

# Characterisation and distribution of calcitonin gene-related peptide in a primitive teleost, the eel, *Anguilla anguilla* and comparison with calcitonin

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## Abstract

Radioimmunoassay (RIA), radioreceptor assay and chromatography were used to study the occurrence of calcitonin gene-related peptide (CGRP) in a primitive teleost, the eel, *Anguilla anguilla*. Immunologically and biologically active CGRP-like molecules were found in brain, heart, kidney, liver, spleen and ultimobranchial body with the higher concentrations in brain, spleen and heart. Gel exclusion chromatography of heart and spleen extracts followed by SDS-PAGE showed that the eel CGRP-like molecules presented a molecular weight between 3.30 and 3.95 kDa similar to that of human CGRP. The wide distribution of CGRP reflects its multiple role as brain neuromediator and peripheral paracrine effector as described in mammals. In comparison, the distribution of calcitonin (CT) was much more restricted, immunologically and biologically active CT-like molecules being localised in the ultimobranchial bodies (UBB) that is the site of CT synthesis in non-mammalian vertebrates. In plasma, CGRP-like concentrations were 10 to 100 higher than those of CT. These high concentrations in a primitive teleost strengthen the possible endocrine role of CGRP in early vertebrates and emphasise the important role of this hormone in evolution.

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**Keywords:** Radioimmunoassay; Radioreceptor assay; Evolution; Endocrine; Paracrine

## 1. Introduction

In mammals, alternative tissue specific processing of initial mRNA transcripts from the calcitonin gene generates two distinct peptides: calcitonin (CT) in the thyroid C cells and calcitonin gene-related peptide (CGRP) in the peripheral and central nerves [1–3]. CT has a hypocalcemic and hypophosphatemic action [4]. CGRP controls mainly cardiovascular function [5] and inhibits gastric acid secretion [6]. At high doses, it presents the same action as CT. In teleosts, alternative splicing was also demonstrated generating two distinct transcripts [7,8]. As in other non-mammalian vertebrates, CT is synthesized in the C cells [9] associated with the ultimobranchial bodies (UBB). As shown in the trout, CGRP is present in many tissues mainly gills and intestine [10]. So far 12 different CT and 20

different CGRP molecules have been identified throughout the evolution in vertebrates [11]. For CT molecules, only 8 amino acids of the 32 residues are conserved. In contrast, CGRP differs in only 9 of the 37 amino acids in chicken, man, rat and salmon [11]. It can be assumed that CGRP is under stronger evolutionary control than calcitonin. The importance of CGRP during evolution was strengthened by studies performed in both vertebrates and invertebrates. Data we obtained in salmonids [12] and molluscs [13] suggested that CGRP may represent the ancestral molecule: both molecules could be detected in vertebrates, in contrast, only CGRP-related molecules were characterised in invertebrates. Studies concerned with the physiological actions of CGRP in teleosts demonstrated that this neuropeptide, by its specific interaction with trout gill membranes [14–16], may control hydromineral metabolism.

In order to further understand the evolution of these two peptides, we investigated their repartition in both tissues and plasma of the European eel, *Anguilla anguilla*, this species is representative of a phylogenetically ancient group (Elopomorphs) among teleost species [17]. This study was

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undertaken both in male and female silver eels in order to detect any change in the hormonal concentrations related to the sex of the animals. The presence of immunoreactive molecules was detected using specific radioimmunoassays (RIA) for salmon CT (sCT) and human CGRP (hCGRP). The immunoreactive molecules were further tested for their functional similarities with CT and CGRP using the ability of these two peptides to interact with their specific receptors. Finally, CGRP immunologically and biologically related molecules present in heart and spleen were partially purified in order to determine their apparent molecular weight.

## 2. Materials and methods

### 2.1. Materials

Human CGRP (hCGRP) and salmon CT (sCT) were obtained from Bachem, Bubendorf, Switzerland. Eel and chicken CT, chicken CGRP were from Sigma, St. Louis, MO.  $^{125}\text{I}$ -labelled hCGRP and sCT (specific activity 2000 Ci/mmol) were from Amersham, Uppsala, Sweden. Aprotinin was from Sigma. Bovine serum albumin (Sigma) was heat inactivated before use in radioreceptor assays (RRA).

