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Studies on localization of calcitonin gene-related peptide (CGRP) in the thyroid-parathyroid complex

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Summary. Calcitonin gene-related peptide (CGRP) was localized by an immunocytochemical technique in the thyroid-parathyroid complexes of rat, guinea pig, rabbit, and in normal human thyroids and parathyroids. Human medullary carcinomas and parathyroid adenomas were also studied. In man and all animal species examined CGRP was present in the parafollicular cell, however, in guinea pigs only in small amounts. Except in rabbits, presence of CGRP was demonstrated in nerves of the thyroid and parathyroid capsule as well as in the nerve fibers of the capsular blood vessels. In the thyroid of guinea pigs CGRP was also noted in nerve fibers and in blood vessel walls between follicles. CGRP was also present in the parathyroid glands of rat and man, in nerve fibers localized between parathyroid cells. In rabbit the parafollicular cells between parathyroid cells also expressed CGRP immunoreactivity. No CGRP was noted in the parathyroids of the guinea pig. The proximity of parathyroid cells and CGRP containing tissue structures suggests a role for CGRP in the modulation of parathyroid hormone secretion. The importance of these regulatory mechanisms appear to be different in individual species.

Key words: Calcitonin gene-related peptide (CGRP) – Thyroid – Parathyroid – Parathyroid hormone – Parafollicular cells

Introduction

CGRP is a polypeptide consisting of 37 amino acids with a N-terminal disulphide ring (Amara et al. 1982; Rosenfeld et al. 1983). This polypeptide and calcitonin are coded by the same gene and alternate RNA processing yields mRNA for either CGRP or calcitonin (Amara et al. 1982). CGRP was demonstrated within the central and the peripheral nervous system in the heart, lungs and alimentary tract (Rosenfeld et al. 1983; Tache et al. 1984; Mulderry et al. 1985). CGRP is also present in thyroid parafollicular cells, in parallel with calcitonin (Tschopp et al. 1984). Thus, CGRP appears to play the role of both neurotransmitter and hormone, like other polypeptide hormone. The physiological role of CGRP has not yet been fully clarified even if several effects are known (Goodman and Iversen 1986). CGRP has been shown to inhibit gastric secretion of acid and pepsin (Kraenzlin et al. 1985) and to affect function of muscle cells of the heart, blood vessels and intestines (Tippins et al. 1984). CGRP inhibits secretion of growth hormone (Tannenbaum and Goltzman 1985) and augments catecholamine secretion (Struthers et al. 1985). CGRP is the most potent vasodilator known (Brain et al. 1985; Struthers et al. 1985). Moreover, CGRP influences the serum level of calcium ions but its effect varies depending upon the species studied (Tippins et al. 1984; Bevis et al. 1986). The presence of receptors for CGRP has been demonstrated in various parts of the central nervous system, in adrenals, exocrine pancreas, hypophysis and, in small amounts in bones and kidneys (Dawbarn et al. 1985; Goltzman and Mitchell 1985; Seifert et al. 1985). The aim of our study was to determined the localization of CGRP in the thyroid-parathyroid complex of several species and man in order to increase our understanding of CGRP function.

Material and methods

Thyroid-parathyroid complexes of 70 rats aged 1 to 720 days, of 10 guinea pigs aged 3 month and 5 rabbits aged 1 year have

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Table 1. Characteristics of applied rabbit antisera

Antiserum to	Optimal dilutions for staining of tissues from:			
	rat	guinea pig	rabbit	man
Rat CGRP (Amersham*)	1:1000	1:500	1:5000	1:1000
Human CGRP (Amersham*)	0	0	1:1000	1:500
Human calcitonin (Calbiochem)	1:6000	1:6000	1:6000	1:6000
Bovine NSE (Dakopatts)	1:300	1:300	0	1:100
Rat NSE (Polyscience)	1:1000	1:1000	0	1:500

CGRP = calcitonin gene-related peptide; NSE = neuron-specific enolase; 0 = lack of cross reactivity; * = cross reactivity with calcitonin was excluded

Table 2. Localization of calcitonin gene related peptide (CGRP) in the thyroid-parathyroid complex

Species	*n*	CGRP presence in			
		Thyroid C-cells[1]	Nerve fibers of		
			Capsules	ptgl[2]	Thyroid
Rat	70	+++	++	+++	+
Guinea pig	10	+	++	−	+++
Rabbit	5	+++[3]	−	−	−
Man	5	+	+	++	−
Human medullary carcinoma	5	++	−	na	−
Human ptgl adenoma	5	na	−	−	na

staining pattern: +++ = intensive; ++ = medium; + = low; na = not applicable

[1] C-cells = parafollicular cells

[2] ptgl = parathyroid gland

[3] C-cells were also presented in parathyroid gland

been investigated. Five parathyroids, 5 parathyroid adenomas, 5 thyroids and 5 medullary carcinomas from humans were also studied (the material orginated from the collection of M. Dietel and S. Schröder (Institut of Pathology, Hamburg, FRG) as well as from B. Zawirska (Department of Pathological Anatomy, Wroclaw, Poland). The material was fixed in Bouin's solution or, exceptionally, in formaldehyde, embedded in paraffin and serially sectioned at 5 μm.

