Korean J Biol Sci 6: 253-261, 2002

Developmental Modulation of Specific Receptor for Atrial Natriuretic Peptide in the Rat Heart

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Key Words:
Atrial natriuretic peptide
Receptor autoradiography
Heart
Endocardium
Development

Although cardiac distribution of specific receptors for atrial natriuretic peptide (ANP) was mainly observed in the ventricular endocardium, the modulation of ANP receptors in relation to cardiac development is not defined. The present study was undertaken to investigate ANP receptor modulation in rat during development. In the developmental stages examined (fetus, after postnatal 3-days, 1-, 2-, 3-, 4-, and 8-week-old Sprague Dawley rats) specific ANP binding sites were localized in the right and left ventricular endocardia by quantitative in vitro receptor autoradiography using ^{125}I-rat ANP as labeled ligand. The specific bindings to endocardium were much higher in the right than the left ventricle. The binding affinities of ANP were much higher in the right than the left ventricular endocardium. The difference of these binding affinities among various developmental stages was not observed in the right ventricle, whereas the binding affinity in left ventricle was gradually increased with aging and reached the peak value at 8 weeks. No significant difference in maximal binding capacities of endocardial bindings was observed in the right and left ventricular endocardia during developmental stages. Also, cGMP production via activation of particulate guanylyl cyclase-coupled receptor subtypes in the ventricular membranes was gradually decreased with close relationship to aging. Therefore, the present study show that the endocardial ANP receptor is modulated with close relationship to cardiac development in the left ventricle rather than the right ventricle, and may be involved in regulating myocardial contractility in left heart.

Atrial natriuretic peptide (ANP) is a 28-amino acid peptide hormone that was originally identified in the heart (De Bold et al., 1981; Flynn et al., 1983). This peptide is primarily synthesized in the atrial myocytes and released into the bloodstream, and exerts various physiological actions such as natriuresis and diuresis in the kidney (De Bold et al., 1981), inhibition of aldosterone synthesis and secretion in the adrenal gland (Atarashi et al., 1984), and vasorelaxation in the vascular smooth muscle (Currie et al., 1983). Two other related peptides have been identified as brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) isolated from the brain (Sudoh et al., 1988, 1990). These peptides have similar structural homology and biological effects to ANP (Silberbach and Roberts, 2001).

These peptides exert various biological actions via activation of their specific receptors which have been classified as three kinds of natriuretic peptide receptor subtypes (NPR-A, NPR-B and NPR-C). Among them, the biological receptors (NPR-A and NPR-B) exhibit intrinsic guanylyl cyclase activity and mediate many of the well-known biological functions of natriuretic peptides. The clearance receptor subtype (NPR-C) has no guanylyl cyclase activity but binds and internalizes all kinds of endogenous natriuretic peptides for clearance (Maack et al., 1987; Chinkers et al., 1989; Koller et al., 1991).

Previously the presence of natriuretic peptide receptors for ANP in the heart was demonstrated by in vitro receptor autoradiography. Specific binding sites for ANP were mainly localized in the cardiac atrial and ventricular endocardium in human and rat (Bianchi et al., 1985; Oehlenschlager et al., 1989; Rutherford et al., 1992). Furthermore, the gene transcripts for natriuretic peptide receptor subtypes have been found in the atrial and ventricular tissues (Wilcox et al., 1991; Nunez et al., 1992; Brown et al., 1993; Lin et al., 1995). And these receptors seem to be more prominent in the endocardium than in the myocardium of the cardiac ventricles (Bianchi et al., 1985; Rutherford et al., 1992). We also have reported that the natriuretic peptide receptors for ANP in the cardiac chambers has

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heterogeneity with much higher affinity for ANP in the right ventricular endocardium, and these receptors are downregulated by cardiac hypertrophy (Kim et al., 1999).

Although the presence and characteristics of natriuretic peptide receptors have been identified in the heart, modulation of these receptors is not established. In the present study, we examined the modulation of natriuretic peptide receptor in rat during the developmental stages.

Methods

Tissue preparation

Sprague Dawley rats, aged fetal (embryonic 20 d), postnatal 3-day, 1-, 2-, 3-, 4-, and 8-week-old, were used. The rats were sacrificed by decapitation. For *in vitro* receptor autoradiography, the hearts were rapidly removed and snap frozen in liquid nitrogen. Sections (20 μm) with transverse plane of right and left ventricles were cut in a cryostat at -20℃, thaw-mounted on gelatin-chrom-alum coated slides, and then dried in a desiccator overnight at 4℃. For determination of concentration of plasma ANP, arterial blood samples were collected into prechilled tubes containing EDTA (1 mg/mL of whole blood), phenylmethylsulfonyl fluoride (PMSF, 0.4 mg%), and soybean trypsin inhibitor (50 *N* α-benzoyl-L-arginine ethyl ester U/mL). Plasma samples were obtained after centrifugation at 10,000 g for 15 min at 4℃.

Preparation of radiolabeled ANP

Radiolabeled ANP was prepared as described previously (Cho et al., 1991, 1995). Synthetic rat ANP (5 μg/5 μL of 0.1 M acetic acid, Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) was introduced into a vial containing 25 μL of 0.5 M phosphate buffered saline (PBS, pH 7.4) followed by addition of 1 mCi of ^{125}I-Na (Amersham International plc, Buckinghamshire, UK). Chloramine-T (10 μg/10 μL) was added to the reaction vial with gentle mixing and 30 sec later bovine serum albumin (BSA, 60 mg/200 μL) solution was added. The reaction mixture was immediately applied to a Sephadex G-25 column (1.0×20 cm) and was eluted with 0.1 M acetic acid containing 0.3% BSA, 0.3% lysozyme, 0.1% glycine, and 200 KIU/ml aprotinin. The iodinated ANP was divided and stored at -70℃ until used. Immediately before using, the iodinated ANP was repurified by HPLC on a reversed phase μ Bondapak column (Waters Associates, Milford, MA, USA) with a linear gradient (20% to 60% acetonitrile) elution. The specific activity (approximately 2,000 Ci/mmol) of ^{125}I-ANP was determined by RIA technique (Joseph et al., 1988). For the RIA of ANP, antibody was prepared from New Zealand White rabbits using a method described previously (Cho et al., 1988).

