VIA MEDICA

SHORT COMMUNICATION

Vol. 62, No. 4, pp. 485–487

ISSN 0015–5659
www.fm.viamedica.pl

The expression of calcitonin, calcitonin gene-related peptide and somatostatin in the thyroids of rats of different ages

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[Received 17 September 2003; Accepted 23 October 2003]

Using immunocytochemistry and in situ *hybridisation expression of calcitonin, calcitonin gene-related peptide (CGRP) and somatostatin was examined in rat thyroids. The immunocytochemical reactions demonstrated the presence of the proteins under investigation at all stages of rat life. Calcitonin and CGRP produced the most numerous parafollicular cells, while somatostatin was present in a few cells only. The number of cells producing the above-mentioned hormones was found to increase in rats with the progressing of age. Hybridocytochemical techniques corroborated the results obtained using immunocytochemical techniques. The most numerous cells were found to contain mRNAs for calcitonin and CGRP. In the case of somatostatin, however, multiple parafollicular cells produced mRNA for the hormone but few cells demonstrated the presence of the corresponding protein.*

key words: calcitonin, CGRP, somatostatin, immunohistochemistry, *in situ* hybridisation, thyroid, rat

INTRODUCTION

Observations on parafollicular cells in the thyroids of various animal species demonstrate certain differences in the cells. This pertains in particular to their number, ultrastructure and the number and types of the secreted hormones [2, 5, 8]. Some authors have expressed the opinion that parafollicular cells undergo alterations in the postnatal period [7]. This study, therefore, was aimed at immunocytochemical and hybridocytochemical evaluation of calcitonin, CGRP and somatostatin expression in the thyroids of rats at various ages.

MATERIAL AND METHODS

The studies were performed on rat thyroids. The thyroids were isolated from male rats of the Wistar strain aged from 14 days to 2 years. The material was fixed in Bouin's solution or in 4% paraformaldehyde and was then embedded in paraffin. In the paraffin sections tests were performed for the presence of calcitonin (Dako), CGRP (Sigma) and somatostatin (Dako) employing the classical avidin-biotinylated peroxidase (ABC) immmunocytochemical reaction [1]. *In situ* hybridisation was also performed to detect mRNAs for the above proteins. mRNAs were detected using digoxigenin-labelled oligonucleotide probes (Sigma-Genosys). In order to increase the sensitivity of the hybridisation reactions *in situ*, the reactions were additionally amplified using biotinylated tyramine [4]. Control reactions were also performed. In ABC reactions specific antibodies were substituted by normal rabbit serum, while in *in situ* hybridisation the appropriate probe was omitted. In both cases the control reactions yielded negative results.

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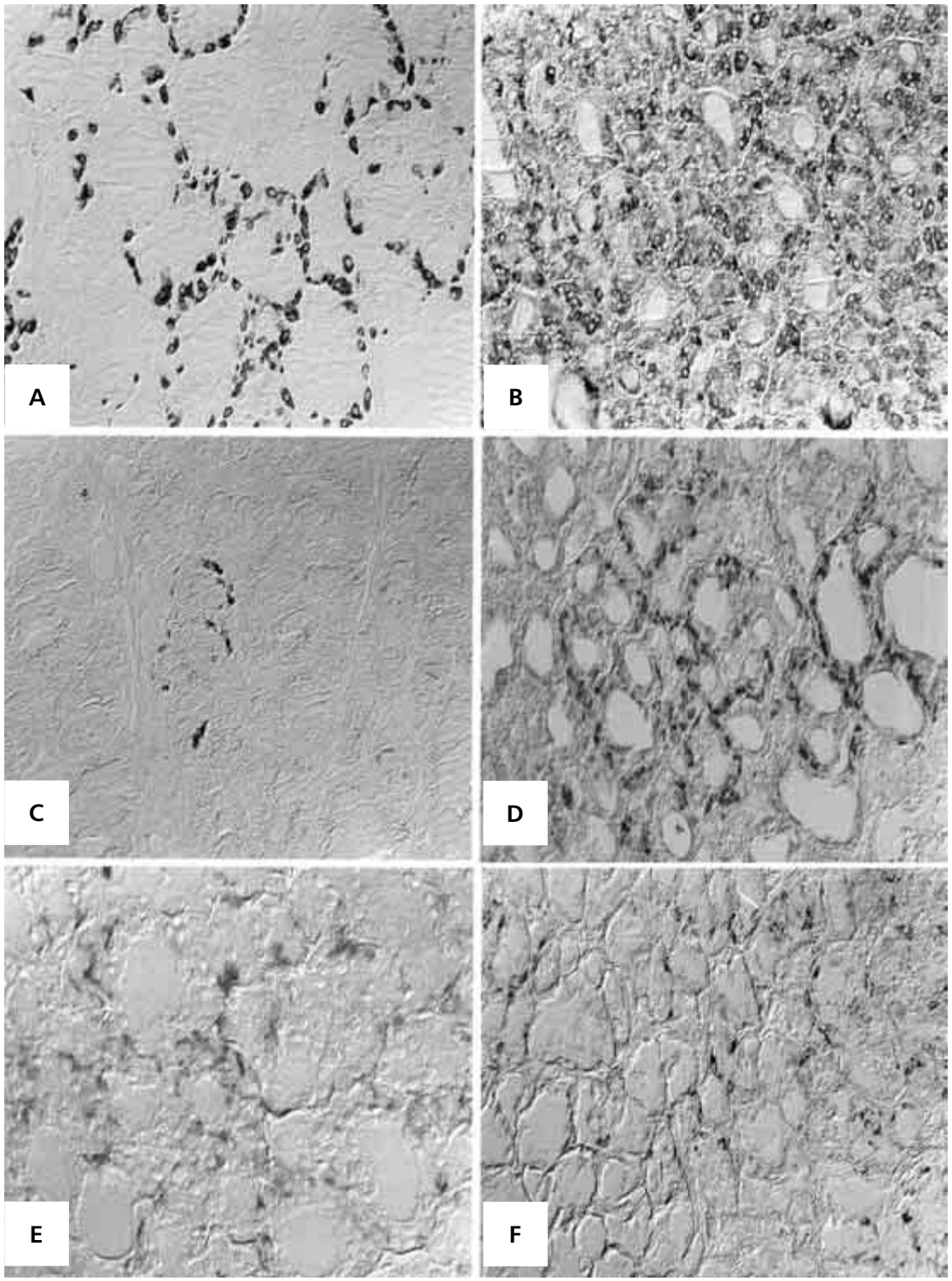

