Ecto-5’-Nucleotidase Deficiency Exacerbates Pressure-Overload–Induced Left Ventricular Hypertrophy and Dysfunction

Xin Xu, John Fassett, Xinli Hu, Guangshuo Zhu, Zhongbing Lu, Yunfang Li, Jurgen Schnermann, Robert J. Bache, Yingjie Chen

Abstract—This study examined whether endogenous extracellular adenosine acts to facilitate the adaptive response of the heart to chronic systolic overload. To examine whether endogenous extracellular adenosine can protect the heart against pressure-overload–induced heart failure, transverse aortic constriction was performed on mice deficient in extracellular adenosine production as the result of genetic deletion of CD73. Although there was no difference in left ventricular size or function between CD73-deficient mice (knockout [KO] mice) and wild-type mice under unstressed conditions, aortic constriction for 2 or 4 weeks induced significantly more myocardial hypertrophy, left ventricular dilation, and left ventricular dysfunction in KO mice compared with wild-type mice. Thus, after 2 weeks of transverse aortic constriction, left ventricular fractional shortening decreased to 27.4±2.5% and 21.9±1.7% in wild-type and KO mice, respectively (P<0.05). Consistent with a role of adenosine in reducing tissue remodeling, KO mice displayed increased myocardial fibrosis and myocyte hypertrophy compared with wild-type mice. Furthermore, adenosine treatment reduced phenylephrine-induced cardiac myocyte hypertrophy and collagen production in cultured neonatal rat cardiomyocytes and cardiac fibroblasts, respectively. Consistent with a role for adenosine in modulating cardiomyocyte hypertrophy, KO mice demonstrated increased activation of mammalian target of rapamycin signaling, accompanied by higher expression of the hypertrophy marker atrial natriuretic peptide. Conversely, the adenosine analogue 2-chloro-adenosine significantly reduced cell size, mammalian target of rapamycin/p70 ribosomal S6 kinase activation, and atrial natriuretic peptide expression in cultured neonatal cardiomyocytes. These data demonstrate that CD73 helps to preserve cardiac function during chronic systolic overload by preventing maladaptive tissue remodeling. (Hypertension. 2008;51:1557-1564.)

Key Words: hypertrophy ■ heart failure ■ fibrosis ■ 5’-nucleotidase ■ adenosine

Adenosine is a nucleoside that is released in response to stresses that increase ATP catabolism. In the heart, adenosine acts on multiple cell types through interaction with 4 different adenosine receptor subtypes.1–3 In addition to its well-known role in ischemic preconditioning,4,5 there is recent evidence that the adenosine analogue 2-chloro-adenosine (CADO) or treatment that increases endogenous adenosine levels (eg, inhibition of adenosine uptake with dipyridamole) can attenuate infarct-induced left ventricular (LV) remodeling.5 Adenosine may protect against heart failure induced by pressure overload, as administration of CADO attenuated hypertrophy, fibrosis, and heart failure in mice exposed to transverse aortic constriction (TAC).6 Increasing interstitial adenosine levels by blocking adenosine uptake using dipyridamole was also reported to reduce hypertrophy in rats exposed to pressure overload.7 The antifibrotic effects of adenosine appear to involve activation of A2b receptors,8,9 whereas a role for cardiac adenosine A1 receptors has been suggested in the adenosine-mediated reduction of cardiomyocyte hypertrophy.6 Interestingly, endogenous adenosine levels rise during compensatory hypertrophy but are diminished as hearts become decompensated.10,11 It has, thus, been suggested that modulation of adenosine levels may be a target for treatment of ventricular dysfunction in failing hearts.11 However, whether the reduction of endogenous extracellular adenosine levels can contribute to the development of heart failure in the overloaded heart is not known.

The membrane-anchored cell-surface enzyme CD73 catalyzes the conversion of extracellular AMP to adenosine, thereby increasing extracellular adenosine production.12 Darvish et al13 reported that CD73 activity accounts for ≈46% of total adenosine production in rat heart homogenates, whereas other studies also demonstrated that CD73-mediated adenosine production is critical to ischemic precon-
CD73 activity and endogenous extracellular adenosine play significant roles in the protection against systolic overload–induced ventricular hypertrophy, fibrosis, and congestive heart failure.

