Angiotensin I-Converting Enzyme Inhibition Increases Cardiac Catecholamine Content and Reduces Monoamine Oxidase Activity via an Angiotensin Type 1 Receptor-Mediated Mechanism

WALTER RAASCH, TORSTEN BARTELS, ANNABELLA GIESELBERG, ANDREAS DENDORFER, and PETER DOMINIAK
Institute of Experimental and Clinical Pharmacology and Toxicology, Medical University of Lübeck, Germany
Received May 10, 2001; accepted October 17, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
Antihypertensive and cardioprotective effects of angiotensin I-converting enzyme (ACE) inhibitors are well established and have usually been attributed to the inhibition of angiotensin II (ANG)-mediated effects at vascular or ventricular (angiotensin type 1) AT1 receptors. One other important mechanism involves ANG-induced interactions with the sympathetic nervous system, which might include alterations of cardiac catecholamine concentrations during ACE inhibition due to a modulation of monoamine oxidase (MAO) activity. Tissue catecholamines were studied in spontaneously hypertensive rats that were long-term treated with captopril (50 or 0.5 mg/kg/day), enalapril (10 or 0.1 mg/kg/day), an AT1 receptor antagonist (candesartan-cilexetil, 3 mg/kg/day), or a calcium antagonist (mibefradil, 18 mg/kg/day). The kinetic parameters of MAO were then determined in vitro in the presence of ANG, captopril, enalaprilat, or candesartan. Noradrenaline and adrenaline contents were doubled in the left ventricle by captopril, enalapril, or candesartan independently of hypotensive potency but not in liver or cortex. In parallel, cardiac MAO activity was reduced by all doses of captopril (49/29%), enalapril (52/24%), or candesartan (38%). Mibefradil, which does not interact with the renin-angiotensin system, did not alter cardiac catecholamines or MAO activity when an equipotent antihypertensive dose was applied. In vitro MAO activity was not influenced by ANG, enalaprilat, or captopril at concentrations of up to 1 mM. It is concluded that diminished AT1 receptor stimulation decreases cardiac MAO activity, probably by regulating MAO expression, since ANG, ACE inhibitors, and AT1 antagonists had no effect on MAO activity in vitro. This action contributes to an increase in cardiac catecholamine content that may improve cardiac sympathetic control during therapy.

Angiotensin I-converting enzyme (ACE) inhibitors are well established in the treatment of hypertension and heart failure. They decrease angiotensin II (ANG) generation by blocking the circulating and local renin-angiotensin systems (RAS) and by preventing the degradation of bradykinin. Both mechanisms seem to be involved in the antihypertensive and cardioprotective effects. Both ANG and bradykinin interact with other neurohumoral systems such as the sympathetic system. ANG increases noradrenaline release from sympathetic nerve endings by stimulating presynaptic AT1 receptors (Brasch et al., 1993). Hence, inhibition of ANG biosynthesis by ACE inhibitors reduces the release of catecholamines (Majewski et al., 1984). In the past, many clinical trials evaluated the pronounced antihypertensive and cardioprotective effects of ACE inhibitors, and in this context, the contributions of diminished plasma noradrenaline levels to their antihypertensive and cardioprotective effects were discussed. The therapeutic significance of this action was revealed for the treatment of heart failure where a pathological sympathetic stimulation was associated with decreased cardiac noradrenaline levels (Regitz et al., 1991) due to an impairment of neuronal catecholamine uptake (Böhm et al., 1995). Kawai et al. (1999) were the first to demonstrate that cardiac noradrenaline content in heart failure is increased by ACE inhibitors due to an enhanced cardiac neuronal uptake of noradrenaline; this should reflect one of the cardioprotective mechanisms of ACE inhibitors. Similar conditions appear to exist in hypertension where ACE inhibitors improved cardiac noradrenaline uptake-1 (Raasch et al., 2001). Since ANG on its own increases MAO activity in cultured cells (Sumners et al., 1987), we hypothesized that this effect may contribute to the reduction of cardiac noradrenaline content seen in hypertension.

