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Age-related peptide production by human thyroid C cells

An immunohistochemical study

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Summary. C cells of thyroid are known to express a variety of products beside calcitonin. These include the peptides PDN21 (katacalcin), calcitonin-gene related peptide (CGRP), bombesin and somatostatin. The expression of these products has been investigated by immunohistochemistry of thyroid tissue in six age ranges from fetal to late adult life. PDN 21 was found to have co-expression with calcitonin. CGRP and bombesin both demonstrated an age-related increase in numbers and intensity of cells stained. Somatostatin immunoreactivity was limited to small numbers of cells.

Key words: Thyroid – C Cells – Peptides – Immunohistochemistry

Introduction

C cells of thyroid form part of the diffuse neuroendocrine system and were initially characterized by secretion of calcitonin. Increasingly, other C cell products have been recognised, particularly in medullary carcinoma of the thyroid. Normal and hyperplastic C cells have also been shown to express a variety of peptides and neuroendocrine-related products. These include the flanking peptide of calcitonin-PDN 21 or katacalcin (Ali-Rachedi et al. 1983), calcitonin gene-related peptide (CGRP) (Sabate et al. 1985) (Cooper et al. 1985), bombesin/gastrin-releasing peptide (Kameya et al. 1983), somatostatin (Van Noorden et al. 1977), chromogranin (O'Connor et al. 1983) and NSE (Lloyd et al. 1983).

Little is known presently of the function or significance of the peptides concerned. PDN 21 was originally thought to have a hypocalcaemic action similar to calcitonin (Hillyard et al. 1983) but this is now uncertain (MacIntyre et al. 1984). By analogy with other sites, the possible function of bombesin and somatostatin might be inferred, but functional significance would depend on the amounts secreted. The role of CGRP in C cells is at present speculative. One means of investigating possible functional significance is to examine age-related changes in peptide production as has been performed in rat C cells (Larsson 1985) and other neuroendocrine cells such as in lung (Wharton et al. 1978; Ghatei et al. 1983). We have therefore investigated C cells throughout a wide age range for immunoreactivity to a panel of antisera raised against peptides and neuroendocrine-related products.

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Materials and methods

The post-natal thyroid specimens were derived from sudden death autopsy cases. This material was chosen in order to avoid the changes to C cells that can occur in protracted illness. The tissue was available usually between 24 and 48 h after death fixed in 10% buffered formalin for up to 5 days. Fetal thyroid specimens were derived from spontaneous miscarriages. The thyroid tissue was exposed to formalin as soon as possible and fixed similarly for up to 5 days. Blocks were taken from the central regions of the lateral lobes where C cells are clustered. 3 cases in each of six age ranges – fetal, under 1 year, 1–15, 16–40, 41–60 and over 60 years were examined. Since C cells numbers in adult thyroid are normally very low (approx. 5 C cells/lpf maximally), cases with at least 20 C cells/lpf were selected to provide adequate examination of immunoreactivity.

Immunohistochemistry. Sections were dewaxed, rehydrated to phosphate buffered saline (pH 7.1–7.4) and endogenous peroxidase was blocked with hydrogen peroxide in methanol. Sections were then exposed to optimally diluted antisera to: calcitonin, PDN 21 (katacalcin), CGRP, bombesin, somatostatin, chromogranin and NSE followed by a standard peroxidase antiperoxidase technique. Control of specificity was obtained by absorp-

tion studies (see Table 1) and substituting normal rabbit serum for the primary antiserum.

Morphometry. In order to provide some degree of comparison of the relative levels of expression of the products being investigated, serial sections of each case were examined following immunostaining with the different antisera. The first and last section in each series were immunostained for calcitonin to ensure adequate numbers of C cells through the block. The proportion of C cells immunostained (0 to 4+) and the intensitive staining (0 to 4+) with the different antisera were compared with calcitonin-stained sections.