Materials and Methods

Mice and TAC Procedure

CD73-KO mice (129 background) and control wild-type (WT) mice were generated as described previously. This study was approved by the institutional animal care and use committee of University of Minnesota. TAC was performed using the minimally invasive suprasternal approach described.16 Detailed methodology is included in the online supplementary data.

Echocardiography and Western blots were performed with methods as described previously.17,18 For details, please see the online data supplement, available at http://hyper.ahajournals.org.

Neonatal Rat Cardiomyocyte Isolation and Culture

Neonatal rat cardiomyocytes were isolated from 2-day–old Sprague-Dawley rats by enzymatic digestion and separated from nonmuscle cells on a discontinuous Percoll gradient as described previously.19 Detailed methodology is included in the online supplementary data.

Figure 1. CD73 KO exacerbates TAC-induced ventricular hypertrophy (A and B), and pulmonary congestion (C and D), KO had no effect on TAC-induced mortality (E) but decreased CD73 activity (F). KO significantly attenuated the 5'-AMP–induced decrease of heart rate (G and H), consistent with the diminished capacity of KO mice to produce extracellular adenosine from 5'-AMP. *P<0.05 vs the corresponding control; #P<0.05 vs WT mice. F, CD73 activity was obtained from 4 to 5 mice per group. The decrease of heart rate in response to 5'-AMP infusion was obtained from KO (n=5), WT littermates of KO mice (n=5), and adenosine A1 receptor KO mice (n=3). The rest of the data were obtained from 11 to 23 mice per group as labeled.

Results

CD73-KO exacerbates TAC-induced myocardial hypertrophy, fibrosis, and dysfunction in the overloaded heart. Under basal conditions, we observed no statistical differences in LV structure or function (Figures 1 to 3 and Table) between CD73-KO mice and their WT control littermates. To examine the role of extracellular adenosine in modulating the response to systolic overload, we exposed KO mice and WT mice to TAC. In response to TAC for 2 weeks, KO mice developed significantly greater increases of ventricular weight and the ratio of ventricular weight:body weight or tibia length than WT mice (Table and Figure 1A and 1B), indicating that KO exacerbated the TAC-induced myocardial hypertrophy. In addition, the ratio of lung weight:body weight or tibia length was significantly greater in KO mice as compared with WT mice 2 weeks after TAC, indicating more pulmonary congestion in the KO mice as compared with WT mice (Figure 1C and 1D and Table). During the 2 weeks of study, the mortality rate after TAC was not different between KO mice and WT mice (Figure 1E).

As anticipated, myocardial CD73 activity was abolished in KO mice as compared with WT mice under control conditions or after 2 weeks TAC (Figure 1F). In addition, KO significantly attenuated 5'-AMP–induced bradycardia, indicating that KO significantly disrupted extracellular adenosine production from 5'-AMP (Figure 1G and 1H). Adenosine A1 receptor KO almost totally abolished 5'-AMP–induced bradycardia (Figure 1G and 1H), consistent with the concept that extracellular adenosine caused bradycardia through activation of the adenosine A1 receptor.

Histological analysis demonstrated that TAC resulted in more ventricular fibrosis (Figure 2) and a greater increase in cardiac myocyte cross-sectional area (Figure 2) in KO mice as compared with WT mice, indicating that the greater
ventricular hypertrophy in the KO mice after TAC was because of both larger cardiomyocytes and an increase of fibrosis. The fibrosis after TAC in both WT and KO mice was more apparent in the perivascular region. In comparison with WT mice, the relative increase in myocardial fibrosis after TAC in the KO mice was much greater than the relative increase in myocyte hypertrophy.

Echocardiographic imaging of the heart 2 weeks after TAC (Figure 3A) demonstrated significant increases of LV wall thickness (Table) and LV end-diastolic diameter (Figure 3). TAC for 2 weeks resulted in significant impairment of LV systolic function in the KO mice, as demonstrated by a greater reduction of LV dP/dt\textsubscript{max} and LV dP/dt\textsubscript{min} as compared with WT mice (Table). After TAC for 2 weeks, mean aortic pressure and LV systolic pressure were significantly lower in KO mice than in WT mice (Table), consistent with the finding of more ventricular dysfunction in KO mice.

