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Distribution of atrial natriuretic peptide in the conduction system and ventricular muscles of the human heart

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Summary. Atrial natriuretic peptide (ANP), a cardiac hormone, is known to be located in the atrial specific granules, but its presence and localization in the ventricular muscle of the human heart has not been examined fully. Using a specific antibody to human ANP, we studied the conduction system and ventricular muscle with immunohistochemical and ultrastructural methods in 30 hearts obtained at autopsy. These included 12 normal and 18 diseased hearts. In the normal hearts, ANP-positive granules, which were regularly observed in the atrial myocytes, were found in small quantities in the cells of the penetrating and branching bundles in 4 of 12, and in the cells of the ventricular free walls in 2 of the 12 hearts. In the diseased hearts, the positivity increased significantly ($P<0.05$), being found in 13 of 18 (72.2%) conduction systems and 10 of 18 (55.6%) ventricular muscles. The granules were confirmed to be immunoreactive with ANP by ultrastructural examination. Furthermore, the presence of ANP mRNA in the conduction system as well as in the ventricular myocytes was demonstrated by Northern blot hybridization for which we used the complementary DNA of human ANP. Thus, a small quantity of ANP appears to be synthesized and stored in the conduction system and ventricles of some normal hearts. However, ANP was shown to be present in a larger percentage of the diseased hearts.

Key words: Atrial natriuretic peptide – Conduction system – Cardiac disease – Immunohistochemistry – Northern blotting

Introduction

Atrial natriuretic peptide (ANP) exerts a wide variety of biological effects, including natriuresis and relaxation of vascular smooth muscles, with a resultant anti-hypertensive action (Ballermann and Brenner 1986; Forssmann 1986). In addition, the recent demonstration of ANP and its analogue in the rat brain suggests another role, that of neurotransmitter (Saper et al. 1985; Sudoh et al. 1988). In man, three subtypes of ANP (hANP) exist: α-, β-, and γ-hANP, which have molecular weights of 3000, 6000, and 13000 daltons, respectively (Kangawa and Matsuo 1984; Kangawa et al. 1984; Miyata et al. 1985). It has been shown by molecular analysis that pre-pro-ANP consists of 151 amino acids; these are processed to yield pro-ANP (γ-ANP, 126 amino acids) which is stored in atrial specific granules, and α-ANP (28 amino acids), which is a circulating form of ANP (Yamaji et al. 1985b). The β form of ANP (56 amino acids) was shown to be an antiparallel dimer of α-ANP (Kangawa et al. 1985). Genes encoding for ANP precursor have been cloned and sequenced (Yamanaka et al. 1984; Maki et al. 1984; Oikawa et al. 1984), providing complementary DNA for detection of ANP mRNA. A specific antibody to ANP has been used for radioimmunoassay, and the plasma level of ANP in various disease conditions has been reported (Nakao et al. 1984; Yamaji et al. 1985a). By immunohistochemical study, ANP, which is usually detected in the atrial muscle, has also been found to localize in the extra-atrial tissues (Cantin et al. 1985; McKenzie et al. 1985; Kawata et al. 1985). ANP has been shown to be present in the ventricular tissues of the heart at concentrations 1000 times lower than those in atrial tissue (Nemer et al. 1986), but in the presence of some cardiac disease conditions, ANP mRNA was detected in higher amounts in ventricular tissue (Saito et al. 1987). However, the exact distribution of ANP in the human heart outside the atrium has not been established. Wharton et al. (1988) have recently reported that on immunohistochemical examination ANP immunoreactivity was found in the fetal ventricular conduction system and in adult ventricular tissues obtained during transplantation surgery.

Our purpose in the present study was to explore ANP and its mRNA in the conduction system and in ventricu-

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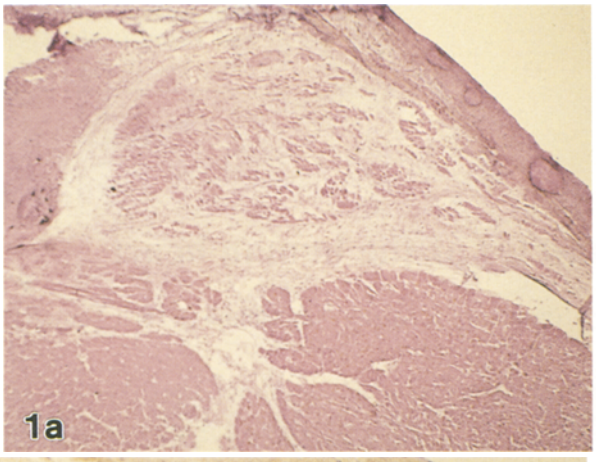
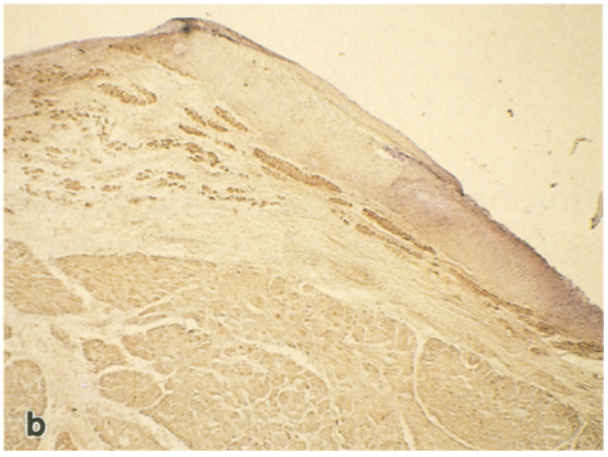