However, it is not clear whether ACE inhibitors exert direct actions on MAO or whether alterations of MAO activ-

ABBREVIATIONS: ACE, angiotensin I-converting enzyme; ANG, angiotensin II; RAS, renin-angiotensin systems; AT1, receptor, angiotensin type 1 receptor; MAO, monoamine oxidase; SHR, spontaneously hypertensive rats; HPLC, high-pressure liquid chromatography; bpm, beats per minute.
ity are due to the hemodynamic consequences of ACE inhibitors; alternatively, they may be dependent on alterations of plasma ANG and therefore AT$_1$ receptor-mediated. To clarify this situation, catecholamine levels and MAO activity were determined in various organs of spontaneously hypertensive rats (SHR) after chronic treatment with one of two ACE inhibitors (captopril, enalapril) or with an AT$_1$ receptor antagonist (candesartan-cilexetil). An additional group was treated with the calcium antagonist mibefradil to elucidate whether any effect on MAO might be related to blood pressure reduction or whether any effect is specific to RAS-interacting substances. SHR were used in this study, since this rat strain has been established as an appropriate animal model that displays the features of human essential hypertension. Furthermore, to detect a direct and structure-dependent influence on MAO activity in vitro, the enzyme kinetics of MAO were determined using isolated rat liver mitochondria in the presence of ANG, candesartan, or one of two ACE inhibitors differing in their structural and lipophilic properties (Raasch et al., 1999a).

**Materials and Methods**

**Reagents and Chemicals.** Candesartan, candesartan-cilexetil, enalapril, enalaprilat, and mibefradil were generous gifts from AstaZeneca GmbH (Wedel, Germany), Merck Sharp and Dohme (Munich, Germany), and Hoffmann-La Roche (Grenzach-Wyhlen, Germany), respectively. All other chemicals (HPLC or analytical grade) were obtained either from Sigma Chemie (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

**Animal Treatments.** This study was conducted according to the declaration of Helsinki, following guidelines set for the care and use of laboratory animals as adopted by the “Ministerium für Natur und Umwelt des Landes Schleswig Holstein, Deutschland”, animal protocol numbers 9/p/93 and 9/A25/98. Male SHR (Charles River, Sulzfeld, Germany) were used in all experiments. The animals were kept under controlled conditions in Makrolon (Tecniplast Deutschland GmbH, Hohenpeissenberg, Germany) plastic cages at room temperature and with a 12 h/12 h dark/light cycle. They received a standard diet and water ad libitum. SHRs were treated for a period of 3 months with either a high or low dose of captopril (2 × 25 or 2 × 0.25 mg/kg of body weight/day) or enalapril (10 or 0.1 mg/kg of body weight/day) by gavage. Controls were given an identical volume of water. A second group of animals with individual controls were treated with candesartan-cilexetil (3 mg/kg of body weight/day), mibefradil (15 mg/kg of body weight/day), or a combination of both at reduced doses (0.99 mg/kg of body weight/day). Systolic blood pressure and heart rate were determined at the beginning and end of each study by tail plethysmography.

**Determination of Catecholamines.** Endogenous catecholamines in the liver, left ventricle, cortex, brain stem, and hypothalamus were determined by HPLC and electrochemical detection. Tissues were homogenized in 10 parts of buffer (50 mM monosodium phosphate, 1.43 mM EDTA, 5.16 mM reduced glutathione, reduced form, pH 7.4). Four hundred microliters of homogenate were mixed with 50 µl of internal standard solution (10 ng/ml dihydroxybenzylamine, 30 ng/ml N-methyl-2-yldopamine), and proteins were precipitated by using 400 µl of perchloric acid (0.2 N). After incubation (20 min, 4°C), the homogenate was centrifuged (4 min, 6000 g), and 700 µl of the clear supernatant were neutralized with 80 µl of KOH (1 N). This sample was then mixed with 800 µl of buffer (1.5 M Tris, 68 mM EDTA) and 50 µl of glutathione (50 mM) before it was adsorbed to 20 mg of aluminum oxide. Catecholamines were eluted with 100 µl of perchloric acid (100 mM) and quantified by HPLC and electrochemical detection. The pellet was suspended in 0.5 N sodium hydroxide solution to allow protein determination by the Folin-Lowry method.