Consistent with increased hypertrophy in KO mice, myocardial atrial natriuretic peptide protein was significantly higher in KO mice than in WT mice after TAC (Figure 4A and 4B). Consistent with the greater increase of LV fibrosis after TAC, KO mice hearts contained higher myocardial collagen I content than WT mice. Myocardial tumor necrosis factor-\alpha levels were also significantly higher in the KO than in the WT mice after TAC (Figure 4A and 4E), suggesting an increased inflammatory response in the KO mice. This observation is in agreement with reports that adenosine can reduce cardiac tumor necrosis factor-\alpha expression.20

![Figure 2. CD73-KO exacerbates TAC-induced cardiac myocyte hypertrophy (A and B), ventricular fibrosis (A and C), and perivascular fibrosis (A and D). *P<0.05 vs the corresponding control; #P<0.05 vs WT (n=4 mice per group).](image)

![Figure 3. CD73-KO exacerbates TAC-induced ventricular dysfunction (A and B) and dilation (C and D). *P<0.05 vs the corresponding control; #P<0.05 vs WT.](image)
CD73 KO Enhances Akt-mTOR-p70 Ribosomal S6 Kinase Activation

The phosphatidylinositol 3-kinase/AKT signaling pathways target mTOR activity to increase the translation of proteins important for cell growth.\textsuperscript{21} Signaling from mTOR appears critical for cardiac hypertrophy and also promotes the transition to heart failure during chronic pressure overload.\textsuperscript{22} AKT can increase the activation of mTOR indirectly by reducing tuberin activity,\textsuperscript{23} and it is also a direct target of mTOR kinase activity.\textsuperscript{24} Interestingly, Western blot analysis revealed that the mTOR effector phosphorylation sites at Akt\textsuperscript{Ser473} and p70 ribosomal S6 kinase (p70S6K)\textsuperscript{Thr389} were significantly increased in the KO mice above levels found in WT mice even under basal conditions. In WT mice, TAC increased levels of phospho-(p)-Akt\textsuperscript{Ser473} and p-70S6K\textsuperscript{Thr389}, and both were further elevated in the KO mice. Consistent with increased phosphorylation of mTOR targets in the KO mice, KO mice demonstrated higher levels of p-mTOR\textsuperscript{Ser2488} as compared with WT mice 2 weeks after TAC (Figure 5). The increased levels of p-mTOR\textsuperscript{Ser2488} in KO mice were the result of both increased

