Captopril Improves Hypertension and Cardiomyopathy in Rats With Pheochromocytoma

Zhuo-wei Hu, Margaret Billingham, Michael Tuck, and Brian B. Hoffman

Hypertension and cardiomyopathy are prominent findings in humans and rats harboring pheochromocytomas, tumors that can secrete enormous quantities of catecholamines. We have previously found that \( \alpha \)- and \( \beta \)-adrenergic receptor antagonists may ameliorate the hypertension and cardiomyopathy found in New England Deaconess Hospital rats implanted with pheochromocytoma. The present studies were designed to determine the possible action of the angiotensin converting enzyme inhibitor captopril on these changes in rats harboring pheochromocytomas. Rats were implanted with transplantable pheochromocytomas and treated with captopril dissolved in the drinking water (1 mg/ml) for 4–6 weeks. Systolic blood pressure was monitored by using the tail-cuff technique. In the rats with pheochromocytoma, blood pressure progressively increased to 184±3 mm Hg after the tumor was implanted. However, in rats with pheochromocytoma treated with captopril in the drinking water before the development of hypertension, blood pressure did not increase (137±3 mm Hg). In rats with pheochromocytoma with established hypertension, captopril normalized the systolic blood pressure. Plasma norepinephrine was markedly elevated to a similar extent in both groups compared with unimplanted control rats. Plasma renin activities were slightly lower in rats with pheochromocytoma compared with unimplanted control rats. Treatment with captopril of rats with pheochromocytoma did not modify contraction of isolated rings of thoracic aorta exposed in vitro to either phenylephrine or angiotensin II. Treatment with captopril markedly attenuated the cardiomyopathy induced by pheochromocytoma. These results demonstrate that captopril prevents the development of hypertension despite markedly elevated concentrations of catecholamines. In addition, captopril attenuates catecholamine-induced cardiomyopathy in pheochromocytoma. To what extent these effects of captopril reflect actions on local renin-angiotensin systems or other mechanisms requires further study. (Hypertension 1990;15:210–215)

Pheochromocytoma is a rare but dramatic cause of human hypertension.\(^1\) These tumors typically secrete large quantities of norepinephrine. Hypertension in patients with pheochromocytoma may be severe; also, many patients with this tumor have been found to have a cardiomyopathy that is highly suggestive of lesions induced by catecholamine injections.\(^2\) Patients are usually treated with \( \alpha \)-adrenergic receptor antagonists with the subsequent addition of \( \beta \)-receptor antagonists in many cases.\(^1\)

It is generally assumed that hypertension induced by pheochromocytoma is simply due to excess activation of vascular \( \alpha \)-adrenergic receptors by the elevated concentrations of circulating catecholamines typically found in patients with this tumor. We have used New England Deaconess Hospital (NEDH) rats harboring pheochromocytoma to investigate this problem.\(^3\)–\(^5\) We found that the sympathetic nervous system plays an important role in the maintenance of hypertension in NEDH rats harboring pheochromocytoma.\(^6\) Those studies were stimulated by observations in patients with pheochromocytoma that demonstrated that the centrally acting antihypertensive drug clonidine lowered blood pressure in these individuals without changing the circulating concentrations of plasma catecholamines.\(^7\)
There have been several recent reports that angiotensin converting enzyme (ACE) inhibitors may lower blood pressure in patients with pheochromocytoma. Catecholamines regulate the secretion of renin by the kidney, but the mechanism by which ACE inhibitors might lower blood pressure in patients with pheochromocytoma is unknown. In addition, myocardial lesions induced by angiotensin have many morphological similarities with those caused by catecholamines. These similarities have led to the suggestion that the ability of angiotensin II to potentiate catecholamine release from adrenergic nerve endings may at least in part be responsible for some of the cardiac effects of angiotensin II.

The present studies were designed to determine the effect of long-term administration of the ACE inhibitor captopril on the development of hypertension and cardiomyopathy in NEDH rats implanted with pheochromocytoma.

**Methods**

**Materials**

Captopril was a generous gift from Squibb (Princeton, New Jersey). Propranolol, phenylephrine, and angiotensin II were purchased from Sigma Chemical Co. (St Louis, Missouri). All other chemicals were purchased from standard commercial sources.

