Early captopril treatment prevents hypertrophy-dependent gene expression in hearts of SHR

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Chen, Songcang, Jinzi Su, Kegui Wu, Wenyang Hu, David G. Gardner, and Daguang Chen. Early captopril treatment prevents hypertrophy-dependent gene expression in hearts of SHR. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1511–R1517, 1998.—Treatment of spontaneously hypertensive rats (SHR) with captopril (100 mg·kg

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had no significant long-term effect on blood pressure. Wu and Berecek (25) extended this experimental paradigm by treating mating pairs of SHR with captopril (i.e., rat fetuses were exposed to captopril in utero) and maintaining newborn pups on the ACE inhibitor after birth. Some of the latter group were taken off treatment at 2 mo of age, and some of these were mated at 3 mo of age. Mean arterial pressures of conscious captopril-treated rats, rats removed from captopril therapy, and the offspring of rats removed from therapy were significantly lower than those of control SHR at 4 and 9 mo of age. Collectively, these data indicate that ACE inhibition during a critical period early in the life of the SHR can significantly reduce the elevations in blood pressure and end organ damage that characterize this strain.

Cardiac hypertrophy is a predictable sequelae of sustained elevations in blood pressure such as those seen in the SHR. Treatment of the mature SHR with ACE inhibitors results in a substantial reversal of cardiac hypertrophy (15). Furthermore, both Harrap et al. (9) and Wu and Berecek (25), in the studies cited above, provided preliminary data indicating that early treatment of the SHR with ACE inhibitors reduced the extent of cardiac hypertrophy in these animals.

Ventricular hypertrophy is characterized phenotypically by the sequential activation of specific programs of gene expression. The earliest of these includes the protooncogenes c-myc, c-fos, and c-jun. Products of these protooncogenes may be linked mechanistically to the stimulation of other genes that are activated later in the hypertrophic process (3). Included in the latter group are the genes of the fetal gene program, such as atrial natriuretic peptide (ANP), β-myosin heavy chain, and α-skeletal actin. These genes are normally expressed only in the late fetal and early neonatal period and are extinguished in the adult ventricular myocardium. Reactivation of expression does occur, however, in myocardium exposed to hemodynamic overload or other perturbations that lead to hypertrophy. Expression of these genes, and ANP in particular, has come to represent one of the most reliable markers of hypertrophy in animal and human ventricular myocardium (3).

In the present study, we show, in confirmation of previous studies (9, 25), that treatment of the SHR in utero and during the first 16 wk of life with captopril results in reduction in blood pressure and suppression of left ventricular hypertrophy. Coincident with the latter there is a striking reduction in c-myc and ventricu-
lar ANP gene expression that persists, at least in part, for up to 24 wk after discontinuation of therapy.

**MATERIALS AND METHODS**

Animals. Both SHR and Wistar-Kyoto (WKY) rat strains were obtained from the Shanghai Institute of Hypertension and bred in our laboratory. Rats were kept in constant temperature (22 ± 3°C) and humidity (60 ± 5%) with a 12:12-h light-dark cycle. Water and standard laboratory rat chow were provided ad libitum throughout the breeding phase, and male and female SHR (20–24 wk old) were placed together in a cage and given captopril (Sino-American Shanghai Squibb Pharmaceutical) in a mixture with a small amount of milk powder at a dose of 100 mg·kg⁻¹·day⁻¹ (combined weight). Female SHR were maintained on this dose throughout pregnancy and lactation. Weaned male pups were maintained on the same dose until 16 wk of age when treatment was stopped. Age-matched male SHR and WKY rat controls were given only milk powder.