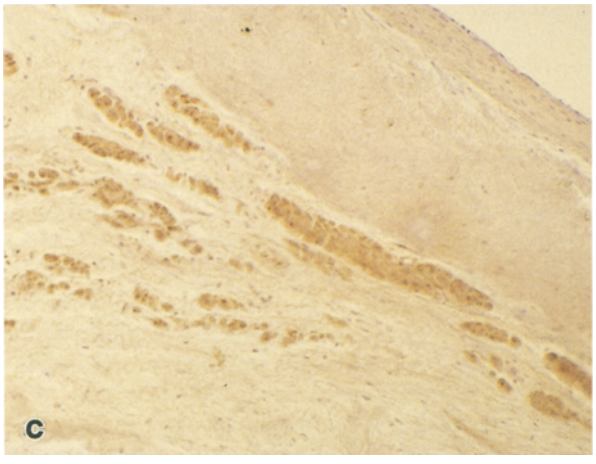
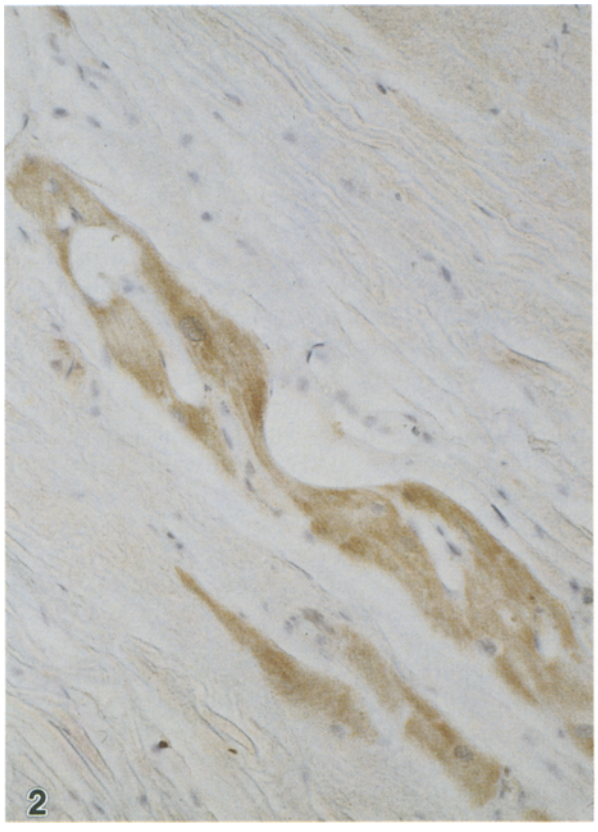


Fig. 1 A–C. The penetrating bundle (His bundle) from case 22. Frozen sections were fixed in cold acetone and stained with haematoxylin and eosin (**A**) or immunostained with anti-ANP by ABC method (**B**), ×40. A higher magnification of the left branching bundle is also shown (**C**). ×200. The immunoreaction is confined to the conduction system of the interventricular septum



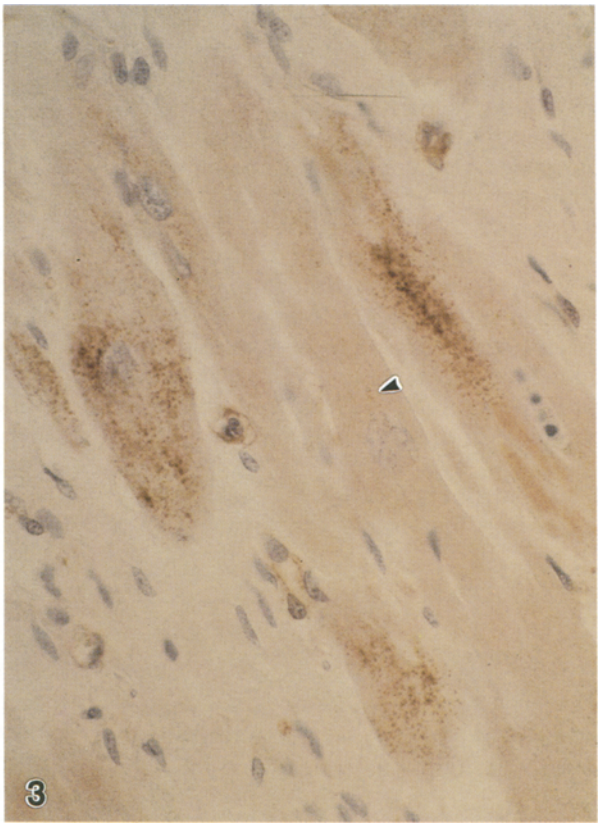


Fig. 2. Left branching bundle from case 8. Diffuse ANP positivity with some granular staining is seen. ×400

Fig. 3. Ventricular muscle from case 12. Brown ANP-positive granules are present in the hypertrophied muscle fibres which are surrounded by fibrosis. *Arrow* indicates lipofuscin granules. ×400

lar muscles of diseased and normal human hearts by performing immunohistochemical, immunoelectron microscopical and Northern blot analyses.