**Preparation of Mitochondria.** Mitochondria were prepared from various organs of untreated or treated male SHR (according to Fowler and Oreland, 1980). Tissue was coarsely minced and homogenized in 10 parts buffer (8.55 g of saccharose, 1.38 g of monosodium phosphate, 0.08 g of Tris, 0.25 g of EDTA in 100 ml) using a tissue potter homogenizer (1 min, 4°C, 800 rpm, 8 strokes), and the homogenate was centrifuged (20 min, 100g, 4°C). The resulting supernatant was recentrifuged (10 min, 20,000g, 4°C). The mitochondrial pellet was washed in buffer, recentrifuged once more, resuspended in 100 (liver, ventricles) or 60 (cortex) parts of 50 mM phosphate buffer (pH 7.2), and used immediately for the determination of MAO activity. Mitochondrial preparations from liver were frozen (~ −80°C) before analysis of MAO activity.

**Influence of Ang, Candesartan, and Various ACE Inhibitors on MAO Activity in Vitro.** MAO activity was determined as described previously (Raasch et al., 1999b) with minor modifications. Briefly, an ACE inhibitor (captopril or enalapril), the AT$_1$ antagonist candesartan, cytochrome, glutathione (reduced), mercaptopoanethol (all 1 µM–10 mM) or ANG (10 pM–1 mM) were preincubated with mitochondrial suspension (final protein content 7–20 µg/ml) in Tris-HCl buffer (0.2 M Tris; 0.1 M HCl; pH 9.2, final sample volume 0.8 ml) for 1 h at 37°C. The reaction was started by adding kynuramine (40 µM final concentration). Complete enzyme kinetics were established for captopril and enalapril (final concentrations at 0.1, 3.3, 10 mM) that were incubated with kynuramine (final concentrations at 0, 2, 5, 10, 20, 40 µM) as described above. After 20 min of incubation at 37°C, the reaction was stopped by adding 200 µl of HCl (1N). After protein precipitation in an ice bath (30 min), the mixture was centrifuged and the supernatant was injected directly into the HPLC system. Kynuramine and the enzymatically generated 4-hydroxy-quinoline were separated on a Nova-Pak C$_{18}$ stainless steel column (5 µm, 3.9 × 150 mm; Waters GmbH, Eichborn, Germany) and detected by absorption at 370 nm. The mobile phase (flow rate 1 ml min$^{-1}$) was a mixture of 500 ml of monosodium phosphate (50 mM), 50 ml of methanol, 5 ml of tetrahydrofuran, and 220 mg of tetrabutylamonium hydrogen sulfate; pH was set to 2.5. The concentrations of kynuramine and 4-hydroxy-quinoline were calculated in relation to the peak heights of external standards. Protein was resuspended in 0.5 N sodium hydroxide solution to allow protein determinations.

**Determination of ACE Activity in the Left Ventricle.** ACE activity in the left ventricle was determined according to a modified method of Carmel and Yaron (1978). Briefly, 100-µl homogenate (1.5, w/v) were incubated with ABz-Gly-p-nitro-Phe-Pro-OH (345 µM; Bachem, Heidelberg, Germany) in phosphate buffer (50 mM, pH 7.4, final volume 300 µl) for 30 min. The enzyme reaction was stopped with perchloric acid (final concentration 0.5 N), and the product (o-aminobenzoyleglycyl) was quantified in the supernatant of the centrifuged sample by HPLC [Nova-Pak C$_{18}$ column, 3.9 × 150 mm (Waters, Milford, MA); mobile phase, 20 mM monosodium phosphate, pH 7.2% methanol, pH 5.6] and fluorometric detection (excitation at 320 nm; emission at 412 nm). Enalaprilat (1 nM) was added to control samples for determining the nonspecific degradation activity, a value which was subtracted from the total activity of each sample.