Results

Calcitonin

Immunostaining for calcitonin gave consistent identification of C cells. In adult specimens, C cell numbers ranged from the lower limit set of 20 cells/lpf to up to 200+cells/lpf in some cases. Those with high numbers also had a tendency to nodule formation but there was no suggestion of medullary carcinoma. Between birth and 15 years, there were generally adequate numbers without selection, usually around 50 C cells/lpf. Fetal specimens also had between 20 and 80 C cells/lpf maximally. The level of expression of the other products examined was compared with that of calcitonin (Fig. 2).

PDN 21

PDN 21 was strongly expressed in C cells and gave results superimposable with calcitonin (Fig. 1). This applied throughout the age spectrum. Lack of cross-reactivity of PDN 21 and calcitonin was confirmed (see above).

Bombesin and CGRP

Immunoreactivity for bombesin and CGRP was absent in fetal cells but first appeared in the under-1 year age group. In post-natal specimens, both were expressed in a variable proportion of C cells. This ranged from no detectable immunoreactivity to weak expression in a minority of C cells (Fig. 1). Serial sections showed that the same cells did not usually contain both. The proportion of CGRP reactive cells usually slightly exceeded that of bombesin reactive cells. With increasing age, there was a tendency to increasing expression of CGRP and bombesin, and there also appeared to be some inter-relationship of expression (Fig. 2).

Somatostatin

Immunoreactivity for somatostatin was found in only scattered very occasional C cells in five out of the eighteen cases. These five were spread across the age spectrum, including one fetal specimen.

Table 1. Specificity of antisera used

Peptide used for absorption	Antisera raised against		
	Calcitonin	PDN 21	CGRP
Calcitonin*	+	−	−
PDN 21+	−	+	−
CGRP*	−	−	+
Bombesin*	−	−	−
Somatostatin*	−	−	−

+ = staining prevented by pre-absorption with 1 nmol synthetic peptide/ml diluted antiserum.
− = staining not prevented by pre-absorption with 20 nmol synthetic peptide/ml diluted antiserum.

Source of antisera: * Regulatory peptide unit and + Endocrine unit, Hammersmith Hospital.

Chromogranin

From birth onwards, chromogranin was detected fairly consistently in a minority of C cells. The pattern of cytoplasmic staining suggested possible granularity. Only one of the fetal specimens (16 weeks) showed similar immunoreactivity, the remaining two (18 and 19 weeks) were negative, despite repeating immunostaining on further serial sections.

NSE

NSE was detected in C cells at all ages. No apparent granularity of staining was detected. With the method used, expression was, however, only moderate and seen in up to half of C cells present.

Discussion

Previous studies of age-related changes in peptide production by neuroendocrine cells have highlighted striking alterations that occur particularly between fetal and neonatal life. In the lung, for example, neuroendocrine cells express bombesin in fetal life but cease to do so in postnatal life (Wharton et al. 1978; Ghatei et al. 1983). In neonatal rats, thyroid C cells were found to be reactive for somatostatin up to 6 to 8 days, but this was then rapidly replaced by CCK-4 like immunoreactivity (Larsson 1985). Similar examples of the transient expression of different substances by endocrine cells have often been described previously (Larsson 1977; Larsson and Morch-Jorgenssen 1978; Teitelman et al. 1981). The transitory peptides or amines