Materials and methods

Thirty human hearts obtained at autopsies performed within 11 h after death were used for immunohistochemical and immunoelectron microscopical examination. Two additional cases were used for extraction of mRNA from the conduction system (see below). For immunostaining, frozen sections from 6 of the 30 cases were examined. The other 24 hearts were perfused through the coronary arteries with freshly prepared 4% paraformaldehyde, and the ventricles were sectioned horizontally parallel to the coronary sulcus at the upper two-thirds level, with serial sections down to the apex at 1 cm intervals. They were postfixed in the same fixative overnight at 4° C. From the atrium, three serial sections, including the sino-atrial node, were prepared; these contained the base of the superior vena cava and the right auricle. The septal portions of the hearts, which included the entire conduction system, that is, the atrioventricular node, His bundle (penetrating bundle), and major branching bundle, were serially sectioned as described by Anderson et al. (1987). In the horizontal sections of the ventricles, the anterior, lateral, and posterior walls were examined. The material was embedded in paraffin after dehydration through the graded alcohol.

For immunohistochemistry the avidin-biotin peroxidase complex (ABC) method was applied to the paraffin-embedded tissues (Hsu et al. 1981). The conduction systems in the six frozen-section cases (cases 1, 3, 7, 18, 21, and 22) were fixed with either cold acetone for 10 min or 4% paraformaldehyde for 20 min. Antibody against human ANP (hANP) was prepared by immunization of rabbits with a conjugate of synthesized α-hANP (Peptide Institute, Osaka) to porcine thyroglobulin (Sigma, St. Louis, Mo.) according to the method described by Skowsky and Fisher (1972). The antibody obtained was shown to react with α, β-, and γ-hANP on immunoblots; it did not react with porcine thyroglobulin.

After deparaffinization, the preparations were pre-treated with H_2O_2-methanol, which blocked endogenous peroxidase, and they were reacted with the ANP antibody at a 500-fold dilution at 4° C overnight, followed by a second reaction with biotinylated anti-rabbit IgG (Dako, Kyoto). They were reacted with ABC solution (Vector, Burlingame, Calif.) and were visualized with 3,3′ diaminobenzidine tetrahydrochloride (DAB). To test the cross-reactivity

Table 1. Immunohistochemical findings on atrial natriuretic peptide in human hearts

Case no.	Diagnosis	Age (years)/sex	Time post-mortem (h:min)	RA	CS	LV	NYHA
Cardiac disease							
1	AMI+OMI	61/M	4:00	++	−	−	I
2	AMI+OMI	71/M	2:00	++	−	−	III
3	AMI+OMI	79/F	1:20	++	+	+	IV
4	OMI	57/M	5:00	++	+	+	I
5	OMI	69/M	2:30	++	+	+	I
6	OMI	72/F	6:00	++	−	−	III
7	MS	58/M	1:00	++	−	−	IV
8	AS	58/M	5:00	++	+	+	IV
9	AR+MS	61/M	2:40	++	+	−	III
10	MVP	64/M	2:00	++	+	+	I
11	DCM	23/M	2:00	++	−	−	I
12	DCM	39/F	2:00	++	+	+	IV
13	DCM	45/F	3:00	++	+	+	IV
14	DCM	63/F	2:00	++	+	+	III
15	HOCM	40/F	2:00	++	+	+	III
16	HNCM	64/M	11:00	++	+	−	I
17	HHD	45/M	2:00	++	+	+	III
18	HHD	73/M	1:50	++	+	−	I
No cardiac disease							
19	Lung cancer	56/M	2:50	++	−	−	I
20	Lung cancer	74/M	3:00	++	−	−	I
21	Lung cancer	76/M	3:00	++	+	+	I
22	Malignant lymphoma	63/M	3:00	++	+	−	I
23	MDS	72/M	1:00	++	−	−	I
24	Cholangiocarcinoma	79/M	2:00	++	−	−	I
25	Hepatoma	62/M	4:00	++	+	−	I
26	Hepatoma	68/M	5:00	++	−	−	I
27	Ovarian carcinoma	70/F	4:50	++	−	−	I
28	Old tuberculosis	79/F	2:00	++	−	−	I
29	Aortic aneurysm	47/F	1:30	++	+	+	I
30	AML	73/M	2:00	++	−	−	I

RA, Right atrium; CS, conduction system in the ventricle; LV, left ventricle; NYHA, score for cardiac failure according to New York Heart Association; AMI, acute myocardial infarction; OMI, old myocardial infarction; MS, mitral stenosis; AS, aortic stenosis; AR, aortic regurgitation; MVP, mitral valve prolapse; DCM, dilated cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; HNCM, hypertrophic non-obstructive cardiomyopathy; HHD, hypertensive heart disease; MDS, myelodysplastic syndrome; AML, acute myelogenous leukaemia; ++, more than half of the myocytes in a low-power field (×100) were positive; +, single or few myocytes in a high power-field (×400) were positive. −: no staining