In vitro receptor autoradiography

Binding conditions of ^{125}I-ANP to the heart section with transverse plane of right and left ventricles were prepared according to a method described elsewhere (Brown and Chen, 1995; Kim et al., 1999, 2000). Briefly, the sections were washed with 150 mM NaCl-0.5% acetic acid (pH 5.0) at room temperature for 10 min in order to remove endogenous ANP, and then preincubated with 30 mM phosphate buffer (pH 7.2) containing 120 mM NaCl and 1 mM phenanthroline at room temperature for 8 min. They were then incubated with 250 pM of ^{125}I-ANP in fresh preincubation buffer containing 40 μg/mL bacitracin, 100 μg/mL PMSF, 10 μg/mL leupeptin, and 0.5% BSA at room temperature for 60 min.

To characterize receptor for ANP, competitive inhibition of ^{125}I-ANP binding of the ventricular endocardium was examined in consecutive sections from eight or nine rats in each developmental stages by coincubating with various concentrations of unlabelled rat ANP (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA). To test specificity of the binding of radioligands, adjacent sections were incubated in the presence of unrelated peptides, angiotensin II or arginine vasopressin (Peninsula Laboratories) (all 10 μM). After incubation, the sections were rinsed and washed with fresh preincubation buffer for 5 min at 4℃. Subsequently, they were rinsed three times in cold distilled water at 4℃ and quickly dried under a stream of cold air.

Quantitative analysis of autoradiograms

Autoradiographic images were generated by exposing the sections to Hyperfilm-^{3}H (Amersham International) in X-ray cassettes together with ^{125}I-labeled polymer standard strips (Amersham International) at room temperature for 7 d. Autoradiograms were developed in Kodak D-19 (Eastman Kodak, Rochester, NY) for 3 min and fixed in Kodak rapid fixer for 5 min at room temperature. The sections were then counterstained with hematoxylin and eosin for tissue localization (Brown and Chen, 1995; Kim et al., 1999, 2000).

Autoradiographic images were viewed with a Leica Wild M420 Macroscope, and captured using a Sony video camera with CCD iris and a Hamamatsu AC adaptor connected to a Power Macintosh 8100/80AV computer. Regional binding of ^{125}I-ANP in the right and left ventricular endocardium was analysed using the PRISM image program (Version 3.6-1, Improve Vision, Coventry, UK). Optical densities were measured as disintegrations per minute (dpm) per square millimeter, based on comparison with a calibration curve derived from the autoradiograms of the ^{125}I standard microscales included in each X-ray cassette. These data were converted into femtomoles ^{125}I-ANP bound per square millimeter, as described elsewhere (Benfenati et al., 1986).

The number of ligand binding sites of different

affinities, apparent dissociation constants (K_d), and maximal binding capacities (B_{max}) on particular structures were derived separately in each individual using the LIGAND iterative model-fitting computer program (Munson and Rodbard, 1980).

Determination of plasma ANP concenration

The plasma ANP was extracted using Sep-Pak C18 cartridges (Waters Associates) as described previously (Cho et al., 1988). Briefly, the plasma was applied on a Sep-Pak C18 cartridge previously activated with 4 mL of 100% acetonitrile and 0.1% trifluoroacetic acid (TFA) and the adsorbed peptide was eluted with 3 mL of 60% acetonitrile in 0.1% TFA. The eluates were dried under vacuum using a Speed-Vac evaporator (Savant, Farmingdale, NY, USA). The recovery rate of ANP was 70.5±1.2%. ANP was measured by radioimmunoassay as described previously (Cho et al., 1991). The lyophilized samples were reconstituted with 100 mM Tris-acetate buffer (pH 7.4) containing 0.2% neomycin, 10 mM EDTA, 50 BAEE U/mL soybean trypsin inhibitor, 200 KIU/mL aprotinin, 0.4 mg% PMSF, 0.02% sodium azide and 1% BSA, and then diluted ANP antiserum and iodinated ANP were added and incubated for 24 h at 4℃. Separation of the free form from the bound form was achieved by addition of second antibody. RIA for ANP was done on the day of experiments, and all samples in an experiment were analyzed in a single assay. Nonspecific binding was <3.0%. The 50% intercept was at 26.6±2.9 pg/tube (n = 7). The intra- and interassay coefficients of variation were 6.3 (n = 9) and 7.8% (n = 11), respectively.

Particulate guanylyl cyclase activation

The ventricular endocardial tissues from each rat were homogenized at 4℃ in 30 mM phosphate buffer (pH 7.2) containing 120 mM sodium chloride and 1 mM phenanthroline by five 30-second bursts of 27,000 rpm using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). The homogenate was centrifuged at 1,500 g for 10 min at 4℃, and the supernatant was recentrifuged at 40,000 g for 60 min at 4℃. The membrane pellet was washed three times with 50 mM Tris-HCl (pH 7.4) and resuspended in this solution. Protein contents were determined by a bicinchoninic acid assay kit (Sigma-Aldrich Fine Chemicals, St. Louis, MI, USA). Particulate GC activity was measured according to the method described elsewhere (Brown and Chen, 1995; Kim et al., 1998, 1999). Aliquots of 10 μg of membrane protein were incubated for 15 min at 37 ℃ in a final volume of 125 μL of 50 mM Tris-HCl (pH 7.6) containing 1 mM 3-isobutyl-1-methylxanthine, 1 mM GTP, 0.5 mM ATP, 15 mM creatine phosphate, 80 μg/mL creatine phosphokinase, and 4 mM $MgCl_2$, plus 1 M ANP. Incubations were stopped by adding 375 μL of ice cold 50 mM sodium acetate (pH 5.8) and boiling for 5 min. Samples were then centrifuged at 10,000 g for 5 min at 4℃.

