Acute Antihypertrophic Actions of Bradykinin in the Rat Heart

Importance of Cyclic GMP

Anke C. Rosenkranz, Sally G. Hood, Robyn L. Woods, Gregory J. Dusting, Rebecca H. Ritchie

Abstract—The antihypertrophic action of angiotensin (Ang)-converting enzyme (ACE) inhibitors in the heart is attributed in part to potentiation of bradykinin. Bradykinin prevents hypertrophy of cultured cardiomoyocytes by releasing nitric oxide (NO) from endothelial cells, which increases cardiomoyocyte guanosine 3′,5′-cyclic monophosphate (cyclic GMP). It is unknown whether cyclic GMP is essential for the action of bradykinin, or whether findings in isolated cardiomoyocytes apply in whole hearts, in the presence of other cell types and mechanical/dynamic activity. We now examine the contribution of cyclic GMP to the antihypertrophic action of bradykinin in cardiomyocytes and perfused hearts. In adult rat isolated cardiomoyocytes cocultured with bovine aortic endothelial cells, the inhibitory action of bradykinin (10 μmol/L) against Ang II (1 μmol/L)–induced [3H]phenylalanine incorporation was abolished by the soluble guanylyl cyclase inhibitor 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one (10 μmol/L). In Langendorff-perfused rat hearts, Ang II (10 nmol/L)–induced increases in [3H]phenylalanine incorporation and atrial natriuretic peptide mRNA expression were prevented by bradykinin (100 nmol/L), the NO donor sodium nitroprusside (3 μmol/L), and the ACE inhibitor ramiprilat (100 nmol/L). The acute antihypertrophic action of bradykinin was accompanied by increased left ventricular cyclic GMP, and the ramiprilat effect was attenuated by HOE 140 (1 μmol/L, a B2-kinin receptor antagonist) or [1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (100 nmol/L). In conclusion, bradykinin exerts a direct inhibitory action against the acute hypertrophic response to Ang II in rat isolated hearts, and elevation of cardiomoyocyte cyclic GMP may be an important antihypertrophic mechanism used by bradykinin and ramiprilat in the heart. (Hypertension. 2002;40:498-503.)

Key Words: angiotensin-converting enzyme • angiotensin II • bradykinin • cyclic GMP • hypertrophy • myocytes • nitric oxide

Angiotensin-converting enzyme (ACE) inhibitors are often used to treat cardiac hypertrophy,1 a significant cardiovascular risk factor in hypertensive patients.2 The antihypertrophic action of ACE inhibitors in vivo is abolished by the B2-kinin receptor antagonist HOE 140,3 indicating a significant contribution by bradykinin. In addition, B2-kinin receptor deficiency promotes the development of cardiac hypertrophy in vivo,4 whereas kallikrein overexpression prevents cardiac growth.5 However, the mechanism by which bradykinin counteracts hypertrophic stimuli in the heart is not fully understood. We have shown that bradykinin prevents hypertrophy of isolated cardiomoyocytes by stimulating the release of NO from endothelial cells6 and that this action is associated with elevation of cardiomoyocyte cyclic GMP.7 An analogue of cyclic GMP, 8-bromo-cyclic GMP, also prevents hypertrophy of isolated rat cardiomoyocytes,8 but whether stimulation of cyclic GMP is essential for the antihypertrophic action of bradykinin has not been addressed.

Cardiomoyocyte hypertrophy is characterized by increased protein synthesis and cell size and induction of the immediate early (eg, c-fos, c-jun, and c-myc) and fetal genes (eg, β-myosin heavy chain, skeletal α-actin, and atrial natriuretic peptide, ANP).9 These cellular changes are established in vitro markers for cardiomoyocyte hypertrophy in neonatal10 and adult cardiomoyocytes,11 as well as in isolated whole hearts stimulated with mechanical load or neurohumoral factors (eg, norepinephrine or angiotensin II, Ang II).12,13 The intact rat heart expresses a functional kallikrein-kinin system,14 but the antihypertrophic action of bradykinin has not been investigated in this setting, where autocrine/paracrine factors (eg, Ang II, endothelin-1, transforming growth factor-β) may influence cardiac growth at the same time.15 Our objective was to examine the acute antihypertrophic effect of bradykinin in the rat isolated heart, compared with an ACE inhibitor and nitric oxide (NO) donor, and to examine the contribution of cyclic GMP to the bradykinin response in vitro.

