Presence of Atrial Natriuretic Peptide in Rat Thyroid Medullary Carcinoma Cell Line

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Atrial natriuretic peptide (ANP) is involved in water and electrolyte homeostasis. It was first discovered in atrial myocytes (1), but since then it has also been demonstrated in non-cardiac tissues such as brain (2), adrenal gland (3), pituitary (4), and colonic enterochromaffin cells (5) as well as in small cell lung cancer (6). In these tissues, a paracrine role of ANP has been suggested (7). In the thyroid gland, ANP has been reported to reduce thyroglobulin secretion through ANP receptors present on follicular epithelial cells (8). However, it is not clear which kinds of cells in the thyroid gland produce ANP and regulate the functions of follicular cells. In this study we therefore used a rat medullary thyroid carcinoma cell line (rMTC 6-23) to investigate whether thyroid C cells, neuroendocrine cells of the thyroid gland, also produce ANP.

MATERIAL AND METHODS

The rat thyroid medullary carcinoma cell line rMTC 6-23 was purchased from an American type culture collection and cultured in DMEM medium supplemented with 10% horse serum. It was seeded on a 10-cm culture dish and incubated at 37°C under humidified conditions in a 5% CO_2 atmosphere. Total RNA was extracted from the cells by the single-step guanidium thiocyanate phenol chloroform method (Isogen, Nippon Gene, Toyama, Japan). Specimens of normal thyroid and whole cerebrum total RNAs were obtained from 7-week-old male SD rats. Northern blot analysis was used to detect ANP mRNA. The probe used for Northern blot analysis was a 0.38 kb cDNA fragment of rat ANP which was synthesized in our laboratory by the reverse transcription polymerase chain reaction (RT-PCR) method, and using an automatic DNA sequencing system, the sequence was confirmed to be identical to that reported previously. Radiolabeling of the probe was performed with [α-^{32}P] dCTP using a DNA labeling kit (Megaprime DNA labeling system, Amersham, UK). The radiolabeled DNA probe was detected using a BAS 2000 bioimage analyzer (Fujix, Tokyo, Japan).

rMTC 6-23 cells were seeded in a Lab-Tek chamber (Nunc Inc., Illinois, USA), and fixed in 4% paraformaldehyde in PBS for 1 h at room temperature followed by rinsing with PBS. Human thyroid medullary carcinoma was obtained by surgical resection, and fixed in formaldehyde for immunohistochemistry. After pretreatment with 1% goat serum in PBS, it was incubated with a rabbit polyclonal antibody against human α (1-28) ANP (diluted 1:500; Affiniti Research Products, UK) for 2 h at 4°C. The specific immunostaining of this antibody was confirmed using human atrial tissue. The specimens were washed with PBS, and then incubated with biotin conjugated anti-rabbit immunoglobulin antibody (diluted 1:300; Vector Laboratories, CA, USA) for 30 min at room temperature. After washing with PBS, the bound antibody was detected using the avidin-biotin peroxydase method (ABC Elite kit; Vector Laboratories, CA, USA). Peroxydase activity was subsequently demonstrated by incubation with 3,3′diaminobenzidine (Sigma Chemical Co., St. Louis, MO, USA) in 0.05 mol/l Tris-HCl for 1 min at room temperature.

RESULTS AND DISCUSSION

Northern blot analysis showed ANP gene expression in the rMTC 6-23 thyroid medullary carcinoma cell line (Fig. 1). Surprisingly, expression of this gene was stronger than that in the brain. Although ANP gene expression in normal thyroid was detected by the RT-PCR method (data not shown), it was not detected by Northern blotting. Immunohistochemical examination also showed that ANP production was localized near the nucleus (Fig. 2a, b). Moreover, human thyroid medullary carcinoma was also stained (Fig. 3). These data showed that ANP mRNA was actually transcribed to ANP protein. There have been a number of studies of ANP production in thyroid gland (7, 9), but these have detected only a weak expression of ANP

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by immunofluorescence or the RT-PCR method. Although an immunofluorescence study showed that ANP production was detected in human thyroid follicular cells but not C cells (9), our data confirmed strong gene expression and production of ANP in rat thyroid medullary carcinoma, which is C cell neoplasm. Although it is difficult to reconcile these conflicting data, a change in C cell characteristics following carcinogenesis may be responsible.

The function of ANP in thyroid gland is not well known. Recently, ANP receptor was detected in thyroid follicular cells, and ANP was shown to reduce thyroglobulin production dose-dependently (8). At least, in the case of thyroid medullary carcinoma, C cell may be a source of intrathyroidal ANP. Further examination of the role of intrathyroidal ANP is required.