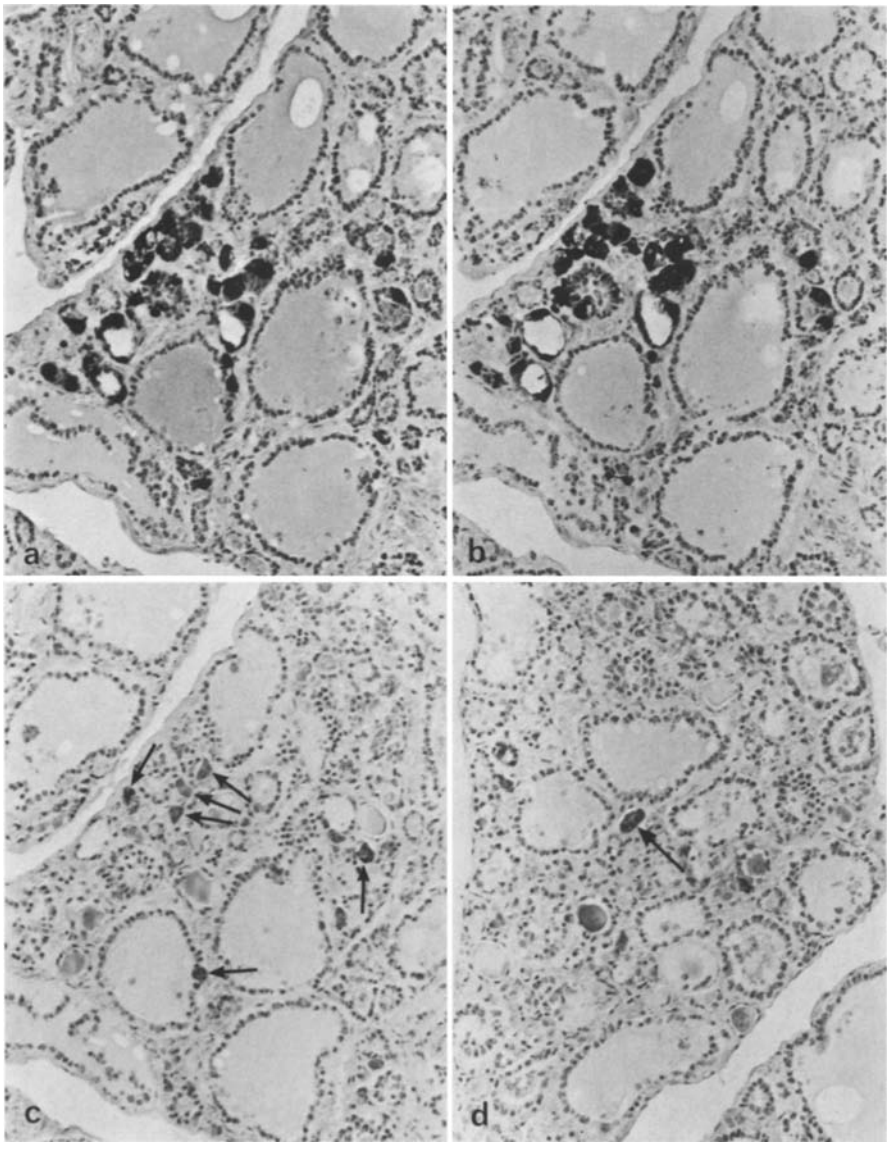

Fig. 1. Serial sections of (**a**) Calcitonin and (**b**) PDN 21 showing co-distribution of staining, and (**c**) CGRP showing smaller numbers of reactive cells. (**d**) Bombesin-another area in the same thyroid specimen showing weak reactivity for bombesin in a small number of C cells

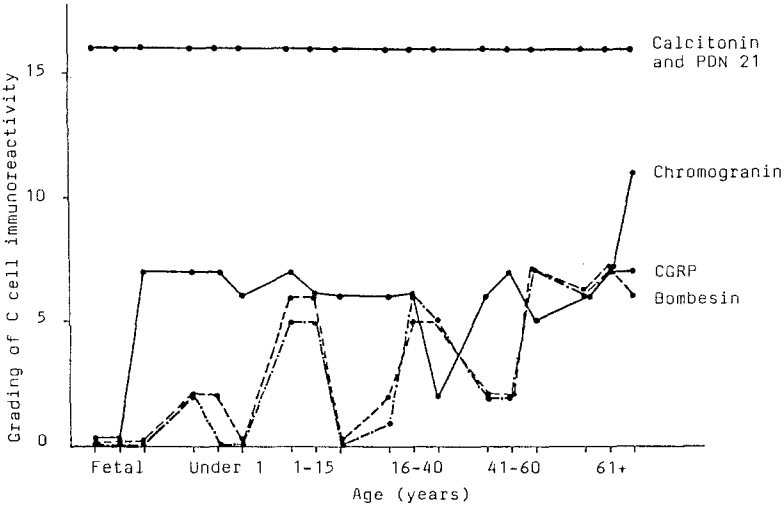

Fig. 2. C cell immunoreactivity (individual cases) for the examined products as a function of age and comparative to the number and intensity of calcitonin immunoreactive cells

which appear during fetal life may play important roles, the most obvious one being some kind of growth-regulating function. This expression of peptides/amines during development is also of interest as some tumours of endocrine tissue contain cells which store substances not found in the cells of the normal adult organ. Our study has therefore investigated peptide expression by human C cells in order to examine developmental and age-related changes.