Methods

Materials
Ang II, sodium nitroprusside (SNP), 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), L-amino acids, bovine serum albumin
Rosenkranz et al

(BSA, fraction V), and d,l-lactic acid were obtained from Sigma Biochemicals. Bradykinin was from Auspep; ramiprilat and HOE 140 were a gift from Hoechst AG (Frankfurt, Germany). Real time polymerase chain reaction (PCR) reagents were purchased from Applied Biosystems. All other reagents were from Sigma unless otherwise stated. All protocols were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Isolation of Adult Rat Cardiomyocytes

Male Sprague-Dawley rats (180 to 280 g) were terminally anesthetized by intraperitoneal bolus of ketamine hydrochloride (100 mg/kg) and xylazine (12 mg/kg). Cardiomyocytes were isolated as previously described and resuspended in serum-free medium 199 (M199, Trace Scientific), containing 0.2% BSA, 2 mmol/L L-carnitine, 5 mmol/L creatine, 5 mmol/L taurine, 25 μg/mL gentamicin (Life Technologies), 100 U/mL penicillin, and 100 μg/mL streptomycin (CSL Biosciences). Cardiomyocytes were plated into laminin (10 μg/mL; Collaborative Biomedical Products)-coated 6-well plates and equilibrated at 37°C (5% CO2,95% O2) for 2 to 24 hours.

Bovine Aortic Endothelial Cell (BAEC) Cultures

BAEC were isolated as described and maintained at 37°C (5% CO2,95% O2) in DMEM (Life Technologies) containing 1% fetal calf serum (CSL Biosciences), 4 mmol/L L-glutamine (Life Technologies), 66 μg/mL gentamicin, 660 U/mL penicillin, and 660 μg/mL streptomycin (CSL Biosciences). BAEC were grown to confluence on 30-mm tissue-culture inserts (0.4-μm membrane, Millipore) and washed in M199 immediately before use. BAEC were used at passages 4 to 6 and at passage 12 in one experiment. No difference in the response to drug treatments was observed at the higher passage.

1H]Phenylalanine Incorporation by Cardiomyocytes

Cardiomyocytes were incubated with BAEC-coated inserts for 2 hours at 37°C in serum-free M199 containing 1H]phenylalanine (2 μCi/mL, NEN Lifesciences) ± Ang II (1 μmol/L) ± bradykinin (10 μmol/L) ± ODQ (10 μmol/L). Solutions containing bradykinin were supplemented with ramiprilat (1 μmol/L) to limit degradation; this concentration does not influence 1H]phenylalanine incorporation. Cardiomyocyte protein and DNA were trichloroacetic acid (TCA)-precipitated before resuspension in 0.3 N NaOH. 1H]Phenylalanine was determined by liquid scintillation, and DNA content was determined fluorometrically using PicoGreen® reagent (Molecular Probes). 1H]Phenylalanine incorporation was normalized to nanograms of DNA per sample to correct for cell number per sample.

Perfusion of Isolated Rat Hearts

Male Sprague-Dawley rats (280 to 300 g) were anesthetized as above. Hearts were removed and arrested in ice-cold Kreb-Henseleit buffer containing the following (all in mmol/L): NaCl 118, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25, glucose 5.5, CaCl2 2.0, and d,l-lactic acid 1.0, and previously equilibrated with 5% CO2,95% O2 at 37°C. The aorta was cannulated and attached to a modified Langendorff rodent heart perfusion system (Petersen Scientific Glass Blowing Pty Ltd) for retrograde perfusion under constant pressure (60 mm Hg). Heart rate and coronary flow were monitored manually (collection of efflux over 60 seconds) throughout the perfusion. Hearts perfused by this method maintained regular contractions for 2.5 to 3 hours.

Experimental Protocol

Following equilibration (15 minutes), hearts were perfused for 90 minutes with buffer alone or buffer containing study drugs. Ang II (acute hypertrophic stimulus) was added after 30 minutes. Seven groups were studied: (1) vehicle, (2) Ang II (10 nmol/L), (3) bradykinin (100 nmol/L) + Ang II, (4) SNP (3 μmol/L) + Ang II, (5) ramiprilat (100 nmol/L) + Ang II, (6) ramiprilat + HOE 140 (1 μmol/L) + Ang II, (7) ramiprilat + ODQ (100 nmol/L) + Ang II.

