VENTRICULAR ATRIOPEPTIN
Unmasking of Messenger RNA and Peptide Synthesis by Hypertrophy or Dexamethasone

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SUMMARY Left ventricular hypertrophy or treatment with dexamethasone caused a 2.5-fold to threefold increase in both immunoreactive atriopeptin (AP) and AP messenger RNA (mRNA), primarily in left ventricular tissue. The combined treatments increased immunoreactive AP and AP mRNA more than either treatment alone. In the animals in which cardiac hypertrophy had been produced by abdominal aortic constriction, there was a decrease in atrial levels of AP and an increase in plasma levels of immunoreactive AP. The increase in left ventricular immunoreactive AP was confirmed by immunohistochemical staining of tissue from hypertrophied and/or dexamethasone-treated rats. The mRNA accumulated in the left ventricle was identical to atrial AP mRNA, as judged by transcriptional start site and by size on Northern blots. Because the mass of ventricular tissue is substantially greater than that of atrial tissue, the induced mRNA levels may represent a total abundance approaching one third of the total AP mRNA in the atria. High performance liquid chromatographic purification of ventricular extracts primarily demonstrated the presence of the high molecular precursor and small amounts of C-terminal peptide AP. Induction of ventricular AP (mRNA and peptide) may represent regression of the tissue to an earlier developmental form. These data provide a unique example of regulation of AP biosynthesis in nonatrial tissue.

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KEY WORDS • hypertrophy • atriopeptin • glucocorticoid

A TRIOPEPTIN (AP), a recently discovered peptide hormone involved in fluid, electrolyte, and vascular homeostasis, is synthesized, stored, and secreted from mammalian myocytes. A 126 amino acid prohormone, AP126 is stored in granules in both atria. Following pharmacological stimulation or atrial stretch, AP is released into the circulation primarily as the 28 amino acid peptide AP28.5,6 Release of AP appears to be most closely associated with changes in right atrial pressure, suggesting that the right atrium is the primary source of the circulating hormone released by stimulation. 3 Long-term manipulations of dietary salt and water intake have been shown to affect the levels of AP and AP messenger RNA (mRNA).6,7 Water deprivation increases immunoreactive AP in the atrium, while it decreases the level of AP mRNA. In contrast, animals on a high salt diet have decreased AP stores and increased AP mRNA levels in the atria. Plasma levels of AP in these animals are inversely related to the cardiac stores. AP has previously been reported to occur at low concentrations in nonatrial tissues, including brain, kidney, eye, and ventricle.8-12 In many of these tissues (i.e., brain, kidney, and eye) the low levels of peptide observed are consistent with the number of AP receptors13,14 and are likely to result from binding of circulating hormone rather than from synthesis. The normal concentration of AP in ventricle is at least 300-fold lower than in atrium,4 but it is still significantly higher than in other tissues.

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The human gene encoding for AP contains a sequence in its second intron that has been reported to be similar to a consensus glucocorticoid receptor binding site. There is no significant similarity to reported consensus sequences in the rat gene; however, functional binding sites can vary widely and may yet be shown to exist. Untreated rats have low levels of ventricular AP mRNA (see Discussion). Administration of pharmacological doses of dexamethasone can elicit the synthesis and release of immunoreactive AP from the atrium and the synthesis of AP mRNA in several tissues, including lung, pituitary, and ventricle.

Cardiac hypertrophy, produced by banding of the abdominal aorta, is associated with an increase in left ventricular mass and total protein synthesis as well as subcellular changes, including increased myofibril density, proliferation of the Golgi apparatus, and the appearance of electron-dense granules. The similarity of these granules to those found in normal atrial myocytes suggest that hypertrophy may induce the formation of AP within ventricular myocytes. In the present study we examined the effects of cardiac left ventricular hypertrophy and dexamethasone on cardiac AP mRNA and peptide content.