Radioimmunoassay of cGMP

Production of cGMP was measured in the supernatants by equilibrated radioimmunoassay. In brief, standards or samples were taken up in a final volume of 100 μL of 50 mM sodium acetate buffer (pH 4.8), and then 100 μL of diluted cGMP antiserum (Calbiochem-Novabiochem, San Diego, CA, USA) and iodinated cGMP (10,000 cpm/100 μL, Specific activity = 2,200 Ci/mmole, Du Pont-New England Nuclear, Wilmington, DE) were added and incubated for 24 h at 4℃. The bound form was separated from the free form by charcoal suspension. RIA for cGMP was done on the day of experiments, and all samples in an experiment were analyzed in a single assay. Nonspecific binding was <2.5%. The 50% intercept was at 0.39±0.03 pmol/tube (n = 15). The intra- and interassay coefficients of variation were 6.7 (n = 12) and 8.6% (n = 9), respectively. Average results of determinations were expressed as picomoles of cGMP generated per milligram protein per minute.

Statistical analysis

Comparisons of results were performed by paired Student's *t*-tests and ANOVA with Duncan multiple range test, accepting $P < 0.05$ as the criterion of significance.

Results

As shown in Fig. 1, specific ^{125}I-ANP binding sites were demonstrated in transverse ventricular section of rat heart using *in vitro* receptor autoradiography. The comparison of autoradiograms with their corresponding hematoxylin-eosin stained sections revealed specifically reversible binding of ^{125}I-ANP to endocardia of right and left ventricles (Fig. 1A). No such binding sites were observed in the myocardium of ventricles. In the presence of 1 μM unlabelled ANP, the dense endocardial binding was completely displaced, but the diffuse background binding of radioligand to myocardium was not significantly affected (Fig. 1B). The unrelated peptides including angiotensin II or arginine vasopressin did not inhibit any binding of ^{125}I-ANP (data not shown).

In all developmental stages used, these specific ^{125}I-ANP binding sites were found, and the binding densities of ^{125}I-ANP between the right and left ventricular endocardia were different as shown in Fig. 2. Density of specific binding to the endocardium was much higher in the right than the left ventricle: the binding densities on the endocardia of the free wall- and septal-sides in fetal, 3-day, 1-, 2-, 3-, and 4-week-old rats were 6447.16±576.61 and 5331.95±858.41, 10108.80±572.86 and 9493.30±513.56, 8349.90±1054.35 and 8080.03±1144.60, 9410.94±650.23 and

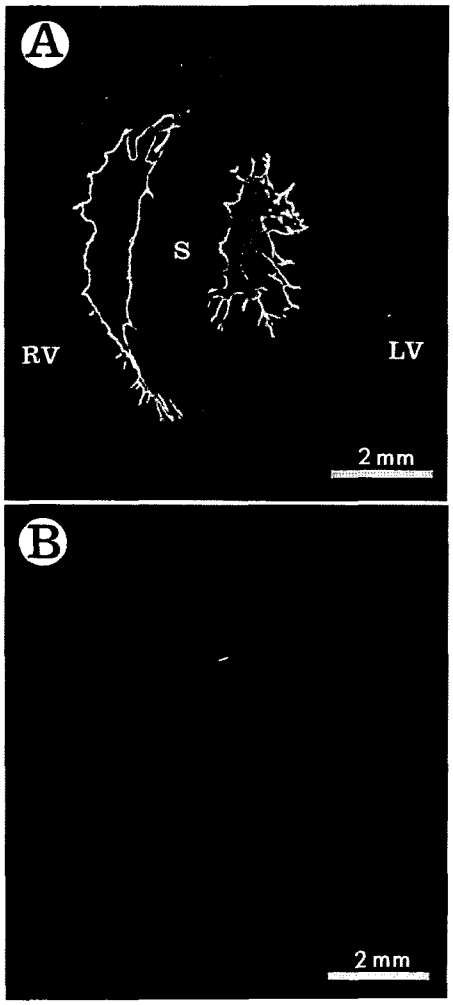

Fig. 1. Dark-field photomicrograph of autoradiograms of the rat cardiac ventricular transverse sections incubated in the presence of 250 pM ^{125}I-ANP (A) and its adjacent section incubated in 250 pM ^{125}I-ANP plus 1 μM unlabelled ANP (B). Specific ^{125}I-ANP binding sites appear as white silver grains in the right (RV) and left ventricular (LV) endocardia of the heart. S, ventricular septum.

