Hypercholesterolemia Stimulates Angiotensin Peptide Synthesis and Contributes to Atherosclerosis Through the AT\textsubscript{1A} Receptor

Alan Daugherty, PhD, DSc; Debra L. Rateri, BS; Hong Lu, PhD; Tadashi Inagami, PhD; Lisa A. Cassis, PhD

Background—Hypercholesterolemia-induced atherosclerosis is attenuated by either pharmacological antagonism of AT\textsubscript{1} receptors or AT\textsubscript{1A} receptor deficiency. However, the mechanism underlying the pronounced responses to angiotensin II (Ang II) antagonism has not been determined. We hypothesized that hypercholesterolemia stimulates the production of angiotensin peptides to provide a rationale for the profound effect of AT\textsubscript{1A} receptor deficiency on atherogenesis.

Methods and Results—Atherosclerotic lesions were analyzed in LDL receptor–deficient mice. Immunocytochemical analysis demonstrated that atherosclerotic lesions contained all the components of the conventional pathway for Ang II synthesis. AT\textsubscript{1A} receptor deficiency caused a marked decrease in atherosclerotic lesion size in both the aortic root and arch of male and female mice, without a discernible effect on composition. AT\textsubscript{1A} receptor deficiency–induced reductions in atherosclerosis were independent of systolic blood pressure and measurements of oxidation and chemoattractants. Aortic AT\textsubscript{2} receptor mRNA expression was not altered in AT\textsubscript{1A} receptor–deficient mice, and AT\textsubscript{2} receptor deficiency had no effect on lesion area or cellular composition. Hypercholesterolemia greatly augmented the systemic renin-angiotensin system, as demonstrated by large increases in plasma concentrations of angiotensinogen and angiotensin peptides (Ang II, III, IV, and 4–8). These increases were ablated in hypercholesterolemic AT\textsubscript{1A} receptor–deficient mice.

Conclusions—AT\textsubscript{1A} receptor deficiency had a striking effect in reducing hypercholesterolemia-induced atherosclerosis in LDL receptor–negative mice. Hypercholesterolemia was associated with increased systemic angiotensinogen and angiotensin peptides, which were reduced in AT\textsubscript{1A} receptor–deficient mice. These results demonstrate that hypercholesterolemia-induced stimulation of angiotensin peptide production provides a basis for the marked effect of AT\textsubscript{1A} receptor deficiency in reducing atherosclerosis. (Circulation. 2004;110:3849-3857.)

Key Words: atherosclerosis ■ blood pressure ■ angiotensin ■ hypercholesterolemia

Hypercholesterolemia accelerates the development of atherosclerosis. Interactions between hypercholesterolemia and the renin-angiotensin system (RAS) in lesion formation have been suggested.\textsuperscript{1} For example, evidence demonstrates that hypercholesterolemia increases AT\textsubscript{1} receptor density\textsuperscript{2,3} and functional responsiveness.\textsuperscript{2} Moreover, ACE has been localized to atherosclerotic lesions, suggesting the capacity for local generation of angiotensin II (Ang II) in a hyperlipidemic environment.\textsuperscript{4,5}

Further evidence for a functional role of the RAS in atherosclerosis comes from a consistent literature demonstrating that inhibition of ACE reduces atherosclerotic lesions in a wide variety of experimental models. Studies using AT\textsubscript{1} receptor antagonists in atherosclerosis have not been consistent. Several studies failed to demonstrate any effect on atherosclerosis,\textsuperscript{6,7} whereas others found a decreased size of hypercholesterolemia-induced atherosclerotic lesions.\textsuperscript{8,9} Losartan has been the predominant AT\textsubscript{1} receptor blocker used in studies that demonstrate a reduction in atherosclerosis. Although an effective AT\textsubscript{1} receptor antagonist, this drug has several ancillary properties that may confound mechanistic interpretation.\textsuperscript{10} Therefore, pharmacological approaches have provided intriguing, but not compelling, evidence for a role of AT\textsubscript{1} receptors in hypercholesterolemia-induced atherosclerosis. More convincingly, a recent study demonstrated that male apolipoprotein E (apoE)–deficient mice that lack the AT\textsubscript{1A} receptor exhibit reduced atherosclerosis compared with wild-type mice.\textsuperscript{11}

There is substantial evidence that Ang II promotes the development of atherosclerosis. There are many suggestions...
for potential mechanisms by which Ang II promotes lesion formation. These mechanisms include indirect consequences of changes in blood pressure, although this does not appear to be a major factor. Potential direct mechanisms include effects on oxidative stress and enhanced inflammation mediated via elaboration of chemoattractants and adhesion molecules.