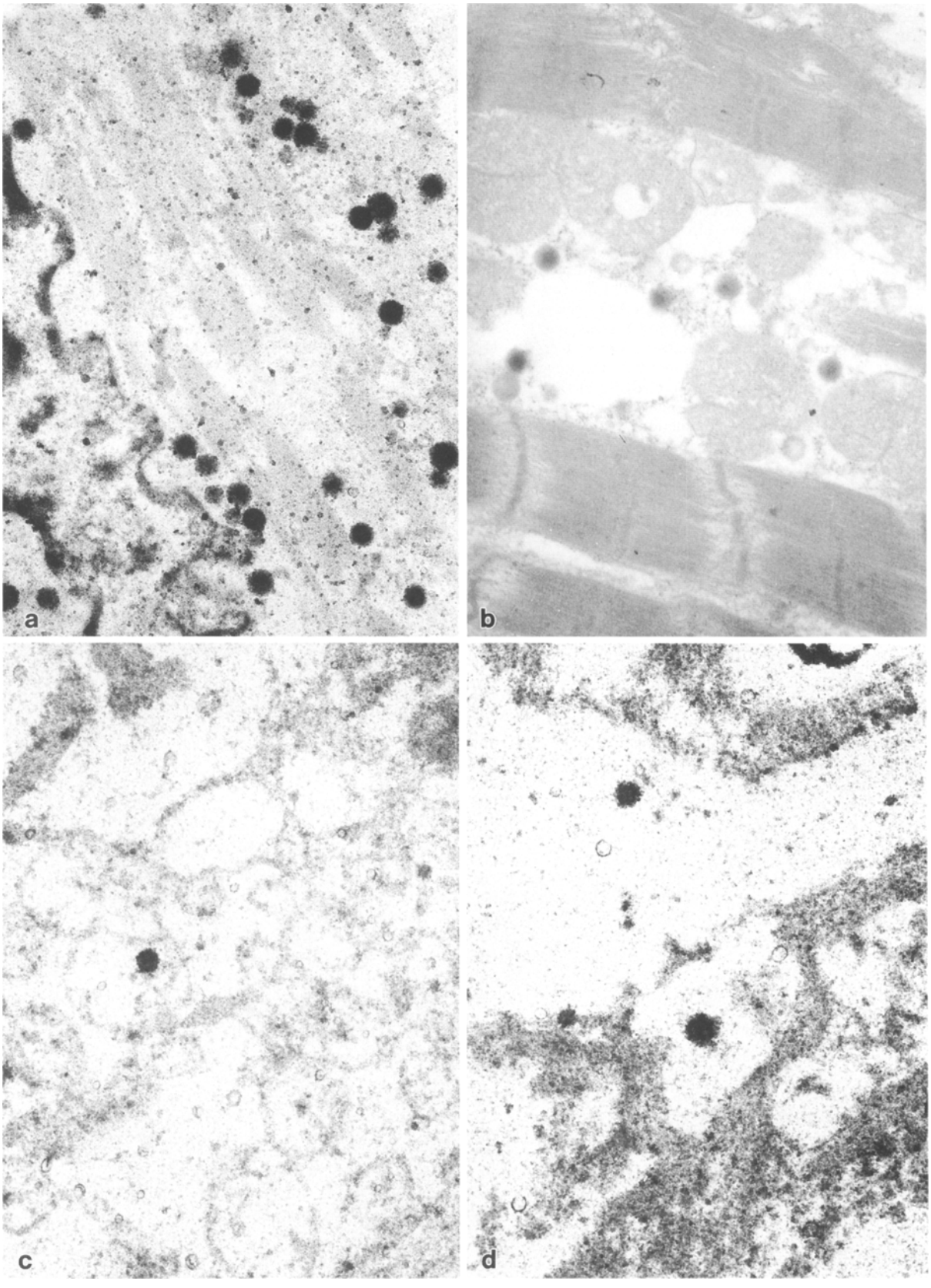


Fig. 4A–D. Immunoelectron microscopic features of ANP-positive granules from case 15. There are many positive granules in the atrial myocytes (**A**). Before the immunoreaction, the granules are grey (**B**). The granules in the penetrating bundle (**C**) and left ventricular wall (**D**) are also immunolabelled. ×25000

with thyroglobulin, which was used as a carrier protein for the immunogen, we applied anti-human thyroglobulin antibody (Dako, Kyoto) instead of the primary antibody.

For immunoelectron microscopy we used material from one well-perfused diseased heart (case 15). Small fragments of the atrial tissue, conduction system, and ventricular muscles were dehydrated in graded alcohols and acetone and were polymerized in epoxy resin. Ultrathin sections were loaded on to nickel grids, etched by a solution of absolute alcohol and saturated NaOH diluted 1:3, and immunostained according to the method used for immunohistochemistry, by means of a post-embedding procedure. After reacting with ABC solution, the sections were fixed with 2.5% glutaraldehyde. After visualization with the DAB reaction, they were post-fixed with 1% osmium tetroxide, counterstained with uranyl acetate, and examined with the electron microscope (Hitachi-600).