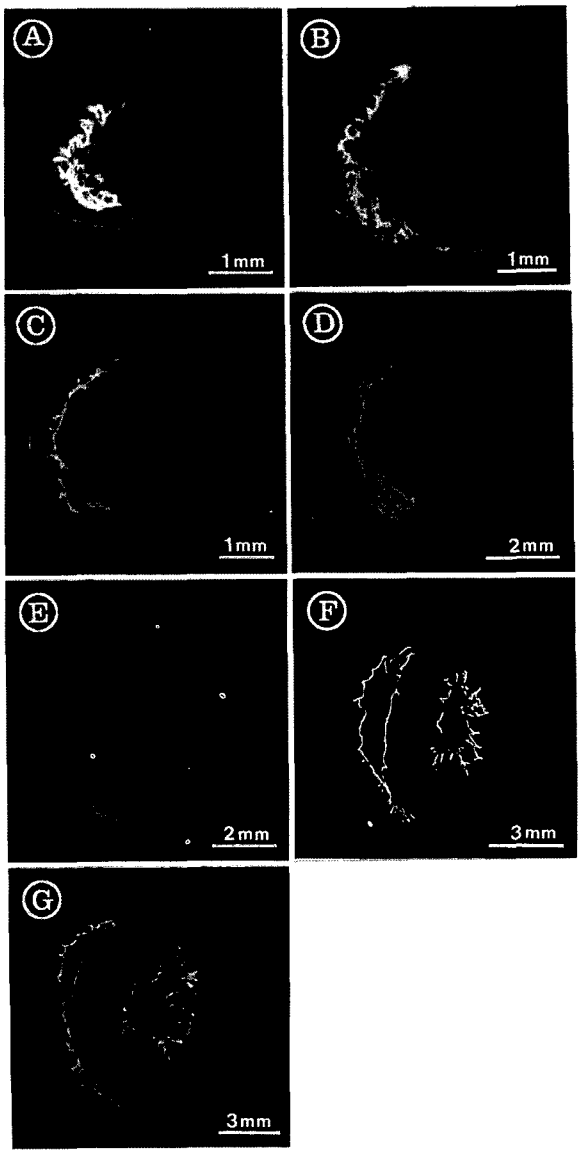

Fig. 2. Comparison of ^{125}I-ANP binding sites in the ventricular endocardia of rat hearts in fetal (A), 3-day old (B), 1-week old (C), 2-week old (D), 3-week old (E), 4-week old (F), and 8-week old stages.

8808.73±492.51, 9956.48±626.07 and 8737.29±378.60, and 9987.17±459.16 and 9045.81±443.27 dpm/unit area in the right ventricles, respectively, and 64.71±13.73 and 52.87±11.87, 162.58±29.59 and 113.33±41.38, 462.42±170.95 and 888.37±347.62, 5028.06±1513.52 and 3276.24±1035.61, 3255.13±1529.78 and 3202.89±1459.08, and 7246.18±1103.92 and 816.86±171.95 dpm/unit area in the left ventricles, respectively. As shown Fig. 3, the binding densities in left ventricular endocardium were gradually increased with aging. However, the difference of these binding densities in the right ventricular endocardium was not significant in various developmental stages except in the fetal rat.

Based the analysis of competitive inhibition of binding of ^{125}I-ANP by unlabelled ANP on the right and left ventricular endocardia, binding affinities of ^{125}I-ANP in the wall and septal sides of the right ventricles were much higher than those in the left ventricular endocardia: apparent dissociation constants (K_d) on the endocardia of the free wall- and septal-sides in fetal, 3-day, 1-, 2-, 3-, 4-, and 8-week-old rats were 0.55±0.13 and 1.29±0.27, 2.21±1.23 and 2.72±0.70, 0.86±0.25 and 1.29±0.44, 2.51±1.29 and 2.60±1.12, 7.62±2.03 and 2.65±0.66, 4.12±0.75 and 1.91±0.53, and 3.34±1.47 and 1.69±1.00 nM in the right ventricles, respectively, and 54.93±24.63 and 52.99±19.40, 15.76±6.44 and 19.31±7.35, 14.10±6.65 and 15.48±8.60, 8.23±2.35 and 10.66±1.46, 8.34±2.56 and 9.25±1.60, 5.46±1.39 and 7.08±2.10, and 1.19±0.35 and 0.83±0.17 nM in the left ventricles, respec-

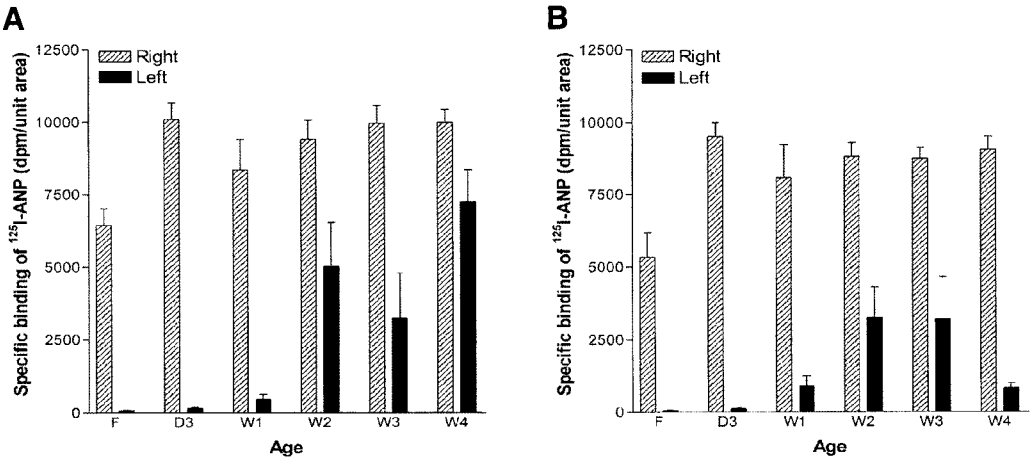

Fig. 3. Comparison of specific ^{125}I-ANP binding densities to free wall (A) and septal (B) sides of the right and left ventricular endocardia of fetal, 3-day (D3), 1- (W1), 2- (W2), 3- (W3), and 4-week-old (W4) rats.