Materials and Methods

Animals

Left ventricular hypertrophy was induced in male Sprague-Dawley rats (weight, 250–275 g; SASCO, Omaha, NE, USA) by constriction of the abdominal aorta between the renal and superior mesenteric arteries. The animals were anesthetized with chloral hydrate and their hearts were exposed through a midline abdominal incision. The aorta was ligated adjacent to a blunted 20-gauge needle with surgical silk, and the needle then was immediately removed, leaving the vessel constricted to the diameter of the needle. Aortas of the sham-operated animals were not constricted. Within each group, animals were divided into two subgroups that either received or did not receive (control) dexamethasone (350 mg/kg) intraperitoneally, and their aortas were exposed through a midline abdominal incision. Seven days after aortic banding, the animals were anesthetized with chloral hydrate and their hearts were immediately frozen and stored at −70°C until assayed. Before the animals were killed, blood was collected from the abdominal aorta into one-tenth volume 0.11 M sodium citrate and centrifuged, and plasma was immediately frozen and stored at −70°C until assay. AP immunoreactivity (APir) of plasma samples and of the extracts was determined with a guinea pig antisera as previously described. APir was calculated in comparison to a standard curve of authentic AP24. The cross-reactivity of the antibody is indicated by the 50% binding for various atrial peptides as follows: rat AP24, 21 fmol; rat AP28, 29 fmol; prohormone AP128, 100 fmol. The intraassay and interassay variation was 4.7% and 9%, respectively. Protein content was determined by the fluorescamine method using a standard of bovine serum albumin.

High Performance Liquid Chromatographic Purification of Ventricular Atriopeptin

Pooled left ventricular extract from hypertrophied and/or dexamethasone-treated animals was partially purified on an octadecylsilane (Waters) column. Pooled extract (2.4 ml) was applied to the column and washed with 10 ml of 20 mM triethylamine, pH 4. APir was eluted with 3 ml of methanol/20 mM triethylamine (80:20). The eluate was lyophilized, reconstituted with 200 µl of 15% acetonitrile, and 0.05% trifluoroacetic acid, and applied to a Vydac C18 reversed-phase column (E.J. Colbert Co., St. Louis, MO, USA) at a flow rate of 1.0 ml/min (A = 0.05% trifluoroacetic acid and B = 100% acetonitrile and 0.05% trifluoroacetic acid). The following linear gradients in B were applied: from 0 to 40% in 30 minutes; from 40 to 60% in 40 minutes.

RNA Isolation and Characterization

Left and right ventricular samples were immediately frozen in liquid nitrogen and stored at −80°C until RNA was extracted. RNA was prepared in guanidine thiocyanate and centrifuged through a 5.7 M cesium chloride cushion, extracted with phenol/chloroform/isoamyl alcohol, and precipitated with ethanol. Total ventricular and atrial RNAs were fractionated on 1.5% agarose gels containing 1.2 M formaldehyde. The RNA was transferred to nitrocellulose filters by capillary blotting and probed with a 760 bp base pair (bp) fragment of AP complementary DNA (cDNA). After autoradiography, the AP cDNA was removed by heating the filter to 95°C for 5 minutes in distilled water. Elimination of this AP cDNA was confirmed by additional autoradiography. The filters and RNA then were reprobed with the dog heart creatine phosphokinase (CPK) cDNA. The mRNA for dog heart CPK migrates in the region of 1.6 kb. Both cDNA probes were labeled to 2 × 10⁶ cpm/µg with ³²P by nick-translation. A 760 bp rat AP cDNA containing the entire coding region of the prohormone was used as a probe to assay total mRNAs from rat cardiac tissue. This cDNA was isolated from a rat atrial cDNA library constructed in λgt10 using two sets of mixed synthetic oligonucleotide probes prepared against amino acids Glu⁴—Val²³.
and Glu-Pro
(M. L. Day and R.C. Wiegand, unpublished observations, 1986). The sequence of the cDNA clone throughout the coding region was identical to that reported by others.31-34

**Primer Extension**

Total RNA (20 µg) was mixed with 2.0 pmol of either primer A or primer B, which had been labeled previously to 1.5 × 10^7 cpm/pmol using [32P]adenosine 5'-triphosphate (ATP) and polynucleotide kinase. Following extraction with phenol and precipitation from ethanol, the mixture was incubated at 42°C in 5 µl of 2 M NaCl, 5 mM EDTA, 0.2 M piperazine-N,N'-bis(2-ethanesulfonic acid), pH 6.4 for 3 hours. After incubation, 5 µl of 0.25 M Tris base, 80 mM MgCl₂, and 4 mM dithiothreitol; 5 µl of 10 mM each dATP, dCTP (cytidine 5'-triphosphate), dGTP (guanosine 5'-triphosphate), and dTTP (thymidine 5'-triphosphate); and 100 units of reverse transcriptase (Life Sciences, St. Petersburg, FL, USA); and H₂O to a final volume of 50 µl were added. After an additional 1 hour at 42°C, the extension reaction was terminated by precipitating the product with ethanol. The product was size-separated by electrophoresis on a gel of 8% polyacrylamide containing 8 M urea, 90 mM Tris borate, 0.25% Triton X-100; and PBS with Triton and 3% acrylamide. The precipitate was collected by centrifugation, washed thoroughly with ethanol, and dried in a vacuum centrifuge. The dried pellets were dissolved in H₂O and analyzed by autoradiography (Leitz microscope (12 filter; Rockleigh, NJ, USA).

