Cardiac Angiotensin II Receptors Are Upregulated by Long-Term Inhibition of Nitric Oxide Synthesis in Rats

Makoto Katoh, Kensuke Egashira, Makoto Usui, Toshihiro Ichiki, Hideharu Tomita, Hiroaki Shimokawa, Hiromi Rakugi, Akira Takeshita

Abstract—It has been shown that nitric oxide (NO) may regulate angiotensin II (Ang II) receptors in vitro. To determine whether the chronic inhibition of NO synthesis upregulates cardiac Ang II receptors in a rat model, we evaluated the in vivo effect of Nω-nitro-L-arginine methyl ester (L-NAME) on several Ang II receptors and on the expression of AT1 receptor mRNA in heart tissue. The chronic administration of L-NAME to normal rats increased the arterial blood pressure. The number of AT1 and AT2 receptors was increased, with no change in affinity, during the first week of L-NAME administration but returned to control levels after 4 weeks of treatment. The AT1 receptor mRNA was changed parallel to AT1 receptor number. Inflammatory changes (monocyte infiltration and myofibroblast formation) in perivascular areas surrounding coronary vessels and myocardial interstitial spaces were observed during the first week. The immunohistochemistry revealed that myofibroblasts expressed AT1 receptor. AT1 receptor blockade or cotreatment with L-arginine, but not cotreatment with hydralazine, prevented the L-NAME–induced increase in Ang II receptors and inflammatory changes. In conclusion, rat cardiac Ang II receptors are upregulated at an early phase of chronic inhibition of NO synthesis. This may contribute to cardiovascular inflammatory changes in an early phase and to remodeling at the later phase, which occurs after inhibition of NO synthesis. (Circ Res. 1998;83:743-751.)

Key Words: angiotensin II ■ angiotensin receptor ■ nitric oxide ■ vascular remodeling

Nitric oxide (NO), which is synthesized in the vascular endothelium, is a major mediator of endothelium-derived relaxing factor.1,2 In addition to its role in controlling vascular tone and platelet aggregation, evidence suggests that NO inhibits proliferation of blood vessels3,4 and prevents monocyte chemotaxis and adhesion.5–10 Pathological conditions associated with endothelial dysfunction such as hypertension, hypercholesterolemia, atherosclerosis, and heart failure have been demonstrated to lead to the abnormal synthesis and/or release of NO.11–14 Thus, these observations suggest that constitutively produced NO may play an important role in attenuating chronic vascular disorders such as atherosclerosis and atherosclerosis.

We15,16 and others17,18 have reported that chronic administration of Nω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, produces systemic arterial hypertension, vascular remodeling (ie, fibrosis and medial thickening) and myocardial remodeling (ie, fibrosis and hypertrophy) in animals. We also showed that long-term inhibition of NO synthesis activates cardiovascular angiotensin-converting enzyme (ACE).19,20 and that both ACE inhibitor and angiotensin II (Ang II) receptor antagonist, but not hydralazine, prevent L-NAME–induced vascular and myocardial remodeling.20 These observations support the hypothesis that a defect in endothelial NO synthesis may lead to the activation of local ACE, which may contribute to vascular and myocardial remodeling.

Ang II has been shown to cause vasoconstriction and promote cell hypertrophy, proliferation, and chemotaxis.21–24 The transcript levels of genes encoding 3 renin-angiotensin system components, angiotensinogen, ACE, and Ang II receptors, have been found to be elevated in cardiovascular tissues from animals with hypertension and left ventricular hypertrophy and/or failure.25–30 Inhibition of ACE has been found to prevent vascular and myocardial remodeling after myocardial infarction in animals and humans.31–33 These results suggest that the local renin-angiotensin system may produce cardiovascular remodeling in hypertension and left ventricular hypertrophy and/or failure.

It has been reported recently that long-term treatment of cultured vascular smooth muscle cells with NO-generating drugs decreased the number of Ang II receptors,34 which suggests that NO may regulate Ang II receptors. It has not been determined whether inhibition of NO synthesis increases the number of Ang II receptor in an in vivo model. We therefore examined the in vivo effect of chronic NO inhibition on the number of cardiac Ang II receptors and on the expression of AT1 receptor mRNA.