### 2.2. Animals and sampling procedure

These experiments were carried out in accordance with the current legislation on animal experiments in France. Male ( $104 \pm 10$  g BW) and female ( $340 \pm 60$  g BW) fresh water silver European eels, *A. anguilla*, were caught in the Loire river, France, by a professional fisherman at the time of the downstream migration. At this stage, male and female eels were still sexually immature with a gonado-somatic index of  $0.16 \pm 0.02$  and  $1.98 \pm 0.07\%$ , respectively. Eels were killed by decapitation. Blood was collected on heparin. Various tissues (brain, gill, heart, intestine, kidney, liver, spleen, stomach and UBB) were collected, frozen in  $\text{N}_2$  liquid and stored at  $-80$  °C until extraction. Plasma was separated by centrifugation and stored at  $-20$  °C until assayed.

### 2.3. Extraction procedure

Plasma was purified on Sep Pak  $\text{C}_{18}$  (Waters, Milford, MA) reverse phase sample cartridge. After adsorption, peptides were eluted with  $\text{CH}_3\text{COOH}/\text{acetone}/\text{H}_2\text{O}$  (20:30:50 v/v); after acetone evaporation, extracts were freeze-dried and stored at  $-20$  °C until processed [10]. Using this extraction procedure, 71% and 75% of synthetic sCT or hCGRP added to eel plasma extract were recovered, respectively.

Tissue samples were homogenised in 0.1 M  $\text{CH}_3\text{COOH}$  (20 vol/g) using an ultra-turrax. After centrifugation, the supernatant was freeze-dried and stored at  $-20$  °C until processed.

### 2.4. Radioimmunoassays of CT and CGRP-like molecules

Screening of CT and CGRP in plasma and various tissues (brain, gill, heart, intestine, kidney, liver, spleen, stomach and UBB) was performed using male and female silver eels. Tissue samples and plasma were assessed using RIAs specific for hCGRP and sCT. Anti-hCGRP and anti-sCT antibodies were kindly donated by Dr. A Jullienne U 349 INSERM Paris.

Both RIAs were performed as previously described [12]. Antibodies were first incubated with tissue extracts for 18 h at 30 °C, then  $^{125}\text{I}$ -labelled hormones were added and the incubation continued for 24 h at 4 °C. Bound and free hormones were separated by charcoal/dextran precipitation. Results were expressed as the percentage of maximal binding ( $B_0$ ) where  $B_0$  represents the binding of the labelled peptide in the absence of unlabelled hormone. Logit–Log linearization of the standard curves were obtained by plotting  $\text{Ln} [(B/B_0)/(1 - B/B_0)]$  as a function of the  $\text{Ln}$  of hormone concentration. Each tissue extract or plasma sample was processed at serial dilutions and the dose–response curves compared to the standard curve in order to estimate their parallelism. Results were expressed as ng of immunoreactive peptides per organ or per ml of plasma.

### 2.5. Radioreceptor assays

Receptor binding ability for immunoreactive molecules was developed using rat kidney and liver membranes for CT and CGRP, respectively. Rat kidney membranes were prepared according to the technique described by Fitzpatrick [18]. The method described by Neville [19] was used to obtain rat liver membranes. Incubations in a 400- $\mu\text{l}$  final volume, were performed at 22 °C for 1 h [20] or 4 h [21] for hCGRP and sCT RRA, respectively. Bound and free hormones were separated by centrifugation. Data were expressed as specific binding, this is obtained by subtracting the nonspecific binding (amount of radioactivity associated to the membranes in the presence of 0.3 and 2  $\mu\text{g}/\text{ml}$  of unlabelled hCGRP and sCT, respectively) from the total binding. Receptor binding ability of eel tissue extract was performed using brain, heart, kidney, liver, spleen and UBB from four female silver eels. Logit–Log transformation was used to compare the slope obtained with serial dilutions of tissue extract to that of the standard curve. Results were expressed both per organ and per  $\mu\text{g}$  of protein. Protein content in tissue extracts was quantified using BSA as the standard and the bicinchoninic acid (BCA) protein assay from Pierce, Rockford, IL [22].

### 2.6. Molecular sieving

Heart and spleen of 20 female silver eels were pooled and acid (acetic acid) extracted. Heart (6.5 mg) and spleen (36 mg) of protein were subjected to gel exclusion chromatography on a TSK HW 50 toyopearl (Sigma) column

(2.5 × 44 cm) using ammonium acetate 0.2 M, pH 5 as eluant. The flow rate was 44 ml/h. Standard hCGRP and various molecular weight markers (Biorad) were used to calibrate the column. CGRP-like immunoreactivity of each column fraction was determined on a 100- $\mu$ l aliquot. RRA of immunoreactive fractions were performed using protein concentrations ranging from 50 to 150  $\mu$ g. Fractions containing bioactive CGRP were subjected to SDS PAGE on 10–20% tris tricine gels (ready gels from Biorad, Hercules, CA). Gels were calibrated with SDS-PAGE polypeptide standards (Biorad) and hCGRP. Protein bands in the gels were stained using Coomassie blue G 250.

