15. I. U. Ali, R. Lidereau, C. Theillet, R. Callahan, *Science* **238**, 185 (1987).
16. A. L. Hartley, J. M. Birch, H. B. Marsden, M. Harris, *Br. J. Cancer* **54**, 819 (1986).
17. W.-H. Lee *et al.*, *Nature* **329**, 642 (1987).
18. E. Y.-H. P. Lee *et al.*, unpublished results.
19. R. Bookstein *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2210 (1988).
20. H.-J. S. Huang *et al.*, in preparation.
21. R. Cailleau, M. Olive, Q. V. Cruciger, *In Vitro* **14**, 911 (1978).
22. H. T. Lynch *et al.*, *Cancer Genet. Cytogenet.* **13**, 43 (1984).
23. J. Toguchida, personal communication; D. Slamon, personal communication.
24. F. P. Li and J. F. Fraumeni, *Ann. Intern. Med.* **71**, 747 (1969); *J. Am. Med. Assoc.* **247**, 2692 (1982).
25. E. Y.-H. P. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
26. All mammary cell lines were obtained from ATCC. Cells were grown in Dulbecco's minimum essential medium supplemented with 10% fetal calf serum and 10 μg of insulin per milliliter.
27. R. Cailleau, R. Young, M. Olive, W. J. Reeves, Jr., *J. Natl. Cancer Inst.* **53**, 661 (1974).
28. R. K. Young, R. M. Cailleau, B. Mackay, W. J. Reeves, Jr., *In Vitro* **9**, 239 (1974).
29. E. Y. Lasfargues, W. G. Coutinho, E. S. Redfield, *J. Natl. Cancer Inst.* **61**, 967 (1978).
30. H. D. Soule, J. Yazquez, A. Long, S. Albert, M. Brennan, *ibid.* 51, 1409 (1973).
31. F. P. Polanowski, E. V. Gaffney, R. E. Burke, *In Vitro* **12**, 328 (1976).
32. E. Y.-H. P. Lee and W.-H. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6337 (1986).
33. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
34. Cell line LAN-1 was kindly provided by R. Seeger. E.L. thanks M. J. Bissell for guidance and inspiration in mammary research, and L. Y. Pan for encouragement. This study was funded by grants from the National Institutes of Health (EY05758, EY00278, and CA39537) and the American Cancer Society.

6 May 1988; accepted 2 June 1988

Isoform-Specific Modulation of Na^+,K^+-ATPase α-Subunit Gene Expression in Hypertension

VICTORIA L. M. HERRERA, ARAM V. CHOBANIAN, NELSON RUIZ-OPAZO

Sodium, potassium–adenosine triphosphatase (Na^+,K^+-ATPase) is hypothesized to be involved in systemic vascular hypertension through its effects on smooth muscle reactivity and myocardial contractility. By means of RNA blot analyses of cardiac, aortic, and skeletal muscle RNAs in two rat hypertensive models, Na^+,K^+-ATPase α-subunit messenger RNA isoforms (α2 and α3) were shown to be deinduced in response to increased intravascular pressure. The changes were observed after 48 hours or more of experimental hypertension. Under these conditions, there is coordinate induction of another α isoform (α1) and of β-subunit messenger RNAs, probably in response to alterations in sodium flux rather than to elevated blood pressure.

THE NA-, K-DEPENDENT ADENOSINE triphosphatase (Na^+,K^+-ATPase) maintains the Na^+ and K^+ electrochemical gradient across the cell membrane to which is coupled other vectorial transport mechanisms important for cell homeostasis and specialized functions (*1*). Because Na^+,K^+-ATPase maintains the Na^+ gradient, this enzyme has been hypothesized to be involved in the pathogenesis of hypertension through its effects on vascular smooth muscle reactivity (*2–4*) and myocardial contractility (*5*). Conflicting data have been reported regarding changes in Na^+,K^+-ATPase activity in various types of experimental and human hypertension (*6, 7*). At least three α-subunit isoforms, α1, α2, and α3 (α, α+, αIII) (*8*) have been characterized in rats (*8, 9*) and humans (*10*). These isoforms have a complex pattern of expression (*9, 11*) and significant functional diversity (*12, 13*). Identification of isoform-specific differential regulation of expression during hypertension could give insight into the roles of the isoforms and the molecular mechanisms involved in hypertension.