Immunocytochemical studies were performed by PAP technique, according to Sternberger (1979). Details concerning the antibodies applied in the study are listed in Table 1. In control reactions the primary antibody was omitted or substituted by normal rabbit serum. Control reactions always yielded negative results. Special attention was payed to the controls of rabbit sections since binding of the second antiserum (anti-rabbit IgG) may appear. In the preparations used incubations with the second antiserum only followed by the detection system showed no non-specific immunostaining.

Results

To identify parafollicular cells unequivocally, immunocytochemical reactions were performed in serial sections applying antisera to calcitonin and to neuron-specific enolase.

The localization of CGRP in the specimens investigated is summarized in Table 2.

In the rat CGRP was present in all thyroid parafollicular cells. This was demonstrated on serial sections, which have been stained for calcitonin and neuron-specific enolase (Fig. 1 a, b). Individual cells varied with regard to the intensity of staining. CGRP presence was also noted in nerve fibers in the connective tissue capsule (Fig. 1 c), in the wall of capsule blood vessels and occasionally in the nerve fibers between thyroid follicles. In parathyroid glands the immunoreaction specific for CGRP resulted in a positivity of nerve fibers localized between the endocrine chief cells (Fig. 2). No differences in localization or amount of CGRP were noted which could be related to the age of the animals.

In guinea pigs the majority of parafollicular cells contained only small amounts of CGRP while some individual single cells showed more reactivity (Fig. 3 a). CGRP presence was also disclosed in nerve fibers of the thyroid parenchyma (Fig. 3 b), and in nerves of blood vessels and in the connective tissue capsule. No CGRP could be found within parenchyma of parathyroids.

In rabbits, the parafollicular cells were diffusely distributed in thyroid and parathyroid glands. In either localization, these cells apparently stored large amounts of CGRP (Fig. 4 a). No CGRP was demonstrated within nerves of the thyroid or the parathyroid. The parafollicular cells of the parathyroid were visualized by proving calcitonin production (Fig. 4 b) with a calcitonin specific antiserum.

In normal human thyroids small amounts of CGRP was present in parafollicular cells which were also characterized by positive immunoreaction to calcitonin and neuron-specific enolase. In the nerves of thyroid connective tissue capsule, individual nerve fibers contained CGRP. Within the parathyroids, CGRP was observed in the intraglandular nerve fibers directly apposed to parathyroid cells (Fig. 5). In human medullary carcinoma, consisting of calcitonin and neuron-specific enolase positive parafollicular cells, large amounts of CGRP were observed in the vast majority of the