### 2.7. Statistical analysis

Statistical analysis was performed with a statistic package (Staview from Biosoft, Cambridge, UK). All data are shown as means  $\pm$  S.E.M. Differences between groups were assessed by Student's *t*-test, and a probability of  $p < 0.05$  was considered statistically significant. Slope comparison of the different dilution curves was performed using the Statgraphics software.

## 3. Results

### 3.1. Specificity of CGRP and CT RIA

In the RIA for hCGRP (Fig. 1, left panel), chicken CGRP showed a lower cross reactivity than the standard hormone: 20% inhibition of initial CGRP binding was observed with 20 and 170 pg of hCGRP and chicken CGRP, respectively; the comparison of the regression lines between hCGRP and chicken CGRP demonstrated signifi-

cant differences ( $p < 0.05$ ). In this RIA, salmon and eel CT showed no cross reactivity.

Plasma and various tissues extracts (brain, gill, heart, intestine, kidney, liver, spleen, stomach and UBB) were analysed in this RIA. Each tissue extract except stomach was able to displace the binding of CGRP to its antiserum. The dose–response curves were parallel to that of the standard curve. Two examples (heart, plasma) are shown in the Fig. 1, left panel.

In the RIA for sCT, eel CT and chicken CT showed 100% cross reactivity. Fifty-percent inhibition of sCT binding was observed with 151, 261 and 419 pg for salmon, eel and chicken CT, respectively. No significant difference could be demonstrated between the slopes of the dose–response curves for sCT and either chicken CT or eel CT ( $p > 0.05$ ). No cross reactivity was observed with chicken or hCGRP. Eel plasma and tissue extracts were analysed in this RIA. Plasma and some tissue extracts (brain, kidney, liver and UBB) were able to displace the binding of sCT to its antiserum with response curves parallel to that of the standard curve. Two examples (plasma and UBB) are given in Fig. 1, right panel. In contrast, no displacement was observed with gill, heart, intestine, spleen and stomach extracts until the maximal amount of proteins analysed (400  $\mu$ g).

So, in the CGRP RIA, no cross reactivity was observed with the teleostean (eel, salmon) CT. Plasma and tissue extracts cross reacted in this RIA allowing the quantification of CGRP in eel samples by reference to this RIA. In the CT RIA, eel and chicken CT showed 100% cross reactivity, no cross reaction was observed with either human or chicken CGRP, the cross reactivity of plasma and tissue extracts in this RIA allows the quantification of CT in eel samples by reference to this RIA.

