pH 7.4, 0.2% bovine, heat denatured albumin, and 0.1% sodium azide) for 4 days at 4 °C. Free and bound hormone were separated by the dextran coated charcoal method; nonspecific binding of calcitonin to the diluted hemolymph samples was minimized by addition of 0.05 ml hemolymph from which endogenous calcitonin had been removed by affinity chromatography[11]. The calcitonin contents of the hemolymph samples were calculated from the sCT bound to antibodies from a standard curve which was linearized by logit-log transformation[12].

Molecular sieving. Hemolymph was chromatographed on a Sephadex G-50 superfine (Pharmacia) column (0.6 × 140 cm), equilibrated with 10 mM ammonium acetate, pH 8.5, at a flow rate of 2 ml/h. Absorbance was monitored at 230 nm, and 1.3 ml fractions were collected, lyophilized, redissolved in radioimmunoassay buffer and the calcitonin content assayed. The chromatography column was calibrated with albumin, RNAse, *Carcinus* hyperglycemic hormone, insulin, glucagon, salmon calcitonin and bacitracin.

Results. Similar displacement of (125 I)-sCT bound to antibody was observed with serial dilutions of shrimp hemolymph or synthetic salmon calcitonin (fig. 1).

Gel filtration analysis of hemolymph revealed two predominant

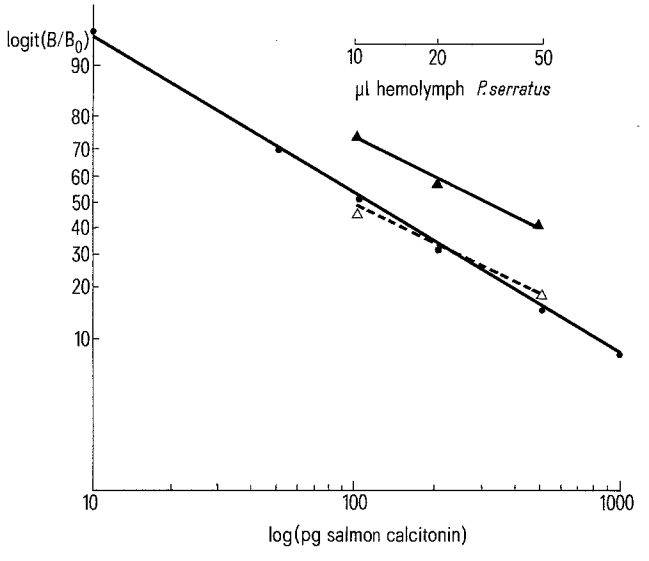

Figure 1. Immunoreactive salmon calcitonin-like material in hemolymph of *Palaemon serratus* (△ stage A and ▲ stage B, ● synthetic sCT).

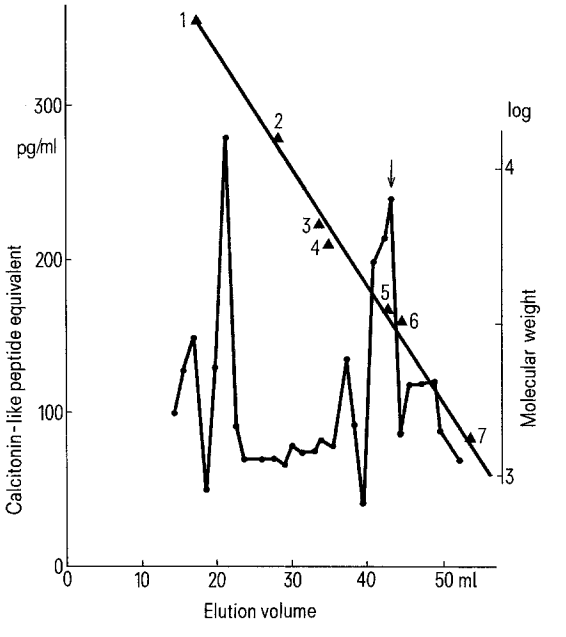

Figure 2. Gel filtration profile of calcitonin-like immunoreactivity in the hemolymph (0.6 ml) of *Palaemon serratus* on Sephadex G-50. Calibration substances are shown by closed triangles: *1* albumin = Vo, *2* RNAse; *3* *Carcinus* hyperglycemic hormone; *4* insulin; *5* glucagon; *6* salmon calcitionin; *7* bacitracin. The elution position of (125I) sCT is indicated by an arrow.

immunoreactive components, one peak eluting close to the exclusion volume and another peak coeluting with (125 I) sCT.