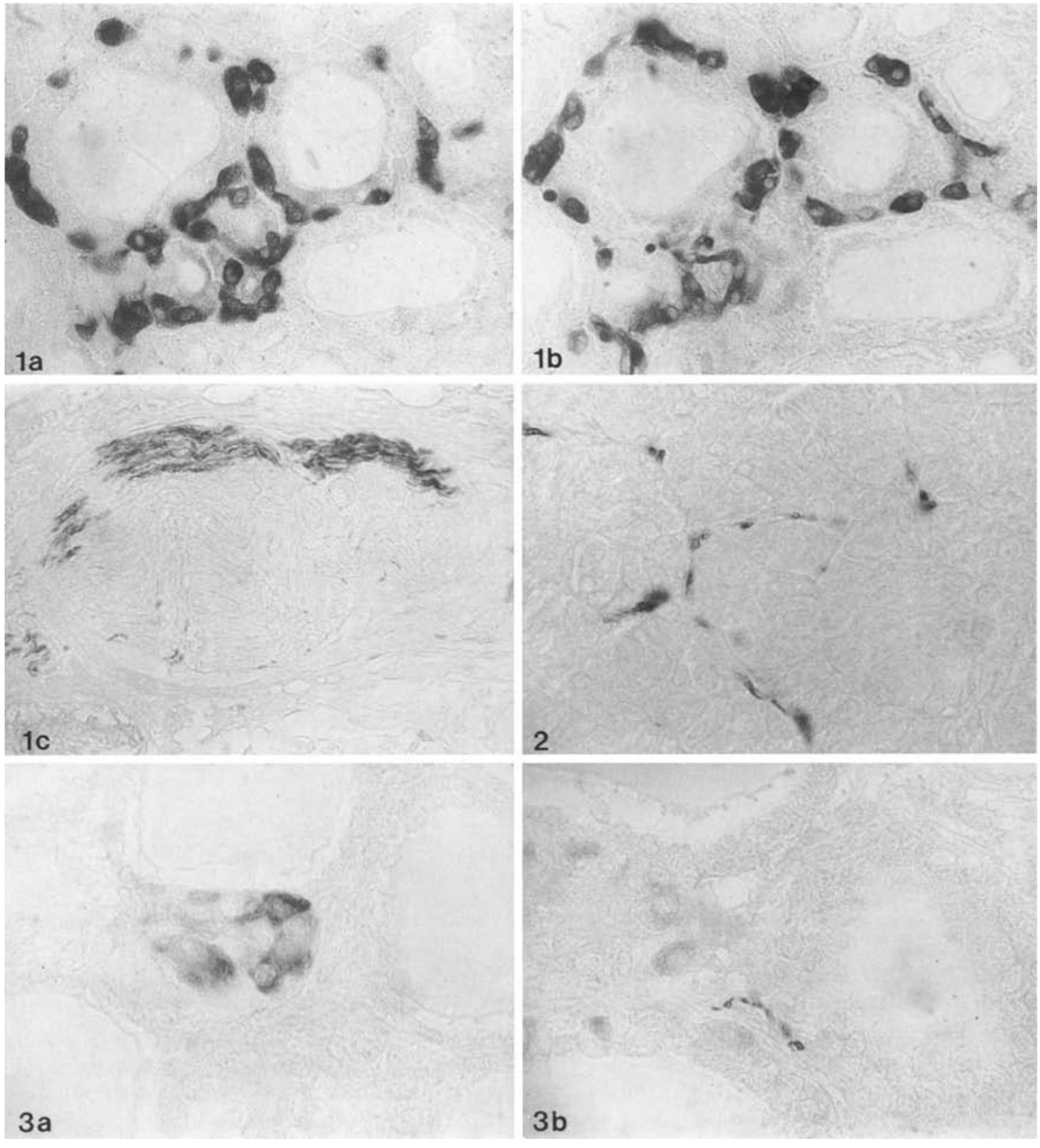

Fig. 1a–c. Thyroid gland of a 90 day old rat. Parafollicular cells stained for calcitonin (**a**) and CGRP (**b**) in serial sections. Considerable amount of CGRP in a nerve of the connective tissue capsule (**c**). × 290

Fig. 2. Parathyroid gland of a 90 day old rat. Numerous intraglandular individual nerve fibers exhibit a positive reaction with anti-CGRP antiserum. × 630

Fig. 3a, b. Thyroid gland of a 90 day old guinea pig. A group of parafollicular cells contain variable amounts of CGRP (**a**). The group of parafollicular cells store small amount of CGRP (**b**). In the close vicinity a nerve fiber shows CGRP immunoreactivity. × 630

tumour cells (Fig. 6). No CGRP could be demonstrated within human parathyroid adenomas or their connective tissue capsule.

Discussion

The data presented demonstrate for the first time the occurrence of CGRP in parathyroid glands. The peptide was found in some but not all parathyroid nerve fibers of rat and man, as well as in the intra-parathyroid parafollicular cells of rabbit. The netlike tissue distribution of CGRP in parathyroids suggests a paracrine or neurocrine mechanism of action which may be of physiological significance in parathyroid control. Whether CGRP