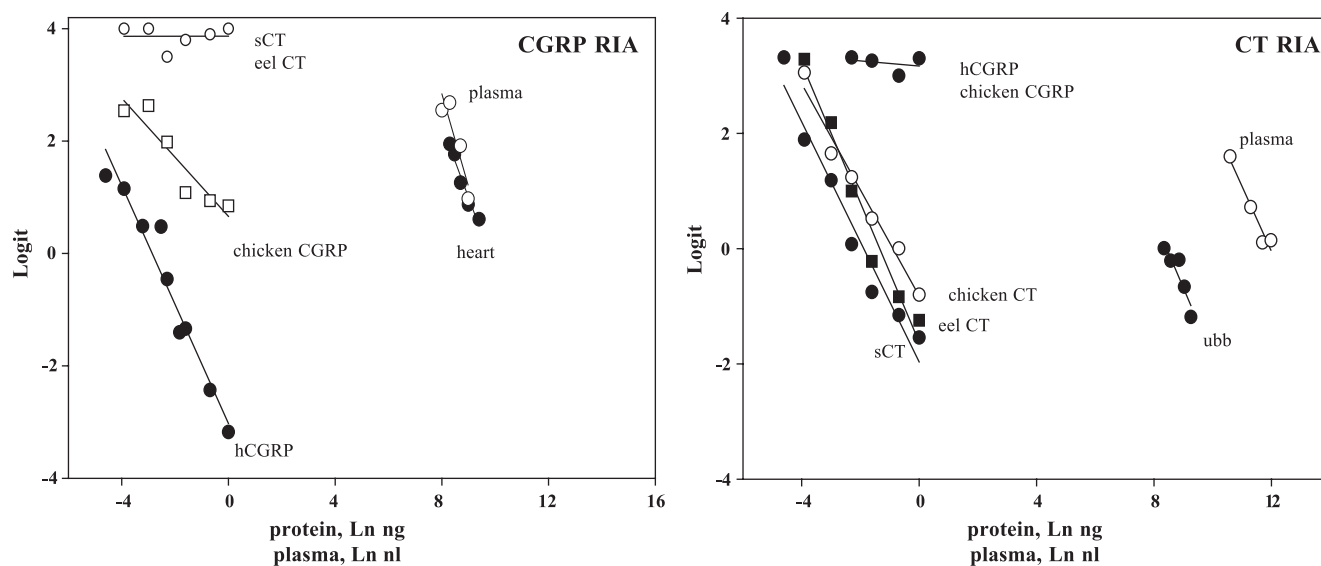


Fig. 1. Effect of increasing quantities of CGRP/ or CT/ analogues and eel tissue or plasma extracts on the binding of  $^{125}$ I-labelled hCGRP (left panel) and  $^{125}$ I-labelled sCT (right panel) to their respective antibodies. Results expressed as Logit:  $\text{Ln} [(B/B_0)/(1 - B/B_0)]$  were plotted as a function of the logarithm of protein concentration (ng) or as a function of the logarithm of plasma volume (nl).

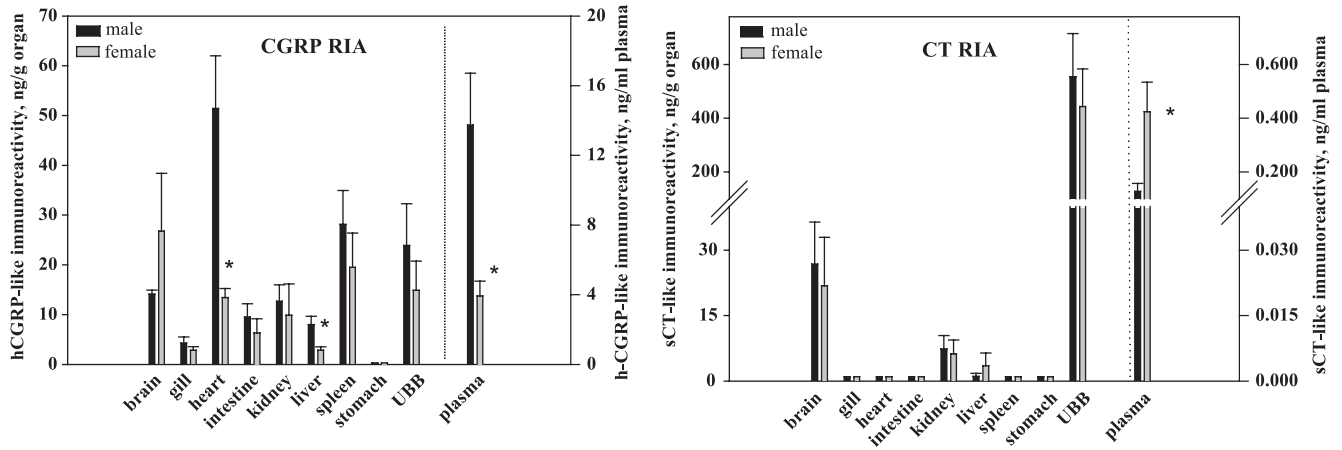


Fig. 2. Concentrations of CGRP (left panel) and CT (right panel) like immunoreactivity in tissues and plasma of male and female silver eels as determined by specific RIA. Each tissue was analysed at multiple dilutions. Results were expressed as ng immunoreactive peptide per g of organ or per ml of plasma. Each bar represents the mean  $\pm$  S.E.M. of five male and six female eels. \* $p < 0.05$  when compared to male eels (Student's *t*-test).

3.2. CGRP and CT-like immunoreactivity in various tissues from female and male eels

These radioimmunoassays were used to quantify the CGRP and CT-like immunoreactivities in various tissues of both male and female eels (Fig. 2). When CGRP was measured in the different organs (brain, gill, heart, intestine, kidney, liver, spleen and UBB), the highest level of CGRP-like peptide was found in brain, heart, spleen and UBB ranging between 15 and 50 ng/g of organ. In gill, liver, intestine and kidney, the CGRP level was lower ranging between 3 and 10 ng/g of organ. A significant difference ( $p < 0.05$ ) was observed between male and female eels in two organs: the heart and the liver. In male, the CGRP-like concentration was three times higher than in female. When CT was measured in the same tissues, a different distribution was observed. The highest levels of CT-like immunoreac-

tivity (Fig. 2, right panel) were detected in the UBB with concentrations of  $554 \pm 161$  and  $443 \pm 140$  ng/g of sample in male and female eels, respectively; no significant difference was observed according to the sex of the animals. In brain, the concentration of immunoreactive CT was about 20 ng/g in male and female, in the kidney the concentration was about 6 to 7 ng in both sexes. A lower immunoreactivity was found in the liver with 3 ng/g in the female and no detectable immunoreactivity in the male.