**Implantation and Monitoring of the Pheochromocytoma**

Pheochromocytomas were implanted as previously described. Briefly, several pieces of pheochromocytoma taken from another NEDH rat harboring the tumor were implanted subcutaneously at the base of the neck in 8-12-week-old NEDH rats (males and females). NEDH rats are an inbred Wistar-derived strain that does not reject this tumor as would likely occur in other strains of rat. A palpable tumor was generally evident 2-4 weeks after tumor implantation. The rat's body weight served as an indication of the progression of the tumor. Tumor-bearing animals gain weight at a rate similar to unimplanted controls for several weeks after tumor implantation. By the time the tumor is palpable, the body weight generally plateaus for 4-7 days and then decreases. At the time of the plateau of body weight, tumor-bearing rats were randomly assigned to either untreated or captopril-treated groups. Captopril was dissolved in the drinking water at a final concentration of 1 mg/ml. The rats drank approximately 20 ml/day. At the time of the initiation of the treatment with captopril, the rats did not have hypertension, and preliminary experiments demonstrated the absence of cardiomyopathy at this time point. Rats were killed when a rapid weight loss was apparent, which generally occurred about 45 days after tumor implantation or about 3 weeks after initiation of the drug treatment. There were two groups of unimplanted control rats that either did or did not receive captopril.

**Measurement of Systolic Blood Pressure**

Systolic blood pressure was generally first measured starting 1 week after tumor implantation and at 5-day intervals subsequently. The tail-cuff technique was used to measure systolic blood pressure. Rats were placed in the restrainer for at least 10-15 minutes before blood pressure determinations were made. Five consecutive readings were obtained at each measurement; variations between readings usually did not exceed 10%. Heart rates were determined from the same tracings.

**Measurement of Plasma Norepinephrine and Renin**

Rats were anesthetized with an intraperitoneal injection of 5% thiopental (2.5–5 ml/kg), and blood was removed directly from the distal inferior vena cava below the level of the renal veins. Blood was placed in ice-cold tubes containing 9% ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and 6% glutathione (final pH was 6.0–7.4) (0.020 ml/ml blood). Samples were centrifuged at 1,000g for 10 minutes at 4°C and plasma frozen at −70°C before analysis. Plasma catecholamines were measured by reverse-phase high-performance liquid chromatography with electrochemical detection as previously described. Plasma renin was measured as previously described.

**Histological Evaluation of Cardiac Samples**

We have previously found that rats harboring pheochromocytomas develop a cardiomyopathy that has the following features: multifocal lesions of enhanced interstitial and replacement fibrosis, granularity of the cytoplasm and contraction band necrosis, and mixed inflammatory infiltrates. To evaluate the cardiomyopathy in the current study, rats were anesthetized as described above, hearts were removed, and one transverse section of the ventricles was placed immediately in 10% buffered formalin. The fixed cardiac tissue was later sectioned and stained with hematoxylineosin or Masson's trichrome. In those rats that died spontaneously, hearts were removed within 24 hours of death and processed as described above. Specimens were graded separately by two independent investigators in a blinded fashion by using a morphological scoring system of 0-3 as previously described. A score of 0 indicated the absence of any morphological abnormalities, whereas a score of 3 indicated lesions scattered throughout almost the entire cross-sectional area. Possible scores were in increments of 0.5 between 0 and 3 with the intermediate scores reflecting partial changes. Samples were evaluated for the presence of acute myocyte degeneration, contraction band necrosis, mixed inflammatory infiltrates, and fibrosis. Preliminary studies demonstrated that there were no differences in morphological score of hearts from pheochromocytoma rats that died spontaneously and those that were killed.
To evaluate the effects of captopril on cardiac hypertrophy induced by pheochromocytoma, morphometric measurements were made of cardiac myocyte size. Transverse measurements through the nucleus in longitudinally oriented cells were made to determine outer cell width. Cells were scanned across the free wall of each ventricle using a Microcomp morphometric system (Southern Micro Assoc., Atlanta, Georgia). Cells were measured until the coefficient of variation of cell width for each specimen decreased to less than 5%; this generally involved measuring at least 20 cells in each ventricle.