Bradykinin Is Antihypertrophic in Rat Hearts

Optimal Sequences (5′–3′) and Concentrations of Taqman Primers and Probes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Probe</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat ANP</td>
<td>GCC CTT GGC CTG GGC GTG TGT CA</td>
<td>TGC AGC TCC AGG AGG GTA TT</td>
<td>6FAM-CAG CTG GTG CGC ATT GCC</td>
<td>100 nM</td>
</tr>
<tr>
<td>ACT-TAMRA</td>
<td>TGGTCGCTGGGAAATCC</td>
<td>TGGTCGCTGGGAAATCC</td>
<td>VIC-TGCTGGCAACGACCTGACCTC</td>
<td>125 nM</td>
</tr>
</tbody>
</table>

Ramiprilat (10 nmol/L) was added to bradykinin-containing solutions to prevent degradation; this concentration did not influence [H]phenylalanine incorporation in our model. The lowest concentrations of ramiprilat and ODQ to elicit a reproducible effect were determined in preliminary studies for use in subsequent experiments; concentrations of other drugs were based on previous reports in isolated hearts. Following 90 minutes perfusion with study drugs, hearts incorporated [H]phenylalanine (0.25 μCi/mL) for a further 60 minutes in the absence of study drugs. [H]Phenylalanine incorporation is linear over this time. Perfusion buffer for [H]phenylalanine incorporation also contained 0.1% BSA and l-amino acids (all in μmol/L): alanine 284, arginine 402, aspartic acid 225, cysteine 220, glutamine 456, glycine 666, histidine 129, isoleucine 153, leucine 457, cysteine 192, methionine 101, phenylalanine 151, serine 241, threonine 252, trans-4-hydroxy-L-proline 348, tryptophan 49, tyrosine 158, and valine 216. Immediately after the perfusion protocol, left ventricles were dissected free, snap-frozen in liquid nitrogen, and stored at −80°C for measurement of left ventricular (LV) [H]phenylalanine incorporation, protein content, ANP mRNA expression and cyclic GMP.

[H]Phenylalanine Incorporation by LV Protein

Protein synthesis was determined as previously described. LV tissue (100 to 150 g) was homogenized in 2 mL ice-cold 6% TCA to denature protein and remove unincorporated [H]phenylalanine. Following centrifugation, pellets were washed in 6% TCA and heated to 80°C for 20 minutes to remove RNA-bound [H]phenylalanine. After centrifugation, pellets were washed in 6% TCA and resuspended in 0.2 N NaOH. Aliquots were taken for liquid scintillation counting and protein assay by the Lowry method.

ANP mRNA Expression

Total RNA was extracted from LV tissue (30 to 50 mg) using RNeasy™ (Ambion) and reverse-transcribed to 10 ng/μL cDNA using the TaqMan® reverse transcription reagents (Applied Biosystems). ANP mRNA was quantified by real time PCR and the ΔΔCt method with 18S ribosomal RNA as the internal standard. Primers and probes were designed from rat-specific sequences published on GenBank (Table). Probes were 5′-labeled with the reporter dye FAM (ANP) or VIC (18S) and 3′-labeled with the quencher molecule carboxytetramethylrhodamine (TAMRA). Optimal primer and probe concentrations were determined in initial studies (Table). All reactions were performed in the ABI Prism® 7700 sequence detection system (Applied Biosystems) as described.

LV Cyclic GMP Content

Pieces of LV tissue (~200 mg) were weighed and homogenized in ice-cold 6% TCA. Following centrifugation, supernatant was extracted in water-saturated diethyl ether and dried under a stream of air at 70°C. The residue was resuspended in sodium acetate assay buffer (0.05 mol/L, pH 6.2), and cyclic GMP content was determined...
with a commercial $[^{3}H]$-cyclic GMP radioimmunoassay (RIA) kit (NEN Dupont).