In plasma (Fig. 2), high circulating levels of CGRP were measured. The circulating CGRP concentration was  $13.74 \pm 2.98$  ( $n = 5$ ) in male and  $4.11 \pm 0.74$  ( $n = 5$ ) ng/ml in female eels. Plasma CT levels were much lower accounting for  $0.13 \pm 0.03$  ( $n = 4$ ) and  $0.42 \pm 0.11$  ( $n = 4$ ) ng/ml, respectively. Sex comparison indicates significantly higher levels of CGRP in male than in female ( $p < 0.05$ ) and inversely higher CT levels in female than in male ( $p < 0.05$ ).

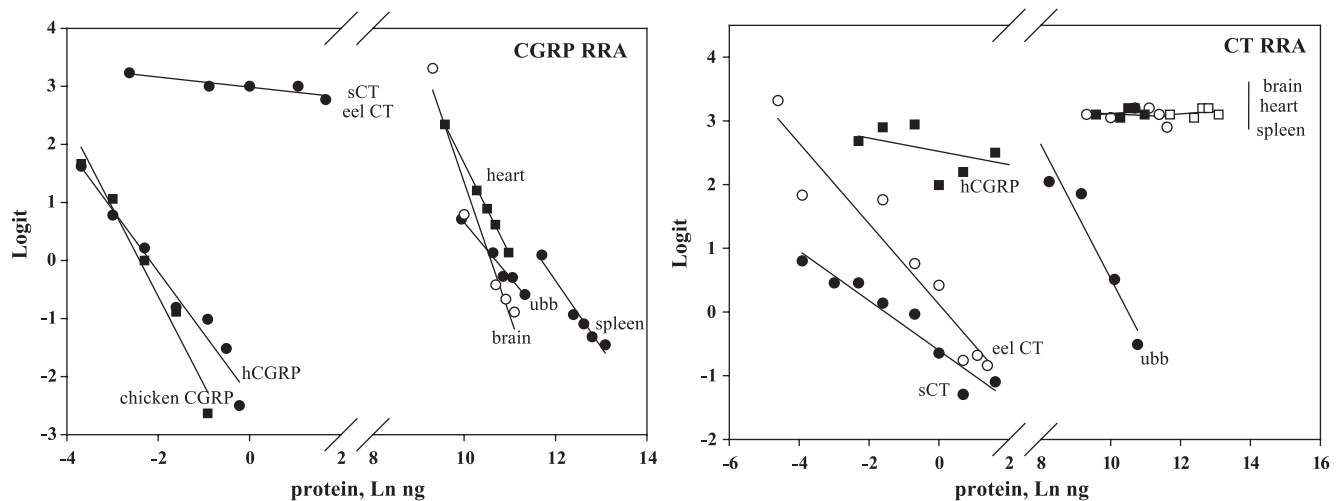


Fig. 3. Effect of analogues and tissue extracts on the  $^{125}\text{I}$ -labelled hCGRP (left panel) and  $^{125}\text{I}$ -sCT (right panel) binding to their specific receptors present in rat liver and kidney membranes, respectively. Data were expressed as in the legend to Fig. 1.

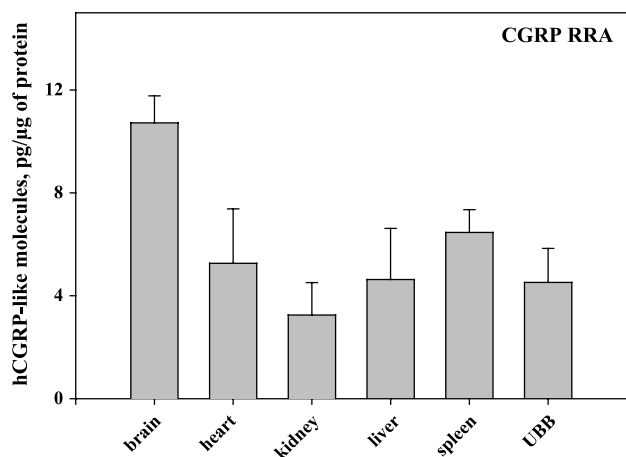


Fig. 4. Concentrations of biologically active CGRP molecules, as determined by RRA, in female silver eels. Each tissue was processed at multiple dilutions. Results were expressed as pg biologically active per  $\mu\text{g}$  of protein. Each bar represents the mean  $\pm$  S.E.M. of four female eels.