**Results**

**Unmasking Ventricular Atriopeptin Synthesis**

Constriction of the abdominal aorta produces an increase in cardiac afterload and left ventricular hypertrophy in proportion to the degree of constriction.26 The effects of this treatment are demonstrated in Table 1. Aortic banding caused an increase in the heart weight/body weight ratio. The hypertrophy was also evident by the increase in left ventricular wall thickness. Two days of treatment with dexamethasone also produced an increase in the heart weight/body weight ratio; however, this increase was due to wasting of the animals with a relative sparing of the cardiac tissue.27 No increases in left ventricular wall thickness were observed. The combination of dexamethasone treatment and aortic banding produced both noncardiac wasting and left ventricular hypertrophy.

The effects of these treatments on levels of APir in samples of left and right ventricular free wall are also presented in Table 1. Extracts of normal ventricle, left or right, contained approximately 100 pg of APir/mg protein. Blood contamination (0.88 ng of APir/ml plasma) of the sample is unlikely to be responsible for the APir levels measured in the ventricular extracts. The values of APir we measured were similar to those reported by Matsuo et al.8 Cardiac hypertrophy or treatment with dexamethasone elevated the levels of APir in the left ventricle 2.5-fold to threefold com-

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**Table 1. Effects of Aortic Banding and Dexamethasone Treatment on Ventricular Dimensions and Cardiac and Plasma Atriopeptin Immunoreactivity**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham-operated control</th>
<th>Hypertrophied control</th>
<th>Sham operation + dexamethasone</th>
<th>Hypertrophy + dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart wt/body wt (mg/g)</td>
<td>3.16 ± 0.06 (5)</td>
<td>3.59 ± 0.15* (6)</td>
<td>3.62 ± 0.07* (5)</td>
<td>3.82 ± 0.19* (6)</td>
</tr>
<tr>
<td>LV wall thickness (µm)</td>
<td>326 ± 5 (7)</td>
<td>383 ± 2* (6)</td>
<td>318 ± 61 (5)</td>
<td>382 ± 9* (6)</td>
</tr>
<tr>
<td>Ventricle (pg APir/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>121 ± 23 (4)</td>
<td>178 ± 27 (5)</td>
<td>131 ± 14 (5)</td>
<td>216 ± 38 (6)</td>
</tr>
<tr>
<td>Left</td>
<td>105 ± 6 (4)</td>
<td>315 ± 62* (4)</td>
<td>235 ± 27* (5)</td>
<td>567 ± 67* (6)</td>
</tr>
<tr>
<td>Atrium (µg APir/atrium)</td>
<td>6.80 ± 1.21 (11)</td>
<td>3.83 ± 0.41* (12)</td>
<td>6.49 ± 1.17* (11)</td>
<td>3.07 ± 0.22* (12)</td>
</tr>
<tr>
<td>Right</td>
<td>2.92 ± 0.39 (11)</td>
<td>1.61 ± 0.18* (12)</td>
<td>2.06 ± 0.21 (11)</td>
<td>1.43 ± 0.22* (12)</td>
</tr>
<tr>
<td>Plasma (ng APir/ml plasma)</td>
<td>0.88 ± 0.07 (14)</td>
<td>1.69 ± 0.39* (15)</td>
<td>0.59 ± 0.07* (14)</td>
<td>0.98 ± 0.14* (13)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Numbers in parentheses indicate number of animals. APir = atriopeptin immunoreactivity; LV = left ventricular.  
*p < 0.05, compared with sham-operated control.  
†p < 0.05, compared with sham operation + dexamethasone.  
‡p < 0.05, compared with hypertrophied control.
pared with levels in the controls. The combination of these treatments appeared to have an additive effect, causing a fivefold increase over control levels. There was no significant effect on right ventricular levels.