To perform Northern blotting we examined the heart of a male patient who died of renal cancer at the age of 77 years. Atrial tissue and left ventricular wall were taken without fixation and used for extraction of RNA. In addition, the conduction system from the His bundle (penetrating bundle) to the branching bundles was carefully dissected. The dissection was performed in a cold room under a stereoscopic wide-field microscope, without contamination by atrial muscles or the atrioventricular nodes, according to the methods described by Kistin (1949) and by Widran and Lev (1951). The conduction system taken from this patient weighed 20 mg in wet tissue, and 10 μg of RNA was extracted. In the present study, we used 20 μg of RNAs from each part of the heart; therefore, we took the conduction system from another male patient who had died of pulmonary cancer at the age of 69 years, and we mixed it with the previous material to obtain a sufficient amount of RNA. Both patients were free of any cardiac disease.

The RNA was extracted by the acid guanidinum thiocyanate phenol chloroform extraction method (Chomczynski and Sacchi 1987). DNA complementary to hANP (cDNA), 670 bp inserted at the *Pst*I site of plasmid phANP (Oikawa et al. 1984), was excised and purified by agarose gel electrophoresis. Preparations of cDNA were labelled with ^{32}P (Feinberg and Vogelstein 1983) with a Random Primer Labeling Kit (Boehringer Mannheim, Calif.). After electrophoretic separation of RNA on formaldehyde/1.5% agarose gel, the samples were transferred to nitrocellulose membranes; the membranes were hybridized with a radiolabelled probe and exposed to Kodak X-Omat AR film for 7 days.

Results

Positive immunostaining with anti-hANP in a granular pattern was observed in the perinuclear areas in all cases of atrial working muscles. Absorption of the antibody by α-hANP abolished the staining. Pre-immune rabbit serum and anti-thyroglobulin did not stain any muscles. In the sino-atrial node, the nodal cells which were grouped together in small interconnecting fasciculi were usually negative, but the cells in the transitional-cell zone, which have characteristics intermediate between those of the packed nodal cells and those of the individual atrial myocardial cells, were often positive. The cells of the atrioventricular node were negative. In the conduction system of the ventricle, the penetrating bundle (bundle of His) and the major branching bundles, immunoreactive granules were less prominent than they were in the atrial muscles. Four of the 12 non-diseased hearts (33.3%) showed staining in the conduction system (Fig. 1), and the number of positive cells was lower than that in the atrial cells. Most of the working muscles of the septum were negative, but in 2 of the 12 normal hearts (16.7%) ANP-positive myocytes were observed in the left ventricular wall near the endocardium.

In the diseased hearts, this ratio became significantly higher both in the conduction system and in the ventricular muscle ($P < 0.05$). The conduction system contained positive granules in 13 of the 18 cases (72.2%). In the cases in which the penetrating bundles were positively stained, the branching bundles were stained consistently (Fig. 2). The ventricular tissue of the diseased hearts contained positive granules in 10 of the 18 cases (55.6%). ANP-positive ventricular cells were frequently found in specimens from hearts with cardiac failure (7/11: 63.6%), which was assessed as grade III and IV according to New York Heart Association (NYHA), but they were also detected in diseased hearts without cardiac failure (3/7: 42.8%). The positive cells were usually distributed in the inner third of the myocardial wall; these cells were hypertrophied (Fig. 3) and surrounded by fibrous tissue, irrespective of the particular cardiac disorder (Table 1).

Frozen sections fixed with acetone generally exhibited a more intense reaction than did paraffin sections, but the immunoreactivity itself was identical to that of paraffin sections obtained by perfusion fixation with 4% paraformaldehyde at autopsy. We were able to distinguish lipofuscin granules from the immunoreaction; the lipofuscin granules were usually large, but irregular in size, yellow and/or greenish-brown, and located in perinuclear spaces, whereas the ANP-positive granules were small and uniform, light brown, and diffusely distributed among the muscle fibres.

On immunoelectron microscopy we found numerous membrane-bound, electron-dense granules, about 100–

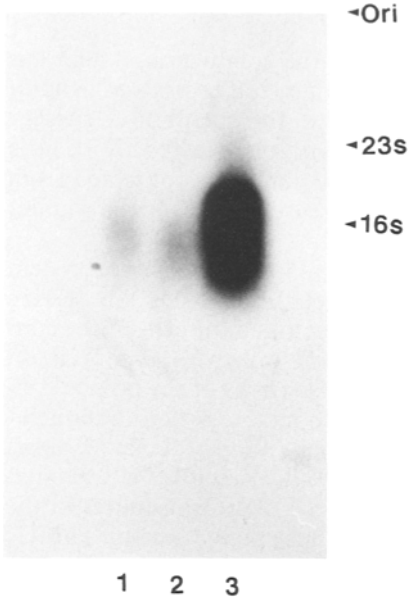


Fig. 5. Northern blot analysis of the normal hearts. RNAs (20 μg) were fractioned on a 1.4% agarose gel, transferred to a nitrocellulose membrane, and hybridized with an ^{35}S-labelled hANP cDNA probe. *Lane 1*: left ventricle. *Lane 2*: conduction system. *Lane 3*: right atrium. Compared to a strong signal of ANP mRNA in the right atrium, a significant amount of the signal is also detected in the conduction system and left ventricular wall

300 nm in diameter, in the perinuclear areas in the atrial cells as well as in the areas between the myofibrils. These granules were labelled with anti-ANP (Fig. 4a). Similar granules stained with anti-ANP, but in very decreased numbers, were found in the cells of the penetrating and branching bundles (Fig. 4c). The granules stained positively in the ventricular muscles of the diseased hearts on immunohistochemical examination were shown by immunoelectron microscopy to be ANP granules (Fig. 4d).