### 3.3. Characterisation by radioreceptor assay of CGRP and CT in various eel tissues

The CGRP and CT-like compounds identified by RIA were further characterised by their ability to displace the specific binding of labelled CGRP and CT to their target organs, that is rat liver and kidney membranes, respectively (Fig. 3).

In the CGRP RRA (Fig. 3, left panel), the displacement obtained with chicken CGRP was parallel to that of the standard peptide (hCGRP). Fifty-percent inhibition of the  $^{125}\text{I}$ -labelled hCGRP binding was obtained with 113 and 101 pg of hCGRP and chicken CGRP, respectively. No displacement was observed with sCT. Among the various tissues containing immunoreactive CGRP-like molecules that is brain, gill, heart, intestine, kidney, liver, spleen, and

UBB, only gill and intestine extract tissues were unable to interact in the CGRP radioreceptor assay. A displacement of the  $^{125}\text{I}$ -labelled hormone binding was observed with the kidney (slope:  $-1.427$ ), the liver (slope:  $-1.00$ ), the UBB (slope:  $-1.658$ ), the brain (slope:  $-0.860$ ), the heart (slope:  $-1.53$ ) and the spleen (slope:  $-0.725$ ) extracts. These slopes were not significantly different from that of the standard curve ( $-1.373$ ). Four examples are shown in Fig. 3, left panel.

In the CT RRA (Fig. 3, right panel), eel CT was able to displace the binding of sCT to its receptors with a slope not significantly different from that of the standard curve with sCT; 50% displacement of the initial CT binding was observed with 213 and 691 pg sCT and eel CT, respectively. No cross reactivity was observed with hCGRP. Among the four tissues that had shown CT-like immunoreactive molecules (brain, kidney, liver and ultimobranchial body), only the ultimobranchial body extract was able to displace the labelled CT binding to its specific receptors (Fig. 3, right panel). The slopes of the lines obtained with the standard peptide ( $-0.769$ ) and serial dilutions of ultimobranchial body extract ( $-1.072$ ) were not statistically significantly different ( $p > 0.05$ ). Extracts from brain, kidney and liver produced no cross reaction up to  $100 \mu\text{g}$  of protein.

The levels of CT-like material in UBB was calculated by reference to the RRA and accounted for  $11 \text{ pg}/\mu\text{g}$  of protein. The level of CGRP biologically active molecules (Fig. 4) showed the highest level in the brain accounting for  $10 \text{ pg}/\mu\text{g}$  of protein, in the other tissues (heart, kidney, liver, spleen and UBB), the CGRP-like level represented between 3 and  $6 \text{ pg}$  per  $\mu\text{g}$  of protein. In contrast with the RIA, the RRA detected higher amounts of CGRP-like molecules; this is probably due to the better interaction of the eel CGRP on this assay (Fig. 3, left panel). So the CT related molecules detected in the UBB were both immunologically and biologically active; the same is true for the

