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# Postnatal Changes in Atrial Compliance and Stretch-Induced ANP Secretion in Rabbits

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To define the postnatal changes in ANP secretion in response to mechanical stretch and atrial compliance, experiments have been done in perfused nonbeating rabbit atria with different ages: 1-day, 1-, 2-, 3-, 4-, and 8-wk-old. In 1-day-old-rabbits, an increase in intraatrial pressure resulted in an increase in atrial volume, which was higher than that in 1-wk-old rabbits. Increases in atrial volume stimulated the secretion of ANP with concomitant translocation of extracellular fluid (ECF) into the atrial lumen. However, mechanically stimulated ECF translocation was lower in 1-day-old rabbits than that in 1-wk-old rabbits. Therefore, positive relationship between mechanically stimulated ECF translocation and ANP secretion was shifted upward in 1-day-old rabbits, as compared to 1-wk-old rabbits. Changes in atrial volume and ECF translocation were gradually increased with aging and reached the peak value at 4 wk. The stretch-induced ANP secretion in terms of ECF translocation (the interstitial ANP concentration) was also increased with aging and reached the peak value at 4 wk. The interstitial ANP concentration was dependent on the atrial content of ANP. These data suggest that the higher level of atrial ANP secretion is related to the postnatal changes in atrial volume and unidentified factor.

Key Words: Atrial natriuretic peptide, Atrium, Compliance, Development, ECF translocation, Postnatal life, Stretch

## INTRODUCTION

Atrial natriuretic peptide (ANP), a peptide hormone, synthesized predominantly in atrial cardiomyocytes, is stored and secreted into the blood stream in response to atrial distension (Dietz, 1984; Lang et al, 1985; Cho et al, 1988). ANP causes diuresis and natriuresis, and decreases blood pressure, thus lowering cardiac workload (De Bold et al, 1981). The primary site of ANP synthesis is cardiac atria, although other tissues such as ventricle, brain, lung, adrenal gland, gastrointestinal tract, reproductive system and immune system also produce ANP (Gutkowska et al, 1984; Gardner et al, 1986; Nemer et al, 1986; Gutkowska & Nemer, 1989; Vollmar, 1990; Kim et al, 1997a).

Expression of ANP mRNA is developmentally regulated in the mammalian heart (Bloch et al, 1986; Nemer et al, 1986; Cantin et al, 1987; Kikuchi et al, 1987; Calycomb, 1988; Wu et al, 1988; Semmekrot & Guignard, 1991). Before birth, ANP mRNA is expressed in both atrium and ventricles of the heart even though the content is lower in ventricles. After birth, atrial ANP mRNA increases, while ventricular ANP mRNA decreases with aging (Nemer et al, 1986; Wu et al, 1988). In normal adult ventricular cardiomycytes, small quantities of ANP mRNA have been detected, of which levels may be approximately 100-fold to 150-fold lower than that of atrial cardiomycytes. However, ANP mRNA in ventricle is reactivated by increased cardiac workload (Lattion et al, 1986; Nemer et al, 1986; Franch et al, 1988). Species differences are observed in the rate of appearance in

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atrial ANP mRNA and in the rate of decline in ventricular ANP mRNA during development (Kikuchi et al, 1987; Wei et al, 1987; Claycomb, 1988). Therefore, the regulation of ANP gene expression may be related to the functional role of cardiac development (Wei et al, 1987). Although atrial ANP mRNA and its content are low in fetus and neonate, plasma concentration of ANP is high and becomes to decline with age (Wei et al, 1987; Wu et al, 1988; Semmekrot & Guignard, 1991). It is not clear what kinds of mechanisms are involved in the maintenance of high level of plasma ANP in fetus and neonate.

Stretch, hypoxia and vasoconstrictive agents are known as strong stimuli of ANP secretion during early life (Cheung & Brace, 1988; Robillard & Winer, 1988; Kojima et al, 1989; Cheung, 1994). The increase in plasma ANP concentration after volume expansion (Robillard & Winer, 1988), as well as close correlations between ANP concentration and cardiothoracic ratio (Kojima et al, 1989), atrial size (Fesonen et al, 1990) and ductal flow (Fesonen et al, 1990) also indicate that atrial stretch is an important stimulus for the secretion of ANP in neonates. During postnatal stage, both atrial stretch (compliance) and tissue content of ANP may change remarkably with cardiac growth. However, there are few reports about postnatal changes in atrial compliance. The aim of the present study was to evaluate changes in stretch-induced ANP secretion related to atrial compliance and atrial content of ANP during early postnatal stage of life. Therefore, we measured atrial compliance and the responsiveness of ANP secretion to stretch with age using isolated perfused rabbit atria.

## METHODS

### *Animals*

New Zealand White rabbits, aged fetal, 1-day, 1-, 2-, 3-, 4-, and 8-wk, were used.

### *Blood collection and tissue preparation*

After anesthesia with pentothal sodium (20 mg/kg), polyethylene tubing was cannulated into the carotid artery, and blood pressure and heart rates were measured. Blood was collected from carotid artery into a prechilled tube containing aprotinin (200 kallikrein inhibitory units, KIU/ml), soybean trypsin inhibitor (SBTI, 50 N-a-benzoyl-L-arginine ethyl ester units, BAEE units/ml), phenylmethylsulfonyl fluoride (PMSF, 600 M/ml), and ethylenediaminetetraacetic acid (EDTA, 2.7 mM/ml). Blood was centrifuged at 10,000 g for 15 min at 4°C and plasma was kept at −70°C. Plasma ANP was extracted using a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) as described previously (Cho et al, 1989). Briefly, 700 $\mu$l of plasma was applied on a Sep-Pak C18 cartridge previously activated with 4 ml of 100% acetonitrile followed by 4 ml of 0.1% trifluoroacetic acid (TFA). The cartridge was washed with 4 ml of 0.1% TFA and adsorbed peptide was eluted with 60% acetonitrile in 0.1% TFA. The eluant was dried using Speed-vac evaporator (Savant, Hicksville, NY).