Systolic blood pressure was measured by tail-cuff sphygmomanometry in conscious animals. Ambient temperature was maintained at 30°C. The animals were acclimated to the restraining cages and the tail-cuff apparatus before blood pressure was determined. Blood pressure measurements were made at 6-wk intervals after drug withdrawal. Three separate determinations of blood pressure were made over a 10-min interval (i.e., 3 determinations separated by 2 5-min rest periods). The three determinations were averaged to provide each measurement recorded. At the termination of the experiment (16 and 40 wk of age) rats were weighed and euthanized. Organs were quickly excised; left ventricles were blotted dry and weighed.

RNA preparation and analysis. Both kidneys and left ventricles were removed and homogenized with a Polytron Tissuemizer at 4°C. Total RNA was extracted from the homogenates using the acid guanidinium thiocyanate-phenol-chloroform method (4). Sixty micrograms of total RNA was separated by electrophoresis on 1% agarose and transferred to nylon membranes by capillary blotting. The quality of the RNA was verified by ethidium bromide staining before and after blotting. Sample concentrates was determined using the Coomassie protein reagent (Pierce Biochemicals). Equal amounts of protein (200 µg/sample) were resolved by denaturing polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Nonspecific binding sites were blocked with 5% nonfat milk powder in PBS-T (15 mM NaH₂PO₄, 80 mM Na₂HPO₄, 1.5 mM NaCl, pH 7.5; 0.5% Tween 20) for 1 h at room temperature. Membranes were incubated with a mouse monoclonal antibody directed against c-Myc (dilution 1:300; Santa Cruz Biotechnology) or a rabbit polyclonal antiserum directed against c-Fos (dilution 1:400; Santa Cruz Biotechnology) overnight at 4°C. Blots were washed with PBS-T and incubated with biotinylated anti-mouse or anti-rabbit IgG followed by streptavidin-horseradish peroxidase complex. Membranes were then washed with PBS-T extensively, and individual bands were visualized using a diaminobenzidine colorimetric detection system (Bio-Rad). Signals were quantitated by laser densitometry.

Statistical analysis. Data are presented as means ± SD. Analysis of the data was performed using one-way analysis of variance with the Newman-Keuls test for significance.

**RESULTS**

Rats treated with captopril continuously from conception through the first 16 wk of postnatal life demonstrated a significant decrease in systolic blood pressure (Fig. 1). Although the level was still increased relative to the WKY control, it remained well below that of the age-matched untreated SHR (WKY: 120 ± 10 vs. SHR-captopril: 142 ± 11 mmHg, P < 0.05; SHR-captopril vs. SHR: 192 ± 14 mmHg; P < 0.01). Blood pressure rose in the SHR-captopril 24 wk after discontinuation of therapy (i.e., 40 wk of age), but levels remained intermediate between untreated, age-matched SHR and the WKY controls (WKY: 125 ± 13 vs. SHR-captopril: 171 ± 13, P < 0.01; SHR-captopril vs. SHR: 217 ± 18 mmHg, P < 0.05).

The reduction in blood pressure was accompanied by a decrease in left ventricular weight (adjusted for total body weight) as shown in Fig. 2. Left ventricular weights in the 16-wk-old, captopril-treated SHR were equivalent to those of the WKY rat and significantly diminished relative to the untreated SHR. There was a partial rebound in left ventricular weight after discontinuation of captopril treatment (see captopril-treated SHR vs. WKY rats at 40 wk), but this remained well below the untreated SHR.

The pattern of ventricular ANP gene expression paralleled that of hypertrophy. As shown in Fig. 3, captopril treatment reduced ANP mRNA levels dramatically at 16 wk of age to a level approximating that seen in the normotensive WKY rat. Cessation of ACE inhibition led to an increase in ANP transcript levels at 40
wk; however, again, levels were substantially lower than those found in the untreated, age-matched SHR.

The reduction in ANP gene expression was not uniformly seen in all ANP expressing tissues. As shown in Fig. 4, renal ANP gene expression was no different in the SHR vs. WKY rat strains and was unaffected by captopril treatment, presumably reflecting selective activation of hypertrophy in the heart vs. kidney of the SHR.