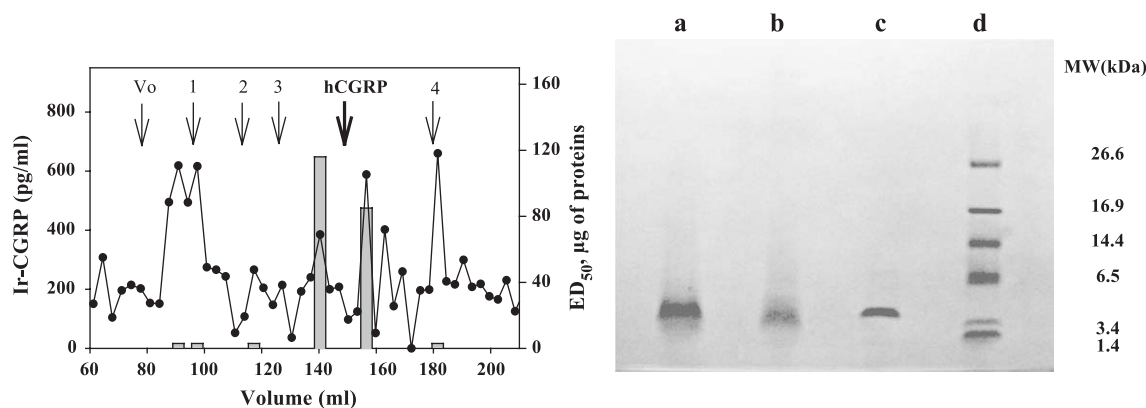


Fig. 5. Partial purification of an acid extract of heart and spleen. The molecular sieving of spleen extract is shown on the left panel. Numbers indicate the elution position of the different markers: 1: bovine serum albumin, 2: lactalbumin; 3: aprotinin; 4: bacitracin. The immunoreactive profile is indicated by closed circles. The bars indicate for each fraction tested in the hCGRP RRA the quantity of protein that induced a 50% displacement of the CGRP binding to its specific receptors ( $\text{ED}_{50}$ ). In the right panel, the SDS-PAGE of heart and spleen extracts is shown. Immunoreactive and biologically active materials were loaded on a 10–20% tris tricine gel and proteins revealed by brilliant blue G 250 staining. Lane a:  $6 \mu\text{g}$  of spleen proteins; lane b:  $8 \mu\text{g}$  of heart proteins, lane c: hCGRP, lane d: polypeptide markers.

CGRP-related molecules characterised in the brain, heart, kidney, liver, spleen and UBB.

#### 3.4. Partial purification of the CGRP-like molecules present in the heart and spleen extract

In order to determine the apparent molecular weight of the CGRP-like molecules, extracts of eel heart and spleen were purified by exclusion chromatography. When 36 mg of eel spleen protein was subjected to gel filtration on a TSK HW 50 column (Fig. 5, left panel), immunoreactivity was detected in most fractions eluting from 60 to 200 ml. Immunoreactive fractions were further analysed in the CGRP RRA. Only fractions eluting from 130 to 150 ml were able to displace this binding. Fifty-percent inhibition was obtained with protein levels between 80 and 120  $\mu\text{g}$ . No displacement of the CGRP binding to its receptors was observed with the other fractions up to 150  $\mu\text{g}$  of protein. Similar results were obtained with eel heart extract (data not shown).

The fractions from heart and spleen extracts containing both immunologically and biologically active CGRP-related molecules eluted for a similar volume as that of standard hCGRP. The molecular weight of the fractions containing CGRP-like biological activity from heart and spleen extract was further determined using SDS gel electrophoresis (Fig. 5, right panel). The apparent molecular weight determined for partially purified heart and spleen fractions was in the range of 3300–3950 Da, that approximates that of hCGRP: 3750 Da.

## 4. Discussion

The present study reports the first data on CGRP in a primitive teleost, the eel. This peptide was partially characterised and its distribution compared to that of CT in order to provide information concerning the potential role of these two peptides in a primitive teleost. RIAs for hCGRP were used to investigate the presence of immunoreactive CGRP molecules in various eel tissues. Displacement curves obtained with the different eel tissues (brain, gill, heart, intestine, kidney, liver, spleen and ultimobranchial body) and with plasma were parallel to the standard curve and enabled us to use these RIAs to quantify the CGRP-like immunoreactivity in eels. The data obtained show a wide repartition of CGRP with concentrations in tissues ranging from 2 to 50 ng/g of organ. No CGRP-like immunoreactivity was found in the stomach. This repartition has some similarities with that which we reported in salmonids where CGRP immunologically related molecules were found in brain, gill, heart, intestine and stomach; the highest concentrations being detected in gill and intestine at concentrations around 10 ng/g of organ [10]. In eels, both sexes were compared and a significant difference was observed in the heart and liver CGRP concentrations in relation with the sex of the animals.

In order to investigate the biological activity of these immunoreactive molecules, specific RRAs were used. With that aim, we tested the ability of the various tissue extracts to compete with hCGRP in a specific RRA (rat liver membranes). Using this specific RRA, biologically active CGRP-like molecules could be identified only in brain, heart, kidney, liver, spleen and ultimobranchial body, while gill and digestive tract extracts were unable to displace the binding of CGRP to its specific receptors. This indicates that the immunoreactivity found in the gill and the digestive tract was not related to CGRP-like molecules. This leads us to select eel tissues that showed the presence of both immunoreactive and biologically active molecules in order to perform the partial purification of the CGRP molecules. Molecular sieving of heart and spleen extract demonstrated that the CGRP molecules, bioactive in the RRA, corresponded to a molecular weight in the range of 3300–3950 Da similar to that of hCGRP (3750 Da).

The presence of CGRP immunologically and biologically related molecules in eel brain at high concentrations is in good agreement with the situation found in mammals where high densities of CGRP mRNA expressing neurons were observed in the rostral part of the lateral hypothalamic area as well as in the lateral portion of the ventral and dorsal parabrachial nuclei [3]. This suggests the involvement of CGRP in various brain functions, especially in specific sensory, motor and integrative systems. Immunologically CGRP-like molecules were also detected in the brain of various vertebrates from cyclostomes to mammals [23] and in some invertebrates, the cockroach [24] and freshwater snail [25]. The data suggest that the role of CGRP as a brain neuromediator could be an ancient function expressed both in invertebrates and vertebrates.