Materials and Methods

These experiments were reviewed and approved by the Committee on Ethics of Animal Experiments, Kyushu University Faculty of Medicine, 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan. E-mail egashira@cardiol.med.kyushu-u.ac.jp © 1998 American Heart Association, Inc.
Nitric Oxide and Angiotensin II Receptors

Medicine, and were conducted according to the Guidelines for Animal Experiments of the Kyushu University Faculty of Medicine.

Drugs

This study used L-NAME, l-arginine (Sigma Chemical Co), hydralazine (Ciba-Geigy Pharmaceutical Co), TCV116, a produg that specifically antagonizes Ang II type 1 (AT1) receptors after oral administration, CV11974, a specific Ang II type 1 (AT1) receptor antagonist (Takeda Chemical Industries Ltd), and PD123319, a specific Ang II type 2 (AT2) receptor antagonist (Parke-Davis, Warner-Lambert Co).

Animal Model of Chronic Inhibition of NO Synthesis

Twenty-week-old male Wistar-Kyoto rats were housed singly in a pyrogen-free facility and fed a normal diet. The rats were separated randomly into 5 groups. The first group (control) received untreated drinking water. The second group (L-NAME) received L-NAME in drinking water (1 mg/mL). At this concentration, the daily intake of L-NAME for the latter group was 30 to 40 mg/day. The third group (L-NAME plus hydralazine (0.12 mg/mL). The fourth group (L-NAME plus l-arginine (80 mg/mL)). The fifth group (L-NAME plus TCV116 (1 mg · kg−1· d−1)). We measured the actual volume of water drunk by each rat on a daily basis and confirmed that all animals drank 30 to 40 mL of the water containing drugs. Systolic arterial pressure and heart rate of each rat were measured on the third day, and after 1 and 4 weeks of treatment. On each of these days, some rats in each group were anesthetized with intraperitoneal pentobarbital and killed by exsanguination. The chest was opened, the heart was rapidly removed, and the atria and the great vessels were trimmed away. The heart was snap frozen in liquid nitrogen and stored at −80°C.

Radiolabeled Receptor Binding Assay

To prepare cardiac membrane fractions, heart tissues were homogenized in 0.25 mol/L sucrose, 5 mmol/L Tris (pH 7.5), 1 mmol/L MgCl2, in a Polytron. The homogenates were sedimented at 5000 g for 30 minutes at 4°C. The pellets were washed twice with incubation buffer (50 mmol/L Tris [pH 7.5]; 1 mmol/L MgCl2, by using a Brandel 24R cell-harvester (Brandel). The filter that had been presoaked in incubation buffer containing 2 mg/mL BSA, by using a Brandel 24R cell-harvester (Brandel). The reaction mixtures were rapidly incubating 200 µL of a membrane suspension (approximately 180 to 250 µg protein) with 50 µL of 125I-Sar,1 Ile-Ang II (specific activity, 2200 Ci/mmol; DuPont-New England Nuclear; final concentration, 0.1 to 3 nmol/L) and 50 µL incubation buffer for 60 minutes at 25°C. Nonspecific binding was assayed by competition with 1 µmol/L unlabeled Sar,1 Ile-Ang II (TGF-β1),36 human skeletal α-actin37 and mouse GAPDH, labeled with [32P]dCTP by a random primer labeling kit (Takara Shuzo). After hybridization and washing, autoradiography was performed with Kodak XARS film at −70°C with intensifying screens for 24 hours. Relative amounts of AT1, TGF-β1, and α-actin mRNA were normalized against GAPDH mRNA.

Histopathology and Immunohistochemistry

The hearts were perfused with oxygenated Krebs-Henseleit solution. The coronary vasculature was fixed with methacarn solution, and the tissues were dehydrated, embedded in paraffin, cut into 5-µm thick slices, and mounted on slides. For histopathology, sections were stained with Hematoxylin-eosin staining solutions. For immunohistochemistry, heart was perfused without fixation. The tissues were embedded immediately in the OCT compound, frozen, cut into 5-µm thick slices, and mounted on slides. The slices were preincubated with 3% low-fat milk to decrease nonspecific binding and incubated anti-human Ang II type 1 receptor polyclonal antibody (TGF-β1), human skeletal α-actin and mouse GAPDH, labeled with [32P]dCTP by a random primer labeling kit (Takara Shuzo). After hybridization and washing, autoradiography was performed with Kodak XARS film at −70°C with intensifying screens for 24 hours. Relative amounts of AT1, TGF-β1, and α-actin mRNA were normalized against GAPDH mRNA.