Table. Anatomic and Functional Data for WT and CD73-KO Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT Control</th>
<th>CD73-KO Control</th>
<th>WT TAC</th>
<th>CD73-KO TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>25.8±0.4</td>
<td>26.1±0.6</td>
<td>25.8±1.0</td>
<td>25.8±0.7</td>
</tr>
<tr>
<td>Ventricular mass, mg</td>
<td>116±2.0</td>
<td>115±2.4</td>
<td>161±4.9†</td>
<td>178±5.7†</td>
</tr>
<tr>
<td>Ratio of ventricular mass:body weight, mg/g</td>
<td>4.52±0.08</td>
<td>4.44±0.10</td>
<td>6.33±0.23†</td>
<td>6.91±0.14†</td>
</tr>
<tr>
<td>Lung mass, mg</td>
<td>145±2.4</td>
<td>146±1.6</td>
<td>164±8.4†</td>
<td>211±18.5†</td>
</tr>
<tr>
<td>Ratio of lung mass:body weight, mg/g</td>
<td>5.63±0.08</td>
<td>5.65±0.15</td>
<td>6.43±0.36°</td>
<td>8.20±0.73†</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>17.5±0.14</td>
<td>17.5±0.14</td>
<td>17.5±0.13</td>
<td>17.6±0.12</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>499±37</td>
<td>511±17</td>
<td>466±15°</td>
<td>444±7.8°</td>
</tr>
<tr>
<td>LV end systolic diameter, mm</td>
<td>2.21±0.15</td>
<td>2.09±0.14</td>
<td>2.85±0.16°</td>
<td>3.44±0.14†</td>
</tr>
<tr>
<td>LV end diastolic diameter, mm</td>
<td>4.01±0.12</td>
<td>3.90±0.12</td>
<td>3.91±0.11°</td>
<td>4.38±0.10†</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>82.2±2.6</td>
<td>84.0±2.2</td>
<td>60.6±3.7°</td>
<td>51.3±3.0°</td>
</tr>
<tr>
<td>LV posterior wall thickness at end diastole, mm</td>
<td>0.69±0.01</td>
<td>0.68±0.03</td>
<td>0.97±0.03°</td>
<td>0.94±0.03°</td>
</tr>
<tr>
<td>LV posterior wall thickness at end systole, mm</td>
<td>1.14±0.03</td>
<td>1.09±0.02</td>
<td>1.25±0.02°</td>
<td>1.26±0.23°</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>87.1±5.9</td>
<td>85.5±2.7</td>
<td>96±3.3</td>
<td>92±3.6</td>
</tr>
<tr>
<td>Systolic LV pressure, mm Hg</td>
<td>106±4.6</td>
<td>103±2.5</td>
<td>153±3.5°</td>
<td>148±4.7†</td>
</tr>
<tr>
<td>LV end diastolic pressure, mm Hg</td>
<td>9.5±1.07</td>
<td>8.8±1.2</td>
<td>20±2.9°</td>
<td>25±4.4°</td>
</tr>
<tr>
<td>LV dP/dt\textsuperscript{min}, mm Hg/s</td>
<td>8791±652</td>
<td>7181±390</td>
<td>7711±286°</td>
<td>5208±350†</td>
</tr>
<tr>
<td>LV dP/dt\textsuperscript{max}, mm Hg/s</td>
<td>−7638±743</td>
<td>−6526±284</td>
<td>−7984±271</td>
<td>−6036±496†</td>
</tr>
</tbody>
</table>

Data are mean±SE. The anatomic data were obtained from all of the mice studied (11 to 23 mice per group). The hemodynamic data were obtained from 8 to 9 mice per group after TAC and 5 mice per group under control conditions.

*P<0.05 as compared with corresponding control conditions.
†P<0.05 as compared with WT mice.
mTOR expression and increased phosphorylation relative to total levels. The lipid phosphatase known as phosphatase and tensin homologue on chromosome 10 (PTEN) can downregulate phosphatidylinositol 3-kinase signaling by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate, and mutations that inhibit PTEN or cardiac-specific deletion of PTEN result in constitutive AKT activity. Under basal conditions, phosphorylation at serine 380 (serine 380 phosphorylation of PTEN involved in reducing membrane association and activity) was significantly increased, whereas total PTEN levels were slightly, but not significantly ($P < 0.05$ relative to sham; $#P < 0.05$ vs WT ($n=4$ to 6 samples per group)).

Adenosine or Adenosine Analogue Attenuates Cardiac Myocyte Hypertrophy and Activation of mTOR and p70S6K

Because in vivo adenosine can increase blood flow, reduce inflammatory responses, inhibit norepinephrine release from nerve endings, and decrease ET-1 production, it was important to determine whether the amplified mTOR/p70S6K signaling in the KO mice was the result of indirect effects of adenosine that caused paracrine regulation of these signaling pathways or a direct effect of adenosine on cardiomyocytes. Therefore, we examined the effect of the adenosine analogue CADO on phenylephrine (PE)-induced hypertrophy and ac-

Figure 5. Systolic overload produced by TAC for 2 weeks increased phosphorylated AKT$^{\text{Ser}473}$, p70S6 kinase$^{\text{Thr}389}$, and mTOR$^{\text{Ser}2448}$ in heart lysates, as measured by Western blot and scanning densitometry. Phosphorylation of these sites in KO was significantly elevated above levels WT animals. The results indicate that KO increased mTOR signaling after TAC. KO also increased PKC$^\alpha$ under both control conditions and after TAC. $^*P<0.05$ relative to sham; $#P<0.05$ vs WT ($n=4$ to 6 samples per group).
tivation of p-mTOR$^{\text{Ser2488}}$ and p70S6K$^{\text{Thr389}}$ in isolated neonatal cardiomyocytes. PE significantly increased the size of the cardiac myocytes and expression of the hypertrophy marker atrial natriuretic peptide, whereas CADO significantly attenuated the PE-induced increase in cell size and reduced atrial natriuretic peptide expression (Figure S1). PE treatment also significantly increased phosphorylation of mTOR$^{\text{Ser2448}}$ and p-70S6K$^{\text{Thr389}}$, and this activation was dramatically reduced by CADO (Figure 6). Similarly, adenosine attenuated the PE-induced increase of cardiac myocyte size and activation of p-70S6K$^{\text{Thr389}}$ and p-mTOR$^{\text{Ser2488}}$ (data not shown).