We therefore have analyzed Na^+,K^+-ATPase α- and β-subunit mRNAs in different tissues during hypertension in two rat models—rats uninephrectomized and treated with deoxycorticosterone (DOC)-salt (*14*) and rats infused with angiotensin-II (A-II) for short periods (*15*) (Table 1). RNA blot analyses of Na^+,K^+-ATPase mRNA were done with RNA from aortic, cardiac left ventricular, and skeletal muscle from hypertensive and control rats. In aorta, a two- to threefold increase (*16*) in amounts of α1-subunit mRNA was noted only in rats treated with DOC-salt (Fig. 1A) and persisted from 2 to 8 weeks of DOC-salt administration. The amounts of aortic α1 mRNA in normotensive, uninephrectomized rats treated with DOC–low salt (DOC-LS) were equivalent (*16*) to those in uninephrectomized controls, thus eliminating the possibility that our results were due to an up regulation of α1 mediated by DOC. The α1 mRNA in aortas from the hypertensive rats treated with A-II for short periods, however, did not show any up regulation (*16*) as compared to aortas from control normotensive rats infused with A-II medium (acetic acid, 0.01*N*) or with subpressor doses of A-II (Fig. 1B, C1 and C2, respectively). These results along with the twofold increase (*16*) in α1 mRNA in the skeletal muscle of hypertensive rats treated with DOC-salt (Fig. 2) demonstrate that the α1 up regulation is not a direct response to increased intravascular pressure. This α1 up regulation is not a nonspecific phenomenon, since the amounts of mRNA of other

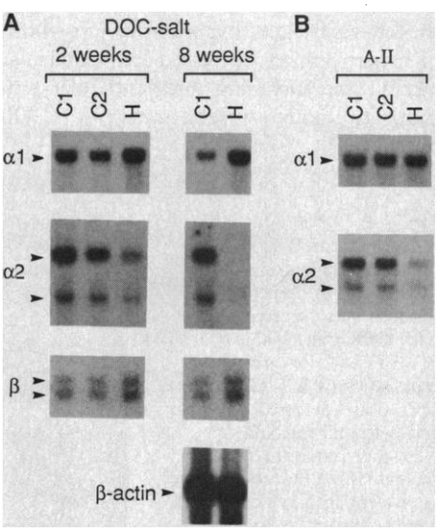

Fig. 1. Na^+,K^+-ATPase α- and β-subunit mRNAs in aortas from hypertensive rats. (**A**) The mRNA of Na^+,K^+-ATPase subunits α1, α2, and β (α3, none detected) were analyzed in separate RNA blots with equivalent amounts of total cellular RNA derived from pooled aortas from hypertensive, uninephrectomized rats treated with DOC-salt (H), control uninephrectomized rats (C1), and control normotensive DOC-LS rats (C2). As a control, β-actin mRNA was also detected. The specific hybridizing bands to the respective probes are indicated by arrowheads. Two hybridizing bands are detected in α2 and β, representing two sizes of mRNAs with different lengths of 3′UT generated by differential utilization of polyadenylation signals (*8, 21*). (**B**) Na^+,K^+-ATPase α-subunit mRNAs, α1 and α2, were analyzed in aortic total cellular RNA from rats made hypertensive by 1-week intraperitoneal A-II infusion. C1, control normotensive rats with pump implanted to infuse medium. C2, control normotensive rats with subpressor dose of A-II infused (75 ng/min). H, hypertensive rats with pressor dose of A-II infused (200 ng/min). Specific hybridizing bands to respective probes are noted with arrowheads. Total cellular RNA was isolated from pooled aortic samples ($n = 3$) and RNA blots were done (*9*). Random-primed ^{32}P-labeled cDNA probes were prepared (*22*) and represented comparable amino acid coding regions: α1, 5′ untranslated region (UT)–3′UT; α2, amino acid 27 to 3′UT; α3, amino acids 10 to 940 (*23*); β, 5′UT–3′UT (*24*); β-actin 5′UT–3′UT. Under stringent hybridization and washing conditions (*9*), the α-subunit cDNA probes used do not cross-hybridize with each other.