In contrast to the effects on ventricular tissue, hypertrophy (in the normal or dexamethasone-treated animal) decreased APir content in both left and right atria. Dexamethasone alone appeared to have no significant effect on atrial APir levels. Because changes in atrial levels of APir are inversely related to the changes in circulating peptide levels, we measured plasma APir levels associated with hypertrophy and dexamethasone treatment. Hypertrophy increased plasma APir almost twofold over control values. Dexamethasone, in both normal and hypertrophied animals, decreased plasma APir.

**Ventricular Immunohistochemistry**

We examined the pattern of immunohistochemical staining in sections of cardiac tissue from normal and hypertrophic and/or dexamethasone-treated animals. In normal atria this staining procedure revealed dense clusters of granular immunofluorescent material with a characteristic perinuclear distribution (Figure 1A).23 In the ventricles of normal animals, we observed small clusters of fine punctate immunofluorescent staining. This staining was relatively sparse and largely confined to the surface of the endocardium and the adjacent myocardium. Staining of the deeper ventricular tissue or the epicardial surface of normal hearts was rare (Figure 1B). In contrast, hearts from hypertrophied and/or dexamethasone-treated animals exhibited diffuse, immunofluorescent staining extending in from the left ventricular endocardium within the wall of the ventricle and along the epicardial surface (Figure 1C). Staining of the ventricles and the atria from both groups of animals was abolished by preabsorption of the antiseraum with the purified fragment of AP126 at a concentration of 5 μg/ml of diluted serum. There was no detectable increase in right ventricular immunoreactivity in the aortic-banded or dexamethasone-treated rats.

**Molecular Form of Ventricular Atriopeptin**

Atriopeptin is stored in granules in the atria as the 126 amino acid prohormone AP126. In contrast, AP isolated from the hypothalamus, eye, and kidney exists in the low molecular weight form of the peptide. Extracts of two hypertrophied and dexamethasone-treated ventricles were pooled, partially purified over octadecylsilane (Waters) minicolumns, and purified by reversed-phase high performance liquid chromatography (HPLC). The APir of the left ventricular extract applied to the reversed-phase HPLC column was 12 ng, and the total APir recovered in all the HPLC fractions was 7.6 ng. A total of 1.4 ng of APir was present in column fraction 24, which comigrates with either AP24 or AP28 under the column conditions employed. A total of 5.7 ng of APir comigrated with standard purified prohormone AP126 (fraction 31–32). The immunoreactivity in this assay is determined against an AP24 standard, but this guinea pig antibody has only one fifth the cross-reactivity for the prohormone. Thus, correction for total amounts present indicates the presence of 28.5 ng of prohormone and 1.4 ng of AP (i.e., a 20:1 ratio). This experiment was repeated with three separate samples of pooled ventricular extract (hypertrophy plus dexamethasone) with comparable results. HPLC purification of pooled ventricle extracts from the other treatment groups have qualitatively identical results (data not shown).

**Ventricular Atriopeptin mRNA**

Relative RNA levels were measured in the left and right ventricles of rats with left ventricular hypertrophy, rats administered dexamethasone, 2.5 mg/kg, at 24 and 48 hours before death, rats subjected to the combined treatments and sham-operated controls. Figure 2 shows a representative Northern blot analysis of such an experiment. Lanes 1, 3, 5, and 7 contain 20-μg samples from left ventricles of hypertrophied, dexamethasone-treated rats; hypertrophied controls; and dexamethasone-treated and untreated sham-operated controls, respectively. All of the treated animals showed an increase in relative AP mRNA levels in the left ventricle compared with controls. The AP mRNA level in each of the right ventricular samples (Lanes 2, 4, 6, and 8) was low; no detectable change occurred in response to the treatments. AP mRNA was seen occasionally in the right ventricle of some animals. In general, the levels were below our level of detection and therefore showed no statistically significant increase. Lane 9 contained 0.1 μg of total atrial RNA. A summary of a number of such experiments is shown in Table 2. RNA samples from each animal were visualized by Northern blotting, and the relative AP mRNA levels were quantitated by densitometry of the autoradiograms. RNA quantitation was determined by measuring A260 and checked by inspection of the ethidium
bromide-stained gels. Dexamethasone or hypertrophy alone resulted in a twofold to fourfold increase in AP mRNA abundance in the left ventricle. The combination of hypertrophy and dexamethasone treatments appeared to have an additive effect.

