Early Genes Induction in Spontaneously Hypertensive Rats Left Ventricle with Angiotensin-Converting Enzyme Inhibitors but Not Hydralazine (43948)

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Abstract. **Spontaneously hypertensive rats were given an angiotensin-converting enzyme (ACE) inhibitor (benazepril or quinapril) or hydralazine and were left for up to 6 hr. To examine whether administration of antihypertensive agents affects expression of immediate early genes in left ventricular myocardium, groups of rats were sacrificed at 1, 3, and 6 hr after dosing; total RNA was extracted from left ventricular tissue and analyzed by blot hybridization technique using labeled probes for c-*myc*, c-*fos*, and GAPDH mRNA. All three antihypertensive agents reduced pressure similarly, and treatment with the two ACE inhibitors increased c-*fos* and c-*myc* mRNA expression in left ventriculum. By contrast, hydralazine did not increase steady-state mRNA expression of either proto-oncogene. Thus, in parallel with the pressure fall, acute administration of the ACE inhibitors induced expression of c-*fos* and c-*myc* mRNAs in the left ventricle. Since the equidepressor dose of hydralazine did not affect expression of these proto-oncogenes, this effect of ACE inhibitors is independent of their hemodynamic action.** [P.S.E.B.M. 1995, Vol 210]

Hypertensive left ventricular hypertrophy (LVH) is an adaptive response to sustained increase in afterload (1). Its development is an active process which involves changes in gene expression and cardiac protein composition of the myocardium and its interstitium (2, 3). However, it remains unresolved as to whether LVH reversal is also an active process that involves changes in expression of cardiac genes or merely represents disuse muscle atrophy in response to reduction in arterial pressure. Many studies have shown that all antihypertensive drugs reverse LVH when given for sufficient time (1, 4, 5), although they differ greatly, even within the same class, in their ability to reduce LVH within short period of time (6–9). Such variability in early responses relate to differences in ventricular mass, collagen content, coronary hemodynamics, and myocardial function. This study was designed to determine whether angiotensin I–converting enzyme (ACE) inhibitors, which rapidly reduce LVH within 3 weeks clinically and experimentally (10, 11), can modify gene expression early in reversal of LVH. To this end, the effects of a single dose of either of two ACE inhibitors were assessed on the immediate expression of two early genes (c-*myc* and c-*fos*) in the left ventricle of spontaneously hypertensive rats (SHR). These genes are associated with every step in growth regulation from signals at the cell surface to the control of RNA transcription (12). Additionally, this early gene induction was disassociated from the concomitant reduction in arterial pressure.

Materials and Methods

Male, 19-week-old SHR, obtained from Charles River Laboratories (Wilmington, MA) were given standard rat chow (Purina, St. Louis, MO) and tap water *ad libitum*. The protocol has been approved by our institutional animal care committee.

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In the first study, systolic pressure was determined plethysmographically in all rats after their initial procurement (13); and they were then divided randomly into three groups of 12 SHRs each. The control SHR group received only the vehicle (tap water); rats of the other two groups received an ACE inhibitor, either quinapril (3 mg/kg) or benazepril (10 mg/kg), by gastric gavage. These drugs were provided by Parke Davis (Pentypool, U.K.) and Ciba-Geigy Research Institutes (Summit, NJ), respectively. Doses of each agent were employed that reduced arterial pressure to the same extent. Indirect pressure measurements, obtained at least in triplicate, were repeated 3 and 6 hr after each rat received their respective treatment. At these time intervals, six rats from each group were sacrificed with an overdose of pentobarbital (100 mg/kg). Their hearts were removed immediately, washed in cold, buffered saline, and blotted dry. The atria were excised, and the right ventricular lateral wall was separated from the left ventricle and septum. Ventricular tissue was frozen instantly in liquid nitrogen and stored at −70°C until analysis. The whole procedure, from chest opening until freezing the ventricles, lasted less than 1 min.

A second series of studies was performed to determine whether proto-oncogene induction by ACE inhibitors was the consequence of ACE inhibitor pressure reduction or of a more specific and nonhemodynamic action. The effect of quinapril on early genes expression was compared with that of an equidepressor dose of hydralazine, a direct-acting smooth muscle vasodilator, and a nonhypotensive dose of quinapril. Rats were anesthetized with ether in order to place catheters (PE-50) into right carotid artery and jugular vein, which were then exteriorized at the nape of the neck. All rats were housed in restraining cages for 3 hr to recover, and arterial pressure was recorded on a physiography recorder attached to a Statham (P-23Db) strain gauge connected to the arterial catheter. The rats then received their respective treatments: the control group was given 0.3 ml of 1% NaCl solution; and the other three groups received either equihypotensive doses of quinapril (3 mg/kg) or hydralazine (1 mg/kg) or a nonhypotensive (0.3 mg/kg) dose of quinapril dissolved in 0.3 ml of saline. Preliminary experiments had shown that this quinapril dose did not affect arterial pressure, cardiac output or total peripheral resistance. Arterial pressure was monitored for the next 1 or 3 hr and, at those respective time periods, six rats from each group were sacrificed by a pentobarbital overdose. Their hearts were removed and processed as described above.

