Immunoreactive brain natriuretic peptide in human adrenal glands and adrenal tumors*

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The presence of brain natriuretic peptide (BNP) in tissues of human adrenal glands and adrenal tumors was investigated by radioimmunoassay. Immunoreactive BNP concentrations were 0.203 ± 0.061 pmol/g wet tissue (mean ± s.e.) in normal parts of adrenal glands (cortex and medulla, N = 8). 0.205 ± 0.037 pmol/g wet tissue in pheochromocytomas (N = 8), 0.230 ± 0.062 pmol/g wet tissue in aldosteronomas (N = 11) and 0.180 ± 0.054 pmol/g wet tissue in adrenocortical adenomas with Cushing’s syndrome (N = 4). Sephadex G-50 superfine column chromatography and reverse-phase high-performance liquid chromatography showed that most (> 70%) of the immunoreactive BNP in the normal part of adrenal glands was eluted in the position of human BNP-32. Sephadex G-50 superfine column chromatography of immunoreactive BNP in the pheochromocytoma and aldosteronoma showed four peaks: one in the position of γ-BNP, one in the position of BNP-32, one between γ-BNP and BNP-32 and one in the smaller molecular weight region. The present study has shown that immunoreactive BNP is present both in normal human adrenal glands and in adrenal tumors. Multiple molecular forms of BNP were found to be present in the tumor tissues of pheochromocytoma and aldosteronoma.

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The natriuretic peptide family consists of at least three peptides: atrial natriuretic peptide (ANP), brain or B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). Brain natriuretic peptide was originally isolated from porcine brain (1). Subsequently, rat BNP and human BNP were identified (2–5). Human BNP-32 is generated from the precursor of BNP consisting of 108 amino acids (γ-BNP or pro-BNP) by proteolytic cleavage. Brain natriuretic peptide is thought to be a cardiac hormone rather than a neuropeptide because high concentrations of immunoreactive (ir-) BNP were found mainly in the heart (6–8), while concentrations of ir-BNP in the human brain are very low (9).

Brain natriuretic peptide mRNA is expressed in the human adrenal gland (10, 11). High concentrations of ir-BNP are present in the human adrenal gland, in particular in the adrenal medulla (11). Plasma concentrations of immunoreactive ir-BNP are elevated in patients with pheochromocytoma or primary aldosteronism (12, 13). However, it was reported that BNP was absent in the tumor tissue of pheochromocytoma (14). Lee et al. (11) reported the expression of BNP and its mRNA in the non-tumorous adrenal medulla obtained from adrenal glands with aldosteronomas, but did not show the presence of BNP in aldosteronomas themselves. Thus the presence of BNP in the adrenal tumor tissues has not been clarified.

Pheochromocytoma is known to produce multiple vasoactive peptides, such as neuropeptide Y (NPY) (15), calcitonin gene-related peptide (CGRP) (16) and endothelin-1 (17). It was reported that ANP was produced and secreted from the adrenal medulla (18–21) and ir-ANP was present in the tumor tissue of aldosteronoma (22). We reported that CNP was present in human adrenal glands, pheochromocytomas and adrenocortical adenomas (23). In the present study, we investigated the presence of ir-BNP in tissues of human adrenal glands and adrenal tumors such as pheochromocytoma and aldosteronoma using a specific radioimmunoassay (RIA) for human BNP-32.

Materials and methods

Tissues and extraction

The study was approved by the Ethics Committee on human research of Tohoku University (No. 90–25),
and the procedures followed were in accordance with the ethical rules of the institutional committee on human experimentation. Eight pheochromocytomas and 17 adrenocortical tumors (11 aldosteronomas, four adrenocortical adenomas with Cushing's syndrome and two non-functioning adenomas) were obtained at surgery. Normal parts of adrenal glands (cortex and medulla) were obtained at surgery from eight patients with adrenal tumors (five patients with primary aldosteronism, two with non-functioning adrenocortical adenoma and one with pheochromocytoma). The tumors and the non-tumorous parts were carefully dissected. Classification of the tumors is based on pathological examinations. All tissues were stored at −80°C prior to extraction.