Both atria and ventricles were separated, weighed and kept in 2 ml of 0.1 N acetic acid at 4°C. Tissues were boiled for 10 min, homogenized with Polytron homogenizer and centrifuged at 10,000 g for 15 min at 4°C. The concentrations of ANP in plasma extracts and tissue homogenates were measured by radioimmunoassay (RIA) as described below.

### *Isolated perfused atrial preparation*

An isolated perfused atrial preparation was made by the method described previously (Cho et al, 1988; Kim et al, 1997b). Briefly, the heart was rapidly removed, placed in oxygenated saline and left atrium was separately dissected from the heart. A Tygon cannula containing three small catheters sealed within it was inserted into the atrium and secured by ligatures. The cannulated atrium was transferred, fitted into the organ chamber containing buffer solution (36.5°C) and fixed with watertight silicone rubber cap. The atrium was immediately perfused with oxygenated HEPES buffer solution at a rate of 0.1 (in case of 1-day- and 1-wk-old rabbits) - 1.0 (in case of 2-, 3-, 4- and 8-wk-old rabbits) ml/min with peristaltic pump. The composition of buffer solution was as follows: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, HEPES 10, glucose 10 mM and bovine serum albumin (BSA) 0.1%. The pericardial buffer solution, which contained [$^3$H] inulin to measure the translocation of extracellular fluid (ECF), was also oxygenated by silicone tubing coils located inside the organ chamber (Cho et al, 1990). The pericardial space of organ chamber was sealed and connected with a calibrated microcapillary tube, by which changes in atrial volume were monitored. The

atrium was perfused for 30 min to stabilize the secretion of ANP and to maintain the [$^3$H] inulin level in the extracellular space at a steady state. The perfusate was collected every 2 min interval at 4°C. After two collection periods, atrial distension was induced for 2 min by elevating the position of outflow catheter tip to 2 cm$H_2O$ and atrial contraction was induced by lowering the position of the catheter tip to the basal level. Atrial pressure was subsequently increased from 0 to 2, 4, 6, or 10 cm$H_2O$ for 2 min every 8 min.

*RIA of ANP*

The concentration of immunoreactive ANP in atrial perfusates, plasma extracts and tissue homogenates was measured using specific RIA as described previously (Cho et al, 1988; 1989; 1990). RIA was performed in Tris-acetate buffer (0.1 M, pH 7.4) containing neomycin (0.2%), EDTA (1 mM), SBTI (50 BAEE units/ml), aprotinin (200 KIU/ml), PMSF (0.4 mg%), sodium azide (0.02%) and BSA (1%). Standard and samples were incubated with anti-ANP antibody and [$^{125}$I] ANP for 24 h at 4°C. Bound form was separated from free form using charcoal suspension or second antibody. RIA for ANP was done on the day of experiments and all samples in an experiment were analyzed in a single assay. The secreted amount of ANP was expressed as ng ANP per min per g of tissue wet weight. The molar concentration of ANP release was calculated as follow (Cho et al, 1988; 1990; 1993):

ANP released ($\mu$M) =
ANP (pg/min/g) / ECF translocation ($\mu$l/min/g) · 3063

The denominator 3063 refers to the molecular mass for ANP$_{(1\text{-}28)}$ (Da) since the ANP secreted was found to be mainly the processed ANP (Cho et al, 1988; 1990).

*Measurement of ECF translocation*

The ECF translocated from the atria was measured as described previously (Cho et al, 1988; 1990; 1993). Radioactivity in perfusate and pericardial buffer solution was measured with a liquid scintillationcounter and the amount of ECF translocated through atrial wall was calculated as follows:

ECF translocation (l/min/g) =
total radioactivity in perfusate (cpm/min) · 1000/ radioactivity in pericardial reservoir (cpm/$\mu$l) · atrial wet weight (mg)

*Statistical analysis*

The results were given as means±SEM. Statistical significance of differences was performed by the use of unpaired Student's t-test and AVOVA with Duncan multiple range test. The critical level of significance was set at a p-value of less than 0.05.

## RESULTS

*Postnatal changes in weight and hemodynamics*

With increment of body weight, atrial weight gradually increased (Fig. 1E). The ratio of atrial weight to body weight was 0.38±0.03 and 0.26±0.01 at fullterm and 1-day-old rabbits, respectively. After 1 wk, it was gradually declined and became 0.12±0.01 at 8 wk. Atrial weight of right side was similar to the left side but became lighter after 4 wk (data not shown). Heart rates increased from 225±23 at 1 day to 277±11 beat/min at 1 wk and reached peak value at 2 wk (Fig. 1D). Mean arterial pressure was persistently increased from 26 3 mmHg at 1 day to 87.5± 1.5 mmHg at 8 wk (Fig. 1C).

