Despite intense investigation in the last decade, many questions concerning the etiology of atherosclerosis remain unanswered. Hypercholesterolemia and hypertension are clearly risk factors for the development of vascular lesions, although the precise molecular steps between elevated lipid or blood pressure levels and the development of atherosclerotic lesions are not completely defined.\textsuperscript{1,2} Hypertension is frequently associated with additional risk factors such as hypercholesterolemia, estrogen deficiency, or hyperinsulinemia. This clustering of risk factors greatly enhances the probability to develop atherosclerosis.\textsuperscript{3} Nevertheless, the cellular events responsible for the mutual appearance of several risk factors are poorly understood. A series of recent studies have addressed the hypothesis that enhanced AT\textsubscript{1} receptor activation could explain the association of various hormonal and metabolic disorders with hypertension, and ultimately, with accelerated progression of vascular lesions.

**Regulation of AT\textsubscript{1} Receptor Expression and Function**

As early as 1980, Alexander and colleagues discovered that the vasoconstriction caused by angiotensin II in resistance vessels was variable.\textsuperscript{4} Further investigations revealed that AT\textsubscript{1} receptor activation is subject to a negative feedback, in that increased levels of angiotensin II diminish and decreased angiotensin II concentrations enhance AT\textsubscript{1} receptor activation.\textsuperscript{5–8} More recently, it has been shown that multiple agonists other than angiotensin II modulate AT\textsubscript{1} receptor expression. This phenomenon, referred to as heterologous AT\textsubscript{1} receptor regulation, is induced by various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), or fibroblast growth factor (FGF), all of which downregulate AT\textsubscript{1} receptor expression.\textsuperscript{9} Numerous other factors, including glucocorticoids, aldosterone, forskolin, TNF, cytokines, nitric oxide, insulin, LDL, estrogen, progesterone, sodium, free radicals, IGF-1, and isoprenaline are known to influence AT\textsubscript{1} receptor expression (Table).\textsuperscript{10–38}

**Hypercholesterolemia-Associated AT\textsubscript{1} Receptor Overexpression**

Hypercholesterolemia and in particular elevated LDL plasma concentrations play a fundamental role in the pathogenesis of atherosclerosis, as shown by numerous epidemiological and interventional studies.\textsuperscript{1,2} Moreover, hypercholesterolemia is frequently associated with hypertension, another potent cardiovascular risk factor.\textsuperscript{3} Despite this large body of epidemiological evidence, the molecular events leading from hypercholesterolemia to hypertension and atherosclerosis are only partially understood. Recent studies have provided insight into these interactions. For example, it has been shown that exposure of vascular smooth muscle cells to LDL markedly augments AT\textsubscript{1} receptor mRNA and protein expression.\textsuperscript{39} Consistent with these findings, the functional response of these cells on angiotensin II stimulation (eg, calcium release, cell proliferation) is also enhanced. The underlying mechanism for this upregulation of the AT\textsubscript{1} receptor was found to be stabilization of its mRNA rather than an alteration of its transcription rate. These studies in cultured cells provided a mechanism that could link hypercholesterolemia to enhanced sensitivity of the vessel wall to angiotensin II stimulation.

Subsequent studies have extended these observations to the in vivo situation. In rabbits with diet-induced atherosclerosis and in rabbits with heritable hyperlipidemia, aortic AT\textsubscript{1} receptor expression is increased by 2-fold.\textsuperscript{40,41} This has functional significance, in that angiotensin II–induced vasoconstriction is markedly increased in these animals. Interestingly, vascular superoxide production is also increased in hypercholesterolemia and this is associated with profound alteration of endothelium-dependent vasodilation (Figure 1). These abnormalities are normalized by blockade of the AT\textsubscript{1} receptor, despite the fact that blood pressure and lipoprotein levels are not changed by this treatment. Most importantly, development of atherosclerotic lesions can be greatly inhibited by AT\textsubscript{1} receptor antagonism. These findings strongly support the concept that hypercholesterolemia increases AT\textsubscript{1} receptor expression and illustrate how this phenomenon can contribute to atherosclerosis.

Subsequent work has provided evidence that hypercholesterolemia increases AT\textsubscript{1} receptor expression in humans.\textsuperscript{42} In hypercholesterolemic subjects, angiotensin II infusion produced more than twice the increase in blood pressure as that observed in normocholesterolemic subjects. In keeping with

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**Current Perspective**

**The AT\textsubscript{1}-Type Angiotensin Receptor in Oxidative Stress and Atherogenesis**

**Part II: AT\textsubscript{1} Receptor Regulation**

Georg Nickenig, MD; David G. Harrison, MD

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this finding, the expression of AT\(_1\) receptors was increased by 2- to 3-fold in hypercholesterolemic subjects. Of profound importance, treatment with statins for a 4-week period normalized the pressor response to angiotensin II infusion and completely normalized AT\(_1\) receptor expression (Figure 1). These results have been confirmed in another study of normocholesterolemic individuals\(^4\) and provide a molecular link between hyperlipidemia and hypertension. Further, these studies provide insight into why lipid lowering may have antihypertensive effects.