Although there is convincing evidence of a major role for Ang II in atherogenesis, no rationale has been presented for the profound effect of inhibition of RAS on hypercholesterolemia-induced atherosclerosis that forms in the absence of exogenous Ang II delivery. One area of speculation has focused on Ang II receptors. AT1 receptors have 2 subtypes of separate gene products that are called AT1A and AT1B. Deficiencies of either receptor subtype cause relatively modest phenotypes, although compound deficiency leads to retarded growth and renal defects similar to that seen in ACE- and angiotensinogen-deficient mice. A single gene encodes the AT2 receptor. Several studies have suggested that the effects of AT1 receptors oppose those of AT1 receptors. Therefore, there is the potential for enhanced AT1 receptor stimulation in AT1A receptor–negative (-/-) mice. However, the role of the AT2 receptor in hypercholesterolemia-induced atherosclerosis has not been defined. In addition, interactions between hypercholesterolemia and the RAS, which may have contributed to effects from AT1A receptor deficiency, have not been defined.

On the basis of suggestions of interactions between hypercholesterolemia and the RAS, we hypothesized that hypercholesterolemia would augment specific components of the RAS. These mechanisms were studied in LDL receptor–/- mice of both sexes. Feeding these mice a diet enriched in saturated fat leads to hypercholesterolemia associated with obesity and insulin resistance. These studies demonstrate that hypercholesterolemia greatly promotes angiotensin peptide concentrations and provide a basis for the profound effects of AT1A receptor deficiency on atherogenesis in hypercholesterolemic states.

Methods

Development of Chicken Antibodies

Antibodies were developed against mouse angiotensinogen, Ang II, and renin by use of the peptides EEEQPTTSVQPGSPE, DRVYI-HPF, and RKFYTEFDRHNNR, respectively (Aves Laboratory). Peptides were conjugated to keyhole limpet hemocyanin and injected into chickens. Purified IgY was isolated from egg yolks by affinity chromatography. The sequence used for angiotensinogen was at the carboxyl terminal and did not react with other angiotensin peptides. Conversely, the IgY against Ang II also reacted with angiotensinogen, Ang I, Ang III, Ang IV, Ang 4–8, and Ang 5–8.

Immunocytochemistry

Immunocytochemistry was performed on frozen sections, with appropriate negative controls, as described previously. Macrophages were detected with rabbit anti-mouse macrophage serum (Accurate Chemicals; AI-AD31240).

ACE was detected by use of a goat anti-mouse IgG (Santa Cruz Biotechnology; sc-12184). Angiotensinogen, Ang II, and renin were detected by use of the chicken IgYs discussed above. CD106 (vascular cell adhesion molecule 1, VCAM-1) was detected by use of a rat anti-mouse IgG (PharMingen; 01811D). Biotinylated labeled secondary antibodies were detected by use of ABC kits (Vector Laboratories) with AEC as chromogen.

Mice and Diet

C57BL/6 mice were purchased from the National Cancer Institute. LDL receptor–/- mice of either sex (B6.129S7-Ldlrtm1Her; stock no. 002207) and AT1A–/- mice (B6.129P2-Agtr1atm1Unc; stock no. 002682) were obtained from the Jackson Laboratory. AT1–/- deficient mice were obtained from Dr Inagami. All genotypes had been backcrossed 10 times into a C57BL/6 background. Littermates were selected for the studies reported in this article. All mice were maintained in a barrier facility and fed normal mouse laboratory diet.

To induce hypercholesterolemia, mice were fed a diet supplemented with fat (21% wt/wt) and cholesterol (0.15% wt/wt; Harlan Teklad; diet no. TD88137) beginning at 8 weeks of age for a total of 12 weeks. All studies were performed with the approval of the University of Kentucky Institutional Animal Care and Use Committee.