Section of Molecular Genetics, Cardiovascular Institute, Boston University Medical Center, Boston, MA 02118.

genes such as α tropomyosin and β-actin did not show any change (Fig. 1A). Furthermore, the increase is tissue-specific because the α1 mRNA in the left ventricle also remained unchanged (*16*) (Fig. 3A).

To investigate possible modulation of expression of α2 and α3, we then analyzed aortic and left ventricular RNA blots. We used the same preparation of mRNA and the same procedures as we used for α1 analysis. The mRNA of α2 shows a 3- to 15-fold decrease (*16*) in aortic samples from hypertensive animals, whether treated with DOC-salt or short-term with A-II (Fig. 1, A and B). Similarly, we also detected a marked α2 down regulation, 3- to 15-fold (*16*), in the left ventricle of hypertensive rats—both in hypertrophied [increased left ventricular wet weight, including interventricular septum, to body weight ratio × 1000 (LV/BW) ratio] and nonhypertrophied [equivalent LV/BW ratio as in controls] left ventricular myocardial samples (Table 1 and Fig. 3, A to C). Aortas and left ventricles from hormone-treated but nonhypertensive rats (DOC-LS or subpressor A-II) did not show this down regulation (Figs. 1B and 3B). These data suggest that the α2 down regulation in the left ventricular myocardium and aorta is elicited by increased intravascular pressure. Consistent with this hypothesis, the skeletal muscle, which is not exposed directly to increased blood pressure, did not show this down regulation in the hypertensive rats treated with DOC-salt (Fig. 2). The mRNA of α3, which has an unknown function, is not detected in aorta or in hearts of young adult rats (13 weeks old) (*9, 11*). In hearts of older rats (19 weeks old), however, α3 mRNA is detected and is also found to be decreased threefold (*16*) in the left ventricular myocardium of hyperten-

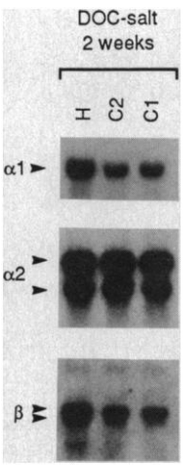

Fig. 2. Na^+,K^+-ATPase α- and β-subunit mRNAs in skeletal muscle in hypertensive rats treated with DOC-salt. Na^+, K^+-ATPase α-subunit isoforms α1, α2 (α3, none detected), and β-subunit mRNAs were analyzed in three separate RNA blots of equivalent amounts of total cellular RNA from skeletal muscle (gluteus muscle group) of a hypertensive rat treated with DOC-salt (H), a control uninephrectomized normotensive rat (C1) and a control normotensive rat treated with DOC-LS (C2). We used the same preparation of mRNA and the same procedures for all three. Specific hybridizing bands to respective cDNA probes are indicated by arrowheads.