Tissues (approximately 0.5 g) were boiled in 2 ml of 1 mol/l acetic acid at 100°C for 10 min. Eight milliliters of 50% methanol in 1 mol/l acetic acid was added to the tissue. The tissue was homogenized and centrifuged at 24 000 g at 4°C for 60 min. The supernatant was separated and dried in air. The resulting materials were dissolved in 1 mol/l acetic acid containing 0.5% (w/v) bovine serum albumin and re-extracted by Sep-Pak C₁₈ cartridges (Waters, Milford, MA). The peptides were eluted with 2 ml of 60% acetonitrile in 0.1% (v/v) trifluoroacetic acid. The eluates were dried by air. The resulting residues were reconstituted in assay buffer and assayed. Assay buffer was 0.1 mol/l phosphate buffer (pH 7.7) containing 0.1% (w/v) bovine serum albumin, 0.2% (v/v) Triton X-100 and 0.1% (w/v) sodium azide. The recovery, determined by adding known amounts of synthetic human BNP-32 to the tissue prior to the extraction, was 73 ± 7% (mean ± s.e., N = 5).

Radioimmunoassay for human BNP

Tissue concentrations of ir-BNP were measured radioimmunologically, as reported previously (9). Briefly, the antiserum against human BNP-32 was raised in a rabbit. Human BNP-32 was used as standard. Iodine-125-labeled human BNP-32 was prepared by the chloramine T method. The assay could detect 1.2 fmol/tube at 95% confidence with duplicate tubes. The assay for human BNP showed less than 0.01% cross-reaction with human ANP-28, porcine BNP-26, rat BNP-32, human CNP-22, human CNP-53, endothelin-1, -2, -3, NPY, CGRP or other peptides tested. Inter- and intrassay coefficients of variation were less than 10% and 12%, respectively.

Chromatography

Immunoreactive BNP in the tissues was characterized by Sephadex G-50 superfine (1 × 56 cm) column chromatography and by reverse-phase high-performance liquid chromatography (HPLC). The tissue extract was reconstituted in 1 mol/l acetic acid containing 0.5% (w/v) bovine serum albumin and loaded onto a Sephadex G-50 superfine column, which was then eluted with 1 mol/l acetic acid containing 0.5% (w/v) bovine serum albumin at a flow rate of 6 ml/h. Fractions (0.8 ml) were collected, dried in air, reconstituted with assay buffer and assayed. The recovery from the column was determined by the ratio of the total ir-BNP in all fractions to the amount of ir-BNP in the extract loaded, and was found to be 70–108%. The elution position of γ-BNP was determined with an extract of human cardiac atrium obtained at autopsy. Immunoreactive BNP in the cardiac atrium was eluted in two positions: soon after the void volume and in the position of human BNP-32 (24). The immunoreactive peak of the cardiac extract eluting soon after the void volume was considered to represent γ-BNP.

Reverse-phase HPLC of the tissue extracts was performed on a µ-Bondapak C₁₈ column (3.9 × 300 mm, Waters). The tissue extract was reconstituted in 0.1% trifluoroacetic acid and loaded onto the column. The column was eluted with a linear gradient of 10–60% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min over 50 min. One milliliter fractions were collected, dried in air, reconstituted with assay buffer and assayed. The recovery from the HPLC column was 71–100%.

Statistical analysis

Data are given as means ± s.e.m., unless indicated otherwise. Statistical analysis was performed by one-way analysis of variance, followed by the Wilcoxon test.

Results

High concentrations of ir-BNP were detected in normal
adrenal glands (cortex and medulla) and adrenal tumors (Fig. 1). Tissue ir-BNP concentrations in normal parts of eight adrenal glands were 0.203 ± 0.061 pmol/g wet tissue. The normal part of adrenal gland obtained from a patient with pheochromocytoma showed the highest ir-BNP concentration. Tissue ir-BNP concentrations in normal parts of adrenal glands obtained from five patients with primary aldosteronism (0.148 ± 0.038 pmol/g wet tissue) were not elevated when compared to the other three cases.

Immunoreactive BNP was detected in eight pheochromocytomas (0.205 ± 0.037 pmol/g wet tissue, N = 8), 7/11 aldosteronomas (0.230 ± 0.062 pmol/g wet tissue, N = 11) and 3/4 adrenocortical adenomas with Cushing's syndrome (0.180 ± 0.054 pmol/g wet tissue, N = 4). There was no significant difference in tissue ir-BNP concentrations among these groups (p > 0.1). Immunoreactive BNP was not detectable in two non-functioning adrenocortical adenomas (< 0.060 pmol/g wet tissue).

Two normal parts of adrenal glands, three tumor tissues of pheochromocytoma and three tumor tissues of aldosteronoma were examined by Sephadex G-50 superfine column chromatography and reverse-phase HPLC. Similar elution profiles were obtained in each group, and representative cases are shown in Figs. 2 and 3.