The Northern blots showed that 20 μg of RNA extracted from the atrium contained an intensely hybridizing RNA band approximately 1.0 kb pair in size (Fig. 5, lane 3). A significant radioactivity of ANP RNA was demonstrated in the 20 μg of RNA extracted from the pooled conduction systems of the two non-diseased hearts (Fig. 5, lane 2). The ventricular muscle was also shown to contain ANP RNA (Fig. 5, lane 1).

Discussion

In this study, we showed that ANP was present not only in the atria, but also in the penetrating and branching bundles in the ventricular conduction system and in the ventricular myocytes of the diseased hearts. We also demonstrated the presence of ANP mRNA in the conduction system and ventricular free wall of the non-diseased heart. In previous studies, the cells of the conduction system in the sino-atrial and atrioventricular nodes lacked specific atrial granules (Sherf and James 1979), and only transitional cells were shown to contain granules (Davies et al. 1983). We obtained similar results, but in the penetrating bundles there were rare granules, and individual fibres that reacted with ANP antibody were occasionally found in 4 of the 12 normal hearts.

The presence of ANP in the conduction system has recently been reported in human fetal hearts and in adult ventricular tissue obtained at transplantation surgery (Wharton et al. 1988). However, there seemed to be little expression of ANP in ventricular tissues obtained from patients without cardiac disease. We obtained similar results in the present study.

In animals, ANP was found in the conduction system of normal hearts (Toshimori et al. 1987, 1988). In bovine hearts, a chemical similarity between the cells of the conduction system and the atrial myocytes has been reported; myosin heavy-chain isozymes of the bovine conduction system were shown to have a pattern intermediate between those of the atrial and ventricular working myocytes (Komuro et al. 1987). Recently, it was suggested that ANP functions as a neurotransmitter in the brain (Saper et al. 1985). Thus, in the human cardiac conduction system, ANP may have the role of an impulse conductor.

In one case without cardiac disease (case 22), a positive reaction was observed in many bundle cells of the conduction system. This case was examined on frozen sections; however, compared to other positive cases (cases 3, 18, and 21) which were also examined on frozen sections, the intensity was more prominent and the distribution of the positive fibres was more widespread. This patient was later found to have been treated over a long period with corticosteroids for malignant lymphoma and HTLV-1 associated myelopathy (Osame et al. 1987). Induction of ANP by corticosteroids has been reported in experimental animals (Gardner et al. 1986) as well as in man (Weidmann et al. 1988). Thus, its strong expression in the conduction system in this case could be attributable to the long-term steroid therapy. For confirmations of this, however, larger numbers of patients who received long-term steroid therapy should be studied.

The working muscles of the ventricles usually lack any ANP-immunoreactive granules. In 2 of the 12 normal hearts, however, there were ANP-positive fibres in the subendocardium. This may indicate that the cells belonged to Purkinje fibres, as has been reported in rats (Tashimori et al. 1988), but we did not examine this possibility, as we have no method for identifying Purkinje fibres histologically. In the diseased hearts, however, ANP-positive granules were observed frequently both in the conduction system (72.2%) and in the left ventricular wall (55.6%). It is sometimes difficult to identify the granular organelles as specific ANP granules by electron microscopy, because of the presence of lysosomes. In this study, however, we were able to demonstrate that the granules were immunoreactive with anti-ANP.

Increased ANP gene expression has been reported in the ventricle of dilated cardiomyopathy (DCM) (Saito et al. 1987). In our series, ANP-positive ventricular cells were found in 3 of 4 cases of DCM. The patient without ANP (case 11) died suddenly, whereas the other three had long-standing cardiac failure. A high serum level of ANP has been reported in the presence of volume overloading and congestive heart failure (Lang et al. 1985; Tikkanen et al. 1985; Ding et al. 1987). This may suggest that ANP expression in the ventricle depends in part on heart failure, rather than on DCM.

A similar interpretation can be applied to other cardiac diseases, because the patients in whom ANP was detected in the ventricles had clinical signs of heart failure. However, in four patients who had suffered moderate to severe cardiac failure (cases 2, 6, 7, 9), we were not able to demonstrate ANP either in the conduction system or in ventricular tissue. This shows that cardiac failure is not an essential factor for ANP induction in the ventricular cells. However, in two patients who had rather small old myocardial infarcts (cases 4 and 5), and died of liver cirrhosis and cerebral contusion, respectively, without obvious signs of cardiac failure (NYHA I), ANP-positive myocytes were found both in the conduction system and in ventricular tissue. In these cases, the ventricular muscles, which, away from the lesions, appeared normal on histological examination, were devoid of ANP granules. In contrast, the myocytes surrounded by fibrosis or hypertrophied myocytes of an old myocardial infarction were stained with ANP. Similarly, muscles showing disarray in a case of hypertrophic cardiomyopathy were negative for ANP, but muscle cells among the fibrotic lesions were positive. Whether ANP expression in the ventricular tissue is related to fibrosis or not remains to be examined further.