We next turned our attention to c-myc, a protooncogene that is activated early in hypertrophy of the cardiac myocyte and is thought to play a pivotal role in the cellular mechanism(s) that leads to increased myocyte growth (11, 13, 20). c-myc mRNA levels were significantly reduced in the captopril-treated SHR relative to the untreated SHR controls at 16 wk, and these levels remained suppressed 24 wk after discontinuation of therapy (Fig. 5). c-myc Transcript levels at 16 and 40 wk were, in effect, not different from levels in the normotensive WKY rats.

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The reduction in c-myc transcript levels was accompanied by a reduction in levels of the encoded protein in captopril-treated ventricular myocardium. As shown in Fig. 6, Western blot analysis of cell extracts from the...
three groups of rats at 16 wk of age showed that captopril reduced c-Myc protein in the SHR to levels found in the WKY rat. The reduction was not seen at the level of the c-fos gene transcript. As shown in Fig. 7, c-fos mRNA was readily detectable and equivalent in SHR and WKY rats. Treatment of the SHR with captopril had no effect on c-fos mRNA levels. Similar findings were obtained in Western blot analyses of c-Fos protein in these tissues (Fig. 6).

**DISCUSSION**

The findings presented here confirm and extend earlier observations (9, 25) identifying a critical period during the development and maturation of the SHR that is particularly amenable to pharmacological intervention. In the present study, administration of ACE inhibitors throughout intrauterine development and during the first 16 wk of life reduced blood pressure in a fashion that was at least partially sustainable for as long as 24 wk after the drug was discontinued.

The fall in blood pressure was accompanied by a reduction in left ventricular hypertrophy. Our studies suggest that normalization of the hypertrophic response after treatment was, if anything, more complete than the observed decrease in blood pressure.

The mechanism(s) underlying the curtailment of hypertrophy remains undefined. The fall in blood pressure seen after ACE inhibition would be expected to lead to reduced tension in the ventricular wall and, by inference, to a decrease in the "driving force" toward hypertrophy. There is also growing evidence for the involvement of locally produced autocrine/paracrine ...
factors that may contribute to cardiac hypertrophy in the setting of hemodynamic overload (2, 10, 19). Locally produced ANG II, in particular, represents a prime candidate for such a role.

ACE-dependent reductions in circulating ANG II are unlikely to account for the inhibition of cardiac hypertrophy. Elevations in circulating ANG II levels are frequently seen in situations (e.g., intravascular volume contraction) where hypertrophy is not present. However, reduction of locally produced (i.e., cardiac) ANG II levels could account for the observed inhibition. Owens (18) postulated a role for ANG II in the generation of the vascular smooth muscle hypertrophy that is seen in the SHR, independent of its pressor effects. Equally hypotensive doses of propranolol had no effect on medial hypertrophy in these animals. Similar findings were reported by Kim et al. (12) for the heart. They found that losartan, enalapril, and amlodipine each lowered blood pressure effectively in the stroke-prone SHR; however, losartan and the ACE inhibitor were much more effective in promoting regression of left ventricular hypertrophy than was amlodipine. Wahlander et al. (24), on the other hand, found that antihypertensive, but not subhypertensive, doses of either enalapril or ramipril were required to effect regression of cardiovascular changes in the SHR. This may imply that the antihypertrophic properties of the ACE inhibitors are exclusively tied to their capacity to reduce blood pressure and afterload. Alternatively, it may suggest that the antihypertensive and antihypertrophic properties (i.e., those operative directly in the heart) of the ACE inhibitors require similarly high (i.e., therapeutic) doses for maximal effectiveness. Collectively, the data point out a potential role for locally produced ANG II in the generation and/or maintenance of cardiovascular hypertrophy in the SHR.