PDN 21 is a 21 amino acid peptide predicted from sequencing calcitonin mRNA, and is located at the C-terminal end (Craig et al. 1982). It has been shown previously to be co-distributed with calcitonin in normal and neoplastic C cells, and calcitonin and PDN 21 are co-localised to the same granules (Ali-Rachedi et al. 1983). Our studies confirm that PDN 21 is co-distributed with calcitonin throughout the age spectrum in C cells. Equimolar amounts of calcitonin and PDN 21 have been demonstrated in the blood and it has been postulated that calcitonin and PDN 21 may have a synergistic role in skeletal maintenance (Hillyard et al. 1983).

Bombesin and somatostatin are known to be expressed in C cells, and this raises questions of possible local control of C cell function. In our study, the absence of bombesin from fetal C cells and subsequent expression in postnatal life is in marked contrast to lung neuroendocrine cells which show the reverse (Wharton et al. 1978). Bombesin is known to have a stimulatory/trophic action but this is apparently not required for maturation of C cells. The subsequent trend of an age-related increase in bombesin expression in post-natal life suggests an increasing requirement for a local agonist action, presumably on C cells. Somatostatin, conversely, is known to exert an antagonist paracrine effect. However, the very limited expression of somatostatin in C cells, analogous to that seen in studies on rat C cells (Van Noorden et al. 1977), suggests that somatostatin is unlikely to be producing any significant local regulatory effect.

CGRP arises by alternative splicing of calcitonin gene-derived mRNA (Amara et al. 1982) and it was initially thought that this was tissue-specific with calcitonin occurring in C cells and CGRP in neural tissue (Amara et al. 1982). However, the recent demonstration of CGRP in C cells (Sabate et al. 1985) has been matched by the finding of calcitonin in neural tissue (Fischer et al. 1983) although doubt has been cast on the latter by some workers (Rosenfeld et al. 1983). Our findings confirm CGRP expression in C cells in post-natal life. No CGRP expression was, however, seen in fetal C cells. As with bombesin, there was a trend of age-related increase in the expression in post-natal life and also an apparently inter-related expression with that of bombesin. The possible function of CGRP in C cells is at present speculative. Gene switching with a change from production of calcitonin to CGRP is thought to be associated with a worse prognosis in medullary carcinoma of thyroid (Nelkin et al. 1984).

Chromogranin and neuron-specific enolase are both known to be present in neuroendocrine cells. NSE is a general marker of neuroendocrine cells (Schmechel et al. 1978), Chromogranin has a specificity similar to that of Grimelius stain (Facer et al. 1985). NSE has been demonstrated previously in C cells (Lloyd et al. 1983) and this was confirmed at all ages. Chromogranin was seen to be expressed consistently in C cells except for absence in two fetal specimens, detection probably being hampered by difficulties with fixation in these specimens.

In conclusion, the results support co-expression of PDN 21 with calcitonin and highlight the possible role of CGRP and bombesin in C-cell regulatory control. The advent of in-situ hybridization techniques capable of demonstrating specific mRNA's may well help to pursue such investigations further.