RNA Isolation and Northern Blot Analysis

Total RNA was extracted from hearts by the acid guanidinium thiocyanate-phenol-chloroform extraction method (ISOGEN, NipponGene), and Poly(A)+RNA was purified on oligo (dT)-cellulose columns (Takara Shuzo). Five micrograms of each Poly(A)+RNA sample was fractionated electrophoretically on agarose gels, transferred to nylon membranes (Hybond N+, Amersham), and immobilized by UV irradiation. The membranes were hybridized overnight with specific cDNA probes for rat AT1 receptor,36 rat transforming growth factor-β1 (TGF-β1), human skeletal α-actin,37 and mouse GAPDH, labeled with [32P]dCTP by a random primer labeling kit (Takara Shuzo). After hybridization and washing, autoradiography was performed with Kodak XARS film at −70°C with intensifying screens for 24 hours. Relative amounts of AT1, TGF-β1, and α-actin mRNA were normalized against GAPDH mRNA.

Systolic Blood Pressure and Heart Rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>Heart Rate, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>142±3</td>
<td>390±9</td>
</tr>
<tr>
<td>Day 0</td>
<td>142±4</td>
<td>390±9</td>
</tr>
<tr>
<td>Day 3</td>
<td>143±3</td>
<td>389±15</td>
</tr>
<tr>
<td>Week 1</td>
<td>141±4</td>
<td>412±7</td>
</tr>
<tr>
<td>Week 4</td>
<td>139±3</td>
<td>396±14</td>
</tr>
<tr>
<td>L-NAME (n=10)</td>
<td>Day 0 142±4</td>
<td>390±9</td>
</tr>
<tr>
<td>Day 3</td>
<td>173±8*</td>
<td>320±16†</td>
</tr>
<tr>
<td>Week 1</td>
<td>186±4†</td>
<td>341±14‡</td>
</tr>
<tr>
<td>Week 4</td>
<td>211±4†</td>
<td>356±17‡</td>
</tr>
<tr>
<td>L+Hyd (n=10)</td>
<td>Day 0 144±3</td>
<td>392±6</td>
</tr>
<tr>
<td>Day 3</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Week 1</td>
<td>145±2</td>
<td>371±13</td>
</tr>
<tr>
<td>Week 4</td>
<td>145±6</td>
<td>376±15</td>
</tr>
<tr>
<td>L+L-arg (n=10)</td>
<td>Day 0 144±3</td>
<td>392±6</td>
</tr>
<tr>
<td>Day 3</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Week 1</td>
<td>175±4†</td>
<td>349±8*‡</td>
</tr>
<tr>
<td>Week 4</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>L+AT1RA (n=10)</td>
<td>Day 0 143±3</td>
<td>400±14</td>
</tr>
<tr>
<td>Day 3</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Week 1</td>
<td>151±5</td>
<td>369±14</td>
</tr>
<tr>
<td>Week 4</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT indicates not tested.

*p<0.05; †P<0.01 vs control group; ‡P<0.05 vs day 0.
rabbit IgG (Zymed Laboratory Inc) overnight at 4°C. This AT1 antibody is prepared against synthetic peptide of human angII AT1 receptor and is shown to be specific for human AT1 receptor and to also react with rat AT1 receptor. Samples were incubated subsequently with biotinylated, affinity-purified goat anti-rabbit IgG (Nitritei), avidin-biotin, and 39,39-diaminobenzidine. Tissue samples were counterstained with hematoxylin.

To determine the cell type of the AT1 expressing cells, immunohistochemical double staining was performed. The slices were incubated with an antibody against AT1 receptor and a monoclonal antibody against aSM actin (1:500) or ED1 (1:1000) overnight at 4°C. The samples were subsequently incubated with swine anti-rabbit IgG and goat anti-mouse IgG, then with mouse alkaline phosphatase anti-alkaline phosphatase immune complex. This was followed by an incubation with rabbit peroxidase-antiperoxidase immune complex. Bound alkaline phosphatase was visualized by Fast red and levamisole to yield a red reaction product. Bound horseradish peroxidase substrate was visualized by 39,39-diaminobenzidine and hydrogen peroxide to appear brownish-black.