**Determination of ANG in Blood.** For the determination of ANG, blood (2 ml) was collected into an inhibitor solution containing 11 µM quinapril and 1 g/l neomycin sulfate (final concentrations). After centrifugation (5000g, 10 min, 4°C), 1 ml of plasma was precipitated with 4 ml of ethanol and stored at ~ −80°C until ANG analysis. For the extraction of peptides, the ethanol supernatant was lyophilized and reconstituted in 0.1% trifluoroacetic acid. Lipids were removed with 5 ml of chloroform, and the aqueous phase was adsorbed onto 100 mg of phenylsilyl silica (Isolute PH; International Sorbent Technology, Mid Glamorgan, UK; Pellacani et al., 1994). The sorbent was washed with isotonic phosphate buffer and 10% methanol (2 ml each), and the retained peptides were eluted into 50% acetonitrile, 0.1% trifluoroacetic acid. This fraction was lyophilized and analyzed by radioimmunoassay as described previously (Den-
Cardiac Catecholamine Content and MAO Activity in Vivo. Noradrenaline content in controls was 226 ± 21 ng in total left ventricle, a figure that was approximately doubled by either dose of captopril or enalapril (Fig. 1). Moreover, ventricular adrenaline content was increased to a similar extent under both dose regimes of captopril and enalapril. In contrast, long term treatment with ACE inhibitors did not affect noradrenaline or adrenaline content in cortex, brain stem, hypothalamus, or liver (Table 1). An elevation in ventricular noradrenaline (75%) and adrenaline (92%) content was also observed under candesartan but not during mibefradil treatment (Fig. 2). However, addition of a third of the full dose of candesartan-cilexetil to a low-dose mibefradil regime had similar effects on ventricular catecholamine content compared with the full dose single treatment with candesartan-cilexetil (Fig. 2). The calculation of the myocardial catecholamine content was not normalized to tissue protein or wet weight but was instead given as the total amount detected in the left ventricles, since ACE inhibitors have been demonstrated to reduce left ventricular hypertrophy (Table 2). This more conservative estimation considers the fact that the number of cardiac adrenergic neurons is not changed during the development or regression of hypertrophy (Gerova et al., 1996).

After chronic treatment of SHR, MAO activity was significantly attenuated in left ventricles after high-dose treatment with captopril (49%) or enalapril (52%). Low doses of both ACE inhibitors also diminished MAO activity (29% and 24%), still representing a significant difference compared with controls (35.8 ± 1.1 nmol/h/mg, wet weight) and the corresponding high-dose regimes (Fig. 3). In parallel to tissue noradrenaline content, MAO activity also remained unaffected in the cortices and livers of the same animals (Table 1). Furthermore, MAO activity was diminished by candesartan (38%) and the low-dose combination of candesartan and mibefradil (21%) compared with controls (23.2 ± 0.9 nmol/h/mg, wet weight) but not by mibefradil treatment alone (Fig. 4). Since treatment with captopril or enalapril as well as with candesartan or mibefradil were performed in separate studies, comparisons were made with control groups, specifically established for these two separate study groups.

Effects on Cardiovascular Parameters. For comparison of captopril and enalapril, a first study was performed on SHR with basal body weights of 164 g, systolic blood pressures of 187 mm Hg, and heart rates of 356 bpm. Parameters determined after 3 months of treatment are listed in Table 2. Systolic blood pressure was decreased to the same extent in SHR treated with high doses of either captopril or enalapril, whereas heart rate was not influenced (Table 2). In contrast, low doses of captopril or enalapril failed to alter blood pressure compared with controls (Table 2). Left ventricular weights of SHR treated with high doses of captopril or enalapril were significantly and equally reduced (17.8 and 21.7%, respectively), and significant reductions were also seen with low-dose treatment (9.3 and 12.5%, respectively). Similarly, left ventricular ACE activity was lowered compared with controls in high-dose-treated animals and those treated with low-dose enalapril (Table 2), indicating a blood pressure-independent mechanism (Linz et al., 1989; Raasch et al., 2001).