*Postnatal changes in ANP concentration*

To evaluate changes in ANP synthesis and secretion during postnatal period, ANP concentrations in plasma and tissue were measured. Plasma concentrations of ANP in full-term fetus and 1-day-old rabbits were 229.7±63.0 and 177.3±55.7 pg/ml, respectively, which were higher than that in 8-wk-old rabbits (Fig. 1A). Thereafter, it was declined to 82.4± 12.5 pg/ml at 1 wk and maintained at the similar levels.

ANP content in left and right atria of full-term fetus was similar (12.2±4.0, 13.5±3.4 ng/mg wet weight). However, at 4 wk, ANP content of right atria became significantly lower than left atrium (20.9±4.4 vs 32.8±6.6 ng/mg, $p<0.05$). Total atrial content was markedly increased and reached the peak value at 3 wk (Fig. 1B). Ventricular content of ANP was 56.0±8.0 pg/mg (12.3±1.4 ng/total ventricles) be-

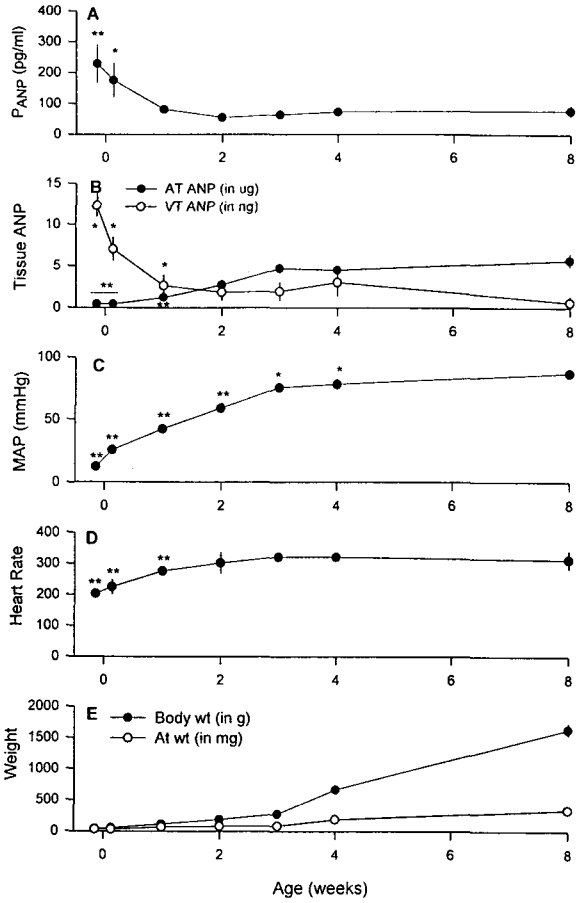


**Fig. 1.** Postnatal changes in weight, hemodynamics and ANP level in rabbits. (A) Plasma concentration of ANP was decreased after birth and maintained constant after 1 wk. (B) Total atrial content of ANP was markedly increased (closed dot, expressed in ug/both atria) and total ventricular content of ANP was markedly decreased (open dot, expressed in ng/both ventricles) with aging. (C) Mean arterial pressure (MAP) was progressively increased with aging. (D) Heart rate was increased and reached peak value on 2 wk. (E) Body weight (closed dot, expressed in g) and atrial weight (open dot, expressed in mg) were markedly increased. *, **, Significantly different from that in 8-wk-old rabbits, $p<0.05$ and $p<0.01$, respectively.

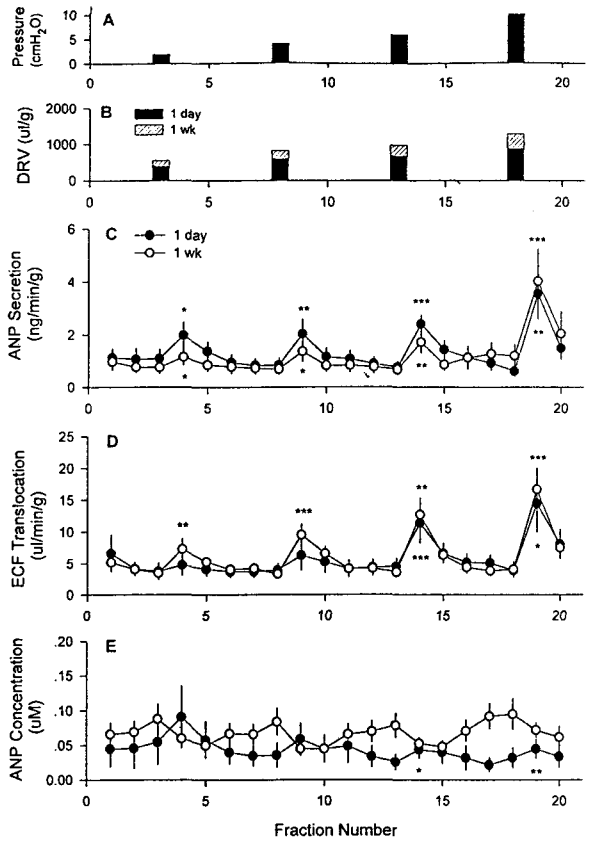


**Fig. 2.** Changes in atrial volume (B), the secretion of ANP (C), translocation of the extracellular fluid (ECF) (D) and interstitial ANP concentration (E) were proportional to intraatrial pressure in isolated perfused nonbeating atria in 1-day- and 1-week-old rabbits. Closed and open dots indicate 1-day- and 1-wk-old rabbits, respectively. *, **, ***, Significantly different from basal value, $p<0.05$, $p<0.01$ and $p<0.005$, respectively.

fore birth, but declined after birth and finally was not detectable at 4 wk.