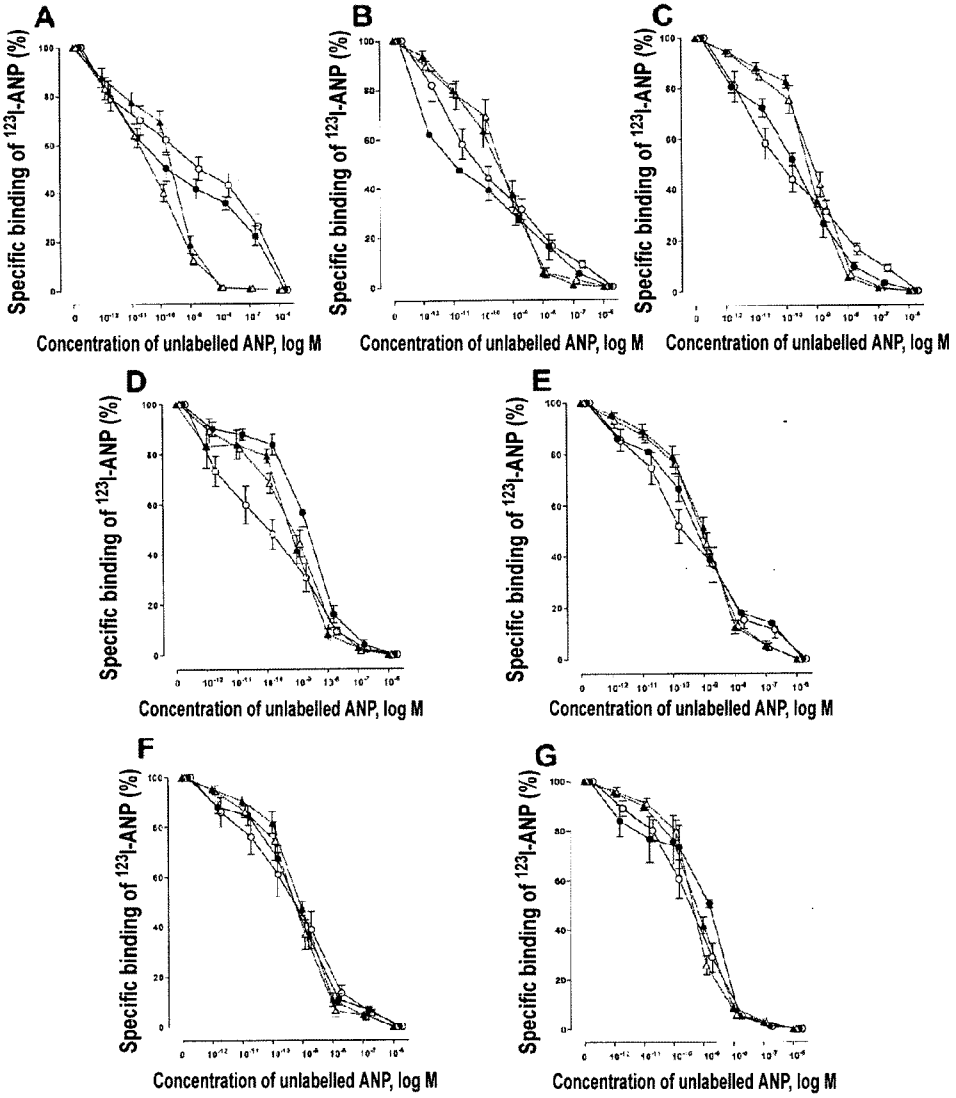

Fig. 4. Comparison of competitive inhibition curves of specific ^{125}I-ANP binding densities to free wall and septal sides of the right and left ventricular endocardia of fetal (A), 3-day (B), 1- (C), 2- (D), 3- (E), 4- (F), 8-week-old (G) rats. ▲, free wall side of RV; △, septal side of RV; ●, free wall side of LV; ○, septal side of LV.

Table 1. Mean values of binding constants for regionally specific reversible bindings of ^{125}I-ANP in ventricular endocardium of rat

Site	Group	Constants	
		K_d(nM)	B_{max}(fmol/mm^2)
Right ventricle			
Wall side	F (n=9)	0.55±0.13	0.61±0.22
	D3 (n=8)	2.21±1.23	1.46±0.38
	W1 (n=8)	0.86±0.25	1.45±0.25
	W2 (n=8)	2.51±1.29	2.17±1.16
	W3 (n=8)	7.62±2.03	5.98±1.57
	W4 (n=8)	4.12±0.75	6.58±1.39
	W8 (n=8)	3.34±1.47	2.93±0.73
Septal side	F (n=9)	1.29±0.27	0.62±0.31
	D3 (n=8)	2.72±0.70	1.29±0.18
	W1 (n=8)	1.29±0.44	0.92±0.25
	W2 (n=8)	2.60±1.12	0.79±0.25
	W3 (n=8)	2.65±0.66	4.91±0.86
	W4 (n=8)	1.91±0.53	2.63±0.78
	W8 (n=8)	1.69±1.00	1.24±0.32
Left ventricle			
Wall side	F (n=9)	54.93±24.63	1.18±0.60
	D3 (n=8)	15.76±6.44	1.00±0.27
	W1 (n=8)	14.10±6.65	1.77±0.69
	W2 (n=8)	8.23±2.35	4.59±1.71
	W3 (n=8)	8.34±2.56	5.07±1.18
	W4 (n=8)	5.46±1.39	6.60±1.80
	W8 (n=8)	1.19±0.35	1.48±0.41
Septal side	F (n=9)	52.99±19.40	1.08±0.45
	D3 (n=8)	19.31±7.35	0.81±0.18
	W1 (n=8)	15.48±8.60	1.29±0.49
	W2 (n=8)	10.66±1.46	2.03±0.43
	W3 (n=8)	9.25±1.60	7.16±1.52
	W4 (n=8)	7.08±2.10	3.81±1.01
	W8 (n=8)	0.83±0.17	0.46±0.10

Apparent dissociation constants (K_d), and maximum binding capacities (B_{max}) were assessed from competitive inhibition of 250 pM ^{125}I-ANP bindings by various concentrations of unlabelled ANP.

tively (Fig. 4, Table 1). As shown in Fig. 5, the binding affinities in the wall and septal sides of the left ventricular endocardium were gradually increased with a close correlation between binding affinity and aging. However, these binding affinities in the right ventricular endocardium were not modulated during the developmental stages. On the other hand, no significant difference in the maximal binding capacities (B_{max}) of endocardial binding was observed in the right and left ventricular endocardia as shown in Table 1.

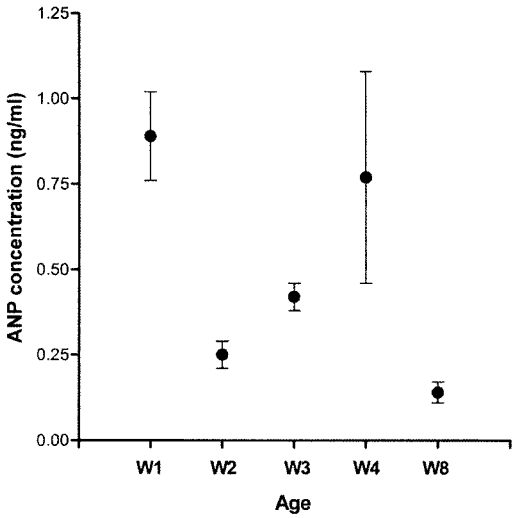

Fig. 6. Relationship between plasma ANP concentrations and age of rats.