**Fig. 2.** Sephadex G-50 superfine column chromatography of tissue extracts of: (A) normal part of adrenal gland; (B) pheochromocytoma; (C) aldosteronoma. Arrows indicate the elution positions of void volume (V₀), 𝛾-BNP (r) and human BNP-32 (BNP), respectively.

**Fig. 3.** Reverse-phase HPLC of the extracts of: (A) normal part of adrenal gland; (B) pheochromocytoma; (C) aldosteronoma. Arrows indicate the elution position of human BNP-32 (BNP). The dotted line indicates the gradient of acetonitrile (ACN).
Sephadex G-50 superfine column chromatography of the tissue extract from the normal adrenal gland showed a peak in the position of BNP-32 (Fig. 2A). Reverse-phase HPLC of the normal adrenal gland revealed a major immunoreactive peak co-migrating with BNP-32 (Fig. 3A). On the other hand, Sephadex G-50 superfine column chromatography of ir-BNP in the tumor tissue extracts of pheochromocytomas and aldosteronomas showed four immunoreactive peaks, one in the position of γ-BNP, one between γ-BNP and BNP-32, one in the position of BNP-32 and one in the smaller molecular weight region (Fig. 2B, 2C). High-performance liquid chromatography of these tumor extracts showed a peak eluting in the position of BNP-32, the other materials eluting later (Fig. 3B, 3C).

Discussion

The present study has demonstrated the presence of high concentrations of ir-BNP in the human adrenal gland and in pheochromocytomas and adrenocortical tumors. The levels were comparable with the levels in human brain (9) and human kidney (25), although far less than those of the cardiac atrium.

Chromatographic studies using a Sephadex G-50 superfine column revealed that the ir-BNP consisted of four components in tumor tissues of pheochromocytomas and aldosteronomas: one with a molecular form identical to human BNP-32, two with larger molecular weight than human BNP-32 and one with a smaller molecular weight, which may be a fragment of BNP-32. The fraction eluting soon after the void volume may represent γ-BNP. Material eluting in the second peak may be a processed form of γ-BNP or β-BNP. On the other hand, the main molecular form of ir-BNP in non-tumorous adrenal glands was BNP-32. This difference between the normal parts of adrenal glands and adrenal tumors may consist of differences in post-translational processing in the BNP synthesis.

The presence of ir-ANP and ir-BNP, and the expression of their mRNAs, was demonstrated in the adrenal medulla by immunocytochemistry and in situ hybridization (11, 18, 20). The ir-BNP in the adrenal gland shown in the present study may therefore mainly derive from the adrenal medulla, and ir-BNP may be produced by the pheochromocytoma. Discrepant results of Ohhashi et al., who found no BNP in pheochromocytoma (14), may be due to the higher sensitivity of their assay. In addition, we have found high concentrations of ir-BNP in tumor tissues of some aldosteronomas and cortisol-producing adrenocortical adenomas.

It was reported that the mean plasma ir-BNP concentration is elevated in patients with pheochromocytomas and with primary aldosteronism (12, 13). We confirm elevated plasma ir-BNP concentrations in five patients with pheochromocytoma (6.6 ± 0.7 pmol/L, p < 0.05 to normal subjects) (normal subjects: 4.2 ± 0.3 pmol/L, N = 27) and eight patients with primary aldosteronism (10.5 ± 1.9 pmol/L, p < 0.05 to normal subjects) (unpubl. obs.). Immunoassay BNP produced by adrenal tumors may partly contribute to the elevated plasma ir-BNP concentrations in patients with these tumors. But we did not find clinical differences between aldosteronomas with and without detectable tumor tissue ir-BNP. Another possible explanation for the elevated plasma ir-BNP concentrations in these patients is an increased BNP secretion from the heart stimulated by cardiovascular changes, such as volume overload due to primary aldosteronism.

Specific receptors for ANP, BNP and CNP are present in bovine and human adrenal glands, and these peptides have an inhibitory effect on adrenal steroidogenesis (26–32). Brain natriuretic peptide stimulated cyclic GMP accumulation and tyrosine hydroxylase activity in cultured bovine adrenal medullary cells (33). Brain natriuretic peptide produced in the adrenal gland and adrenal tumors may modulate adrenal steroidogenesis and catecholamine synthesis.

In conclusion, ir-BNP is present in normal adrenal glands and adrenal tumors. While BNP-32 was the main molecular form in the normal part of the adrenal gland, multiple molecular forms of BNP were present in the tumor tissues of pheochromocytomas and aldosteronomas. Brain natriuretic peptide in the adrenal and adrenal tumors may have a local action in an autocrine and paracrine manner, both in cortex and medulla.

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References


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