*Characteristics of stretch-induced ANP secretion in 1-day-old rabbits compared to 1-wk-old rabbits*

To evaluate changes in stretch-induced ANP secretion from the atria during early stage of life, isolated perfused nonbeating left atria were used. The basal rate of ANP secretion was similar in 1-day- and 1-wk-old rabbits: $1.07\pm0.45$ pg/min/g (n=10) and $0.79\pm0.31$ pg/min/g (n=11), respectively (Fig. 2C). When atrial pressure was increased from basal level to 2, 4, 6, or 10 $cmH_2O$ for 2 min by the elevation of outflow tip and then decreased to basal level, atrial volume was changed. In 1-day-old rabbits, atrial volumes at each pressure were increased by $359.4\pm111.3$, $512.7\pm125.5$, $671.3\pm145.1$ and $984.6\pm185.5$ l/g, which were higher than those in 1-wk-old rabbits (Fig. 2B). ANP secretion was increased after reduction of atrial volume from stretch with peak values of $1.98\pm0.54$, $1.98\pm0.61$, $2.41\pm0.37$ and $3.56\pm1.03$ ng/min/g, which were similar to those in 1-wk-old rabbits (Fig. 2C).

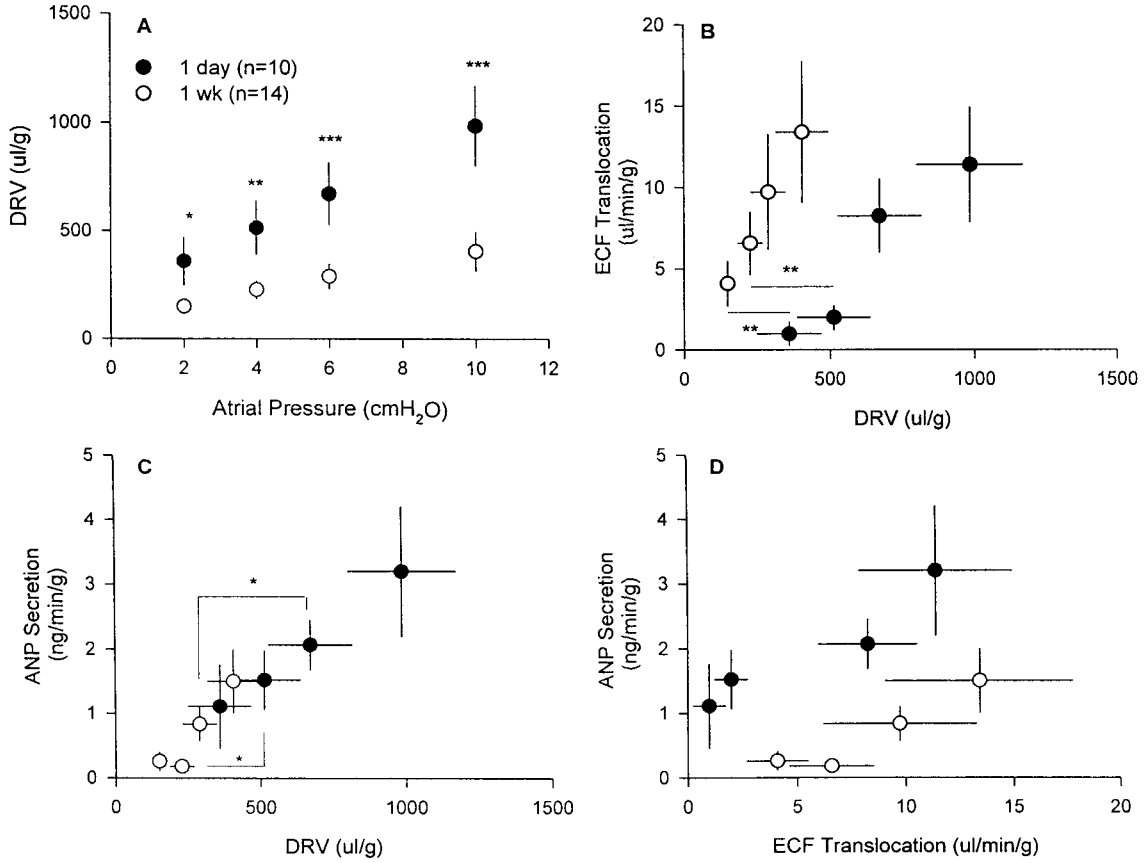


**Fig. 3.** Relationships between atrial volume change (distension-reduction volume, DRV) and intraatrial pressure (A), ECF translocation and DRV (B), ANP secretion and DRV (C), ANP secretion and ECF translocation (D) in 1-day-old and 1-wk-old rabbits. Data were derived from Fig. 1. *, **, ***, Significantly different from 1-wk-old rabbits, $p<0.05$, $p<0.01$ and $p<0.005$, respectively.

In order to compare quantitatively the responsiveness of ANP secretion and ECF translocation to stretch in 1-day- and 1-wk-old rabbits, mechanically stimulated ECF translocation and ANP secretion were calculated by subtracting mean values of previous two observations from the peak values from Fig. 2 and replotted in Fig. 3. Proportional increases in atrial volume were significantly higher, and changes in ECF translocation by stretch were lower in 1-day-old rabbits than those in 1-wk-old rabbits. Therefore, the relationship between atrial volume and mechanically stimulated ECF translocation in 1-day group was shifted downward, as compared to 1-wk-old rabbits (Fig. 3B). In contrast, the relationship between atrial volume and ANP secretion was similar in both groups (Fig. 3C). This relationship shows DRV-dependent higher secretion of ANP in 1-day rabbits. The ANP secretion in a function of ECF translocation was shifted upward, as compared to 1-wk-old rabbits (Fig. 3D). Therefore, the secretion of ANP divided by ECF translocation (interstitial ANP concentration) was markedly increased in 1-day-old rabbits ($0.08\pm0.02$ vs $0.03\pm0.01$ $\mu$M at 6 $cmH_2O$, $p<0.025$).