In a second experiment, SHR were treated with candesartan-cilexetil, mibefradil, the combination of both, or water (controls). Before treatment, the rats had body weights of 279 g, systolic blood pressures of 185 mm Hg, and heart rates of 344 bpm. Parameters at the end of 3 months of treatment are listed in Table 3. All treatments uniformly and equivalently reduced systolic blood pressure over the 3-month test period, although heart rate was not affected. Left ventricular weight was reduced by candesartan (27.6%) and by the combination with mibefradil (18.7%) but not by mibefradil alone (Table 3). ACE activity in left ventricles was not influenced by mibefradil and the combination but was significantly diminished by candesartan (14%, Table 3). Plasma ACE activity did not differ between the groups. Compared with controls, circulating ANG levels were approximately 5- and 2.3-fold increased in candesartan-cilexetil- and candesartan-cilexetil/mibefradil-treated SHR, respectively, but no differences were observed in mibefradil-treated animals.

In Vitro Effects of ANG, Candesartan, and Various ACE Inhibitors on MAO Activity. The in vitro enzyme activity in cardiac mitochondria was not altered by enalapril but was increased by 10 mM captopril to 182 ± 4% of control levels (49.1 ± 1.7 nmol/h/mg of protein) and almost completely inhibited by candesartan at 10 mM (IC50 of 2.18 ±
The influence of ACE inhibitors on MAO activity and noradrenaline content in liver and different areas of the brain of SHR after 3 months of treatment with a high or low dose of captopril (50 or 0.5 mg/kg/day) or enalapril (10 or 0.1 mg/kg/day).

Values are expressed as means ± S.E.M. (n = 8–30).

<table>
<thead>
<tr>
<th>MAO activity</th>
<th>Captopril</th>
<th>Enalapril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (nmol/h/mg of protein)</td>
<td>7.12 ± 0.09</td>
<td>7.12 ± 0.16</td>
</tr>
<tr>
<td>50 mg/kg/day</td>
<td>0.5 mg/kg/day</td>
<td>10 mg/kg/day</td>
</tr>
<tr>
<td>Cortex (nmol/h/mg of protein)</td>
<td>9.84 ± 0.48</td>
<td>10.75 ± 1.20</td>
</tr>
<tr>
<td>NA</td>
<td>112.1 ± 11.0</td>
<td>149.8 ± 15.4</td>
</tr>
<tr>
<td>Liver (pg/mg of protein)</td>
<td>10.08 ± 0.28</td>
<td>10.26 ± 0.55</td>
</tr>
<tr>
<td>Cortex (ng/mg of protein)</td>
<td>18.64 ± 1.95</td>
<td>19.87 ± 0.96</td>
</tr>
<tr>
<td>Hypothalamus (ng/mg of protein)</td>
<td>22.25 ± 0.94</td>
<td>21.56 ± 1.26</td>
</tr>
<tr>
<td>Brain stem (mg/mg of protein)</td>
<td>6.05 ± 0.30</td>
<td>6.07 ± 0.41</td>
</tr>
</tbody>
</table>

NA, noradrenaline.

0.04 mM; Fig. 5A). The same influences on in vitro MAO activity were also observed in rat liver mitochondria (Fig. 5B). The two ACE inhibitors modulated MAO activity in vitro in a structure-related manner (Figs. 5B and 6A); whereas captopril (containing a sulfhydryl group) increased the \( V_{\text{max}} \) (476.5 ± 4.7 nmol/h/mg of protein) significantly to 143% of control levels (334.1 ± 5.1 nmol/h/mg of protein), enalaprilat (containing a carboxyl group) had no effect on enzyme activity (\( V_{\text{max}} \) 385.0 ± 7.8 nmol/h/mg of protein; \( K_m \) 8.45 ± 0.35 \( \mu \)M at 40 \( \mu \)M kynuramine). MAO activity was stimulated by captopril without any effect on \( K_m \) (8.70 ± 0.24 \( \mu \)M) compared with controls (7.26 ± 0.18 \( \mu \)M; Fig. 5A). Such a dose-dependent increase of \( V_{\text{max}} \) was also observed using other sulfhydryl group-containing substances such as cysteine (maximal 117% at 10 mM) and glutathione (maximal 116% at 1 mM). However, when glutathione concentrations were increased further, the MAO activity decreased significantly below control levels (Fig. 6B). Only mercaptoethanol, which was also used as a sulfhydryl group-containing reference substance, failed to influence MAO activity over the whole concentration range tested. ANG at concentrations between 10 pM and 1 mM failed to alter MAO activity (477–502 nmol/h/mg of protein) in rat liver mitochondria compared with controls (497.1 ± 6.5 nmol/h/mg of protein; not depicted).