**Statistical Comparisons**
For isolated cell studies, treatment groups were studied at least in triplicate and the average result taken for each experiment; “n” for each set of results represents the number of myocyte preparations studied. $[^{3}H]$Phenylalanine incorporation per nanogram of DNA was expressed as a percentage of the paired control, mean±SD of the least square mean for treatment. Comparative statistical analyses used 2-way ANOVA or paired $t$ test as indicated. Bonferroni correction for multiple comparisons was applied where appropriate.

Whole hearts were perfused in batches of 6 to 12 hearts. Each set included at least one negative (vehicle) and one positive hypertrophic control (Ang II). LV $[^{3}H]$phenylalanine incorporation per milligram of protein and cyclic GMP content per wet tissue weight for each set of results represents the number of myocyte preparations used 2-way ANOVA or paired $t$ test as indicated. Bonferroni correction for multiple comparisons was applied where appropriate.

Resting heart rate remained at 181±8 beats/min in vehicle-perfused hearts (n=20). Heart rate was not significantly altered by any of the drug treatments (range 165 to 210 beats/min). Coronary flow in vehicle hearts averaged 11.4±1.0 mL/min (n=20). Bradykinin and ramiprilat (before addition of Ang II) tended to increase coronary flow to 12.9±0.7 (n=8, P=0.06) and 14.1±1.9 mL/min (n=8, P=0.07), respectively. Addition of Ang II restored flow to 9.6±1.0 (n=7, P<0.05 versus bradykinin alone) and 9.9±1.1 (n=8, P=0.07 versus ramiprilat alone). Ang II alone

**Figure 1.** Bradykinin (10 μM/L, n=6) prevented Ang II (1 μM/L, n=6)–stimulated $[^{3}H]$phenylalanine incorporation (% of control, n=6) in cardiomyocytes cocultured with BAEC. This bradykinin action was abolished by the soluble guanylyl cyclase inhibitor ODQ (10 μM/L, n=6). *P<0.05 versus control (paired t test); †P<0.05 versus Ang II; ‡P<0.05 versus Ang II + bradykinin (2-way ANOVA).

**Results**
Cyclic GMP Mediates the Antihypertrophic Action of Bradykinin in Cardiomyocytes
In cardiomyocyte/BAEC cocultures, Ang II–stimulated $[^{3}H]$phenylalanine incorporation (to 135±3% of control, P<0.001, n=6) was abolished by bradykinin (98±3% of control, n=6, Figure 1). ODQ prevented the antihypertrophic action of bradykinin (131±3% of control, P<0.05 versus Ang II + bradykinin, n=6).

Heart Rate and Coronary Flow in Rat Isolated Hearts
Resting heart rate remained at 181±8 beats/min in vehicle-perfused hearts (n=20). Heart rate was not significantly altered by any of the drug treatments (range 165 to 210 beats/min). Coronary flow in vehicle hearts averaged 11.4±1.0 mL/min (n=20). Bradykinin and ramiprilat (before addition of Ang II) tended to increase coronary flow to 12.9±0.7 (n=8, P=0.06) and 14.1±1.9 mL/min (n=8, P=0.07), respectively. Addition of Ang II restored flow to 9.6±1.0 (n=7, P<0.05 versus bradykinin alone) and 9.9±1.1 (n=8, P=0.07 versus ramiprilat alone). Ang II alone

**Figure 2.** Compared with vehicle alone (n=20), Ang II (10 nmol/L, n=22) significantly increased LV $[^{3}H]$phenylalanine incorporation (% vehicle). This acute hypertrophic response was prevented by bradykinin (100 nmol/L, n=11) and the NO donor sodium nitroprusside (SNP, 3 μmol/L, n=10). *P<0.001 versus vehicle (unpaired t test); †P<0.001 versus vehicle (paired t test); ‡P<0.001 versus Ang II (2-way ANOVA).

Bradykinin Prevents the Acute Hypertrophic Response to Ang II in Whole Hearts
Compared with hearts perfused with vehicle alone, Ang II (10 nmol/L) completely abolished Ang II–stimulated LV $[^{3}H]$phenylalanine incorporation from 182±19% (P<0.001, n=10) to 99±7% of vehicle (n=15, P<0.001 versus Ang II, Figure 2); similar inhibition of Ang II–induced $[^{3}H]$phenylalanine incorporation was observed with the NO donor SNP (3 μmol/L): 92±10% of vehicle (P<0.001, n=10).