*Characteristics of stretch-induced ANP secretion in 2-, 3- and 4-wk-old rabbits*

In 2-wk-old rabbits, increases in atrial volume and ECF translocation were not significantly different from 1-wk-old rabbits (Figs. 4A & 4B). However, change in stretch-induced ANP secretion was slightly higher than that in 1-wk-old rabbits (Figs. 4C & 4D). The concentration of interstitial ANP was markedly increased ($0.16\pm0.05$ vs $0.03\pm0.01$ $\mu$M at 6 $cmH_2O$, $p<0.005$).

In 3-wk-old rabbits, atrial volume dramatically increased, as compared to 2-wk-old rabbits (all $p<0.01$, Fig. 4A). Simultaneously, changes in ANP secretion

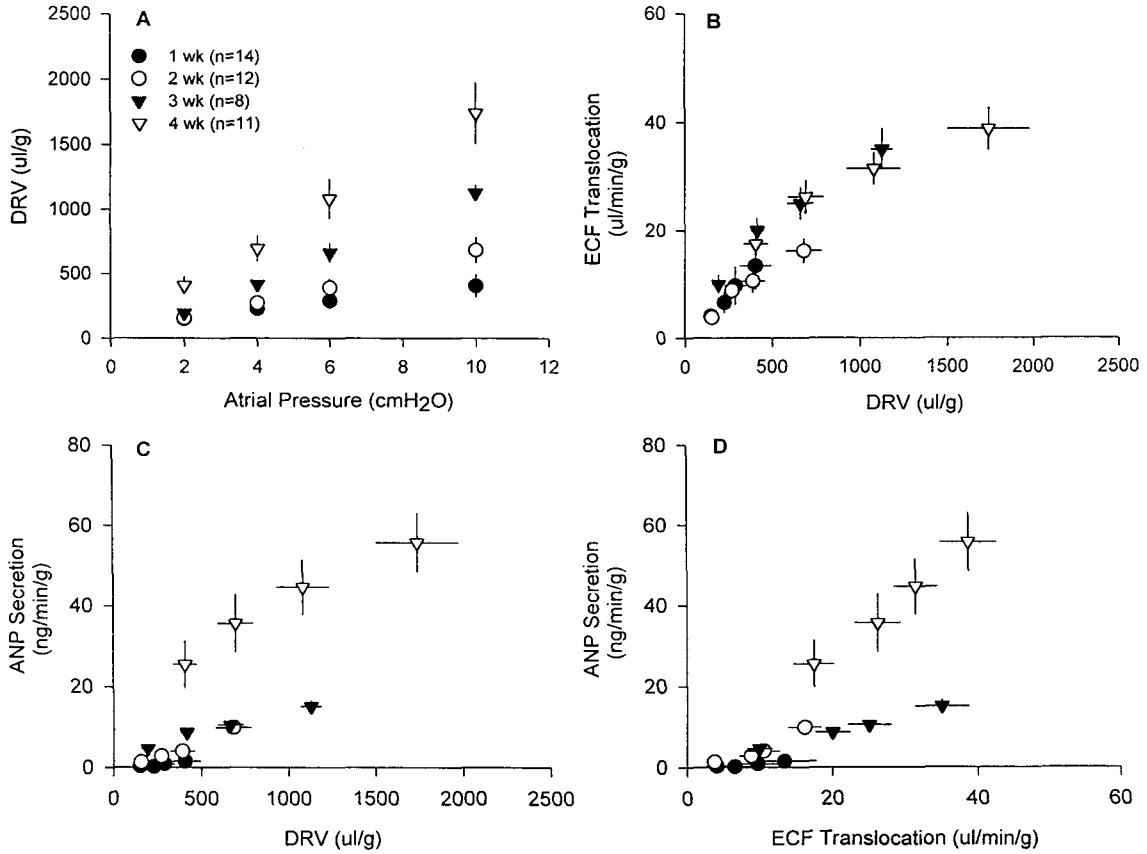


**Fig. 4.** Relationships between atrial volume change (DRV) and intra-atrial pressure (A), ECF translocation and DRV (B), ANP secretion and DRV (C), ANP secretion and ECF translocation (D) in 1-, 2-, 3-, and 4-wk-old rabbits, respectively. Other legends are the same as in Fig. 3.

and ECF translocation were significantly higher than those in 2-wk-old rabbits (Figs. 4B & 4C). An increase in ANP secretion was related to the change in ECF translocation (Fig. 4D). The ANP concentration in the interstitium was not different from that of 2-wk-old rabbits ($0.13 \pm 0.03$ vs $0.16 \pm 0.05$ $\mu$M at 6 $cmH_2O$).