**Measurement of Vascular Reactivity**

Measurement of vascular reactivity in ring segments isolated from rats was done essentially as previously described. In these studies, rats were decapitated, and the thoracic aortas were removed and placed in a physiological buffer of the following composition (mM): NaCl 118.2, KCl 4.6, CaCl$_2$ 2.5, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, glucose 10.0, and NaHCO$_3$ 24.8, which was bubbled with 95% O$_2$ and 5% CO$_2$. Loose connective tissue and fat were removed and two 4 mm-wide ring segments were cut from each aorta. The aortic rings were mounted in muscle baths under 1 g of resting tension. After the rings had equilibrated for 90 minutes in the buffer, cumulative dose-response curves of isometric contraction were conducted with the $\alpha$-adrenergic agonist phenylephrine or with angiotensin II.

**Data Analysis**

The individual dose-response curves of vascular smooth muscle contraction were analyzed using a least-squares fit of the four parameter logistic function, which gave estimates of maximum contraction ($E_{max}$) and the dose of the drug that gave a 50% of maximum contraction ($E_{50}$). Statistical significance in these studies was determined with analysis of variance and Student's unpaired $t$ tests using geometric means, with a $p<0.05$ taken as significant. In the measurements of cardiac cell size, results were analyzed using analysis of variance and Fishers probability of the least significant difference test with the program STATVIEW II (Abacus Concepts, Berkeley, California). Results are expressed as mean±SEM.

**Results**

The systolic blood pressures of control rats and rats harboring pheochromocytoma are shown in Figure 1. As expected, the NEDH rats harboring pheochromocytoma showed a progressive rise in blood pressure several weeks after the tumor was implanted; at the end of the experiment their systolic blood pressure was 184±3 mm Hg compared with 134±2 mm Hg in normal controls ($p<0.001$). However, when rats harboring pheochromocytoma were treated chronically with captopril before hypertension developed, the blood pressure was significantly less (137±3 mm Hg) than in the untreated rats harboring pheochromocytomas ($p<0.001$). There was no significant difference in blood pressures between the normal control and the treated rats harboring pheochromocytoma ($p>0.05$). Long-term treatment of unimplanted control rats with captopril had no effect on systolic blood pressure (122±2 mm Hg) ($p>0.05$). However, long-term treatment with captopril did not attenuate the rise in heart rate that occurred in rats harboring pheochromocytoma (Figure 2). After the development of hypertension, orally administered captopril promptly decreased blood pressure from 168±3 to 136±2 mm Hg ($p<0.01$, $n=3$). Although treatment with captopril markedly attenuated the rise in blood pressure that occurred with the progression of the pheochromocytoma, captopril had no effect in these rats on the circulating concentrations of norepinephrine, which were markedly elevated compared with those of unimplanted controls (Figure 3). Plasma renin activity was somewhat lower in the rats harboring pheochromocytoma; in
both groups there was a similar, marked rise in plasma renin activity with long-term treatment with captopril (Figure 4).

Because captopril attenuated the rise in blood pressure in rats harboring pheochromocytoma without having any effect on the plasma concentration of norepinephrine, we wondered if long-term treatment of these rats with captopril might have modified vascular responsiveness to catecholamines. As shown in Table 1, phenylephrine-induced contraction was markedly desensitized in aortic ring segments from rats harboring pheochromocytoma compared with normal control rats, in agreement with our earlier findings. Treatment of rats harboring pheochromocytoma with captopril did not modify the responsiveness of aortic ring segments to the α-agonist compared with implanted rats that did not receive captopril (Table 1). Smooth muscle contraction induced by angiotensin II was also desensitized in the aortas of rats harboring pheochromocytoma compared with normal controls (Table 2) indicating that desensitization of contraction was heterologous. As in the case of phenylephrine-induced contraction, captopril treatment did not modify aortic smooth muscle responsiveness to angiotensin II (Table 2).

The effect of captopril treatment on the cardiomyopathy induced by pheochromocytoma is shown in Figure 5. In agreement with our previous results, rats harboring pheochromocytoma exhibited a cardiomyopathy characterized by multifocal areas of interstitial and replacement fibrosis, mixed inflammatory infiltrates, and contraction band necrosis. The long-term treatment with captopril markedly attenuated the development of cardiomyopathy with a cardiomyopathy score of 1.3±0.2 in the untreated rats harboring pheochromocytoma compared with 0.5±0.2 in those treated with captopril (p<0.01). The cardiomyopathy score in the unimplanted control group was 0.1±0.1 and the control treated with captopril was 0±0 (p>0.05).