<table>
<thead>
<tr>
<th>Cell/Tissue</th>
<th>Agonist</th>
<th>Regulation</th>
<th>Mechanism</th>
<th>Signal Transduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSMC (rat)</td>
<td>IL (1\alpha)</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSMC (rat)</td>
<td>TNF(\alpha) + IFN(\gamma)</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSMC (rat)</td>
<td>LDL</td>
<td>↑</td>
<td>Posttranscriptional</td>
<td></td>
</tr>
<tr>
<td>VSMC (rat)</td>
<td>angiotensin II</td>
<td>↓</td>
<td>Posttranscriptional and transcriptional</td>
<td>Phenylarsine oxide</td>
</tr>
</tbody>
</table>
| VSMC (rat)  | ATP
| VSMC (rat)  | forskolin | ↓ | Posttranscriptional | PKA |
| VSMC (rat)  | forskolin
| VSMC (rat)  | isoproterenol | ↓ | Posttranscriptional | |
| VSMC (rat)  | IFN\(\gamma\) | ↓ | | |
| VSMC (rat)  | insulin | ↑ | Posttranscriptional | tyrosine kinase, calcium |
| VSMC (rat)  | estrogren progestrone | ↑ | Posttranscriptional | NO-synthase PI-3 kinase |
| VSMC (rat)  | NaCl | ↑ | | |
| VSMC (rat)  | free radicals | ↓ | Posttranscriptional | P38-MAP kinase |
| VSMC (rat)  | all-trans retinoic acid (vitamin A) | ↓ | Transcriptional | |
| VSMC (rat)  | Statins | ↓ | Posttranscriptional | Geranylgeranylation |
| VSMC (rat)  | EGF | ↓ | Posttranscriptional and transcriptional | |
| VSMC (rat)  | FGF | ↓ | | |
| VSMC (rat)  | PDGF | ↓ | | |
| Heart (rat) | dexamethasone | ↑ | | |
| Heart (rat) | deoxycorticosterone | ↓ | | |
| Cardiac fibroblasts | TNF\(\alpha\) | ↑ | | Inositol phosphate |
| Cardiac fibroblasts | IL\(1\beta\) | ↑ | | |
| Cardiac fibroblasts | IL2, IL6 | | | |
| Neuronal cells (WKY and SHR) | phorbol ester | ↑ | Transcriptional | |
| Neuronal cells (WKY and SHR) | forskolin | ↑ | | |
| Brain (mouse) | Haloperidol | ↑ | | |
| Astrocytes (rat) | GH | AT1A | ↑ | Transcriptional |
| Pituitary, brain (rat) | Estrogen | ↓ | | |
| Neuroblastoma cells (mouse) | Angiotensin II | ↓ | | |
| Pituitary, adrenal gland, uterus | Estrogen | ↓ | Posttranscriptional | |
| Bovine zona glomerulosa cells | ACTH | ↓ | | |
| Bovine zona glomerulosa cells | cAMP | ↓ | | |
| Bovine zona glomerulosa cells | IGF1 | ↑ | | |
| Bovine zona glomerulosa cells | KCl | ↑ | | |
| Bovine zona glomerulosa cells | Cortisol | ↓ | | |
| Bovine zona glomerulosa cells | Aldosterone | → | | |
| Mesangial cells (rat) | Glucose | ↓ | PKC | |
| Mesangial cells (rat) | dexamethasone | AT1A | → | |
| Mesangial cells (rat) | dexamethasone | AT1B | ↓ | |
| Placenta, trophoblast cells (human) | progestrone | ↓ | | |
| Placenta, trophoblast cells (human) | estradiol | → | | |

Various agonists regulate AT\(_1\) receptor expression in different tissues or cell types. Few studies have delineated the participating signal transduction pathways and have characterized the mechanism of regulation.
Because statin treatment is capable of reducing expression of AT1 receptors in hypercholesterolemic individuals without normalizing LDL levels, further studies have been performed in normocholesterolemic spontaneously hypertensive rats and cultured vascular smooth muscle cells. These experiments demonstrated that statins directly downregulate AT1 receptor expression in vitro as well as in vivo.44,42,45

Because statin treatment is capable of reducing expression of AT1 receptors in hypercholesterolemic individuals without normalizing LDL levels, further studies have been performed in normocholesterolemic spontaneously hypertensive rats and cultured vascular smooth muscle cells. These experiments demonstrated that statins directly downregulate AT1 receptor expression in vitro as well as in vivo.44,42,45