In 4-wk-old rabbits, increases in atrial volume and ANP secretion were significantly higher at all pressure levels but changes in ECF translocation were similar, as compared to 3-wk-old rabbits (Figs. 4A, 4B & 4C). Increased ANP secretion was a function of ECF translocation (Fig. 4D). The concentration of interstitial ANP was markedly increased from $0.13 \pm 0.03$ to $0.44 \pm 0.06$ $u$M at 6 $cmH_2O$ ($p < 0.001$, n=8). Changes in atrial volume, ANP secretion, ECF translocation and the concentration of interstitial ANP observed in 4-wk-old rabbits were similar to those in 8-wk-old rabbits (data not shown).

In order to compare postnatal changes in atrial volume, ECF translocation and ANP secretion, we replotted those parameters observed at 6 $cmH_2O$ of atrial pressure (Fig. 5). Atrial volume was gradually increased with aging except in 1-day-rabbits and reached the peak value in 4-wk rabbits (Fig. 5A). Mechanically stimulated ECF translocation, which was dependent on atrial volume change, also reached the peak value at 4 wk (Fig. 5B). The secretion of ANP in terms of ECF translocation, which reflects the concentration of interstitial ANP, gradually increased except in 1-day-rabbits and reached the peak value at 4 wk (Fig. 5C).

Atrial content of ANP was changed developmentally. Therefore, we replotted the concentration of ANP in the interstitium against atrial content of ANP in all groups (Fig. 6). The secretion of ANP was dependent on atrial content of ANP and a close correlation between them was observed ($y=0.4x-0.06$, $r=0.73$, n=35, $p < 0.001$).

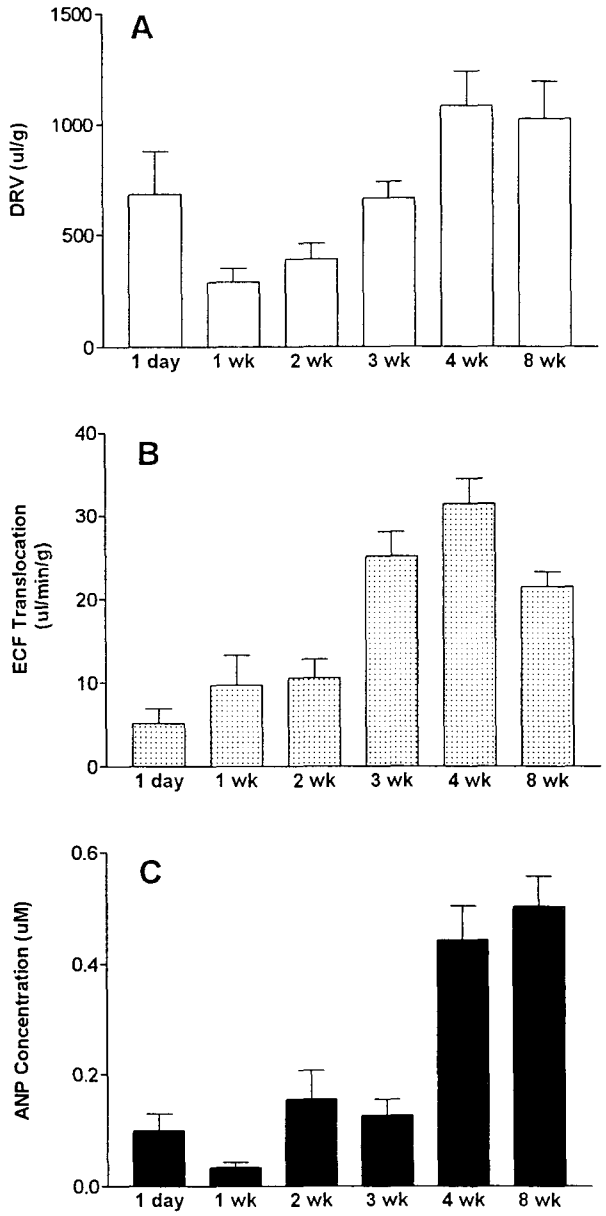


**Fig. 5.** Developmental changes in atrial volume (A), mechanically stimulated ECF translocation (B) and interstitial ANP concentration (C) observed at 6 $cmH_2O$ of atrial pressure.

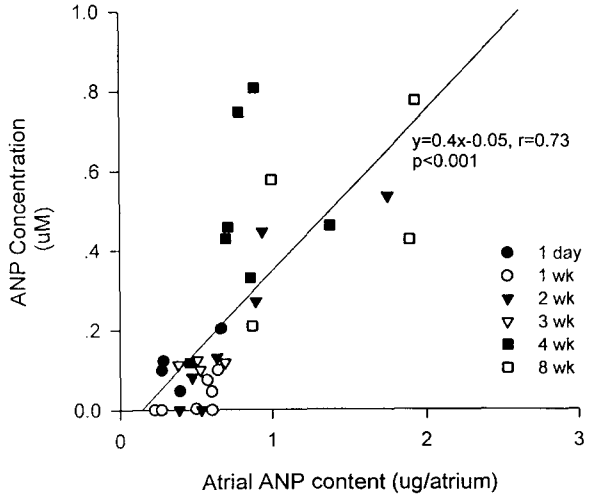


**Fig. 6.** Relationship between the interstitial ANP concentration and atrial ANP content in all age groups. A closely positive relationship between them was observed ($y=0.4x-0.05$, $r=0.73$, $p<0.001$).

## DISCUSSION

The present study clearly shows that higher atrial compliance is a cause of an accentuation of ANP release from atria in 1-day-old rabbits and both compliance and ANP content are regulatory factors for the postnatal changes in ANP secretion.