Expression of ANP mRNA and ANP synthesis has been reported to be high in the ventricules of hypertensive animals (Day et al. 1987; Ruskoaho and Leppäluoto 1988). However, not all patients with essential hypertension have high serum levels of ANP (Yamaji et al. 1986; Sugawara et al. 1988). There were two cases of hypertensive heart disease in our series, in one of which (case 17) the heart contained ANP-positive myocytes. The patient had a polycystic kidney, and he died of heart failure; the ANP-negative patient (case 18) died of a brain abscess without exhibiting signs of heart failure. Further cases will need to be studied for determination whether ANP expression is related to hypertension or heart failure.

Overall, these findings suggest that ANP is probably produced by diseased ventricular muscle, to compensate for loss of atrial ANP. The presence of ANP mRNA in the ventricular muscles and the compensatory role of ANP in heart disease have been well established in animals (Day et al. 1987; Hamid et al. 1987). However, the mechanism of expression of ANP in diseased ventricular cells remains unknown.

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References

Anderson RH, Ho SY, Becker AE (1987) Gross anatomy and microscopy of the conduction system. In: Mandel WJ (ed) Cardiac arrhythmias, 2nd edn. Lippincott, Philadelphia, pp 13–52

Ballermann BJ, Brenner BM (1986) Role of atrial peptides in body fluid homeostasis. Circ Res 58:619–630

Cantin M, Gutkowska J, Thibault G, Milne RW, Ledoux S, Min Li S, Chapeau C, Garcia R, Hamet P, Genest J (1985) Immunocytochemical localization of atrial natriuretic factor in the heart and salivary glands. Histochemistry 80:825–832

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Davies MJ, Anderson RH, Becker AE (1983) Ultrastructural investigation of the atrioventricular conduction tissues. In: Davies MJ (ed) The conduction system of the heart. Butterworths, London, pp 48–52

Day ML, Schwartz D, Wiegand RC, Stockman PT, Brunnert SR, Tolunay E, Currie MG, Standaert DG, Needleman P (1987) Ventricular atriopeptin. Unmasking of messenger RNA and peptide synthesis by hypertrophy or dexamethasone. Hypertension 9:485–491

Ding J, Thibault G, Gutkowska J, Garcia R, Karabatsos T, Jasmin G, Genest J, Cantin M (1987) Cardiac and plasma atrial natriuretic factor in experimental congestive heart failure. Endocrinology 121:248–257

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Forssman WG (1986) Cardiac hormones. I. Review on the morphology, biochemistry, and molecular biology of the endocrine heart. Eur J Clin Invest 16:439–451

Gardner DG, Hane S, Trachewsky D, Schenk D, Baxter JD (1986) Atrial natriuretic peptide mRNA is regulated by glucocorticoids in vivo. Biochem Biophys Res Commun 139:1047–1054

Hamid Q, Wharton J, Terenghi G, Hassall CJS, Aimi J, Taylor KM, Nakazato H, Dixon JE, Burnstock G, Polak JM (1987) Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human appendage. Proc Natl Acad Sci USA 84:6760–6764

Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Kangawa K, Matsuo H (1984) Purification and complete amino acid sequence of α-human atrial natriuretic polypeptide (α-ANP). Biochem Biophys Res Commun 118:131–139

Kangawa K, Fukuda A, Kubota I, Hayashi Y, Matsuo H (1984) Identification in rat atrial tissue of multiple forms of natriuretic polypeptides of about 3000 daltons. Biochem Biophys Res Commun 121:585–591

Kangawa K, Fukuda A, Matsuo H (1985) Structural identification of β- and γ-human atrial natriuretic polypeptides. Nature 313:397–400

Kawata M, Ueda S, Nakao K, Morii N, Kiso Y, Imura H, Sano Y (1985) Immunohistochemical demonstration of α-atrial natriuretic polypeptide-containing neurons in the rat brain. Histochemistry 83:1–3

Kistin AD (1949) Observations of the anatomy of the atrioventricular bundle (bundle of HIS) and the question of other muscular connections in normal human hearts. Am Heart J 37:849–867

Komuro I, Nomoto K, Sugiyama T, Kurabayashi M, Takaku F, Yazaki Y (1987) Isolation and characterization of myosin heavy chain isozymes of the bovine conduction system. Circ Res 61:859–865

Lang RE, Thölken H, Ganten D, Luft FC, Ruskoaho H, Unger T (1985) Atrial natriuretic factor – a circulating hormone stimulated by volume loading. Nature 314:264–266

Maki M, Takayanagi R, Misono KS, Pandey KN, Tibbetts C, Inagami T (1984) Structure of rat atrial natriuretic factor precursor deduced from cDNA sequence. Nature 309:722–724

McKenzie JC, Tanaka I, Misono KS, Inagami T (1985) Immunocytochemical localization of atrial natriuretic factor in the kidney, adrenal medulla, pituitary and atrium of rat. J Histochem Cytochem 33:825–832

