Release of calcitonin gene-related peptide from a human medullary carcinoma of the thyroid in vitro

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Summary. The secretion of human calcitonin gene-related peptide was examined in perifusates of medullary carcinoma of the thyroid with a sensitive radioreceptor assay. Calcitonin gene-related peptide was released after the addition of calcium (25–100 mM), in a dose-dependent manner. The results indicate that human medullary carcinomas of the thyroid secrete the calcitonin gene-related peptide as well as calcitonin.

Key words. Calcitonin gene-related peptide; calcitonin; calcium; perifusion; medullary carcinoma of thyroid.

Calcitonin gene-related peptide (CGRP) is a novel neuropeptide, predicted by analysis of the complementary DNA sequence of the rat calcitonin gene[2]. Tissue-specific RNA processing results in the production of a precursor of calcitonin in the C-cells of the thyroid, and of another precursor of CGRP in both thyroid C-cells and nervous tissues[3]. Medullary carcinoma of the thyroid (MCT), a tumor of the C-cells of the thyroid, produces large amounts of calcitonin and CGRP. High concentration of CGRP have been observed in the plasma of MCT patients[4]. Here we demonstrate the secretion of CGRP by human MCT tissue, in vitro.

Materials and methods. Fresh MCT tissue was obtained from a patient (25 y, female) with multiple endocrine neoplasia type 2b. The plasma level of calcitonin was 7200 pg/ml and it was stimulated by pentagastrin. MCT tissue fragments were packed into a 1 × 7 cm polypropylene column (Sepacol column, Seikagaku Kogyo Co., Tokyo, Japan), which was then submerged in a water bath kept at 37 °C. The column was connected to a peristaltic pump with silicon tubing. The tissues were perifused at a flow rate of 0.5 ml/min with Krebs-Ringer bicarbonate solution, pH 7.4, supplemented with 0.1 % BSA, 0.2 % glucose, 2 U/ml Bacitracin and saturated with 5 % CO_2 and 95 % O_2. $CaCl_2$ was dissolved in the freshly gassed perifusion medium and introduced into the column through side arm tubing. The effluent was collected in 3-min periods through polyethylene tubing and stored at −20 °C until assay.

A radioreceptor assay for human CGRP (hCGRP) was performed by the method of Tschopp et al.[5]. Homogenates were prepared from the human cerebellar cortex. The assay mixture contained (2-[^{125}I]-iodohistidyl)hCGRP (20,000 cpm), human cerebellar membrane fractions (1 mg protein/tube), and effluent sample in 0.4 ml of 50 mM HEPES/Tris containing 1 % BSA and 0.2 % NaN3. The mixtures were incubated for 6 h at 4 °C and centrifuged at 12,000 × g for 3 min. The pellet was washed with 1 ml 0.9 % NaCl and the radioactivity was counted. Immunoreactive somatostatin was measured in the perifusate by the method of Conlon et al.[6]. Anti-somotostatin-14 serum was donated by Dr Roger H. Unger (University of Texas, Southwestern Medical School, Dallas, Texas, USA). The content of calcitonin in the perifusate was measured by a radioimmunoassay using a commercially available kit (Eiken Immunochemical Company, Tokyo, Japan).

Results. Figure 1 shows a displacement curve of [^{125}I]-hCGRP with human cerebellar tissue homogenates. The specific binding of [^{125}I]-hCGRP was decreased by the addition of unlabeled hCGRP and the MCT extracts. The amount of synthetic hCGRP required to prevent 50 % of the binding of [^{125}I]-hCGRP to the membranes was approximately 600 pg. Human calcitonin (5 μg/ml) did not inhibit the binding of [^{125}I]-hCGRP. Using this radioreceptor assay, the content of hCGRP in the perifusates from the MCT tissue was estimated. Calcium (25, 50 and 100 mM) stimulated the release of hCGRP, in a dose-dependent manner (fig. 2). After the addition of 25 mM calcium immunoreactive calcitonin was also increased from 60 ng/ml to a peak level of 300 ng/ml, and somatostatin from 100 pg/ml to 378 pg/ml.