Adenosine Attenuates Collagen Synthesis and Fibroblast Proliferation

We also determined the effect of adenosine on cardiac fibroblast proliferation and collagen production, and the results showed that adenosine significantly reduced cardiac fibroblast proliferation and collagen production (Figure S2), which is in agreement with previous reports.9

Discussion

The major finding in this study is that deletion of CD73 significantly exacerbates LV hypertrophy, dilation, and dysfunction in the TAC model of LV pressure overload. These results suggest that conversion of extracellular AMP to adenosine plays a significant role in modulating maladaptive tissue remodeling and hypertrophic signaling pathways activated in response to systolic overload. The greater ventricular hypertrophy in CD73 KO mice after TAC was the result of both increased fibrosis and greater cardiomyocyte hypertrophy. The more prominent hypertrophy and dysfunction in the KO hearts were associated with increased activation of the mTOR signaling pathway. Moreover, the demonstration that adenosine or the stable adenosine analogue CADO attenuated phenylephrine-induced cardiomyocyte mTOR/p70S6 kinase signaling and myocyte hypertrophy, as well as fibroblast collagen production, suggests that the in vivo effects of CD73 deletion can be attributed to the loss of adenosine production. Taken together, these findings provide the first direct evidence that endogenous extracellular adenosine exerts a protective effect against ventricular tissue remodeling and cardiac myocyte hypertrophic signaling pathways during chronic systolic overload.

Although no previous reports have directly examined the effect of CD73 on pressure-overload–induced ventricular remodeling, there is evidence that increased endogenous adenosine can attenuate the development of cardiovascular disease.29 Adenosine is known to inhibit norepinephrine release from presynaptic vesicles,27 reduce the production of ET-1,28 and reduce tumor necrosis factor-$\alpha$ production.20 Recently, we also found that, whereas 8-sulfophenyltheophylline significantly increased myocardial oxygen consumption in dogs with failing hearts, it had no effect on oxygen consumption in normal dogs,26 suggesting that endogenous adenosine may help reduce myocardial oxygen demand, particularly in the failing heart. Studies in rats in which extracellular adenosine was increased by blockade of adenosine uptake with dipyridamole7 also reported attenuation of pressure-overload–induced myocardial hypertrophy. Interestingly, a mutation of the adenosine monophosphate deaminase 1 gene (which results in increased adenosine production) predicted a better clinical outcome in patients after myocardial infarction,30 implying that increased endogenous adenosine levels can exert a protective effect on the diseased human heart. Myocardial adenosine concentrations increase during the compensated phase of ventricular hypertrophy but then decrease when there is evidence of decompensation,10,11 suggesting that a decrease of extracellular adenosine levels might be a contributing factor in the transition to heart failure. Our
finding that a genetically engineered loss of CD73 activity exacerbated TAC-induced ventricular hypertrophy and dysfunction provides further evidence that endogenous adenosine production protects against progression to heart failure under conditions of pressure-overload.