Genotyping by Polymerase Chain Reaction

AT1A receptor genotyping used the following primers: antisense, 5'-AAATGCCCTAACCCTCTCCTG-3' and sense, 5'-ATTAGGGAAAGGGAAACAGGAAGC. Resultant wild-type and deficient allele bands were 650 bp and 1.1 kb, respectively (Data Supplement Figure I). AT1 receptor genotyping used the following primers: antisense, 5'-GGATTCTCCTCTTGTGAGAC and sense, 5'-GTAAGAATATTGGAG-TTGC. Resultant wild-type and deficient allele bands were 500 bp and 1.1 kb, respectively (Data Supplement Figure I). LDL receptor genotyping used the following primers: 5'-AGGTGAGATGAGA-GGAGATC, 5'-AGGATGACTCCTCGAGCCAG, and 5'-GCA-GTGCTCCATCTGCTTG. Resultant wild-type and deficient allele bands were 383 and 800 bp, respectively.

Quantification of Atherosclerosis

Atherosclerosis was quantified both on the aortic intima and in the root as described previously.

Blood Pressure Measurements

Systolic blood pressure was measured on conscious, restrained mice by use of the Visitech (Visitech Systems) tail-cuff system as described previously.

Measurement of Serum Components

Lipids

Serum cholesterol concentrations and lipoprotein cholesterol distributions were determined as described previously.

Renin-Angiotensin System

Plasma renin concentration was measured by generation of Ang I during incubation of plasma as described previously. Plasma aldosterone concentrations were quantified by radioimmunoassay by use of a commercially available kit (Diagnostic Systems Laboratories). To quantify angiotensin peptides, plasma was collected directly into an antiproteolytic cocktail to curtail artifactual production or degradation of peptides. Plasma concentrations of angiotensin peptides were resolved by high-performance liquid chromatography (HPLC) and quantified by radioimmunoassay. The anti–Ang II IgY exhibited 100% cross-reactivity to Ang II, Ang III, and Ang IV and 80% with Ang 4–8.

Monocyte Chemotactic Protein-1

Serum monocyte chemotactic protein (MCP)-1 concentrations were quantified by ELISA (JE/MCP-1, R&D).

Oxidation Autoantibody Titers

Serum autoantibodies to malondialdehyde (MDA)-LDL were measured by use of a commercially available kit (Diagnostic Systems Laboratories). To quantify angiotensin peptides, plasma was collected directly into an antiproteolytic cocktail to curtail artifactual production or degradation of peptides. Plasma concentrations of angiotensin peptides were resolved by high-performance liquid chromatography (HPLC) and quantified by radioimmunoassay. The anti–Ang II IgY exhibited 100% cross-reactivity to Ang II, Ang III, and Ang IV and 80% with Ang 4–8.
added and incubated for 4 hours. A biotinylated anti-mouse antibody (1:200; BA-9200, Vector Laboratories), ABC (Vectastain, PK-6100), and ATBS (Sigma, A-9941) were used to detect reactivity of sera.

**mRNA Abundance of Angiotensin Receptors**

RNA was harvested from mouse aortas by use of the SV Total Isolation System (Promega). Polymerase chain reaction (PCR) was performed with 100 ng of total RNA. The following primers were used: AT$_{1A}$ sense, 5'-GACCAACTCAACCCAGAAAAGC and antisense, 5'-CGAAGCGATCTTACATAGGTG; AT$_{1B}$ sense, 5'-GCACATTAGCTAGACAGTTC and antisense, 5'-GCCCTACGAATCTTAACACAC; and AT$_{2}$ sense, 5'-CCCTTTGATAATCTCAACGCAACT and antisense, 5'-GACAACAAACAGTGAGACCACAA. The Access RT-PCR system (Promega) was used to amplify mRNA expression. The annealing temperatures used were 58°C for AT$_{1A}$ and AT$_{1B}$ and 52°C for AT$_{2}$ receptors. Expected amplicon lengths were 340, 488, and 160 bp for AT$_{1A}$, AT$_{1B}$, and AT$_{2}$ receptors, respectively. Products were resolved by agarose gel electrophoresis and visualized with ethidium bromide. Quantitative assessment of band densities was performed with Kodak Image software (Image Station, 440CF, Kodak). mRNA abundance was determined by comparison with ß-actin.