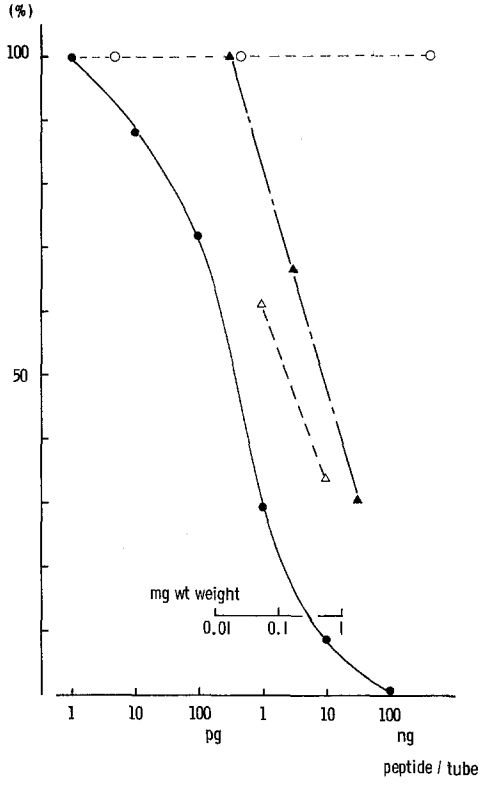

Figure 1. Standard curve of radioreceptor assay for hCGRP. Displacement of [^{125}I]-hCGRP by hCGRP (●), human calcitonin (○), and the extracts prepared from two main tumors of MCT patient (△, ▲) are shown. Values represent means of duplicate determinations of a representative experiment.

Discussion. Immunoreactive hCGRP has been reported to be present in normal human plasma, and it has also been found that the levels are increased in MCT patients[4,7]. Therefore, circulating hCGRP may be a novel tumor marker of MCT, together with calcitonin. However, the release of hCGRP from human MCT has apparently not been reported. Estimations of hCGRP content have been performed by radioimmunoassay[4]. The present radioreceptor method developed by Fischer et al. was comparable to the reported radioimmunoassay, with regard to sensitivity and specificity[5]. Recently, Amara et al. have identified DNA encoding rat β-CGRP, which is different from CGRP (α-CGRP) by a single amino acid[8]. Although human calcitonin did not compete with hCGRP for receptor binding, it is possible that the present assay detects β-CGRP as well as CGRP.

We found that hCGRP is released by the usual secretagogue, calcium ion, which evokes the secretion of calcitonin from human MCT tissues in vivo. Our data suggest that hCGRP may be

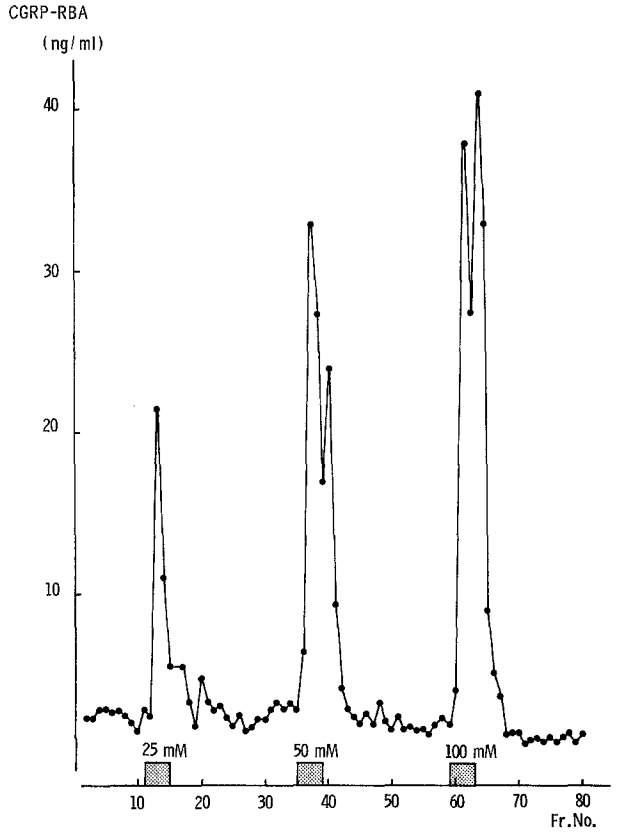

Figure 2. Ca^{++}-evoked hCGRP secretion of perifused human MCT tissue.

secreted concomitantly with the release of calcitonin and somatostatin from the human MCT. Brain et al.[9] recently reported that hCGRP possesses potent vasodilatatory activity in rats and humans[9]. The occasional flushing experienced in patients with MCT may be explained by the transient release of hCGRP[9].
It is tentatively concluded that the abnormally high concentration of hCGRP in the plasma of patients with MCT originates from MCT tissues.