The calcium level in the hemolymph of *Palaemon serratus* showed a maximum in the premolt stage and a minimum at the post-molt stage B (fig. 3). The calcitonin-like material in hemolymph, on the other hand, attained a maximum during stage B of the molt cycle and a minimum at stage C.

No variations of the calcium concentration in hemolymph at different times (0, 15, 30, 60 min) were observed after administration of synthetic salmon calcitonin (100 ng/animal) at the stage D1 or D2.

Discussion. The results demonstrate the presence in the hemolymph of *Palaemon serratus* of immunoreactive salmon calcitonin-like molecules and confirm preliminary results obtained by immunocytochemistry[13]. On gel filtration analysis of hemolymph, two major immunoreactive peaks were detected, one with an apparent mol.wt similar to salmon calcitonin, and a larger mol.wt component. In human plasma, several immunoreactive components with mol.wt larger than those of human calcitonin (1–32) have been recognized[14]. The large immunoreactive form in hemolymph may represent aggregates or the small forms could be breakdown products of the larger form.

Variations of the concentration of the calcitonin-like molecules in hemolymph during the molt cycle were of particular interest. Our findings suggest that calcitonin-like peptides play a role in invertebrates in relation to the molt cycle. The fall in hemolymph calcium levels may be the consequence of the molecules. However, we have been unable to reduce hemolymph calcium levels by the exogenous administration of synthetic salmon calcitonin. In another invertebrate, *Ciona intestinalis,* human calcitonin-like molecules are present[6], and therefore the ancestral calcitonin gene is considered to be of the human type. Duplication of this ancestral gene[15] may give rise to the salmon, eel or chicken calcitonins[16–18] which seem to have appeared later in evolution. Our findings in *Palaemon serratus* of salmon calcitonin-like molecules suggests that in addition to a human calcitonin-like gene, a second salmon calcitonin-like gene may exist in invertebrates. We therefore propose that duplication of an ancestral calcitonin gene gave rise to both the human and teleostean genes at an early stage of evolution.

In conclusion, *Palaemon serratus* is an important animal model for the study of possible roles of calcitonin-like molecules present in invertebrates.

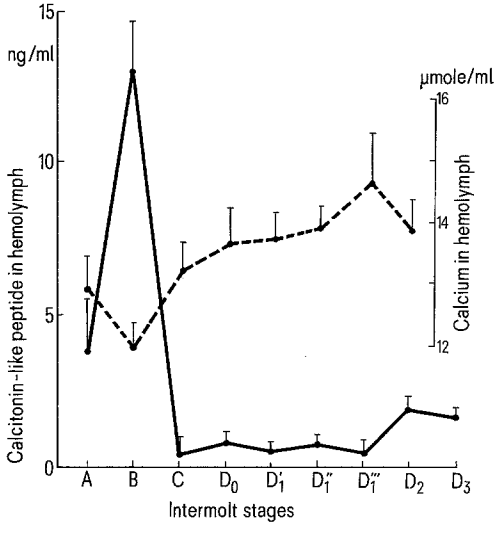

Figure 3. Levels of immunoreactive calcitonin (—) and calcium (– – – –) in the hemolymph during the intermolt cycle of *Palaemon serratus*. Measurement in triplicate of pooled (at least 10 shrimps) hemolymph samples.

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Vasoactive intestinal polypeptide stimulates prolactin release in vivo in the ring dove *(Streptopelia risoria)*

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Summary. I.v. administration of vasoactive intestinal polypeptide (VIP) to ring doves significantly elevated the plasma concentration of prolactin after 10 min in a dose-related manner. The plasma prolactin concentration of nonbreeding doves with low basal levels was increased by a similar amount as in brooding doves whose initially high concentration of plasma prolactin had been reduced by nest deprivation prior to treatment.

Key words. Prolactin release, stimulation of; vasoactive intestinal polypeptide; ring doves; nest deprivation.