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References

Ali-Rachedi A, Varndell IM, Facer P, Hillyard CJ, Craig RK, MacIntyre I, Polak JM (1983) Immunocytochemical localisation of katacalcin, a calcium-lowering hormone cleaved from the human calcitonin precursor. J Clin Endocrinol Metab 57:680–682

Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM (1982) Alternative RNA processing in calcitonin gene expression generates mRNA's encoding different polypeptide products. Nature 298:240–244

Cooper CW, Borosky SA, Peng TC (1985) Secretion of calcitonin gene-related peptide from baby rat thyroid glands in vitro. Proc Soc Exp Biol Med 180:562–566

Craig RK, Hall L, Edbrooke MR, Allison J, MacIntyre I (1982) Partial nucleotide sequence of human calcitonin precursor m-RNA identifies flanking cryptic peptides. Nature 295:345–347

Facer P, Bishop AE, Lloyd RV, Wilson BS, Hennessy RJ, Polak JM (1985) Chromogranin: A newly recognised marker for endocrine cells of the human gastrointestinal tract. Gastroenterology 89:1366–1373

Fischer JA, Tobler PH, Henke H, Tschopp FA (1983) Salmon

and human calcitonin-like peptides coexist in the human thyroid and brain. J Clin Endocrinol Metab 57:1314–1316
Ghatei MA, Sheppard MN, Henzen-Logman S, Blank MA, Polak JM, Bloom SR (1983) Bombesin and vasoactive intestinal polypeptide in the developing lung: Marked changes in acute respiratory distress syndrome. J Clin Endocrinol Metab 57:1226–1232
Hillyard CJ, Myers C, Abeyasekera G, Stevenson JC, Craig RK, MacIntyre I (1983) Katacalcin: a new plasma calcium-lowering hormone. Lancet 1:846–848
Kameya T, Bessho T, Tsumuraya M, Yamaguchi K, Abe K, Shimosato Y, Yanaihara N (1983) Production of gastrin releasing peptide by medullary carcinoma of the thyroid. Virchows Arch [Pathol Anat] 401:99–108
Larsson LI (1977) Ontogeny of peptide-producing nerves and endocrine cells of the gastro-duodeno-pancreatic region. Histochemistry 54:133–142
Larsson LI, Morch-Jorgensen L (1978) Ultrastructural studies on the cytodifferentiation of duodenal endocrine cells. Cell Tiss Res 194:79–102
Larsson LI (1985) Differential changes in calcitonin, somatostatin and gastrin/cholecystokinin-like immunoreactivities in rat thyroid parafollicular cells during ontogeny. Histochemistry 82:121–130
Lloyd RV, Sisson JC, Marangos PJ (1983) Calcitonin, carcinoembryonic antigen and neuron-specific enolase in medullary thyroid carcinoma. Cancer 51:2234–2239
MacIntyre I, Hillyard CJ, Reynolds JJ, Gaines DAS RE, Craig RK (1984) A second plasma calcium lowering peptide from the plasma calcitonin precursor – a re-evaluation. Nature 308:84
Nelkin BD, Rosenfield KI, deBustros A, Leong SS, Roos BA, Baylin SB (1984) Structure and expression of a gene encoding human calcitonin and calcitonin gene related peptide. Biochem Biophys Res Comm 123:648–655
O'Connor DT, Burton D, Deftos LJ (1983) Immunoreactive human chromogranin A in diverse polypeptide hormone producing human tumours and normal endocrine tissues. J Clin Endocrinol Metab 57:1084–1086
Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM (1983) Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304:129–135
Sabate MI, Stolarsky LS, Polak JM, Bloom SR, Varndell IM, Ghatei MA, Evans RM, Rosenfeld MG (1985) Regulation of neuroendocrine gene expression by alternative RNA processing. J Biol Chem 260 (5):2589–2592
Schmechel D, Marangos PJ, Brightman M (1978) Neuron-specific enolase is a marker for peripheral and central neuroendocrine cells. Nature 276:834–836
Teitelman G, Joh TH, Reis DJ (1981) Transformation of catecholaminergic precursors into glucagon (A) cells in mouse embryonic pancreas. PNAS 75:5225–5229
Van Noorden S, Polak JM, Pearse AGE (1977) Single cellular origin of somatostatin and calcitonin in the rat thyroid gland. Histochemistry 53:243–247
Wharton J, Polak JM, Bloom SR, Ghatei MA, Solcia E, Brown MR, Pearse AGE (1978) Bombesin-like immunoreactivity in the lung. Nature 273:769–770

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