**Western Blot Analysis of Plasma Angiotensinogen**

Plasma proteins were resolved by SDS polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore). Western blot analyses were performed as described previously.29 PVDF membranes were incubated with chicken anti-mouse angiotensinogen IgY (1:200). Blots were then incubated with peroxidase-conjugated rabbit anti-chicken IgG (1:5000 dilution, Jackson Immuno-Research). Immunoreactivity was visualized with an enhanced chemiluminescence Western blotting detection kit (Pierce). Angiotensinogen protein was evaluated as the densitometric value/mean control value (C57BL/6 mouse plasma) ratio.

**Statistics**

Data were analyzed with 2-way ANOVA by use of SigmaStat. Data were tested for use of parametric or nonparametric post hoc analysis, and multiple comparisons were performed by use of Tukey tests. Values of $P<0.05$ were considered to be statistically significant. All data are represented as mean±SEM.

**Results**

Hypercholesterolemia-Induced Atherosclerotic Lesions Contain All the Components for Local Synthesis of Angiotensin Peptides

To determine whether the components required for angiotensin peptide production were present in atherosclerotic lesions,
frozen serial sections of aortic root were immunostained by use of commercially available antibodies against macrophages and ACE. In addition, sections were immunostained with newly developed chicken antibodies against angiotensinogen, renin, and angiotensin peptides (Figure 1). As noted previously in humans and monkeys, ACE immunostaining was detected in macrophage-rich regions of the core and shoulder of lesions.\textsuperscript{4} ACE was also detected on regions of the media. Interestingly, angiotensinogen, renin, and angiotensin peptides were present in macrophage-rich areas in abluminal regions. Negative controls included lack of chromogen development during incubation with the same concentrations of preimmune IgY, nonimmune IgG, or dilution of nonimmune serum (data not shown).

**Deficiency of AT\textsubscript{1A} Receptors Strikingly Reduces Atherosclerotic Lesion Size Independently of AT\textsubscript{2} Receptors**

We determined the role of AT\textsubscript{1A} and AT\textsubscript{2} receptors in hypercholesterolemia-induced atherosclerosis of LDL receptor\textsuperscript{+/−} mice fed a saturated-fat diet. Atherosclerosis was quantified on both the aortic intimal surface in the arch and in sections from the aortic root. The effect in the aortic intima was particularly striking, with a 2-fold decrease of atherosclerosis in both sexes of AT\textsubscript{1A} receptor–deficient mice (P<0.001 and P<0.004 for males and females, respectively; Figure 2A). AT\textsubscript{1A} receptor deficiency also profoundly reduced atherosclerotic lesion size throughout the aortic root in both males and females (Figure 2B). The cellular contents of the lesions were predominantly macrophages in both AT\textsubscript{1A} receptor\textsuperscript{+/−} and \textsuperscript{−/−} mice (Data Supplement Figure II).

Although there were sex-specific changes in body weight, plasma cholesterol concentrations, and lipoprotein-cholesterol distribution, these parameters were not affected by AT\textsubscript{1A} receptor genotype (Table 1). Serum titers of MDA-LDL autoantibodies, a marker of oxidative stress, were not affected by AT\textsubscript{1A} receptor genotype (Table 1). In addition, serum concentrations of the chemokine MCP-1 were not affected by AT\textsubscript{1A} receptor genotype (Table 1). VCAM-1 has been implicated in the mechanisms by which Ang II induces atherosclerosis. In mature lesions from AT\textsubscript{1A} receptor wild-type animals, VCAM-1 was present throughout the endothelium, macrophages, and smooth muscle cells underlying lesions. There was no discernible difference in the distribution of VCAM-1 protein in lesions from AT\textsubscript{1A} receptor\textsuperscript{−/−} mice (Data Supplement Figure III).

In agreement with previous studies,\textsuperscript{30} there was an increased concentration of renin in plasma from AT\textsubscript{1A} receptor–deficient mice of both sexes. However, plasma aldosterone concentration was not significantly altered in AT\textsubscript{1A} receptor–deficient mice. Moreover, although there was a trend in males, there were no significant differences in systolic blood pressure among any groups (P=0.3 for genotype; Table 1).

Absence of AT\textsubscript{1A} receptors has the potential to lead to a compensatory change in AT\textsubscript{2} receptor abundance. However, AT\textsubscript{2} receptor mRNA expression in the aorta of AT\textsubscript{1A} receptor–deficient mice was not altered. In contrast, AT\textsubscript{1B} receptor mRNA expression was increased in the aorta of AT\textsubscript{1A} receptor–deficient mice (Figure 3).