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2 Amara, S.G., Jonas, V., Rosenfeld, M.G., Ong, E.S., and Evans, R.M., Nature *298* (1982) 240.
3 Rosenfeld, M.G., Mermod, J.J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W., and Evans, R.M., Nature *304* (1983) 129.
4 Morris, H.R., Panico, M., Etienne, T., Tippins, J., Girgis, S.I., and MacIntyre, I., Nature *308* (1984) 746.
5 Tschopp, F.A., Henke, H., Petermann, J.B., Tobler, P.H., Janzer, R., Hökfelt, T., Lundberg, J.M., Cuello, C., and Fischer, J.A., Proc. natn. Acad. Sci. USA *82* (1985) 248.
6 Conlon, J.M., McCulloch, A.J., and Alberti, K.G.M.M., Diabetes *32* (1983) 723.
7 Girgis, S.I., MacDonald, D.W.R., Steveson, J.C., Bevis, P.J.R., Lynch, C., Wimalawansa, S.J., Self, C.H., Morris, H.R., and MacIntyre, I., Lancet *2* (1985) 14.
8 Amara, S., Arriza, J.L., Leff, S.E., Swanson, L.W., Evans, R.M., and Rosenfeld, M.G., Science *229* (1985) 1094.
9 Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R., and MacIntyre, I., Nature *313* (1985) 54.

0014-4754/86/070829-02$1.50 + 0.20/0
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Inhibition of human pancreatic elastase II activity on human aortic elastin by human α2-macroglobulin

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Summary. Human α2-macroglobulin-human pancreatic elastase II binding were investigated using a homologous substrate, human aortic elastin, in order to test the enzymatic activity. We demonstrated that two moles of α2-M are required to inhibit one mole of HPE_{II} when the enzyme is added to a mixture of elastin and α2-M. In addition, when the elastase-α2-M complex is prepared under some circumstances, it exhibits an elastinolytic activity.
Key words. Pancreatic elastase II; α2-macroglobulin; aortic elastin; human; elastinolysis.

Human pancreatic elastase II ($H.P.E_{II}$) is a protein of 25,000 mol.wt able to hydrolyze insoluble elastin. Plasma α1-antiprotease (α1-AP) and α2-macroglobulin (α2-M) have been shown to inhibit this enzyme[1-3]. The latter protein forms enzymatic complexes with proteinases and in some cases the enzyme may be released from the complex[4-9]. However, recently we have shown that, when homologous reactants were used, the complex between HPE_{II} and α1-AP was able to degrade homologous elastin[10,11]. The interaction between HPE_{II} and α2-M has been studied especially by Gustavson et al.[9] using Benzyloxycarbonyl-alanine-p nitrophenyl ester as a substrate.
We decided to investigate the human α2-M-human $P.E._{II}$ binding using a homologous substrate to test the enzymatic activity. We demonstrate here that two moles of α2-M are required to inhibit one mole of HPE_{II}, when the enzyme was added to a mixture of elastin and α2-M. In addition, when the elastase – α2-M complex is prepared, under some circumstances, it exhibits an elastinolytic activity.

Materials and methods. Human pancreas and aorta were extracted from brain-death patients, together with the kidneys later used for transplantation by the 'monobloc' technique at the University Hospital Center of Bordeaux[12]. Human pancreatic elastase: HPE_{II} was chosen for this study because under physiological conditions, it is the enzyme secreted in greatest quantities by the exocrine region of the pancreas. Several authors have shown that it reaches the general circulation by the lymphatic pathway[13] or by crossing the intestinal barrier[14-16]. The enzyme was purified by the procedure of Largman et al.[17] and labeled with ^{125}Iodine as described in Rabaud et al.[11]. Human aortic elastin was prepared according to the method of Leppert et al.[18]. The fibers were swollen by equilibration in the appropriate buffer.
Human α2-Macroglobulin was purified as previously described[8]. It was kept at 4 °C in a solution buffered by 10 mM Tris HCl 500 mM NaCl, pH 7.2. The protein was homogeneous in polyacrylamide gel electrophoresis and had no detectable ami-