Adenosine Effects on Cardiomyocyte
Hypertrophic Signaling
The present data identify a major hypertrophic signaling pathway targeted by extracellular adenosine. KO mice demonstrated increased phosphorylation of p-mTORSer2488, p-70S6KThr389, and p-AKTSer473, suggesting that adenosine normally acts to downregulate these signaling pathways. Activation of mTOR and its downstream targets results in increased cell size and is commonly associated with cardiac hypertrophy. Furthermore, overexpression of p70S6 kinase resulted in cardiac hypertrophy, whereas inhibition of mTOR signaling with rapamycin attenuated the development of type 2A adenosine receptors, which are highly expressed in the reduced contractility found in CD73 KO mice during signaling. Interestingly, PKCε cardiomyocytes confirms that adenosine regulates mTOR hypetrophy and mTOR/p70S6 kinase activation in isolated cardiac myocytes,6 transgenic overexpression of A1 or A3 receptors in the heart actually promotes cardiac hypertrophy and dilation. The role of the A2b receptor is slightly more well defined, as most published data suggest a role in reducing cardiac fibroblast proliferation and collagen synthesis. The A2b receptor also plays a role in ameliorating pathological LV tissue remodeling after infarct. Activation of type 2A adenosine receptors, which are highly expressed in the coronary vasculature, can downregulate vascular cell adhesion molecule expression to reduce monocyte adhesion to endothelial cells and vascular inflammation. The increased vascular inflammation and fibrosis in the CD73-KO mice after TAC suggests that A2A and A2b receptors may not be adequately activated in the absence of CD73-dependent adenosine production. In addition to increased vascular inflammation, CD73-KO has been reported to cause a 15% decrease in basal coronary flow. Although this modest decrease in coronary flow would not likely affect cardiac function under basal conditions, abnormalities of coronary flow might impair oxygen delivery during pressure overload, when oxygen demand is increased and diffusion distances are increased by perivascular fibrosis. It is, therefore, probable that the protective effects of extracellular adenosine against the TAC-induced ventricular hypertrophy and dysfunction are not mediated by activation of any individual adenosine receptor subtype alone but more likely involve complimentary effects of multiple adenosine receptor subtypes on multiple cardiac cell types. Additional studies will be needed to distinguish the specific adenosine receptors and cell types that mediate the protective effects of adenosine in the pressure-overloaded heart.

Limitations
Although we demonstrated that CD73-KO abolished myocardial CD73 activity, and a previous study using the same mouse strain demonstrated that KO abolished extracellular adenosine production in other tissues, we were not able to collect extracellular fluid from the mouse heart for adenosine analysis because of the small size of the heart. Secondly, because all of the adenosine receptors are expressed in the heart, future studies will be needed to determine the specific adenosine receptor(s) responsible for the protective effect against pressure-overload-induced ventricular hypertrophy.

Perspectives
Previous studies have demonstrated that adenosine analogues and selective adenosine A1 or A3 receptor agonists protect the heart from ischemia/reperfusion-induced myocardial damage. However, the effect of endogenous extracellular adenosine on chronic pressure-overload–induced ventricular hypertrophy and heart failure has not been studied previously. Here we demonstrated that loss of CD73 activity exacerbates the ventricular hypertrophy, fibrosis, and dysfunction that occur in the heart exposed to chronic hemodynamic overload. This study also identifies, for the first time, a specific hypertrophic signaling pathway (mTOR-p70S6K) that is targeted by adenosine and that may explain the antihypertrophic effects of adenosine. These findings provide the first direct evidence that endogenous extracellular adenosine plays an important role in regulating pressure-overload–induced ventricular remodeling, indicating that increasing extracellular adenosine production or activation of specific adenosine receptors may be a therapeutic approach for treating the pressure-overloaded heart.

Sources of Funding
This study was supported by National Heart, Lung, and Blood Institute grants HL71790 (to Y.C.) and HL21872 (to R.J.B.) from the National Institutes of Health and a Scientist Development Grant 0730451N (to J.F.) and a postdoctoral fellowship 0725795Z (to X.X.) from the American Heart Association.

Disclosures
None.
References


Ecto-5′-Nucleotidase Deficiency Exacerbates Pressure-Overload–Induced Left Ventricular Hypertrophy and Dysfunction
Xin Xu, John Fassett, Xinli Hu, Guangshuo Zhu, Zhongbing Lu, Yunfang Li, Jurgen Schnermann, Robert J. Bache and Yingjie Chen

Hypertension. 2008;51:1557-1564; originally published online April 7, 2008; doi: 10.1161/HYPERTENSIONAHA.108.110833

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/6/1557

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2008/04/07/HYPERTENSIONAHA.108.110833.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension 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 Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/