Miyata A, Kangawa K, Toshimori T, Hatoh T, Matsuo H (1985) Molecular forms of atrial natriuretic polypeptides in mammalian tissues and plasma. Biochem Biophys Res Commun 129:248–255

Nakao K, Sugawara A, Morii N, Sakamoto M, Suda M, Soneda J, Ban T, Kihara M, Yamori Y, Shimokura M, Kiso Y, Imura H (1984) Radioimmunoassay for α human and rat atrial natriuretic polypeptide. Biochem Biophys Res Commun 124:815–821

Nemer M, Lavigne J-P, Drouin J, Thibault G, Gannon M, Antakly T (1986) Expression of atrial natriuretic factor gene in heart ventricular tissue. Peptides 7:1147–1152

Oikawa S, Imai M, Ueno A, Tanaka S, Noguchi T, Nakazato H, Kangawa K, Fukuda A, Matsuo H (1984) Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide. Nature 309:724–726

Osame M, Matsumota M, Usuku K, Izumo S, Ijichi N, Amitani H, Tara M, Igata A (1987) Chronic progressive myelopathy associated with elevated antibodies to human T-lymphotropic virus type I and adult T-cell leukemia-like cells. Ann Neurol 21:117–122

Ruskoaho H, Leppäluoto J (1988) Immunoreactive atrial natriuretic peptide in ventricles, atria, hypothalamus, and plasma of genetically hypertensive rats. Circ Res 62:384–394

Saito Y, Nakao K, Arai H, Sugawara A, Morii N, Yamada T, Itoh H, Shiono S, Mukoyama M, Obata K, Yasue H, Ohkubo H, Nakanishi S, Imura H (1987) Atrial natriuretic polypeptide (ANP) in human ventricle. Increased gene expression of ANP in dilated cardiomyopathy. Biochem Biophys Res Commun 148:211–217

Saper CB, Standaert DG, Currie MG, Schwartz D, Geller DM,

Needleman P (1985) Atriopeptin-immunoreactive neurons in the brain. Presence in cardiovascular regulatory areas. Science 227:1047–1049

Sherf L, James TN (1979) Fine structure of cells and their histologic organization within internodal pathways of the heart: clinical and electrocardiographic implications. Am J Cardiol 44:345–369

Skowsky WR, Fisher DA (1972) The use of thyroglobulin to induce antigenicity to small molecules. J Lab Clin Med 80:134–144

Sudoh T, Kangawa K, Minamino N, Matsuo H (1988) A new natriuretic peptide in porcine brain. Nature 332:78–81

Sugawara A, Nakao K, Kono T, Morii N, Yamada T, Itoh H, Shiono S, Saito Y, Mukoyama M, Arai H, Imura H (1988) Atrial natriuretic factor in essential hypertension and adrenal disorders. Hypertension 11 [Suppl I]:212–216

Tikkanen I, Fyhrguist F, Metsärinne K, Leidenius R (1985) Plasma atrial natriuretic peptide in cardiac disease and during infusion in healthy volunteers. Lancet II:66–69

Toshimori H, Toshimori K, Oura C, Matsuo H (1987) Immunohistochemistry and immunocytochemistry of atrial natriuretic polypeptide in porcine heart. Histochemistry 86:595–601

Toshimori H, Toshimori K, Oura C, Matsuo H, Matsukura S (1988) The distribution of atrial natriuretic polypeptide (ANP)-containing cells in the adult rat heart. Anat Embryol 177:477–484

Weidmann P, Matter DR, Matter EE, Gnädinger MP, Uehlinger DE, Shaw S, Hess C (1988) Glucocorticoid and mineralocorticoid stimulation of atrial natriuretic peptide release in man. J Clin Endocrinol Metab 66:1233–1239

Wharton J, Anderson RH, Springgall D, Power RT, Rose M, Smith A, Espejo R, Khaghni A, Wallwork J, Yacoub MH, Polak JM (1988) Localization of atrial natriuretic peptide immunoreactivity in the ventricular myocardium and conduction system of the fetal and adult heart. Br Heart J 60:267–274

Widran J, Lev M (1951) The dissection of the atrioventricular node, bundle and bundle branches in the human heart. Circulation 4:864–867

Yamaji T, Ishibashi M, Nakaoka H, Imataka K, Amano M, Fujii J (1985a) Possible role for atrial natriuretic peptide in polyuria associated with paroxysmal atrial arrhythmias. Lancet I:1211

Yamaji T, Ishibashi M, Takaku F (1985b) Atrial natriuretic factor in human blood. J Clin Invest 76:1705–1709

Yamaji T, Ishibashi M, Sekihara H, Takaku F, Nakaoka H, Fujii J (1986) Plasma levels of atrial natriuretic peptide in primary aldosteronism and essential hypertension. J Clin Endocrinol Metab 63:815–818

Yamanaka M, Greenberg B, Johnson L, Seilhamer J, Brewer M, Friedemann T, Miller J, Atlas S, Laragh J, Lewicki J, Fiddes J (1984) Cloning and sequence analysis of the cDNA for the rat atrial natriuretic factor precursor. Nature 309:719–722