The effects of ACE inhibition on ANP and, even more impressively, on c-myc gene expression seem clearly tied to the reduction in ventricular hypertrophy. c-myc Gene expression was reduced to close to WKY levels by ACE inhibition despite the fact that blood pressure and, by inference, ventricular afterload in these animals were intermediate between the untreated SHR and the WKY rat. Thus c-myc gene expression may prove to be even more sensitive to ACE inhibition in...
this setting than cardiac hypertrophy itself. c-fos, an early marker of hypertrophy in the myocyte (3), was neither increased in the SHR (vs. WKY) nor affected by captopril treatment. This implies that c-fos gene expression is a poor marker for myocyte growth late in hypertrophy.

This study, as well as several previous studies (9, 25), demonstrates a prolonged reduction in blood pressure and suppression of cardiac hypertrophy with early captopril treatment that persists, to some degree, after discontinuation of the drug. The etiology of this prolonged reduction in blood pressure remains incompletely understood. It does not appear to be related to the antihypertensive properties of the ACE inhibitors alone. Pre- and postnatal administration of hydralazine did not alter the structural vascular changes seen in the untreated SHR at 21 wk of age, and cessation of the drug resulted in a prompt return of hypertension (22). Freslon and Giudicelli (8) showed that treatment of SHR with captopril resulted in inhibition of cardiac hypertrophy that was sustained for up to 7 wk after cessation of the drug, whereas treatment with equivalent doses of dihydralazine had no effect. A similar lack of efficacy in modulating cardiovascular structure in the SHR has been reported for felodipine (16), again, despite an equivalent decrease in blood pressure. Harrap et al. (9) reported that the reduction in blood pressure seen after ACE inhibition in the SHR resulted from a decrease in peripheral vascular resistance without changes in circulating renin or angiotensin levels. They interpreted the fall in blood pressure as being secondary to suppression of remodeling in the vascular tree (analogous to that which occurs in the heart), perhaps reflecting reduced levels of locally generated ANG II in the vessel wall.

Amplified production of or sensitivity to circulating or locally produced ANG II during the relatively short developmental window identified in these studies could provide the momentum that leads inextricably to the full hypertensive phenotype in the adult SHR. By inference, ACE inhibition would result in abrogation of the structural vascular hypertrophy, which typically leads to further increments in blood pressure and, ultimately, end organ damage. It should be noted that this hypothesis remains controversial, because the correlation between a drug’s ability to suppress cardiovascular remodeling and sustain the fall in blood pressure after cessation of treatment is not absolute (5).

Alternatively, accumulation of antihypertensive factors (vs. depletion of ANG II) during the critical period could result in the sustained fall in blood pressure that is observed. O’Sullivan and Harrap (17) reported that accumulation of bradykinin after ACE inhibition could account for the sustained decrease in blood pressure and amelioration of the structural changes found in the heart and vasculature of the SHR.

In summary, ACE inhibition during a relatively short period of development in the SHR results in long-standing reductions in blood pressure, cardiac hypertrophy, and hypertrophy-dependent gene expression. An analogous developmental window, should it exist in hypertensive humans, would represent an obvious therapeutic target because intervention for a relatively short period of time could exert long-lasting suppressant effects on both the blood pressure and its end organ sequelae.

Perspectives

This study confirms and extends earlier findings from other laboratories describing a hypotensive, anti-hypertrophic effect of ACE inhibition in the SHR. Of note, as with the prior studies, the effects of ACE inhibition persist, to some degree, after discontinuation of the drug. This study also demonstrates that expression of some hypertrophy-associated genes (e.g., ANP or c-myc) respond to ACE inhibition in a fashion that parallels the antihypertrophic effect, whereas other genes (e.g., c-fos) are seemingly unaffected by this intervention. This would imply that ACE inhibition, possibly through interference with the local cardiac RAS, has differential effects on individual components of the hypertrophic response. The data would also suggest that the duration of the effects on gene expression, similar to that on blood pressure and hypertrophy, extends well beyond the actual time of administration of the drug.

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