To further evaluate whether AT\textsubscript{2} receptors contributed functionally to hypercholesterolemia-induced atherosclerosis, we developed AT\textsubscript{2} receptor–deficient animals in an LDL receptor\textsuperscript{−/−} background. There was no effect of AT\textsubscript{2} receptor deficiency on the size of atherosclerotic lesions in either male or female mice (Figure 4). The absence of AT\textsubscript{2} receptors had no effect on body weight, serum cholesterol, MCP-1, plasma aldosterone, or systolic blood pressure (Table 2).

**Hypercholesterolemia Increases Plasma Angiotensinogen and Angiotensin Peptide Concentrations**

Under normolipidemic conditions, we determined plasma concentrations of total angiotensin peptides to be 116±9
pg/mL. This was predominantly in the form of Ang II, although measurable concentrations of angiotensin III, IV, and 4–8 peptides were also present, as described previously.\textsuperscript{24,31} In LDL receptor–deficient mice fed a high-saturated-fat diet, there was a highly significant increase in plasma concentrations of the sum of the detectable angiotensin peptides to 990±164 pg/mL (\(P<0.001\)). Under hyperlipidemic conditions, Ang II remained the predominant form in plasma. However, there were also significant increases in angiotensins III, IV, 4–8, and 5–8 peptides. Despite the observed elevations in plasma renin concentration in AT\(_{1A}\) receptor–deficient mice (see Table 1), the plasma concentrations of Ang II, III, and IV were decreased (Figure 5). To define mechanisms for regulation of angiotensin peptide concentrations with hypercholesterolemia and with AT\(_{1A}\) receptor deficiency, we determined relative plasma angiotensinogen concentrations by Western blotting (Figure 6). The pattern of changes in angiotensinogen concentration in plasma closely mirrored changes in plasma angiotensins, with marked elevations in plasma angiotensinogen with hypercholesterolemia that were normalized in AT\(_{1A}\) receptor–deficient mice.

### Table 1. Effects of AT\(_{1A}\) Genotype Deficiency on LDL Receptor\(^{+/−}\) Mice Fed a Fat-Supplemented Diet for 12 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
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<tr>
<td></td>
<td>(+/+, n=13)</td>
<td>(+/−, n=13)</td>
<td>(+/+, n=12)</td>
<td>(+/−, n=11)</td>
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<tr>
<td>Body weight, g</td>
<td>38.8±0.9</td>
<td>34.1±1.5</td>
<td>26.0±1.2†</td>
<td>24.1±1.0†</td>
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<td>Serum cholesterol, mg/dL</td>
<td>1282±63</td>
<td>1351±66</td>
<td>1044±66†</td>
<td>917±68†</td>
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<td>Serum MDA-LDL autoantibody titers</td>
<td>3.84±0.21</td>
<td>3.78±0.24</td>
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<td>ND</td>
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<tr>
<td>Serum MCP-1, pg/mL</td>
<td>390±44</td>
<td>296±43</td>
<td>337±47</td>
<td>313±47</td>
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<tr>
<td>Plasma renin concentra()on, ng/mL</td>
<td>0.057±0.02</td>
<td>0.131±0.02*</td>
<td>0.069±0.02</td>
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<td>Plasma aldosterone, pg/mL</td>
<td>246±22</td>
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<td>242±23</td>
<td>198±24</td>
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<td>Systolic blood pressure, mm Hg</td>
<td>116±5</td>
<td>105±4</td>
<td>119±5</td>
<td>111±5</td>
</tr>
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</table>

Values are represented as mean±SEM. ND indicates not determined.

\*\(P<0.001\) for renin concentrations comparing genotypes within sexes.

†\(P<0.001\) for cholesterol concentrations and body weight comparing sexes within genotypes.