Captopril also attenuated the cardiac hypertrophy induced by pheochromocytoma. In the left ventricle, myocyte width was 17.4±1.3 μM in control rats, which was less than the cell size of 22.4±0.9 μM in rats harboring pheochromocytoma (p<0.05). However, captopril prevented the development of cardiac cell enlargement; in rats treated with the drug, cardiac cell size (19.0±0.5 μM) was not different from controls but was less than untreated rats harboring pheochromocytoma with captopril.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Bar graph showing plasma renin activities in rats harboring pheochromocytoma (pheo). Plasma renin activities in untreated rats harboring pheochromocytoma was slightly lower than control New England Deaconess Hospital rats (p=0.05), and both groups had marked increases in plasma renin activity when treated with captopril (p<0.001).

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** Bar graph showing plasma renin activities in rats harboring pheochromocytoma (pheo). Plasma renin activities in untreated rats harboring pheochromocytoma was slightly lower than control New England Deaconess Hospital rats (p=0.05), and both groups had marked increases in plasma renin activity when treated with captopril (p<0.001).

**Table 1.** Responsiveness of Vascular Smooth Muscle to Phenylephrine

<table>
<thead>
<tr>
<th>Groups</th>
<th>EC_{50}</th>
<th>E_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>-7.12±0.14</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>Control+captopril (n=6)</td>
<td>-7.01±0.24</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>Pheochromocytoma (n=6)</td>
<td>-5.88±0.24*</td>
<td>0.8±0.3*</td>
</tr>
<tr>
<td>Pheochromocytoma+captopril (n=6)</td>
<td>-6.20±0.09*</td>
<td>0.9±0.3*</td>
</tr>
</tbody>
</table>

The effect of long-term treatment with captopril on responsiveness of vascular smooth muscle to phenylephrine. Aortas from both unimplanted control rats and rats harboring pheochromocytoma, captopril-treated or untreated, were prepared as described in Methods. The concentration of phenylephrine inducing 50% contraction (EC_{50}) and maximum contraction (E_{max}) was determined in each group. Captopril had no effect on the response to phenylephrine in either control or pheochromocytoma groups. n refers to the number of experiments in each group.

*Significantly different from control rats, p<0.001.

**Table 2.** Responsiveness of Vascular Smooth Muscle to Angiotensin II

<table>
<thead>
<tr>
<th>Groups</th>
<th>EC_{50}</th>
<th>E_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>-8.24±0.10</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>Control+captopril (n=6)</td>
<td>-8.45±0.04</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>Pheochromocytoma (n=6)</td>
<td>-7.13±0.20*</td>
<td>0.6±0.1*</td>
</tr>
<tr>
<td>Pheochromocytoma+captopril (n=6)</td>
<td>-7.26±0.14*</td>
<td>0.4±0.0*</td>
</tr>
</tbody>
</table>

The effect of long-term treatment with captopril on responsiveness of vascular smooth muscle to angiotensin II. Responses to angiotensin II were desensitized in aortas from rats harboring pheochromocytomas. Long-term treatment with captopril did not change the responsiveness of the desensitized aortas to angiotensin II. EC_{50} concentration inducing 50% contraction; E_{max}, concentration inducing maximum contraction.

*Significantly different from control rats, p<0.01.
boring pheochromocytoma \((p<0.05)\). Analogous results were obtained in the size of cells in the right ventricle: controls, \(15.1 \pm 0.6\) \(\mu M\); pheochromocytoma alone, \(17.6 \pm 0.9\) \(\mu M\); and pheochromocytoma treated with captopril, \(15.5 \pm 0.6\) \(\mu M\).

**Discussion**

The present study demonstrates that long-term treatment with the ACE inhibitor captopril prevented the development of hypertension in rats harboring pheochromocytoma without decreasing the markedly elevated plasma concentrations of norepinephrine found in these rats. The rats with pheochromocytoma did not have elevated plasma renin activity compared with values seen in controls. The fall in blood pressure induced by captopril could not be explained by a further decrease in aortic smooth muscle responsiveness to catecholamines in vessels that were already desensitized to catecholamines. In addition, captopril significantly decreased the degree of cardiomyopathy induced by the pheochromocytoma.