To determine whether the increase seen in AP mRNA was specific and not due to an increase in all mRNA levels in the heart, the mRNA levels of a second unrelated gene, creatine kinase (CK), an enzyme found throughout cardiac muscle, was measured. The AP cDNA probe was washed off, and the same filters were reprobed with a nick-translated CK cDNA. In a number of such experiments (n = 6 animals per treatment) there was no detectable change in levels of CK mRNA in either left or right ventricle in response to dexamethasone, cardiac hypertrophy, or the combined treatments (data not shown).

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Northern blot analysis of total ventricular RNA from rats with hypertrophy or dexamethasone or both treatments. Total ventricular RNA (10 μg in each lane) was size-fractionated on a 1.5% agarose gel and probed for atriopeptin (AP) mRNA. Lanes 1, 3, 5, and 7 are left ventricular total RNA. Lanes 2, 4, 6, and 8 are right ventricular total RNA. Lane 9 is 0.1 μg untreated, total atrial RNA. The treatments are indicated above each pair of lanes (HD = hypertrophy and dexamethasone; HC = hypertrophied control, no dexamethasone; SD = sham operation and dexamethasone, no hypertrophy; SC = sham-operated control, no hypertrophy and no dexamethasone).

**Table 2. Atriopeptin mRNA Levels in Left Ventricle**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>Dexamethasone</th>
<th>Hypertrophy</th>
<th>Dexamethasone + hypertrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00 ± 0.22*  (5)</td>
<td>—</td>
<td>3.66 ± 0.44† (6)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>1.00 ± 0.21*  (4)</td>
<td>3.26 ± 0.36† (5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>1.00 ± 0.35*  (3)</td>
<td>4.11 ± 0.69† (3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>1.00 ± 0.20*  (4)</td>
<td>4.33 ± 0.80† (4)</td>
<td>2.36 ± 0.44†</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>1.90 ± 0.27* (4)</td>
<td>—</td>
<td>1.96 ± 0.23† (4)</td>
</tr>
<tr>
<td>6</td>
<td>1.00 ± 0.05*  (3)</td>
<td>2.45 ± 1.11† (5)</td>
<td>—</td>
<td>5.42 ± 1.66† (5)</td>
</tr>
<tr>
<td>7</td>
<td>1.00 ± 0.51*  (3)</td>
<td>3.97 ± 0.96† (3)</td>
<td>2.12 ± 0.13† (3)</td>
<td>—</td>
</tr>
</tbody>
</table>

Northern blots were performed as described in Methods. In Experiments 1, 5, and 6 a 20-gauge needle was used for the ligation. In Experiments 4 and 7 an 18-gauge needle was used, resulting in a lesser degree of hypertrophy. The relative intensities of the bands on the resulting autoradiograms were determined by densitometric scanning with an EC910 transmission densitometer (E-C Apparatus Corporation, St. Petersburg, FL, USA). The numbers generated are in arbitrary units of density. Within each experiment, values were determined for each animal and the mean and SEM computed. To allow easier comparisons, the numbers in each experiment were then normalized to the control value, except for Experiment 3, which had no sham control group and was therefore normalized to the value for the dexamethasone-treated animals. The values shown are means ± SEM. Numbers in parentheses indicate number of animals.

*Mean value arbitrarily set to 1.00.
†p < 0.05, compared with sham-operated control.
‡p < 0.05, compared with sham operation + dexamethasone.

**Primer Extension Experiments**

Analysis of genomic DNA by Southern blotting indicates that there is a single gene for AP in the rat. However, the possibility exists that alternative transcripts, which might encode the same or a different protein, could be generated in the ventricle. This possibility was examined by primer extension. End-labeled oligonucleotides complementary to known points on the mRNA (diagrammed in Figure 3) were hybridized with the mRNA and extended using reverse transcriptase and cold nucleotide triphosphates. The length of the extended product is a measure of the distance from the primer to the 5' end of the message. The primers used hybridize to the mRNA at points 111 bp apart, near the 5' end of the message. The transcripts from each primer were identical in the ventricular lanes and the atrial lanes, and their lengths were consistent with the known start site of transcription, approximately 90 bases upstream of the AUG start codon. Primer extension studies were performed to verify that the transcriptional start site of the AP mRNA in the dexamethasone-treated, hypertrophied left ventricle is the same as it is in the atria (see Figure 3). This finding, coupled with the fact that, in all cases, both atrial and ventricular messages were the same size when separated by gel electrophoresis, indicates that the transcripts, with regard to the 5' terminus in both atrial and ventricular tissue, are probably identical. The methods employed would not detect heterogeneity at the 3' end or differences in splice sites smaller than 50 bases.