**Discussion**

The most striking result of our study was that catecholamine levels were increased in left ventricles of SHR treated with ACE inhibitors or an AT1 receptor antagonist as a consequence of reduced cardiac MAO activity.

ACE inhibitors have been found to increase cardiac noradrenaline and adrenaline levels in hypertension (Raasch et al., 2001). The present study demonstrates similar effects for an AT1 receptor antagonist and that alterations in catecholamine tissue concentrations were restricted to the left ventricle and did not occur in other organs such as liver, cortex, hypothalamus, or the brain stem. This organ distribution is paralleled by the influence of ACE inhibitor treatment on MAO activity, since enzyme activity was dose dependently inhibited by captopril and enalapril only in the heart and not in the liver or cortex.
Under mibefradil, which served as a negative control, cardiac catecholamine content and MAO activity remained unaffected, even though blood pressure was reduced in the same way as was seen with ACE inhibitors and candesartan. The conclusion that MAO activity is reduced specifically by attenuation of ANG effects rather than hemodynamic alterations is further confirmed by the reduction of cardiac catecholamine content and MAO activity at nonantihypertensive doses. Since candesartan revealed the same diminishing effects on MAO activity as ACE inhibitors, it seems likely that endogenous ANG persistently stimulates MAO via an AT₁

**TABLE 2**
Cardiovascular parameters of SHR treated for 3 months with a high or low dose of captopril (50 or 0.5 mg/kg/day) or enalapril (10 or 0.1 mg/kg/day)

Values are expressed as means ± S.E.M. (n = 8–30).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Captopril 50 mg/kg/day</th>
<th>Captopril 0.5 mg/kg/day</th>
<th>Enalapril 10 mg/kg/day</th>
<th>Enalapril 0.1 mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Systolic blood pressure (mm Hg)</td>
<td>32.9 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-25.5 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+30.0 ± 3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-26.5 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+36.3 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Δ Heart rate (bpm)</td>
<td>16.0 ± 4.6</td>
<td>14.2 ± 7.4</td>
<td>18.1 ± 10.7</td>
<td>16.4 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>Left ventricular weight (mg)</td>
<td>943 ± 15</td>
<td>775 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>855 ± 23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>738 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>825 ± 31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACE-activity (pmol/h/mg of protein)</td>
<td>23.0 ± 0.8</td>
<td>15.8 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.9 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.9 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.9 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>a</sup> p < 0.05 vs. controls.

<sup>b</sup> p < 0.05 high vs. low dose.

<sup>c</sup> p < 0.05 vs. before treatment analysis of variance, Bonferroni’s multiple comparison test.

**TABLE 3**
Cardiovascular parameters of SHR treated for 3 months with candesartan-cilexetil (3 mg/kg/day), mibefradil (18 mg/kg/day), or a combination of both (0.9 and 9 mg/kg/day, respectively)

Values are expressed as means ± S.E.M. (n = 14–15).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Candesartan</th>
<th>Mibefradil</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Systolic blood pressure (mm Hg)</td>
<td>+14.9 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-36.7 ± 4.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-36.7 ± 4.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-28.6 ± 3.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Δ Heart rate (bpm)</td>
<td>13.1 ± 5.9</td>
<td>15.2 ± 7.1</td>
<td>6.9 ± 5.3</td>
<td>22.5 ± 7.9</td>
</tr>
<tr>
<td>Left ventricular weight (mg)</td>
<td>1025 ± 10</td>
<td>742 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>990 ± 13</td>
<td>833 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACE-activity (pmol/h/mg of protein)</td>
<td>27.2 ± 0.8</td>
<td>23.4 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.2 ± 0.7</td>
<td>27.6 ± 0.6</td>
</tr>
<tr>
<td>ANG plasma concentration (pg/ml)</td>
<td>65.5 ± 11.1</td>
<td>335.0 ± 74.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.3 ± 16.5</td>
<td>151.7 ± 28.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>a</sup> p < 0.05 vs. controls.