These findings likely have important clinical implications, in that they help to explain some of the molecular mechanisms leading to the development and progression of hypertension and atherosclerosis in hypercholesterolemia. Further, they demonstrate why statins, which primarily inhibit cholesterol biosynthesis, may have favorable effects on blood pressure regulation, and why AT1 receptor antagonists have a beneficial effect on the atherosclerotic process. Obviously, statins exert many pleiotropic, potentially blood pressure–lowering effects such as enhancement of NO bioavailability. The latter is governed by inhibition of small G-proteins and the reduced expression of caveolin-1.46,47

Importantly, other components of the renin-angiotensin system such as ACE (angiotensin converting enzyme) are also upregulated during atherosclerosis as well as hypercholesterolemia.48 In this context, high abundance of angiotensin II has also been shown in atherosclerotic plaques.49 Therefore, an enhanced production of angiotensin II would coincide with AT1 receptor upregulation leading to an increased efficacy of the renin-angiotensin system and its deleterious consequences. Of note, ACE and AT1 receptors are components of the local renin-angiotensin system in the vessel wall. Regulation of these is likely completely independent of the circulating system, which appears not to be regulated during atherosclerosis.

**Figure 2.** Principal pathways involved in regulation of AT1 receptor expression. AT1 receptor expression is modulated by angiotensin II–induced internalization of the receptor protein or by desensitization of signal transduction pathways downstream of the AT1 receptor. Furthermore, AT1 receptor gene expression is also altered at both transcriptional and posttranscriptional levels. Finally, alternative splicing may modulate AT1 receptor expression.
associated with endothelial dysfunction and increased vascular production of superoxide, and these abnormalities were normalized by either estrogen replacement or AT₁ receptor blockade. These data strongly suggest that abnormal vascular function observed in the estrogen-deficient state is at least in part due to angiotensin II and AT₁ receptor activation. Parallel studies in cultured vascular smooth muscle cells revealed that estradiol causes a time-dependent decrease in AT₁ receptor gene expression. This effect was mediated via nitric oxide release and dependent on posttranscriptional modulation of the AT₁ receptor mRNA. Estrogen-induced downregulation of AT₁ receptor expression could help to explain the association between estrogen-deficiency, hypertension, and atherosclerosis observed in many clinical studies. However, gender differences in risk factor susceptibility could be also explained by upregulation of AT₁ receptor expression and activation of the renin angiotensin system via androgens. These findings suggest that either ACE inhibitors or AT₁ receptor antagonists may be beneficial in the postmenopausal state to antagonize the effect of increased AT₁ receptor expression.

**Mechanisms of AT₁ Receptor Regulation**

Most angiotensin II effects are mediated via AT₁ receptors. Thus, the number of AT₁ receptors defines the biological efficacy of angiotensin II. There are at least 4 different aspects involved in AT₁ receptor regulation. First, activation of AT₁ receptors with angiotensin II evokes internalization of the receptor protein and reduces receptor numbers on the cell surface. Second, prolonged angiotensin II stimulation reduces angiotensin II signaling via protein kinase C-dependent pathways, an event termed desensitization. Third, alternative splicing of the AT₁ receptor pre-mRNA can alter AT₁ receptor protein translation. Fourth, and likely the most important mechanism regulating AT₁ receptors is modulation of its gene expression. These factors are summarized in Figure 2.

Alterations of AT₁ receptor gene expression are generally obvious several hours after agonist stimulation and are sustained for variable periods of time thereafter. Recently, it has been shown that internalization of the AT₁ receptor seems to regulate downregulation of AT₁ receptor mRNA. Gene expression is predominantly modulated by transcriptional and posttranscriptional mechanisms. Among other stimuli, growth factors and angiotensin II are known to reduce the rate of AT₁ receptor mRNA transcription. Although, numerous consensus sequences were discovered within the promotor region of the AT₁ receptor (eg, Ap-1, Sp1, estrogen-response element, cAMP-response element), the exact mechanisms of transcriptional control of AT₁ receptor mRNA synthesis are poorly understood.