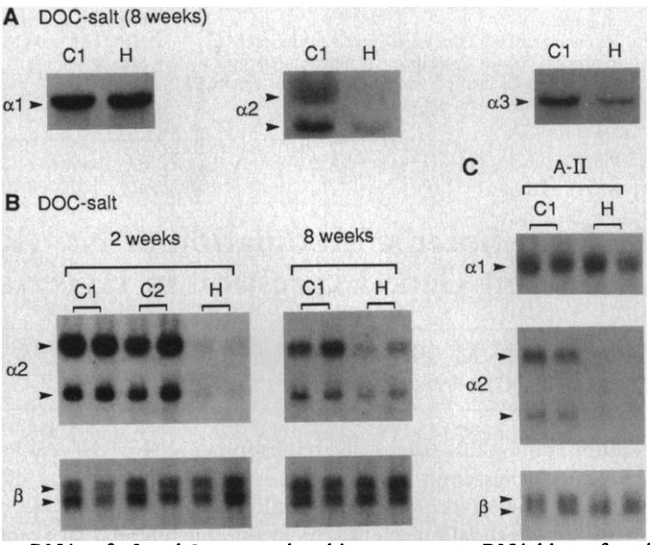

Fig. 3. Na^+,K^+-ATPase α- and β-subunit mRNAs in hypertensive rat left ventricular myocardium. (**A**) Subunit isoforms α1, α2, and α3 mRNAs were analyzed in three separate RNA blots of total cellular RNA from a rat treated with DOC-salt for 8 weeks (Table 1). H, hypertrophied left ventricle from a 19-week-old hypertensive rat treated with DOC-salt; C1, normal left ventricle from age-matched control uninephrectomized rats. We used the same preparation of mRNA and the same procedures for both. Specific hybridizing bands to respective cDNA probes are noted (arrowheads). (**B**) The mRNAs of α2 and β were analyzed in two separate RNA blots of total cellular RNA from individual rat left ventricle (n = 5, two of each group shown here) in the hypertensive rats treated with DOC-salt (H) for two periods of induction, 2 weeks (13-week-old rats) and 8 weeks (19-week-old rats) (Table 1). We used the same preparation of mRNA and the same procedure as before. C1, control uninephrectomized rats; C2, control normotensive rats treated with DOC-LS. Specific hybridizing bands to respective cDNA probes are noted with arrowheads. No hybridizing band to α3 cDNA probe was detected in the 13-week-old rats treated for 2 weeks with DOC-salt (data not shown). (**C**) In the A-II model, Na^+,K^+-ATPase subunit mRNAs, α1, α2 (α3 not detected), and β, were analyzed in three separate RNA blots of total cellular RNA obtained from nonhypertrophied left ventricle from individual (n = 4, two shown) hypertensive rats (H) (infused for 48 hours with A-II, see Table 1). We used the same preparation of mRNA and the same procedure as before. Specific hybridizing bands to respective probes are indicated by arrowheads. Methods are essentially as in Fig. 1, except that total cellular RNA was obtained from individual rat left ventricle samples. Total cellular RNA from brains was used as a positive control in the determination of the nondetectable levels of α3 in 13-week-old rat left ventricles.

Table 1. Physiological parameters of hypertensive and control rats. The hypertensive uninephrectomized rats treated with DOC-salt for 2 weeks (H), age-matched control uninephrectomized rats (C1), and control uninephrectomized rats treated with DOC-LS (C2) were prepared as described (*14*) except that slow-release DOC acetate pellets (100 mg per pellet, Innovative Research of America) were implanted subcutaneously in the hind leg; the pellets delivered approximately 1.5 mg of DOC per 100 g of body weight per day. The DOC–low salt (C2) group received regular drinking water instead of 1% saline. Blood pressure measurements were obtained weekly by the tail cuff method (*14*). The hypertensive uninephrectomized rat treated with DOC-salt for 8 weeks (H) and age-matched control uninephrectomized (C1) rats were prepared essentially as described (*14*) with 1.5 mg DOC per 100 g of body weight (subcutaneous biweekly injections) and 1% saline drinking water for the hypertensive group. In the A-II model, hypertension was induced in some rats via intra-arterial (IA) infusion of isoleucine^5-angiotensin-II (Sigma Chemical) for 48 hours at a dose of 200 ng/min with direct daily arterial blood pressure measurements. In other animals, A-II was administered by intraperitoneal (IP) infusion for 1 week by Alzet osmotic minipumps (Alza Corporation) (*15*) at 200 ng/min for the hypertensive group (H) and 75 ng/min for the control subpressor group (C2). Angiotensin-II medium (0.01*N* acetic acid) was infused in another control group (C1). Blood pressure measurements were obtained daily by the tail cuff method (*14*) and are ± standard deviation. n, number of rats; BP, blood pressure; LV/BW, left ventricular wet weight (including interventricular septum) to body weight ratio × 1000 of the two animals per group whose left ventricular RNA blots are presented.

Measure	DOC-salt model					A-II model				
	2 weeks			8 weeks		48-hour IA infusion		1-week IP infusion		
	C1	C2	H	C1	H	C1	H	C1	C2	H
n	6	6	5	5	5	4	4	2	3	3
BP (mmHg)	117 (±10)	140 (±8)	220 (±15)	122 (±10)	170 (±10)	120 (±19)	168 (±5)	141 (±6)	131 (±15)	213 (±11)
LV/BW	2.1, 2.1	2.2, 2.2	3.0, 3.2	1.7, 1.9	3.0, 2.7	2.1, 2.0	2.1, 2.2	2.3	2.3, 2.2	2.4, 2.3

sive rats after 8 weeks of DOC-salt treatment (Table 1 and Fig. 3A).