With aging, systemic hemodynamics such as blood pressure and heart rates markedly increased with enlargement of body and heart. Heart rate increased to the peak value at 2 wk but blood pressure continuously increased. Atrial content of ANP increased, but ventricular content of ANP decreased. These data are consistent with others (Kikuchi et al, 1987; Claycomb, 1988; Wu et al, 1988). Both atrial contents of ANP were similar until 3 wk after birth, but the left atrial content of ANP became higher than that in the right side. These changes are also observed in human left auricle (Cantin et al, 1987; Kikuchi et al, 1987; Wu et al, 1988). Postnatal changes in relative concentration of atrial ANP may be related to changes in the right and left atrial pressures. In addition, the reverse change in ventricular and atrial content of ANP after birth may also be related to changes in cardiac workload and may have physiological significance in the cardiac growth.

In fetus, plasma concentration of ANP was 229.7 pg/ml, which was much higher than that in maternal circulation (85 pg/ml, n=3), as shown in ovine (Cheung et al, 1987). Plasma ANP level was still higher (177.3 pg/ml) in 1-day-old rabbits and declined to adult level at 1 wk. These data are consistent with the results reported by others (Cheung et al, 1987; Wu et al, 1988; Semmekrot & Guignard, 1991). The data show that during the early stage of life, atrial content of ANP was lower but plasma concentration of ANP was similar or higher, as compared to adult. It is, therefore, of interest to disclose the mechanisms for the maintenance of high levels of plasma ANP in

the early postnatal stage of life. Possible explanations may be an accentuation of stretch-induced ANP secretion from the atria by shunting blood, transient pulmonary hypertension and vasoconstrictor, the secretion of ANP from extraatrial sources such as cardiac ventricles and lungs, and a decrease in clearance receptor or low metabolic rate of ANP (Wu et al, 1988; Dodd et al, 1994).

In the present study, in order to determine whether the responsiveness of ANP secretion to stretch from the atria may change during early postnatal stage, atrial compliance and stretch-induced ANP secretion from the atria were measured in 1-day, 1-, 2-, 3-, 4-, and 8-wk-old rabbits using isolated perfused non-beating atria. In 1-day-old rabbits, changes in atrial volume induced by increased intra-atrial pressure were measurable in this model. Proportional increases in atrial volume caused increases in ECF translocation and ANP secretion. There were close relationships between those parameters, as previously shown in adult rabbits (Cho et al, 1988; 1993; 1995). This means that ANP released from atria in response to stretch is secreted sequentially into atrial lumen along the translocation of ECF in 1-day-old rabbits. As compared to 1-wk-old rabbits, atrial volume changes were higher but mechanically stimulated ECF translocations were lower. These data suggest that the characteristics of the atrium in 1-day-old rabbits are high atrial compliance and the underdevelopment of the paracellular pathway of ECF translocation. Therefore, it is possible that high level of plasma ANP at 1 day may be at least due to an accentuation of ANP secretion by high atrial compliance.

Atrial compliance (increase in atrial volume induced by increased intra-atrial pressure) increased progressively with aging except in 1-day-old rabbits and reached the peak value at 4 wk. Increases in atrial volume caused increases in ECF translocation in all age groups and the relationships between those parameters were similar. However, the relationships between atrial volume and stretch-induced ANP secretion were shifted upward with aging and reached the peak value at 4 wk. In order to rule out changes in ANP secretion caused by an increase in atrial compliance with age, the stretch-induced ANP secretion was corrected by ECF translocation, i.e. ANP concentration in the interstitium. It was progressively increased with aging except in 1-day-old rabbits and reached the peak value at 4 wk.

What is the factor to stimulate the release of ANP from the atria with aging? As shown in Fig. 1, atrial content of ANP dramatically increased with aging. In order to define the relationship between ANP release and tissue content of ANP during the early postnatal period, we replotted the interstitial ANP concentration against atrial content of ANP used in this study. As shown in Fig. 6, the concentration of interstitial ANP was positively related to tissue content. At present, it is difficult to explain the reason for constant low level of plasma ANP after 1 wk despite an accentuation of ANP release with aging. It may be due to developmental changes in body water and NPR population with aging.

In summary, the present study suggests that high atrial compliance is one of factors responsible for the accentuation of ANP secretion from atria of 1-day-old rabbits, and both compliance and ANP content are related to the postnatal changes in ANP secretion.

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## REFERENCES

Bloch KD, Seidman JG, Naftilan JD, Fallon JT, Seidman CE. Neonatal atria and ventricles secrete atrial natriuretic factor via tissue-specific secretory pathways. *Cell* 47: 695−702, 1986

Cantin M, Ding J, Thibault G, Gutkowska J, Salmi L, Garcia R, Genest J. Immunoreactive atrial natriuretic factor is present in both atria and ventricles. *Mol Cell Endo* 52: 105−113, 1987

Calycomb WC. ANF mRNA is developmentally regulated in the heart ventricles and actively expressed in cultured ventricular cardiac muscle cells of rat and human. *Biochem J* 255: 617−620, 1988

Cheung CY, Gibbs DM, Brace RA. Atrial natriuretic factor in maternal and fetal sheep. *Am J Physiol Endocrinol Metab* 252: E279−E282, 1987

Cheung CY, Brace RA. Fetal hypoxia elevates plasma atrial natriuretic factor concentration. *Am J Obstet Gynecol* 159: 1263−1268, 1988

Cheung CY. Regulation of atrial natriuretic factor by endothelin in ovine fetuses. *Am J Physiol Regulatory Integrative Comp Physiol* 267: R380−R386, 1994