Total ventricular RNA was extracted using the acid guanidium thiocyanate phenol chloroform method (14). The ratio of the absorbance at 260/280 nm was 1.8 or more in all samples. Integrity of the RNA was ascertained by the appearance of 18S and 28S rRNA bands after agarose gel electrophoresis and ethidium bromide staining. For Northern blot analysis, 20 μg of total RNA was denatured with formaldehyde and formamide and size fractioned on 1% agarose gel (15). Transfer of RNA to nylon membranes (Gene Screen Plus, DuPont-NEN, Boston, MA) was effected by capillary blotting. Hybridization to cDNA probes for c-*fos* and c-*myc* was then performed. cDNA probes were purchased from Oncor and labeled with α-P^{32} using a nick translation kit (NEN, DuPont). All hybridizations were carried at 42°C for 18 hr in a buffer containing 25 m*M* KH_2PO_4, formamide (50%), 5× SSC, 5× Denhardt's solution, dextran (10%), SDS (1%), and salmon sperm DNA (0.5 mg/ml). After washing (2× SSC, 0.1% SDS), the membranes were autoradiographed. Kodak X-Omat AR film and intensifying screens were used. In order to check for evenness of loading and transfer, membranes were stripped and rehybridized to GAPDH probe. GAPDH oligonucleotides were purchased from Oncogene Science and 5′ end labeled using T4 polynucleotide kinase kit (Gibco, Gaithersburg, MD). Membranes were then processed as already described. Autoradiographs were analyzed densitometrically, and mRNA levels of proto-oncogeneds were normalized for GAPDH.

All data were expressed as mean ± one standard error of the mean. A two-way analysis of variance followed by Bonifferoni's modification of the *t* test was used for multiple comparisons.

Results

The effects of both ACE inhibitors and hydralazine on arterial pressure and heart rate are shown in Table I and II. Both quinapril and benazepril, given by gastric tube, significantly decreased systolic pressure for the full 6-hr follow-up period. There were no changes in heart rate. After intravenous administration, quinapril (3 mg/kg) and hydralazine (1 mg/kg) reduced pressure (Table II); and this was associated with an increased heart rate in only hydralazine-treated rats 1 hr after dosing. No changes in pressure or heart rate were observed in control rats and the SHR given the low dose of quinapril.

Three hours after either quinapril or benazepril were administered by gavage, c-*fos* and c-*myc* were expressed in the SHR left ventricle and this proto-oncogene expression decreased markedly at 6 hr (Fig. 1). Both proto-oncogenes were expressed 1 and 3 hr after intravenous administration of blood pressure–lowering dose of quinapril, but not following hydralazine or the nonhypotensive dose of quinapril (Fig. 2). Transcript levels of c-*myc* were low.

Discussion

The presented data demonstrate that the two ACE inhibitors benazepril and quinapril increased steady-

Table I. Systolic Pressure (SP) and Heart Rate (HR) in SHR Given Vehicle (Control), Quinapril, or Benazepril by Gavage

		Basal	3 hr	6 hr
Control	BP (mm Hg)	198 ± 4.8^a	207 ± 5.2	200 ± 4.9
	HR (b/min)	371 ± 16	387 ± 12	381 ± 13
Quinapril	BP	205 ± 5.2	173 ± 6.2^b	182 ± 4.2^b
(3 mg/kg)	HR	368 ± 14	377 ± 12	375 ± 11
Benazepril	BP	207 ± 4.1	175 ± 5.4^b	173 ± 5.6^b
(10 mg/kg)	HR	378 ± 15	361 ± 12	355 ± 14

^{a} Means ± 1 SEM. Six rats in each group.
^{b} $P < 0.05$.

Table II. Mean Arterial Pressure (MAP) and Heart Rate (HR) in SHR Given Vehicle (Control), Low or High dose of Quinapril, or Hydralazine Intravenously

		Basal	1 hr	3 hr
Control	MAP	172 ± 4.4^a	177 ± 3.8	170 ± 4.2
	HR	360 ± 14	373 ± 17	370 ± 11
Quinapril	MAP	180 ± 4.2	175 ± 3.6	182 ± 4.2
(0.3 mg/kg)	HR	383 ± 12	371 ± 14	385 ± 14
Quinapril	MAP	175 ± 4.6	150 ± 5.4^b	148 ± 4.8^b
(3 mg/kg)	HR	384 ± 13	388 ± 12	390 ± 11
Hydralazine	MAP	179 ± 4.2	144 ± 4.6^b	149 ± 4.7^b
(1 mg/kg)	HR	377 ± 10	437 ± 16^b	401 ± 14

^{a} Means ± 1 SEM. Six rats in each group.
^{b} $P < 0.05$.