To evaluate changes in ANP secretion during the developmental stages, ANP concentrations in plasma were measured. Plasma concentrations of ANP in 1-, 2-, 3-, 4-, and 8-week-old rats were 0.89±0.13, 0.25±0.04, 0.42±0.04, 0.77±0.31, and 0.14±0.03 ng/mL, respectively. As shown in Fig. 6, no linear correlation between the plasma ANP concentration and aging was found. Plasma concentrations of ANP in fetal and 3-day-old rats were not measured because their plasma volumes were not enough for extraction by the Sep-

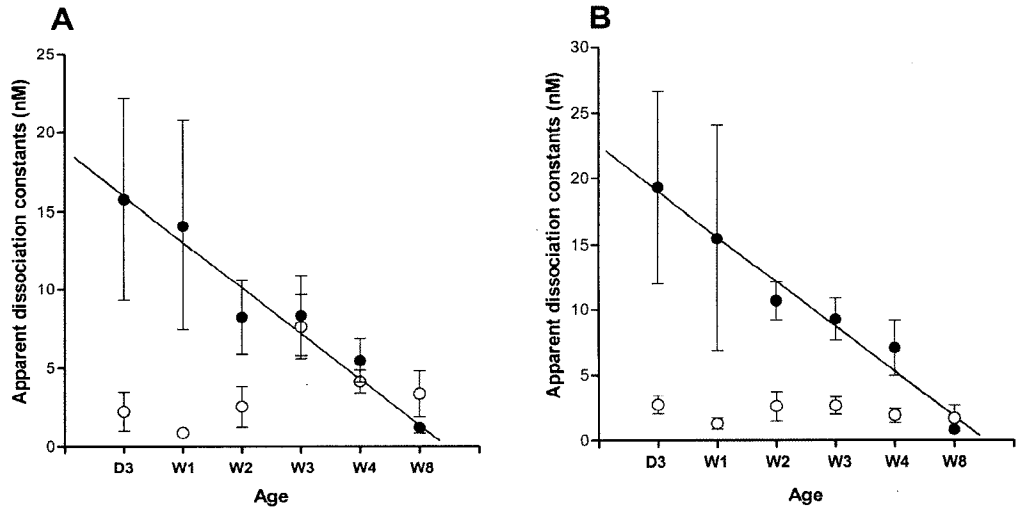

Fig. 5. Relationship between the ^{125}I-ANP binding affinity (apparent dissociation constant) and age of rats. A closely negative relationship between them was observed in free wall (A, y = -2.82x + 18.71, r = 0.98, p < 0.001) and septal side (B, y = -3.40x + 22.34, r = 0.98, p < 0.001) of the left ventricular endocardia of the heart.

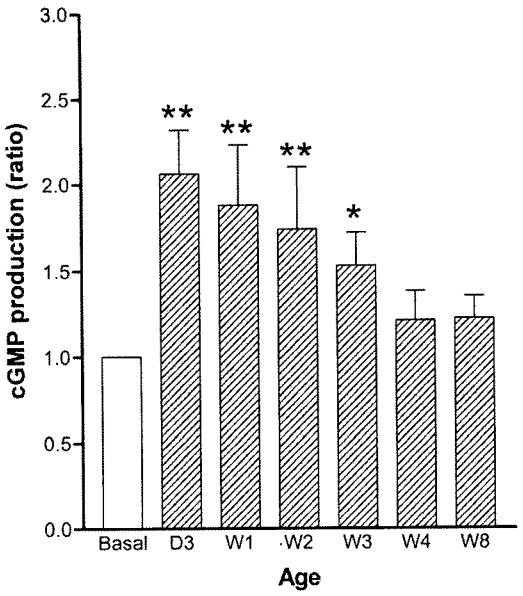

Fig. 7. Comparison of cGMP production in response to ANP (1 μM) in the ventricular membranes from 3-day (D3), 1-week old (W1), 2-week old (W2), 3-week old (W3), 4-week old (W4), and 8-week old (W8) rats. *p < 0.05 and **p < 0.01 for differences between basal and each groups.

Pak method.

The rates of cGMP production by particulate guanylyl cyclase activation of the ventricular membranes from 3-day, 1-, 2-, 3-, 4-, and 8-week-old rats were basally 4.23±0.37, 6.60±0.76, 6.65±1.14, 4.69±0.42, 5.03 ±0.37, and 4.19±0.47 pmol/mg protein/min, respectively. As shown in Fig. 7, ANP (at 1 μM) caused an increment of cGMP production by 2 fold over basal levels in 3-day-old rats. However, this activation was gradually decreased during the developmental stages.

Disucussion

The present study indicates expression of the specific receptors for ANP in the ventricular endocardium of the heart and their modulation during the developmental stages of rats. The specific ^{125}I-ANP binding sites were mainly localized in the right and left ventricular endocardia by *in vitro* receptor autoradiography. This endocardial localization of specific ^{125}I-ANP binding sites was consistent with the previous reports (Bianchi et al., 1985; Rutherford et al., 1992; Kim et al., 1999).