Cho KW, Seul KH, Ryu H, Kim SH, Koh GY. Characteristics of distension-induced release of immunoreac-

tive atrial natriuretic peptide in isolated perfused rabbit atria. *Regul Pept* 22: 333−345, 1988

Cho KW, Kim SH, Koh GY, Seul KS, Huh KS, Chu D, Rapp NS, Moon HB, Kim KK, Kook YJ. Plasma concentration of atrial natriuretic peptide in different phases of Korean-hemorrhagic fever. *Nephron* 51: 215−219, 1989

Cho KW, Seul KH, Kim SH, Koh GY, Seul KM, Hwang YH. Sequential mechanism of atrial natriuretic peptide secretion in isolated perfused rabbit atria. *Biochem Biophys Res Commun* 172: 423−431, 1990

Cho KW, Kim SH, Hwang YH, Seul KH. Extracellular translocation in perfused rabbit atria: Implication in the control of atrial natriuretic peptide secretion. *J Physiol (Lond.)* 468: 591−607, 1993

Cho KW, Kim SH, Kim CH, Seul KH. Mechanical basis of atrial natriuretic peptide secretion in beating atria: atrial stroke volume and ECF translocation. *Am J Physiol Regulatory Integrative Comp Physiol* 268: R1129−R1136, 1995

De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. *Life Sci* 28: 89−94, 1981

Dietz JR. Release of natriuretic factor from rat heart-lung preparation by atrial distension. *Am J Physiol Regulatory Integrative Comp Physiol* 247: R1093−R1096, 1984

Dodd A, Kullama LK, Ervin MG, Leake RD, Ross MG. Ontogeny of ovine fetal renal atrial natriuretic factor receptor. *Life Sci* 15: 1101−1107, 1994

Fesonen E, Merritt AT, Heldt G, Sahn DJ, Elias W, Tikkanen I, Fyhrquist F, Andersson S. Correlation of patent ductus arterisus shunting with plasma atrial natriuretic factor concentration in preterm infants with respiratory distress syndrome. *Pediatr Res* 27: 137−139, 1990

Franch HA, Dixon RAF, Blaine EH, Seigle RKS. Ventricular atrial natriuretic factor on the cardiomyopatic hamster model of congestive heart failure. *Circulation* 62: 31−36, 1988

Gardner DG, Deschepper CF, Ganong WF, Hane S, Fiddes J, Baxter JD, Lewichi J. Extra-atrial expression of the gene for atrial natriuretic factor. *Proc Nalt Acad Sci USA* 83: 6697−6701, 1986

Gutkowska J, Thibault G, Januszewics P, Cantin M, Genest J. Direct radioimmunoassay of atrial natriuretic factor. *Biochem Biophys Res Commun* 122: 593−601, 1984

Gutkowska J, Nemer M. Structure, expression, and function of atrial natriuretic factor in extraatrial tissues. *Endocrine Rev* 10: 519−536, 1989

Kim SH, Cho KW, Kim SZ, Koh GY. Characterization of the atrial natriuretic peptide system in the oviduct. *Endocrinology* 138: 2410−2416, 1997a

Kim SH, Cho KW, Chang SH, Kim SZ, Chae SW. Glibenclamide suppresses stretch-activated ANP secretion: involvement of $K^{+}_{ATP}$ channels and L-type $Ca^{2+}$ channel modulation. *Pflugers Arch-Eur J Physiol* 434: 362−372, 1997b

Kikuchi K, Nakao K, Hayashi K, Morii N, Sugawara A, Sakamoto M, Imura H, Mikawa H. Ontogeny of atrial natriuretic polypeptide in the human heart. *Acta Endo (Copen)* 115: 211−217, 1987

Kojima T, Fukuda Y, Hirata Y, Ono A, Kinoshita Y, Matsuzaki S, Iwase S, Kobayashi Y. Relationship between plasma atrial natriuretic peptide concentrations and cardio-thoracic ratio during the early neonatal period. *Acta Paediatr Scand* 78: 29−33, 1989

Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger T. Atrial natriuretic factor- a circulating hormone stimulated by volume loading. *Nature* 314: 264−266, 1985.

Lattion AL, Michel JB, Arnauld E, Corvol P, Soubrier F. Myocardial recruitment during ANF mRNA increases with volume overload in the rat. *Am J Physiol Heart Circ Physiol* 251: H890−H896, 1986

Nemer M, Laviegne JP, Drouin J, Thibault G, Gannon G, Antakly T. Expression of ANF gene in heart ventricular tissue. *Peptides* 7: 1147−1152, 1986

Robillard JE, Winer C. Atrial natriuretic factor in human fetus: Effect of volume expansion. *J Pediatr* 113: 552−555, 1988

Semmekrot B, Guignard P. Atrial natriuretic peptide during early human development. *Biol Neonate* 60: 341−349, 1991

Vollmar AM. Atrial natriuretic peptide in extraatrial tissue. *Klin Wochensdher* 68: 699−708, 1990

Wei Y, Roid CP, Day ML, Weigand RC, Needleman LD, Cole BR, Needleman P. Developmental changes in the rat atriopeptin hormonal system. *J Clin Invest* 79: 1325−1329, 1987

Wu JP, Deschepper CF, Gardner DG. Perinatal expression of the atrial natriuretic factor gene in rat cardiac tissue. *Am J Physiol Endorinol Metab* 255: E388−E396, 1988