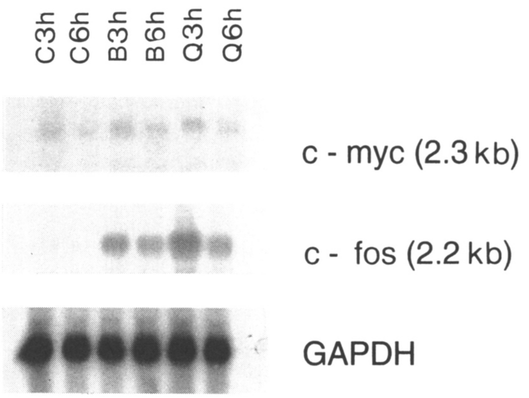

Figure 1. The effect of benazepril (B) and quinapril (Q) on the expression of c-*myc* and c-*fos* mRNA in left ventricular tissue of SHR, 3 and 6 hr after po administration. C, control rats. To check for evenness of loading, membranes were rehybridized with a glyceraldehyde 3-phosphate dehyrogenase (GAPDH) probe.

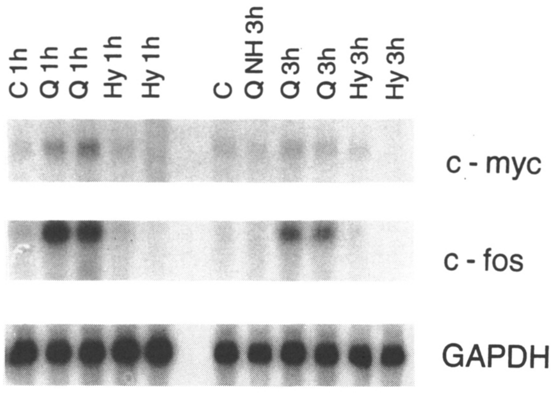

Figure 2. The effect of quinapril (Q) and hydralazine (Hy) on the expression of c-*myc* and c-*fos* mRNA in left ventricular tissue of SHR, 1 and 3 hr after iv administration. C, control rats; NH, nonhemodynamic dose. To check for evenness of loading, membranes were rehybridized with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe.

state mRNAs levels of the two proto-oncogene (c-*myc* and c-*fos*) in left ventricle, most likely due to increased gene transcription. Since an equidepressor dose of hydralazine did not affect proto-oncogene expression, it appears that the induced early genes expression by the ACE inhibitors was independent of their hypotensive action. It seems unlikely that reflex cardiac stimulation accounted for the proto-oncogene induction for two reasons: (i) ACE inhibitors do not promote reflex cardiac stimulation (16); and (ii) hydralazine, an agent that does initiate reflex cardiac stimulation (17), did not induce proto-oncogene expression. It therefore seems likely that the ACE inhibitors had a more specific cardiac effect on proto-oncogene mRNAs induction in the SHR, although the lower quinapril dose did not induce early genes expression. This failure could be explained on the basis that the small dose was ineffective.

Since angiotensin II has been shown to induce proto-oncogene expression in myocardial cells (18), it was somewhat surprising to find that two ACE inhibitors also induced immediate expression of similar proto-oncogenes. It should be noted that stimulation of early genes does not necessarily mean that the later ensuing gene expression should be identical. Furthermore, the effect of ACE inhibitors is not necessarily mediated by the renin-angiotensin system. Linz and

Schölkens have reported that a bradykinin receptor antagonist may abolish the effect of ACE inhibitors on reducing left ventricular mass in aortic-banded rats (19). Thus, it is possible that bradykinin, nitric oxide, or prostacyclin may be increased (20) and that they affected proto-oncogenes expression. Furthermore, in addition to their well-documented role as transcriptional activators, proto-oncogenes may also act as negative regulators. Thus, for example, *fos*-related proteins may inhibit expression of other genes (21–23), and *fos/jun* heterodimers (AP-1 transcription factor) may induce gene expression (24). It is also of interest to note that ACE inhibitors reduce collagen content in hypertrophied myocardium and that gene encoding interstitial collagenase, whose preferred substrates are type I and III collagen, is an oncoprotein (c-*fos*)-responsive gene (25).

Whether or not the observed induction of early genes by ACE inhibitors has a role in LVH reversal induced by ACE inhibitors still remains to be demonstrated; however, it should be noted that c-*fos* protein appears to have an important role in regulation of both collagenase and collagen genes expression (25, 26). Other responses could be induced. For example, we have recently shown that ACE inhibitors not only reduce collagen deposition in the left ventricle (27), but they will also prevent calcium antagonist–mediated collagen deposition in the right ventricle.

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