We also found immunologically and biologically active CGRP in the heart, that is in agreement with the situation described in mammals. Immunocytochemistry has demonstrated the presence of CGRP-related molecules in the nerve fibers of the cardiovascular system of the rat [26] and the guinea pig [27]. This peptide was described as one of the most potent vasodilating substances inducing a decrease of vascular resistance [28] and an increase in the rate and force of contraction of the heart [29].

Our study indicates the presence of immunoreactive and biologically active CGRP in other organs such as kidney, liver and spleen. The present finding may open new axes of investigation concerning the presence and the role of CGRP in these organs. Finally, we also found the presence of immunoreactive and biologically active CGRP in the UBB, which could be related to the well-known presence in this organ of the other calcitropic peptide, CT, in teleosts.

In order to compare the distribution of CGRP to that of CT in the eel, we also tested the same tissue extracts in the RIA for sCT. RIAs for sCT showed a complete cross reaction with standard eel CT. Displacement curves obtained with different eel tissues (brain, kidney, liver and UBB) and with plasma were parallel to the standard curve

and enable us to use these RIAs to quantify the CT-like immunoreactivity in eels. CT immunoreactivity was detected in brain, kidney, liver and UBB. No cross reactivity was observed with other tissues (gill, heart, intestine, stomach and spleen). The highest concentration was found in UBB.

As for CGRP, a specific CT RRA (rat kidney membranes) was used to discriminate biologically active CT. In the CT RRA, only UBB extract was able to displace the binding of sCT to its receptors. This allows us to demonstrate that UBB is the only site of production of biologically active CT. The high concentrations of CT in UBB are not surprising as this organ has been shown to be the site of CT synthesis in non-mammalian vertebrates [9] as the C cells of the thyroid in mammals [30]. In addition, this hormone, in teleosts, is important to maintain extracellular calcium concentrations within a physiological range by increasing the elimination of calcium (or decreasing calcium transport) through the gills (and kidney) since they live in a high calcium environment [31]. The concentration detected in the eel (49 and 72 ng/gland of male and female, respectively) was lower than that previously found in the trout UBB where CT levels increased from 10 to 100 µg/gland at the time of spawning [32]. However, we must notice that in the present experiment, both male and female silver eels were far from their complete maturity and that the CT level may increase in UBB during the reproduction period. Furthermore, these differences in the CT concentration of the UBB between eels and salmonids may be related to a species difference in the UBB physiology.

In this study, we measured very high levels of circulating CGRP in the eel, these levels being 10 to 100 times higher than those of circulating CT: in male, we detected 13.7 and 0.13 ng/ml of immunoreactive CGRP and CT, respectively; in female, 4.11 and 0.42 ng/ml, respectively. The highest concentration of CGRP measured in male eels may be related to the sex variation we observed in some tissues, especially the heart. This situation differs from that found in the trout where circulating CGRP about 0.5 ng/ml are equivalent to that of CT: 0.6 ng/ml [33]. In mammals, the situation is opposite to that observed in the eel with a ratio of circulating CGRP/CT representing only 20%: in humans, the circulating CGRP [34] and CT [35] levels were reported to be 7 and 35 pg/ml, respectively. This suggests an important endocrine role of CGRP in the eel which would be reduced during vertebrate evolution.

In our study, we demonstrated the presence of immunoreactive and biologically active CGRP in the UBB of the eel while we could not detect this peptide in the same organ of the trout [10]. The production of CGRP by the UBB of the eel could be the origin of the higher level of circulating CGRP and reflect an important endocrine role of this neuropeptide in this fish. All these data suggest that the UBB may be an endocrine organ producing not only CT but also CGRP in a primitive teleost, the eel. The wide distribution of CGRP in the other tissues is likely related to a role

as brain neuromediator and peripheral paracrine effector as also found in other vertebrates including mammals [36]. In invertebrates, our ongoing studies [12,13] indicate that only CGRP immunologically and biologically related molecules would be present while no CT biologically active molecules could be identified. This suggests that CGRP may represent the ancestral molecule and appeared early in evolution. In contrast, CT would be a more recent peptide in vertebrates.

Our data in the eel suggest for CGRP both an endocrine and a paracrine role, the role of CT being mainly an endocrine one. The comparison between fishes and mammals suggests that CGRP would have an important endocrine role in a primitive teleost, the eel, a function that may have been lost during vertebrate evolution and possibly replaced by CT. Further, pharmacological, immunohistological and biological studies would lead to a better understanding of the putative role of CGRP and CT in the various vertebrates.

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