<sup>b</sup> p < 0.05 vs. candesartan-cilexetil.

<sup>c</sup> p < 0.05 vs. before treatment.
has to be assumed that the ANG-induced increase in MAO activity in cell culture or after rat pretreatment is due to an induction of MAO expression. This conclusion is strengthened by the fact that ANG progressively increases MAO activity during the course of incubation in neuronal cells (Sumners et al., 1987).

Only high concentrations of structurally different ACE inhibitors showed divergent effects on MAO in vitro, and such effects seem to be substance, and not substance class, specific. Whereas enalapril has no in vitro effect, captopril increases MAO activity, which may indicate an allosteric binding site. The imidazoline I2 binding site has been characterized as exactly such an allosteric site in this context (Tesson et al., 1995; Raasch et al., 1999b; Remaury et al., 2000). However, the binding of guanidine or imidazoline derivatives to this site inhibited, rather than stimulated, MAO activity (Raasch et al., 1999b), and the structure of captopril is so unrelated to these ligands that an important interaction appears unlikely. Due to the chemical structure of captopril, other sulfhydryl reagents were investigated for their ability to influence MAO activity. A slight increase in enzyme activity was observed with cysteine and reduced glutathione but not with mercaptoethanol. When cysteine concentration was increased further, the slight stimulation of MAO activity was converted into a significant inhibition of the enzyme. This inhibition points toward an interaction with flavin adenine dinucleotide or another redox active disulfide at the catalytic center of MAO (Sabin and Ramsay, 1998; Ramsay and Sabin, 1999). However, much lower plasma or tissue captopril concentrations are attained in rats (Drummer et al., 1983) compared with those in the millimolar range required to alter MAO activity in vitro. Therefore, the relevance of the described in vitro effect for the in vivo situation must be questioned.

It should be stated that the observed AT1-mediated regulation of MAO does not represent the only mechanism by which the RAS may alter cardiac catecholamine levels. First, it was recently shown that ACE inhibitors increase uptake-1 in heart failure (Kawai et al., 1999) and hypertension (Raasch et al., 2001), and second, ANG enhances catecholamine release via stimulation of presynaptic AT1 receptors (Brasch et al., 1993; Balt et al., 2001a), so that AT1 antagonists were able to diminish noradrenaline overflow in various in vitro and in vivo models (Dominiaik et al., 1987; Minatoguchi et al., 1992; Dendorfer et al., 1998; Häuser et al., 1998; Balt et al., 2001a,b). However, it should be emphasized that the reversible or irreversible MAO inhibitors viloxazine or pargyline were found to increase biogenic amines in brains and livers due to their MAO inhibitory effects, underlining the importance of MAO for regulating tissue catecholamines (Martinez et al., 1986; Raasch et al., 1999b).

In summary, our results show that cardiac catecholamine levels are doubled in SHR after chronic treatment with blood pressure effective and ineffective doses of ACE inhibitors or an AT1 antagonist. This increase in cardiac catecholamines is specific for the heart and is paralleled by a reduction of left ventricular MAO activity. It is suggested that diminished circulating and local ANG levels decrease MAO activity via an AT1-mediated mechanism, probably by regulating MAO expression. Furthermore, a direct effect of ACE inhibitors and the AT1 receptor antagonist in vivo were excluded, even though modulations of MAO activity were observed after.
using high and therapeutically irrelevant concentrations. Thus, a down-regulation of MAO induced by ACE inhibitors or AT₁ receptor antagonists may contribute to an increase in cardiac catecholamine content and consequently the improvement of cardiac sympathetic control observed with these substances.

Acknowledgments

We thank A. Kaiser for expert technical assistance and Dr. J. P. Keogh for editorial assistance in preparing the manuscript.

References


Address correspondence to: Dr. Walter Raasch, Medical University of Lübeck, Institute of Experimental and Clinical Pharmacology and Toxicology, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: raasch@medinf.mu-luebeck.de