Figure 1. A. Localisation of calcitonin in the thyroid of a 4-month-old rat. Darkly stained parafollicular cells entwine entire thyroid follicles. ABC reaction (× 400); **B.** CGRP in the thyroid of an 18-month-old rat. Note the very high number of parafollicular cells. Most of cells exhibit rather weak immunocytochemical reaction. ABC reaction (× 400); **C.** Somatostatin in the thyroid of a 6-month-old rat. At the time only small groups of somatostatin-containing cells were noted. ABC reaction (× 400); **D.** mRNA for calcitonin in the thyroid of a 6-month-old rat. Biotinylated tyramine amplified *in situ* hybridisation (× 400); **E.** mRNA for CGRP in the thyroid of a 4.5-month-old rat. *In situ* hybridisation (× 600); **F.** mRNA for somatostatin in the thyroid of a 6-month-old rat. A relatively high number of mRNA producing cells. Biotinylated tyramine-amplified *in situ* hybridisation (× 400).

RESULTS AND DISCUSSION

The immunocytochemical reactions demonstrated that at all age periods of rat life the thyroid parafollicular cells produced calcitonin, CGRP and somatostatin. However, a number of the cells underwent changes with the increasing age of the rats. In young rats (14 days of life) the cells were individually present close to the thyroid follicles. With progressing age the number of cells increased and very frequently parafollicular cells entwined the follicles (Fig. 1A). With the progressing age of the rats the cells exhibited a tendency to group into clumps of as many as 10 cells or more. In each case, calcitonin-producing cells were the most numerous. CGRP was present in around 1/2 to 2/3 of the cells that secreted calcitonin. In general, up to the 12th month of life almost all cells developed an intense immunocytochemical reaction (they were dark). In 18-month-old and older rats the intensity of the reaction decreased (Fig. 1B). In case of somatostatin a positive reaction was noted before the 3rd month of rat life only in individual parafollicular cells (3 to 5 cells per section), while in older rats the cell number increased to around 100 positive cells in one-year-old rats. At the beginning individual cells were spread throughout the thyroid, but later (beginning at 3 months) the cells formed groups (Fig. 1C). Hybridocytochemical studies on the expression of mRNAs for calcitonin, CGRP and somatostatin showed that most parafollicular cells contained mRNA for calcitonin (Fig. 1D) and CGRP (Fig. 1E). As far as somatostatin was concerned, many more cells were found to contain mRNA for the protein than the protein itself (Fig. 1C, F). Our studies demonstrated that the number of parafollicular cells as well as the hormonal content of cells changed with the progression of age in rats. Increasing age was accompanied by an increase in the numbers of cells secreting calcitonin and CGRP [7]. The observations are consistent with the data of Wimalawansa who has demonstrated an increase in serum CGRP and calcitonin levels with age progression in rats [6]. In the case of somatostatin the results obtained with immunocytochemistry and by *in situ* hybridisation were divergent. Despite the intense increase in numbers of parafollicular cells, the number of cells which contained somatostatin remained low in all the stages of the rat's life tested [3, 5, 7]. The application of *in situ* hybridisation revealed that a relatively high number of parafollicular cells produced mRNA for somatostatin. It is likely that the parafollicular cells have developed a mechanism to inhibit somatostatin synthesis at the stage of translation. A possible alternative is that two types of parafollicular cells are present, of which some synthesise somatostatin, while the remaining ones store it.

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