**Posttranscriptional AT₁ Receptor Regulation**

In general, the abundance of a particular mRNA transcript and its resulting protein product is not only governed by its transcription rate, but also by its half-life (otherwise referred to as mRNA stability). Of relevance to the AT₁ receptor has been the finding that numerous agonists that regulate AT₁ receptor expression also affect posttranscriptional processing of its mRNA. Estrogens, angiotensin II, and cAMP-stimulating agents decrease AT₁ receptor expression by stimulating degradation of its mRNA. In contrast, progestrone, LDL, and insulin upregulate AT₁ receptor expression by decreasing its mRNA decay. The data concerning growth factors such as PDGF are inconsistent. It is well established that these factors reduce AT₁ receptor mRNA transcription rate. Experiments following mRNA degradation after blockade of transcription with actinomycin D revealed that growth factors induce degradation of the AT₁ receptor mRNA. Other data with recombinant, retroviral approaches have suggested that PDGF may have no effect on AT₁ receptor mRNA stability. These discrepancies are likely because of difficulties in obtaining unambiguous measures of inducible mRNA turnover. Despite these inconsis-
tencies, there is strong evidence that posttranscriptional mechanisms predominate AT₁ receptor regulation. As is the case for many other mRNAs, this process involves binding of proteins to both the 5' and 3' untranslated region of the AT₁ receptor mRNA. Data from recombinant retroviral AT₁ receptor mRNA species and experiments performed in brain tissue suggest that proteins interacting with the 5' untranslated region of the AT₁ receptor mRNA are involved in both cAMP- and estrogen-induced modulation of AT₁ receptor regulation. To date, however, the identity of these proteins remains undefined, as do the precise regions of the AT₁ receptor mRNA involved in binding these proteins. Our own data show that a family of proteins residing in the polysomal compartment bind to the 3' untranslated region of the AT₁ receptor mRNA (Figure 3). Detailed analysis revealed that a region between bases 2175 to 2195 within the AT₁ receptor mRNA, just adjacent to the poly-A-tail, is responsible for the protein-mRNA interaction. Interestingly, this AU-rich region forms a stem loop typical for a mRNA region prone to protein binding (Figure 3). Transfection experiments in vascular smooth muscle cells and in vitro decay assays within the polysomal compartment derived from vascular smooth muscle cells demonstrated that a protein binding to the bases 2175 to 2195 of the AT₁ receptor mRNA mediates AT₁ receptor regulation. However, the participating proteins remain unknown and need further clarification (Figure 3).

Signal Transduction of AT₁ Receptor Regulation

No uniform signal transduction pathway has been defined that inevitably results in modulation of AT₁ receptor expression for all agonists in all cell types. In vascular smooth muscle cells, cAMP participates in isoprenaline and possibly in angiotensin II–induced AT₁ receptor downregulation. Also, superoxide radicals and hydrogen peroxide are involved in angiotensin II–induced AT₁ receptor regulation. The p38 MAP kinase mediates radical-induced AT₁ receptor regulation, whereas p42/44 MAP kinase is presumably involved in insulin-driven AT₁ receptor overexpression. In vascular smooth muscle cells, superoxide as well as hydrogen peroxide downregulate AT₁ receptor mRNA expression mediated through posttranscriptional mechanisms. Again, transcriptional regulation seems to not be of importance. Furthermore, nitric oxide mediates estrogen-dependent AT₁ receptor downregulation, PI-3 kinase has been implicated in progesterone-caused AT₁ receptor upregulation (Table). Thus, various signal transduction pathways have been implicated; however, the detailed cascade between a cell surface impulse and the ultimate modulation of gene expression are only partially understood. Particularly, the steps immediately upstream of the described AT₁ receptor mRNA binding proteins are not known.

Perspectives

AT₁ receptor regulation very likely represents, among others, a molecular switch connecting traditional risk factors such as hypercholesterolemia, estrogen deficiency, and hyperinsulinemia with hypertension and atherosclerosis (Figure 4). From this point of view, AT₁ receptor overexpression is one potential molecular mechanism that links a variety of exogenous risk factors to cellular events in the vascular disease. Presently, it is not certain if increased activity of other local components of the renin-angiotensin system such as ACE and angiotensin II is concomitantly required, or whether AT₁ receptor overexpression itself is sufficient to propagate vascular lesion formation.
The genetic factors that predispose to atherosclerosis are still not precisely defined. It is tempting to speculate that AT_1 receptor overexpression putatively in concert with other upregulated components of the renin-angiotensin system in response to risk factors could be one of the genetically defined predispositions. Overexpression of the AT_1 receptor would likely increase the risk of atherosclerosis regardless of the underlying risk factor profile. Whether variations in the AT_1 receptor regulation occur in the population and whether they are a predictor of vascular risk needs to be evaluated in large-scale clinical studies. Inducible polymorphic proteins, which interact with the AT_1 receptor mRNA in its 3' untranslated region, could be key players in this scenario. We propose that these factors initiate upregulation or downregulation of AT_1 receptor expression via both increased or decreased AT_1 receptor mRNA decay. In this context, differences in activities or expression of the relevant RNA-binding proteins based on polymorphisms of the encoding genes could be very important as a mechanism predisposing to vascular disease. Further characterization of the molecular events related to posttranscriptional AT_1 receptor regulation and identification of these relevant factors will help to determine if these pathways contribute to the genetic susceptibility to atherosclerosis.

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