We also confirmed that the binding characteristics of natriuretic peptide receptor have heterogeneity in the intracardiac chamber. As shown in Figs. 2 and 3, the heterogeneity of specific ^{125}I-ANP binding sites was found in the early developmental stage. In fetal (embryonic 20-day) rat, the specific receptor for ANP with high ^{125}I-ANP binding density was observed in wall and septal sides of right, the but not the left, ventricular endocardium. Also, no difference in binding densities between the wall and septal sides was observed in the right ventricular endocardium. Maximal specific ^{125}I-ANP binding density in the right ventricular endocardium was reached the peak value at 3 d. In the left ventricular endocardium, however, these binding densities were gradually increased with aging. Even though the presence of mRNA transcripts for natriuretic peptide receptors has been noticed in the cardiac ventricular tissues of adult animals (Nunez et al., 1992; Brown et al., 1993; Lin et al., 1995; Kim et al., 1999), gene expression of these receptors in early developmental stages including fetus remains to be clarified. As shown in Table 1 from analysis of the competitive inhibition study, the binding affinities of ^{125}I-ANP were much higher in the right than in the left ventricular endocardium in all of the developmental stages examined. In the wall and septal sides of the left ventricular endocardium, apparent dissociation constants were gradually decreased with a close relationship to aging. This means that the affinity of ANP to its receptor molecule is increased during development. However, their maximal binding capacities were not significantly different between the right and left ventricles, and among the developmental stages examined. Therefore, these results suggest that the heterogeneity of maximal specific ^{125}I-ANP binding densities in the ventricular endocardia is related to the properties of binding affinity of the receptor molecules rather than a difference in receptor populations.

In the previous study, we found that the clearance receptor subtype (NPR-C) is the predominant one among three kinds of subtypes in the ventricular endocardium of rats (Kim et al., 1999). Since an excess concentration of C-ANP, a selective ligand for NPR-C subtype (Schenk et al., 1987; Maack et al., 1987; Leitman et al., 1988), inhibited approximately 90% of the specific bindings of ^{125}I-ANP to ventricular endocardial binding sites, this finding implies that the remaining binding sites of these structures are the biological receptor subtypes NPR-A and/or NPR-B. In the experimental model of cardiac hypertrophy, the ventricular hypertrophy was accompanied by an elevation of plasma levels of ANP (Akimoto et al., 1988; Hirata et al., 1992; Comini et al., 1995; Kim et al., 2001). Previously, we found that increment of plasma ANP levels could downregulate natriuretic peptide receptor population in ventricular endocardium of rat heart (Kim et al., 1999). This downregulation of natriuretic peptide receptors may be related to the high levels of plasma ANP continuously maintained in the ventricular hypertrophy. In perfusion model of isolated atrium of rabbit, ANP secretion and atrial contents gradually increased with age after birth (Kim et al., 2002). However, we have not found any linear correlation between plasma ANP concentration and aging. Even though it is difficult to explain the reason for the non-correlation, it may be due to developmental changes in the body fluids with age.

In the heart, myocardial contractility is a major function for the mechanical work, and modulated by extracardiac neural and hormonal systems. Recently, in vitro and in vivo experimental evidence has indicated that the endocardium is also involved in the regulation of myocardial function (De Hert et al., 1993; Brutsaert et al., 1998). Considering the ventricular endocardium is involved in the regulation of myocyte function as in the vascular system (Brutsaert et al., 1998), it is also possible that modulation of the endocardial natriuretic peptide receptors during cardiac development, which may affect the contractility of the subjacent cardiomyocytes in the heart. Although NPR-C subtype is the predominant receptor subtype, we also found the presence of guanylyl cyclase-coupled natriuretic peptide receptor subtypes in the ventricular endocardium of rat (Kim et al., 1999). As shown in Fig. 7, ANP increased cGMP production via activation of particulate guanylyl cyclase-coupled receptor subtypes of the ventricular membranes. Interestingly, cGMP productions were gradually decreased with close relationship to aging. This finding suggests that the ratio of subtypes (NPR-A and/or NPR-B vs. NPR-C) in total receptor population may be modulated according to cardiac developments.

In conclusion, the present data show that the endocardial natriuretic peptide receptor for ANP is modulated with close relationship to cardiac development in the left ventricle rather than the right ventricle, and may be related to myocardial functions in the left ventricle as the main chamber of the heart.

Acknowledgements

This work was supported by the Korea Research Foundation Grant (KRF-2000-F00022), Republic of Korea.

References

Akimoto K, Miyata A, Hayakawa K, Kangawa K, and Matsuo H (1988) Plasma and atrial levels of atrial natriuretic peptide (ANP) in pulmonary hypertensive rats. *Life Sci* 43: 1125-1132.

Atarashi K, Mulrow PP, Franco-Saenz R, Snajdar R, and Rapp J (1984) Inhibition of aldosterone production by an atrial extract. *Science* 224: 992-994.

Benfenati F, Cimino M, Agnati LF, and Fuxe K (1986) Quantitative autoradiography of central neurotransmitter receptor: methodological and statistical aspects with special reference to computer-assisted image analysis. *Acta Physiol Scand* 128: 129-146.

Bianchi C, Gutkowska J, Thibault G, Garcia R, Genest J, and Cantin M (1985) Radioautographic localization of ^{125}I-atrial natriuretic factor (ANF) in rat tissues. *Histochemistry* 82: 441-452.

Brown J and Chen Q (1995) Regional expression of natriuretic peptide receptors during the formation of arterial neointima in the rabbit. *Circ Res* 77: 906-918.

Brown LA, Nunez DJR, and Wilkins MR (1993) Differential regulation of natriuretic peptide receptor messenger RNAs during the development of cardiac hypertrophy in the rat. *J Clin Invest* 92: 2702-2712.

Brutsaert DL, Fransen P, Andries LJ, De Keulenaer GW, and Sys SU (1998) Cardiac endothelium and myocardial function. *Cardiovasc Res* 38: 281-290.

Chinkers M, Garbers DL, Chang M-S, Lowe DG, Chin H, Goeddel DV, and Schulz S (1989) A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature* 338: 78-83.

Cho KW, Kim SH, Kim CH, and Koh GY (1995) Mechanical basis of ANP secretion in beating atria: atrial stroke volume and ECF translocation. *Am J Physiol* 268: R1129-R1136.

Cho KW, Kim SH, Koh GY, and Seul KH (1988) Renal and hormonal responses to atrial natriuretic peptide and turtle atrial extract in the freshwater turtle, *Amyda japonica*. *J Exp Zool* 247: 139-145.