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**Figure 3.** Abundance of AT\(_{1A}\), AT\(_{1B}\), and AT\(_{2}\) receptor mRNA in aortic tissue from AT\(_{1A}\)^{+/−} and ^{−/−} mice. Top, Examples of gel that show examples of amplicons for AT\(_{1A}\) (340 bp), AT\(_{1B}\) (488 bp), and AT\(_{2}\) (160 bp). Bottom, Quantification of mRNA abundance of AT\(_{1A}\) and AT\(_{2}\) receptor mRNA in AT\(_{1A}\)^{+/−} and ^{−/−} mice. Histobars are means of at least 6 mice, and bars are SEM. \*\(P=0.005\) for ^{+/−} compared with ^{−/−}. For AT\(_{2}\), \(P=0.212\).

**Figure 4.** AT\(_{2}\) receptor deficiency has no effect on atherosclerotic lesion development in aortic arch. Triangles represent values for individual mice, diamonds represent means, and bars are SEM. There was no statistical significance between genotypes or sexes.
Discussion

Hypercholesterolemia-Induced Atherosclerotic Lesions Contain All the Components for Local Synthesis of Angiotensin Peptides

The only protein in the classic synthetic pathway of Ang II that has been detected previously in atherosclerotic lesions is ACE.4,5,32 Using a commercially available antibody, we also demonstrated that ACE protein is widely distributed throughout mouse lesions, as described in human and monkey atherosclerosis.4,33 To detect mouse renin and angiotensinogen, we developed antibodies using peptide sequences specific to the mouse protein to enable the first description of these proteins in atherosclerotic lesions. Both renin and angiotensinogen were detected predominantly in macrophage-rich shoulder regions of atherosclerotic lesions. The epitope of the anti–Ang II IgY was common to both precur-

<table>
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<th>Table 2. Effects of AT2 Genotype Deficiency on LDL Receptor−/− Mice Fed a Fat-Supplemented Diet for 12 Weeks</th>
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<td>Male</td>
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<td>Body weight, g</td>
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<tr>
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</tr>
<tr>
<td>Serum MCP-1, pg/mL</td>
</tr>
<tr>
<td>Plasma aldosterone, ng/mL</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
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Values are represented as mean±SEM. *P<0.001 for male vs female groups. No significant differences were observed between genotypes.

Figure 5. Hypercholesterolemia increases circulating angiotensin peptides, and AT1A receptor deficiency results in reductions. Concentrations of angiotensin peptides were determined in age-matched male mice of the following genotypes of LDL receptors and AT1A receptors, respectively: +/+ (gray column), −/− × +/+ (closed column), −/− × −/− (open column). Blood was drawn and immediately placed in an antiprotease cocktail. Plasma was processed as indicated in text, and angiotensin peptides were resolved by reverse-phase HPLC. Newly developed purified IgY exhibited 100% reactivity against Ang II, III, IV, and 4–8, with more limited reactivity against 5–8. Marked elevations in Ang II and other angiotensins were observed in LDL receptor −/− compared with +/+ mice. In LDL receptor −/− mice with AT1A receptor deficiency, angiotensin peptides were decreased to concentrations of wild-type mice. Histobars represent means of plasma from 4 to 10 individual mice, and bars are SEM. **P<0.01, *P<0.001 for LDL receptor −/− × AT1A receptor −/− group compared with other genotypes.

Figure 6. Hypercholesterolemia increases plasma concentrations of angiotensinogen that are normalized in mice with AT1A receptor deficiency. Western blots were performed using a newly developed chicken IgY developed to carboxyl end of angiotensinogen. Top, Three examples per group. Bottom, Quantitative analysis of relative intensities of different glycosylated forms of angiotensinogen. Angiotensinogen was determined in age-matched male mice of the following genotypes of LDL receptors and AT1A receptors, respectively; +/+ (gray column), −/− × +/+ (closed column), −/− × −/− (open column). Histobars represent means of plasma from at least 6 individual mice and bars are SEM. *P<0.001 for LDL receptor −/− × AT1A receptor −/− group compared with other genotypes.
sors and products of Ang II. Therefore, we have referred to the immunoreactivity as “angiotensin peptides,” which were diffusely distributed throughout lesions.

Although we defined the presence of all components for the synthesis of angiotensin peptides within atherosclerotic lesions, it is not clear that they were synthesized at this locus. For angiotensinogen, renin, and ACE, there is the potential for each of these proteins to be derived from the systemic sources of liver, kidney, and lung, respectively. Future studies will determine the source of lesion RAS components in the development of atherosclerosis.