The twofold to fourfold increases in mRNA we report herein for either dexamethasone treatment or hypertrophy were closely paralleled by the twofold to threefold increases in the stored levels of AP in milligram of total acid-soluble protein in the ventricle (see Table 1), suggesting that this mRNA is functional.

**TABLE 2. Atriopeptin mRNA Levels in Left Ventricle**

<table>
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<tr>
<th>Experiment</th>
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<th>Hypertrophy</th>
<th>Dexamethasone + hypertrophy</th>
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<tr>
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<td>—</td>
</tr>
<tr>
<td>4</td>
<td>1.00 ± 0.20*  (4)</td>
<td>4.33 ± 0.80† (4)</td>
<td>2.36 ± 0.44†</td>
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<td>3.97 ± 0.96† (3)</td>
<td>2.12 ± 0.13† (3)</td>
<td>—</td>
</tr>
</tbody>
</table>
The existence of AP in ventricular tissue is supported by the demonstration that the amount of AP in the tissue is much greater than can be accounted for by blood contamination. In addition, membrane-bound dense granules have been reported in the ventricular (as well as atrial) myocytes of embryonic mice, rats, and humans. These granules disappear in the ventricular cells in later embryonic life, while they become more prominent in atrial cells. In cardiac hypertrophy, there is an apparent regression of the ventricular myocyte toward its fetal type, including hypertrophy and hyperplasia of the Golgi and multiplication of the atrial-like granules. Additional morphological evidence includes immunohistochemical localization of AP in embryonic rat ventricle, and in the ventricles of hypertrophied and dexamethasone-treated adult animals (see Figure 1). Consistent with this evidence, there is substantial AP and AP mRNA in the ventricles of neonatal rats. Both the mRNA and AP rapidly decline in abundance as the rats reach maturity (M. L. Day, C. Rodi, and R. C. Wiegand, unpublished observation, 1986). AP has been demonstrated to have mitogenic activity on adrenal zona glomerulosa cells, and AP could be involved in the normal growth and development of the heart. The changes seen in AP expression support the hypothesis that in hypertrophy the ventricular cells revert to an earlier developmental form.

A variety of experimental treatments have been shown to affect atrial and plasma levels of AP. Acute stimulation (i.e., vasopressin, water immersion, or volume overloading) will increase plasma AP levels at the expense of cardiac stores. Synthesis of new AP prohormone does not occur within the first hour after vasopressin-stimulated release, suggesting that the atrium contains a sufficiently large capacity of stored AP, precluding the need for rapid synthetic replenishment. Chronic alterations in salt and water intake produce changes in circulating AP levels consistent with attempts to maintain plasma homeostasis. Changes in atrial AP stores are inversely related to the plasma levels in these animals. Conditions of experimental hypertension, including the spontaneously hypertensive rat and hypoxia-induced pulmonary hypertension, have also been shown to lower atrial (and increase plasma) AP levels. Interestingly, these two experimental models are also associated with cardiac hypertrophy. The demonstration of the effects of hypertension and dexamethasone on left ventricular levels of AP mRNA and AP represents a unique example of regulation of AP biosynthesis in nonatrial tissue.

The total amount of AP mRNA induced in the ventricle, especially by hypertrophy, is substantial. The total mass of ventricular tissue expressing AP is about 10-fold greater than the mass of the atrium. The increase in AP mRNA therefore serves as a useful biochemical marker of hypertrophy. In the animals with combined dexamethasone and hypertrophy treatment, the level of AP mRNA was at least 5% of the abundance observed in atria, based on the fivefold increase over levels in untreated animals. In untreated control animals, the abundance of AP mRNA in pooled (n = 7) total RNA from ventricle was determined to be 1.1% of that from atrium by quantitative Northern blots. Signals in a range from 0.05 to 10 μg of each RNA were determined by densitometry of the autoradiogram, and relative abundance was compared in the range of the linear response. The total mass of AP mRNA induced in the left ventricle thus approached one third of the mass of AP mRNA in the atria. The total stored AP mRNA ratio appears to be lower in the ventricle than in the atrium. This difference could be due to a lower rate of translation, higher turnover of the protein, or more rapid release of the protein from the ventricle. Further studies will be necessary to explore these possibilities.

Acknowledgments

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References

Ventricular atriopeptin. Unmasking of messenger RNA and peptide synthesis by hypertrophy or dexamethasone.

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