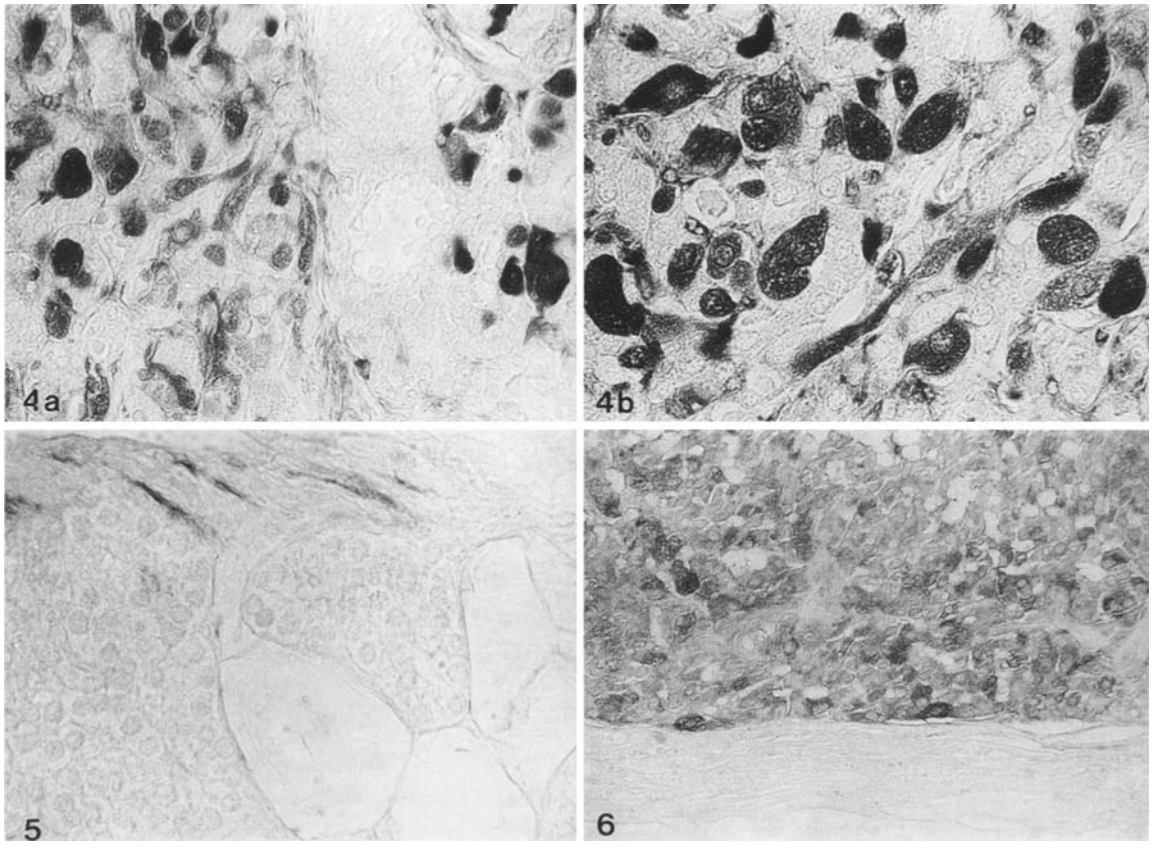

Fig. 4. **a** Thyroid-parathyroid complex of a 1 year old rabbit. Left: parathyroid, right: thyroid. Both contain numerous CGRP immunoreactive parafollicular cells. **b** Parathyroid gland containing calcitonin storing cells. **a** ×290, **b** ×450

Fig. 5. Human parathyroid gland. CGRP immunoreactive nerve fibers adjacent to parathyroid cells. ×450

Fig. 6. Human medullary carcinoma. Tumour cells store variable amount of CGRP. ×110

directly effects parathyroid chief cells or whether it acts by changing the local blood stream or whether there are other mechanisms is unclear.

Several experiments have been performed to elucidate the effect of exogenously added CGRP on serum PTH and calcium levels (Tippins et al. 1984; Kraenzlin et al. 1985; Struthers et al. 1985; Bevis et al. 1986). However, the results were not homogeneous which might in some way reflect the interspecies differences in content and localization of CGRP. Thus, the physiological role of CGRP in the regulation of calcium homoeostasis remains open.

The presence of CGRP in nerve fibers of the thyroid connective tissue, as shown here, has been described previously (Grunditz et al. 1986) and was interpreted to indicate functional importance for thyroid regulation. However, the CGRP containing nerve fibers also demonstrated in parathyroid glands point to a more general mechanism, possibly linking both organs at the level of CGRP. This hypothesis is supported by in vivo studies with demonstrate that the secretion of the two hormones is functionally linked, but inversely (Copp 1970). The CGRP positive nerve fibers may be the neuronal connection for the interaction of parafollicular and parathyroids cells. Since this mechanism cannot be simulated in vitro the results from previous experimental studies are not contradictory although they demonstrate the failure of calcitonin to modulate PTH secretion (Dietel 1982; Dietel and Hölzel 1983; Potts 1984) and vice versa (Cooper et al. 1977; Zabel 1985).

The thyroid/parathyroid link is further substantiated by an early study of Deftos and Parthemore (1974) who described elevated PTH levels in a considerable number of patients suffering from medullary carcinomas. No explanation of this result was possible at that time since CGRP was unknown. The data shown in our study may explain this early observation.

We may speculate that the functions of CGRP

may be a general one in the endocrine system. The appearance of CGRP in endocrine cells and in nerve fibers supplying several endocrine organs (Rosenfeld et al. 1983, Sabate et al. 1985) indicates to a dual function of CGRP as hormone with endocrine or paracrine effects and as neurotransmitter (Grunditz et al. 1986). Condensing the data of our study and those from the literature the existance of CGRP positive nerve fibers may point to a specialization of this type of fibers which, among other functions, may play a special role in the innervation of endocrine organs.

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