This complex modulation of α-subunit mRNA isoforms in hypertension raises a question about the status of Na^+,K^+-ATPase β-subunit mRNA expression. Although all the biochemical properties of Na^+,K^+-ATPase are localized to the α subunit, the vectorial transport of ions is detected only in the intact αβ complex. We therefore assessed the amounts of β mRNA in the aortic and left ventricular RNA blots. We used the same preparation of mRNA and the same procedures as we used for α1 analysis. The β-subunit mRNA is modulated together with α1 mRNA. In the aorta, where a 2- to 3-fold increase in α1 mRNA and a 3- to 15-fold decrease in α2 mRNA are noted, a 2-fold increase in β mRNA (*16*) was detected (Fig. 1A). In skeletal muscle, where the major α isoform, α2, does not change and the minor α isoform, α1, increases twofold, an equivalent increase in β-subunit mRNA (*16*) was likewise detected under stringent hybridization conditions (Fig. 2). In the left ventricle, where the amount of α1 mRNA does not change, amounts of β mRNA likewise did not appear to change (*16*) (Fig. 3, B and C). Thus, the parallel modulation of β and α1 mRNAs suggests coordinate regulation of the β and α1 genes.

These results provide evidence that hypertension induces gene-specific modulation in vascular and cardiac tissues. Furthermore, the modulation of Na^+,K^+-ATPase is isoform-specific. The down regulation of the amounts of α2 mRNA (and probably also that of α3 in the left ventricle) in left ventricle and aorta of hypertensive rats may represent a deinduction of gene expression elicited in response to stimuli from increased intravascular pressure before development of hypertrophy. This deinduction appears to be closely related to pressure increments, since it was detected in left ventricular samples of hypertensive rats that were infused with A-II for 2 days (Table 1). The mechanisms of transduction of a mechanical pressor stimulus to a genetic response are unknown. Because of similar findings in aortas and skeletal muscle of hypertensive rats treated with DOC-salt (Figs. 1A and 2), the up regulation of α1- and β-subunit mRNA expression is clearly not pressure-related, but could be secondary to alterations in Na^+ flux induced by DOC-salt treatment; other studies have documented increased Na^+ influx in these tissues (*17, 18*). The increase in α1 mRNA correlates with increased vascular Na^+,K^+-ATPase activity in hypertensive rats treated with DOC-salt (*19, 20*).

The increase in α1 mRNA may be in response to an increase in Na^+ influx to maintain homeostatic levels of intracellular Na^+, and the decrease in the number of Na^+,K^+-ATPase mRNA molecules in the left ventricle might be an adaptive process to increments in vascular pressure. This decrease in Na^+,K^+-ATPase mRNA may underlie a decrease in the number of Na^+ pumps, which could be an endogenous equivalent of a ouabain-induced Na^+,K^+-ATPase inhibition resulting in cardiac inotropy. These changes in amounts of mRNA could represent transcriptional regulation, although changes in mRNA stability cannot be ruled out. Determination of the mechanisms by which specific Na^+,K^+-ATPase α- and β-subunit genes are modulated in hypertension may provide insight into the molecular mechanisms involved in the pathogenesis or consequences of hypertension.