As a model for studying the production of ANP, cardiac myocytes have an inherent problem with their culture because of their short viability. Therefore, small cell lung carcinoma cell lines which secrete ANP are also used as a model of ANP producing cells (6, 10). This rMTC 6-23 cell line expresses ANP mRNA more strongly, and can therefore be used as a useful material for studies of ANP gene expression.

In conclusion, we have detected the gene expression and immunolocalization of ANP in the thyroid medullary carcinoma cell line rMTC 6-23. Our results suggest that thyroid medullary carcinoma is a major site of extracardiac ANP secretion, and may be a useful material for studies of ANP production.

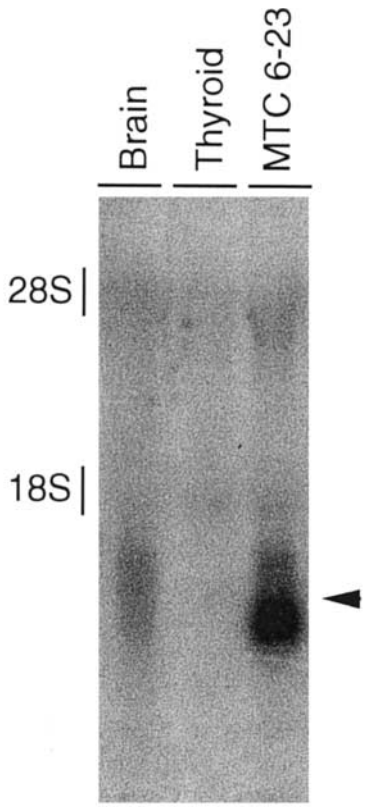

Fig. 1. Northern blot analysis of atrial natriuretic peptide mRNA. 20 μg of total RNA was electrophoresed in each lane.

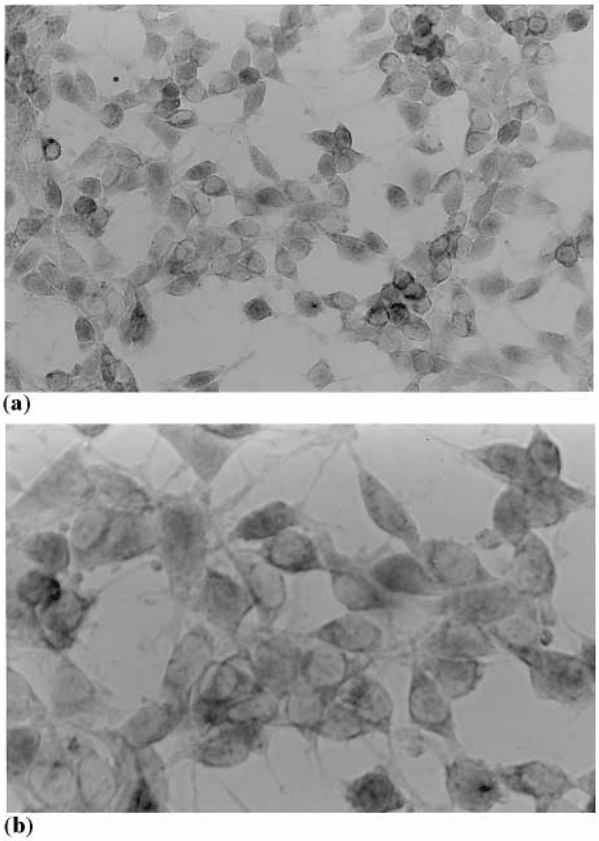

Fig. 2. (a) Immunohistochemical staining of ANP in rMTC 6-23 cells. (b) Higher magnification of photograph. ANP was localized near the nucleus.

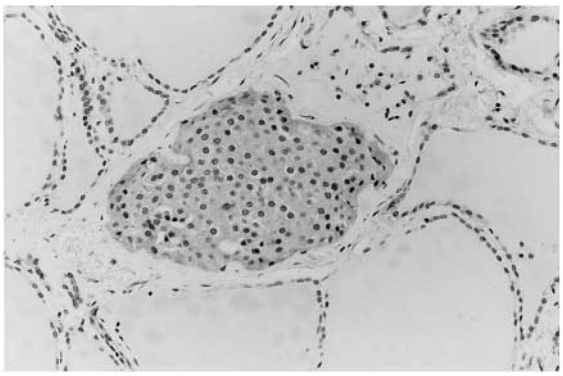

Fig. 3. Immunohistochemical staining of ANP in human thyroid medullary carcinoma. Although normal component of specimen was scarcely stained, carcinoma cells were diffusely stained with anti-ANP antibody.

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