Several reports\(^{8-10}\) that ACE inhibitors lower blood pressure in patients with pheochromocytoma were a stimulus for these studies in NEDH rats harboring this tumor. Although catecholamine-induced activation of \(\beta\)-adrenergic receptors in the kidney are a well-known stimulus of renin release, there is relatively little information on plasma renin activity in patients with pheochromocytoma.\(^{17}\) These clinical reports have raised questions about the mechanism by which pheochromocytoma causes hypertension. Our results in NEDH rats suggest that these animals are a potentially useful model to investigate further the mechanism of hypertension in pheochromocytoma and the means by which captopril attenuates the rise in blood pressure in this disease. The results serve to exclude several possible effects of captopril. Firstly, captopril does not lower blood pressure by suppressing norepinephrine released by the tumor as plasma catecholamines were greatly elevated in the treated rats. In addition, it does not appear that treatment with captopril attenuates smooth muscle responses to either \(\alpha\)-adrenergic receptor–mediated or angiotensin II receptor–mediated smooth muscle contraction. An absence of a change in responsiveness to angiotensin II after treatment with captopril has been found in other experimental forms of hypertension such as renal hypertensive rats.\(^{18}\)

Captopril is an effective antihypertensive drug in many experimental models of hypertension.\(^{19}\) ACE inhibitors are well-known to inhibit the circulating endocrine renin–angiotensin system; these drugs decrease the conversion of angiotensin I to angiotensin II. However, it has become clear that the explanation for the pharmacological effects of these drugs is not simply due to the decrease in circulating concentrations of angiotensin II. For example, many tissues such as the heart, brain, and vascular smooth muscle have local systems of renin–angiotensin that may have important physiological effects and be involved in the pathophysiology of hypertension.\(^{20}\) The activity of these local renin–angiotensin systems in pheochromocytoma is not known. Furthermore, inhibition of the renin–angiotensin system by captopril may also lead to a reduction in sympathetic nervous system activity through a decrease in norepinephrine release from adrenergic nerve endings.\(^{21}\) In addition, captopril may lead to accumulation of the vasodilator bradykinin and modulate the synthesis of prostaglandin \(E_2\). It does not appear that the antihypertensive action of captopril in the NEDH rats with pheochromocytoma can be explained on the basis of a fall in circulating angiotensin II as the plasma renin activity was not elevated. Although there is some evidence that captopril may decrease the responsiveness of vascular smooth muscle to catecholamines, the present studies do not provide any evidence in support of the idea that captopril lowers blood pressure in pheochromocytoma on this basis. It is noteworthy that the fall in blood pressure occurred in the presence of very high circulating concentrations of norepinephrine suggesting the possibility that captopril enhances the action of a vasodilating factor. Alternatively, as we have previously found that sympathetic nervous system plays an important role in the maintenance of hypertension in rats harboring pheochromocytoma,\(^{6}\) it may be that effects of captopril on the autonomic nervous system are of importance in lowering blood pressure in these rats. These possibilities will require further experimental testing.

Captopril attenuates the development of the cardiomyopathy induced by pheochromocytoma. We have previously found that the \(\beta\)-adrenergic antagonist timolol largely prevents the cardiomyopathy in rats harboring pheochromocytoma and is more effective than the \(\alpha\)-adrenergic antagonist phenoxybenzamine.\(^{4}\) Those data suggest that excess \(\beta\)-adrenergic stimulation by norepinephrine plays a major role in the development of the cardiomyopathy. Because we have found that the nonspecific vasodilator hydralazine had no effect on the development of the cardiomyopathy while normalizing blood pressure in rats harboring pheochromocytoma,\(^{4}\) it is unlikely that captopril ameliorates the cardiomyopathy solely because of its antihypertensive efficacy. It is of interest to speculate whether the possible actions of
captopril on sympathetic nervous system activity might be involved in the ability of the drug to attenuate the cardiomyopathy that appears to require activation of β-adrenergic receptors. In addition, there is evidence that captopril may act as a free radical scavenger, which might also tend to ameliorate catecholamine-induced cardiomyopathy. 22

The results of this study demonstrate important effects of captopril on hypertension and cardiomyopathy in rats harboring pheochromocytoma. The mechanism by which captopril mediates these effects require additional study. NEDH rats harboring pheochromocytoma should prove to be an important model to investigate the multiplicity of effects of ACE inhibitors on the cardiovascular system.

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References


KEY WORDS • captopril • pheochromocytoma • catecholamines • cardiomyopathy
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