REFERENCES AND NOTES

1. J. Kyte, *Nature* **292**, 201 (1981).
2. S. M. Friedman *et al.*, *Circ. Res.* **7**, 44 (1959).
3. H. Hendrickx and R. Casteels, *Pfluegers Arch.* **346**, 299 (1974).
4. M. P. Blaustein, *Hypertension* **6**, 445 (1984).
5. H. W. Overbeck *et al.*, *ibid.* **3**, 306 (1981).
6. H. W. Overbeck and D. E. Grissette, *ibid.* **4**, 132 (1982).
7. C. A. Bruner *et al.*, *Am. J. Physiol.* **251**, H1276 (1986).
8. G. E. Shull *et al.*, *Biochemistry* **25**, 8125 (1986).
9. V. L. M. Herrera *et al.*, *J. Cell Biol.* **105**, 1855 (1987).
10. M. M. Shull and J. B. Lingrel, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4039 (1987).
11. R. M. Young and J. B. Lingrel, *Biochem. Biophys. Res. Commun.* **145**, 52 (1987).
12. K. J. Sweadner, *J. Biol. Chem.* **254**, 6060 (1979).
13. J. Lytton, J. C. Lin, G. Guidotti, *ibid.* **260**, 1177 (1985).
14. P. Brecher, C. T. Chan, C. Franzblau, B. Faris, A. V. Chobanian, *Circ. Res.* **43**, 561 (1978).
15. D. I. Diz, P. G. Baer, A. Nasjletti, *J. Clin. Invest.* **72**, 466 (1983).
16. Scanning densitometry of autoradiograms was used to quantitate hybridization signals. Values represent the range of difference between the average densitometry units of samples from hypertensive animals and controls in each study group.
17. A. W. Jones and L. A. Miller, *Blood Vessels* **15**, 83 (1978).
18. R. Nagaoka, S. Yamashita, T. Maruyama, N. Akaike, *Brain Res.* **410**, 283 (1987).
19. T. A. Brock, J. B. Smith, H. W. Overbeck, *Hypertension* **4** (suppl. II), II-43 (1982).
20. R. S. Moreland, F. S. Lamb, R. C. Webb, D. F. Bohr, *ibid.* **6** (suppl. I), I-88 (1984).
21. R. M. Young, G. E. Shull, J. B. Lingrel, *J. Biol. Chem.* **262**, 4905 (1987).
22. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
23. The α1 cDNA probe consisted of a partial Eco RI–Hind III ~3.4-kb restriction fragment from a pUC18 plasmid containing the α1 cDNA, spanning from −260 nucleotide (nt) in the 5′ UT region to the Bam HI site in the 3′ UT, nt 3357 (*9*). The α2 cDNA probe consisted of a ~3.1-kb cDNA clone recently isolated by us, Eco RI–Eco RI, and shown by nucleotide sequence to span from amino acid 27 (*8*) to the polyadenylated 3′ end. The α3 cDNA probe consisted of a partial Sac I/Eco RI ~2.8-kb restriction fragment spanning amino acids 10 to 940.
24. R. W. Mercer *et al.*, *Mol. Cell. Biol.* **6**, 3884 (1986).

25. We thank R. Levenson for the rat Na^+,K^+-ATPase β-subunit cDNA clone and K. Sweadner for critical reading of the manuscript. Supported by NIH grants HL39267, HL01967, and HL18318.

25 February 1988; accepted 9 May 1988

Amyloid β Protein Precursor Is Possibly a Heparan Sulfate Proteoglycan Core Protein

DAVID SCHUBERT, ROLLY SCHROEDER, MONIQUE LACORBIERE, TSUNAO SAITOH, GREG COLE

The amyloid β protein peptide is a major constituent of amyloid plaque cores in Alzheimer's disease and is apparently derived from a higher molecular weight precursor. It is now shown that the core protein of a heparan sulfate proteoglycan secreted from a nerve cell line (PC12) has an amino acid sequence and a size very similar to those of the amyloid β protein precursor and that these molecules are antigenically related. This amyloid β protein precursor–related protein is not found in the conditioned medium of a variant cell line (F3 PC12) that does not secrete heparan sulfate proteoglycan. The synaptic localization and metabolism of this class of proteoglycans are consistent with its potential involvement in central nervous system dysfunction.

FILAMENTOUS AMYLOID STRUCTURES accumulate in the brain of patients with Alzheimer's disease (*1*), Down syndrome (*2*), and a number of infectious encephalopathies (*3*). In Alzheimer's disease, areas of disorganized neuropil called neuritic plaques surround a core of extracellular amyloid. The core has two major components, inorganic aluminosilicate (*4*) and a protein of approximately 4000 daltons termed β protein (*2*) or A4 (*5*). On the basis of a partial amino acid sequence of the β

D. Schubert, R. Schroeder, M. LaCorbiere, Salk Institute for Biological Studies, Post Office Box 85800, San Diego, CA 92138.
T. Saitoh and G. Cole, Department of Neurosciences, M-024, University of California, San Diego, La Jolla, CA 92093.