**Effects of AT\textsubscript{1A} and AT\textsubscript{2} Receptor Deficiency on Atherogenesis**

A recent study has demonstrated that AT\textsubscript{1A} receptor deficiency reduces atherosclerosis in apoE-deficient mice.\textsuperscript{11} There are potential differences in the atherogenic mechanisms between apoE\textsuperscript{−/−} and LDL receptor\textsuperscript{−/−} mice, because the latter respond to high-fat diets with obesity and diabetes.\textsuperscript{23,34} The ability of AT\textsubscript{1} receptor deficiency to decrease lesion size in these different models of atherosclerosis highlights the important role of the AT\textsubscript{1A} receptor subtype in the development of atherosclerosis. In addition, the profound effect of AT\textsubscript{1A} receptor deficiency to reduce lesion size was noted in 2 vascular beds and in both male and female mice, demonstrating that vascular location and sex do not influence AT\textsubscript{1A} receptor–mediated atherosclerosis.\textsuperscript{35}

The mouse aorta has been reported to express both the AT\textsubscript{1A} and AT\textsubscript{1B} receptors. A recent study demonstrated that AT\textsubscript{1A} receptor deficiency had no effect on Ang II–induced contractions of mouse aortic tissue.\textsuperscript{26} These responses were presumed to be caused by AT\textsubscript{1B} receptors, because AT\textsubscript{1} receptor antagonists inhibited Ang II contractions in AT\textsubscript{1A} receptor–deficient mice.\textsuperscript{36} Our results demonstrate that deficiency of AT\textsubscript{1A} receptor results in compensatory upregulation of AT\textsubscript{1B} receptor expression in the aorta. However, profound reductions in the development of atherosclerosis were observed even with increased AT\textsubscript{1B} receptor expression. Thus, the AT\textsubscript{1A} receptor appears to be the predominant receptor involved in the development of atherosclerotic lesions.

Many mechanisms have been proposed for the atherogenic effects of Ang II. One of the most prominent is increased oxidant stress.\textsuperscript{37} Although there is no generally accepted measurement of oxidant stress, titers of autoantibodies against modified LDL have been used as an indication.\textsuperscript{38} The measurement of oxidant stress, titers of autoantibodies involved in the development of atherosclerosis.

**Hypercholesterolemia Increases Plasma Angiotensinogen and Angiotensin Peptide Concentrations**

The present study defined the effect of hypercholesterolemia on plasma concentrations of angiotensin peptides. Despite the intense interest in the physiological and pathological effects of Ang II, there is a relative paucity of data on angiotensin peptide concentrations in plasma of many species, most particularly in mice. The few publications in mice show widely differing concentrations, ranging from 14 to ≈3000 pg/mL.\textsuperscript{31,44,45} The quantification of angiotensin peptides is potentially complicated by their artificial destruction and/or production after acquisition of plasma. In the present study, we used an antiproteolytic cocktail added to freshly removed blood to prevent postacquisition production or degradation of angiotensins.\textsuperscript{24} The measurement of Ang II is also complicated by the antibody recognizing an epitope that is present in many angiotensin peptides. Therefore, we used HPLC to resolve angiotensin peptides before radioimmunoassay. Use of this technique demonstrated that Ang II and IV are the major peptides in the plasma of normolipemic mice, as described previously.\textsuperscript{24} Furthermore, it demonstrates that hypercholesterolemia promotes large increases in angiotensin peptides. This increase was predominantly in Ang II, but we also observed significant increases in III, IV, and 4–8 peptides.

To define mechanisms for increases in systemic angiotensin peptide concentrations, we focused on angiotensinogen as the only known precursor to Ang II. Recent studies demonstrate that, in contrast to humans and rats, in which circulating
In addition to the role of AT1A receptors, there is evidence that AT2 receptors may also contribute to the development of atherosclerosis. AT2 receptors are found in the vascular wall and their activation has been shown to reduce blood pressure and reduce the formation of atherosclerotic plaques. However, the exact role of AT2 receptors in the development of atherosclerosis is not fully understood.

Conclusion

The present study demonstrates that hypercholesterolemia-induced atherosclerosis involves a reduction in AT1 receptors, which contributes to the development of atherosclerotic lesions. Further studies are needed to elucidate the mechanisms involved in the regulation of AT1 and AT2 receptors in the development of atherosclerosis.

Acknowledgments

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