Statistical Analysis
Data are expressed as mean±SEM. Serial time-related changes in parameters of each group were compared by 2-way ANOVA and Bonferroni’s multiple comparison test. Differences between groups were determined by using ANOVA and a multiple comparison test. A P value ≤0.05 was considered statistically significant.

Results
Blood Pressure and Heart Rate
The L group showed a progressive rise in systolic arterial pressure. Increases in systolic arterial pressure were similar between the L and L+L-Arg groups. The systolic arterial pressure showed no significant change in the control, L+Hyd, or L+AT1RA groups. Heart rates did not change in the control, L+Hyd, and L+AT1RA groups, whereas there was a reduction in heart rate in the L and L+Arg groups (Table).

Ang II Receptor Number and AT1 Receptor mRNA Levels
125I-Ang II binding to membrane receptors was saturable in heart tissues of the control and L group rats (Figure 1A); Scatchard plot analysis of data in panel A. C, Bar graphs showing the binding of 125I-Ang II in the presence or absence of PD123319 or CV11974 in the control and L groups (n=6, both groups). *P<0.05; **P<0.01 vs control group.

Figure 1. Radiolabeled receptor binding assay. A, Binding of 125I-Sar1,Ile8-Ang II to heart membranes from a control rat (C) and from rat administered L-NAME for 3 days (L). B, Scatchard plot analysis of data in panel A. C, Bar graphs showing the binding of 125I-Ang II in the presence or absence of PD123319 or CV11974 in the control and L groups (n=6, both groups).

Figure 2. AT1 receptor transcript levels in the control and L groups. A, Autoradiograms of Northern blot hybridizations. B, Densitometric analysis of AT1 receptor RNA normalized relative to GAPDH mRNA in each sample. The normalized values in the L group were compared with those in the control group, which was assigned an arbitrary value of 1. Each bar has n=6. *P<0.05; **P<0.01 vs control group.

Figure 2. AT1 receptor transcript levels in the control and L groups. A, Autoradiograms of Northern blot hybridizations. B, Densitometric analysis of AT1 receptor RNA normalized relative to GAPDH mRNA in each sample. The normalized values in the L group were compared with those in the control group, which was assigned an arbitrary value of 1. Each bar has n=6. *P<0.05; **P<0.01 vs control group.
of both receptor subtypes was higher on day 3 and week 1 of L-NAME administration but decreased to control levels by week 4 (Figure 1C).

In concert with changes in the number of Ang II receptors in the L group, AT1 receptor mRNA increased on day 3 and week 1 of L-NAME administration but decreased to control levels by both the week 4 (Figure 2). The L-NAME–induced increases in both the number of Ang II receptors and the level of AT1 receptors mRNA were markedly attenuated by treatment with L-arginine but not by treatment with hydralazine (Figure 3). This effect, however, was not due to significant changes in receptor affinity (data not shown). The receptor number or the mRNA level was not affected by treatment with either L-arginine alone or hydralazine alone (data not shown).

Effect of Ang II AT1 Receptor Blockade on L-NAME–Induced Expression of Skeletal α-Actin and TGF-β1 mRNA

To test whether increased expression of AT1 receptor mRNA results in an enhancement of its function, we assayed the level of TGF-β1 and skeletal α-actin mRNA in L-NAME–treated rats. The levels of both transcripts were increased at week 1 (Figure 4). Treatment with the AT1 receptor antagonist, TCV116, attenuated the increases induced by L-NAME, whereas hydralazine had no effect (Figure 4). The level of TGF-β1 and skeletal α-actin mRNA or systolic arterial pressure was not affected by treatment with TCV116 alone (data not shown).

Histopathology and Immunohistochemistry

Histopathological examination revealed evidence of an inflammatory process, including infiltration of mononuclear leukocytes and fibroblast-like cells into the perivascular areas immediately surrounding the coronary arteries and myocardial interstitial spaces in the L group on day 3 or 7 of treatment (Figure 5). The areas affected by inflammatory changes declined 4 weeks after L-NAME treatment (Figure 5). In contrast, no such evidence of an inflammatory response was observed in the control group (Figure 5).