Cho KW, Seul KH, Kim SH, Seul KM, and Koh GY (1991) Atrial pressure, distension, and pacing frequency in ANP secretion in isolated perfused rabbit atria. *Am J Physiol* 260: R39-R46.

Comini L, Agnoletti G, Panzali A, Mantero G, Pasini E, Gaia G, Albertini A, and Ferrari R (1995) Activation of ANP synthesis during congestive heart failure in rats treated with monocrotaline. *Am J Physiol* 268: H391-H398.

Currie MG, Geller DM, Cole BR, Boylan JC, Yusheng W, Holmberg SW, and Needleman P (1983) Bioactive cardiac substance: potent vasorelaxant activity in mammalian atria. *Science* 221: 71-73.

De Bold AJ, Borenstein HB, Veress AT, and Sonnenberg H (1981) A rapid and potent natriuretic response to intravenous injections of atrial myocardial extracts. *Life Sci* 28: 89-94.

De Hert SG, Gillebert TC, Andries LJ, and Brutsaert DL (1993) Role of the endocardial endothelium in the regulation of myocardial function: Physiologic and pathophysiologic implications. *Anesthesiology* 79: 1354-1366.

Flynn TD, De Bold M, and De Bold AJ (1983) The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. *Biochem Biophys Res Commun* 117: 859-865.

Genest J and Cantin M (1989) The atrial natriuretic factor: its physiology and biochemistry. *Rev Physiol Biochem Pharmacol* 110: 1-145.

Hirata Y, Suzuki E, Hayakawa H, Matsuoka H, Sugimoto T, Kojima M, Kangawa K, and Matsuo H (1992) Role of endogenous ANP in sodium excretion in rats with experimental pulmonary hypertension. *Am J Physiol* 262: H1684-H1689.

Joseph LJ, Desai KB, Mehta MN, and Mathiyarasu R (1988) Measurement of specific activity of radiolabelled antigens by a simple radioimmunoassay technique. *Nucl Med Biol* 15: 589-590.

Kim SH, Lee KS, Kim SZ, Seul KH, and Cho KW (2000) Postnatal changes in atrial compliance and stretch-induced ANP secretion in rabbits. *Korean J Physiol Pharmacol* 4: 393-401.

Kim SH, Han JH, Lim SH, Lee SJ, Kim SZ, and Cho KW (2001) Attenuation of inhibitory effect of CNP on the secretion of ANP from hypertrophied atria. *Am J Physiol* 281: R1456-R1463.

Kim SH, Han JH, Cao C, Kim SZ, and Cho KW (2002) Postnatal changes in inhibitory effect of C-type natriuretic peptide on secretion of ANP. *Am J Physiol* 282: R1672-R1679.

Kim SZ, Cho KW, and Kim SH (1999) Modulation of endocardial natriuretic peptide receptors in right ventricular hypertrophy. *Am J Physiol* 277: H2280-H2289.

Kim SZ, Kang SY, Lee SJ, and Cho KW (2000) Localization of receptors for natriuretic peptide and endothelin in the duct of the epididymis of the freshwater turtle. *Gen Comp Endocrinol* 118: 26-38.

Kim SZ, Kim SH, Park JK, Koh GY, and Cho KW (1998) Presence and biological activity of C-type natriuretic peptide-dependent guanylate cyclase-coupled receptor in the penile corpus cavernosum. *J Urol* 159: 1741-1746.

Koller KJ, Lowe DG, Bennett GL, Minamino N, Kangawa K, Matsuo H, and Goeddel DV (1991) Selective activation of B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* 252: 120-123.

Leitman DC, Andresen JW, Catalano RM, Waldman SA, Tuan JJ, and Murad F (1988) Atrial natriuretic peptide binding, cross-linking and stimulation of cyclic GMP accumulation and particulate guanylate cyclase activity in cultured cells. *J Biol*

Chem 263: 3720-3728.
Lin X, Hanze J, Heese F, Sodmann R, and Lang RE (1995) Gene expression of natriuretic peptide receptors in myocardial cells. *Circ Res* 77: 750-758.
Maack T, Suzuki M, Almeida FA, Nussenzveig D, Scarborough RM, McEnroe GA, and Lewicki JA (1987) Physiological role of silent receptors of atrial natriuretic factor. *Science* 238: 675-678.
Munson PJ and Rodbard D (1980) LIGAND; a versatile computerized approach for characterization of ligand binding system. *Anal Biochem* 107: 220-239.
Nunez DJR, Dickson MC, and Brown MJ (1992) Natriuretic peptide receptor mRNAs in the rat and human heart. *J Clin Invest* 90: 1966-1971.
Oehlenschlager WF, Baron DA, Schomer H, and Currie MG (1989) Atrial and brain natriuretic peptides share binding sites in the kidney and heart. *Eur J Pharmacol* 161: 159-164.
Rutherford RAD, Wharton J, Gordon L, Moscoso G, Yacoub MH, and Polak JM (1992) Endocardial localization and characterization of natriuretic peptide binding sites in human fetal and adult heart. *Eur J Pharmacol* 212: 1-7.
Schenk DB, Phelps MN, Porter GJ, Fuller F, Cordell B, and Lewicki JA (1987) Purification and subunit composition of atrial natriuretic peptide receptor. *Proc Natl Acad Sci USA* 84: 1521-1525.
Silberbach M and Roberts CT (2001) Natriuretic peptide signaling: Molecular and cellular pathways to growth regulation. *Cell Signall* 13: 221-231.
Sudoh T, Kangawa K, Minamino N, and Matsuo H (1988) A new natriuretic peptide in porcine brain. *Nature* 332: 78-81.
Sudoh T, Minamino N, Kangawa K, and Matsuo H (1990) C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. *Biochem Biophys Res Commun* 168: 863-870.
Wilcox JN, Augustine A, Goeddel DV, and Lowe DG (1991) Differential regional expression of three natriuretic peptide receptor genes within primate tissues. *Mol Cell Biol* 11: 3454-3462.

[Received May 15, 2002; accepted July 31, 2002]