Immunoreactivity for Ang II AT1 receptor was present in the vascular media of the control and L groups (Figure 5). In contrast, AT1 receptor immunoreactivity was seen intensely...
in areas of inflammatory cell infiltration in the L group on days 3 and/or 7 (Figure 5) and reduced to the control level 4 weeks after L-NAME administration (Figure 5). No immuno-reactivity was observed in the control group or the L group when the antibody was replaced with nonimmune IgG (not shown), which served as a negative control.

Immunohistochemical staining revealed that on day 3 or 7, a considerable proportion of inflammatory cells that had infiltrated into the lesion was ED1-positive monocytes or fibroblast-like cells positive for αSM actin (myofibroblast) (Figures 5 and 6). CD3-positive lymphocytes were <10% of inflammatory cells (data not shown). By immunohistochemical double staining on day 3 or 7, AT1 receptor–positive cells were judged to be αSM actin–positive myofibroblasts (Figure 6). Few ED1-positive monocytes expressed immunoreactivity for AT1 receptor (Figure 6D and 6G). Cotreatment with the AT1 receptor antagonist or l-arginine, but not cotreatment with hydralazine, prevented the increases in inflammatory changes and AT1 receptor immunoreactivity seen in the L group on day 3 or 7 (Figure 7).

Discussion
We have demonstrated that the chronic inhibition of NO synthesis by L-NAME increases the number of Ang II receptors (both AT1 and AT2 receptors) as well as the level of AT1 receptor mRNA in rat hearts (Figures 1 and 2). Both increases were noted as early as on day 3 of L-NAME administration, suggesting that NO-deficient hypertension rapidly induces an upregulation Ang II receptors. In addition, our finding that l-arginine, but not hydralazine, reduces the effects of L-NAME (Figure 3) suggests that the inhibition of NO synthesis, but not inhibition of the arterial hypertension induced by L-NAME, is crucial in inducing the upregulation of Ang II receptors in vivo.

We have demonstrated in the present study that inhibition of NO synthesis induces cardiovascular inflammatory changes (monocyte infiltration and myofibroblast formation) during the first week of L-NAME administration. Previously, we also had reported that long-term inhibition of NO synthesis for 4 to 8 weeks activated cardiovascular tissue ACE activity and thus caused cardiovascular structural changes and ACE inhibition or Ang II AT1 receptor blockade prevented such cardiovascular structural changes. We found in the present study that AT1 receptor blockade or treatment with l-arginine, but not treatment with hydralazine, prevented the increase in inflammatory changes and AT1 receptor immunoreactivity in the lesion (Figure 7), suggesting that upregulation of local AT1 receptor also participated in the development of inflammatory changes in this model. Thus, coactivation of ACE and Ang II receptors in the inflamma-
Ang II receptors are normally present in various types of cells in the heart such as vascular smooth muscle cells, myocardial myocytes, fibroblasts, and endothelial cells. Mononuclear leukocytes also have Ang II receptors. Using immunohistochemical experiments, we found a close temporal association between the appearance of cells expressing AT1 receptor and L-NAME-induced increases in receptor number in rat hearts (Figure 5). Immunohistochemical double staining revealed that cells with AT1 receptor activity were myofibroblasts that had infiltrated into the lesion (Figure 6), suggesting that myofibroblasts in the inflammatory lesion are responsible at least in part for AT1 receptor overproduction and activity. Interestingly, cells expressing AT1 receptor in the inflammatory lesion may not be completely identical to cells expressing ACE, because we previously reported that ACE activity is increased in vascular endothelial cells and in the fibroinflammatory lesions in this model. These results are in agreement with those of Sun and Weber, who showed that the predominant cell expressing high-density Ang II receptor was myofibroblast, and the cells expressing ACE were endothelial cells, monocytes, and/or myofibroblasts at the site of myocardial infarction in rats. However, we did not exclude the possibility that our
immunohistochemical methods might have been insensitive to detect AT1 receptor overproduction in other types of cells. For example, Ang II receptors in myocardial myocytes have been shown to increase after hypertension, ventricular hypertrophy, and/or heart failure.26–28

Myofibroblasts normally do not exist in the heart. This cell type is usually transformed from interstitial fibroblasts or pericytes via TGF-β, produces extracellular matrix such as collagen, and thus is responsible for the development of tissue fibrosis.33,42 We recently have reported that the induction of TGF-β via AT1 receptor stimulation plays a major role in the pathogenesis of cardiac fibrosis in this rat model of NO inhibition43; administration of neutralizing antibody against TGF-β prevented the transcript levels of collagen and fibronectin.43 Thus, it is likely that in our in vivo model, inhibition of NO synthesis activates local renin-angiotensin system and induces monocyte infiltration, myofibroblast formation, and expression of TGF-β and thus contributes to cardiac fibrosis.

To determine whether the L-NAME–induced upregulation of Ang II receptors also leads to cardiac fibrosis and/or hypertrophy, we examined the effect of AT1 receptor blockade on changes in TGF-β1 and skeletal α-actin mRNA. Skeletal α-actin, a marker of fetal phenotype in myocardial myocytes, is known to accumulate in hypertrophied myocardial myocytes.44 TGF-β1 induces synthesis of extracellular matrix and thus is responsible for tissue fibrosis.33 Infusion of Ang II induces increases in levels of TGF-β1 and skeletal α-actin mRNA in rat hearts in vivo.44,45 Thus, our finding that the increased level of α-actin and TGF-β1 mRNA after L-NNAME administration was inhibited by AT1 receptor blockade and by treatment with L-arginine (Figure 4) suggests that inhibition of NO synthesis induced the expression of α-actin and TGF-β1 via AT1 receptor stimulation in myocardial myocytes and nonmyocytes. Because most of the effects of Ang II on cellular proliferation, hypertrophy, and chemotaxis are mediated by AT1 receptors,33 we did not examine the effects of L-NNAME on AT2 receptor activity.

Although the precise cellular mechanisms by which Ang II receptor expression is upregulated are not clear, 2 possibilities can be proposed. First, we recently have reported that NO decreases AT1 receptor expression and activity in vascular smooth muscle cells and the AT1 promoter region is responsible for the suppressive effect of NO,47 suggesting that NO may directly regulate AT1 receptor expression. Second, reactive oxygen species may mediate the expression of the Ang II receptor gene. For example, inhibition of NO synthesis has been shown to increase the production of reactive oxygen species in endothelial cells and induces the expression of inflammatory genes under the control of the transcription factor, nuclear factor-κB, in vitro and in vivo. Interestingly, nuclear factor-κB–responsive elements have been found in the promoter region of the AT1 receptor gene,48,49 which suggests that reactive oxygen species may act as intracellular second messengers in the regulation of Ang II receptor gene expression. Thus, roles of oxidative stress and/or redox-sensitive transcription factor in mediating the expression of Ang II receptors remain to be elucidated.

Because inflammatory changes in coronary vessels have been reported in animal models with genetic hypertension50,51 and renovascular hypertension,52 inflammatory changes seen in our experimental model might result at least in part from the rapid increase in systolic arterial pressure induced by L-NNAME administration. In the present study, however, the decrease in systolic loading conditions by the treatment with hydralazine did not prevent the inflammatory changes. Thus, it is unlikely that the change in systolic arterial pressure was responsible for the induction of inflammatory changes in our experimental model.

In conclusion, rat cardiac Ang II receptors are upregulated at an early phase of chronic inhibition of NO synthesis. This may contribute to cardiovascular inflammatory changes in an early phase and to remodeling at the later phase that occurs with inhibition of NO synthesis. Our present findings suggest...
that biological effects of Ang II via AT1 receptor may be enhanced when NO activity is decreased. This may be one of antiatherosclerotic actions of NO.

Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research (Nos. 07557346, 10307019, 10177226) from the Ministry of Education, Science and Culture, Tokyo; a 1996 Grant-in-Aid from Mitsukoshi Foundation, Tokyo; a Research Grant from the Suzuki Memorial Foundation, Nagoya; Ryouiti Naito Foundation for Medical Research, Osaka; and by a Research Grant from the Kanae Foundation of Research for New Medicine, Osaka, Japan.

References


Cardiac Angiotensin II Receptors Are Upregulated by Long-Term Inhibition of Nitric Oxide Synthesis in Rats

Makoto Katoh, Kensuke Egashira, Makoto Usui, Toshihiro Ichiki, Hideharu Tomita, Hiroaki Shimokawa, Hiromi Rakugi and Akira Takeshita

Circ Res. 1998;83:743-751
doi: 10.1161/01.RES.83.7.743

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/83/7/743

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/