The antihypertrophic action of the ACE inhibitor ramiprilat was investigated in a separate series of hearts. Ramiprilat (100 nmol/L) completely abolished Ang II–stimulated LV $[^{3}H]$phenylalanine incorporation from 182±19% (P<0.001, n=10) to 99±7% of vehicle (n=15, P<0.001 versus Ang II, Figure 3). HOE 140 (1 μmol/L) or ODQ (100 nmol/L) restored $[^{3}H]$phenylalanine incorporation to levels not significantly different from Ang II alone, to 141±10% with HOE 140 (n=12, P=0.07 versus ramiprilat + Ang II) and 142±15% with ODQ (n=11, P=0.06 versus ramiprilat + Ang II, Figure 3).

**Figure 3.** Ang II (10 nmol/L, n=10)–stimulated $[^{3}H]$phenylalanine incorporation (% vehicle) was completely abolished by the ACE inhibitor ramiprilat (100 nmol/L, n=15). The protective action of ramiprilat was attenuated by the B2-kinin receptor antagonist HOE 140 (1 μmol/L, n=12) or the soluble guanylyl cyclase inhibitor ODQ (100 nmol/L, n=11). *P<0.001 versus Ang II alone (unpaired t test); †P<0.001 versus vehicle (unpaired t test); ‡P<0.001 versus ramiprilat + Ang II (2-way ANOVA).
ANP mRNA to 9.4 ± 4.5-fold above vehicle (*P < 0.05, n = 9, compared with 10 nmol/L, n = 12). Sodium nitroprusside (SNP, 3 μmol/L, n = 9) and the ACE inhibitor ramiprilat (100 nmol/L, n = 6) failed to significantly elevate cyclic GMP. *P < 0.05 versus Ang II alone (2-way ANOVA).

In addition to its effects on [3H]phenylalanine incorporation, Ang II also significantly increased LV expression of ANP mRNA to 9.4 ± 4.5-fold above vehicle (P < 0.05, n = 8, Figure 4). This was completely prevented by bradykinin (1.7 ± 0.5-fold, n = 6, P < 0.05 versus Ang II). A similar protective action against Ang II–induced expression of ANP mRNA was observed with SNP (2.0 ± 0.6-fold, P < 0.05 versus Ang II, n = 9) and ramiprilat (2.7 ± 1.1-fold of control, n = 6, P < 0.05 versus Ang II, Figure 4).

Bradykinin Stimulates Cyclic GMP in the Isolated Perfused Rat Heart

Compared with perfusion with vehicle alone, Ang II exerted negligible effects on cyclic GMP content (118 ± 15% of vehicle, n = 12, Figure 5). In contrast, bradykinin markedly stimulated cyclic GMP accumulation to 202 ± 46% of vehicle (n = 6, P < 0.05 versus Ang II). Addition of SNP or ramiprilat failed to elevate cyclic GMP significantly (142 ± 20% and 135 ± 17% of vehicle, respectively, both n = 6, Figure 5).

Discussion

The key finding to emerge from the present study is that bradykinin exerts a marked antihypertrophic action in the isolated perfused rat heart, which more closely resembles the in vivo setting than do isolated myocytes. This action was mimicked by an ACE inhibitor and an NO donor. Furthermore, the antihypertrophic action of bradykinin was associated with elevation of LV cyclic GMP in whole hearts and was abolished by soluble guanylyl cyclase inhibition in isolated cardiomyocytes. Thus, we demonstrate for the first time that bradykinin elicits a potent, direct, antihypertrophic action in the rat heart and that cyclic GMP appears to be an important mediator of this response.

In the Rat isolated heart, Ang II–induced increases in LV [3H]phenylalanine incorporation and ANP mRNA expression were abolished by bradykinin and SNP (Figures 2 and 4). This is in agreement with findings that nitrates can preventing cardiac remodeling and hypertrophy and supports our earlier report that activation of endothelial bradykinin receptors with subsequent release of NO prevents hypertrophy in cardiomyocytes cocultured with endothelial cells. The ACE inhibitor ramiprilat was similarly protective in whole hearts (Figures 3 and 4) and a large component of this effect was sensitive to HOE 140 (Figure 3). Thus, bradykinin contributes significantly to the antihypertrophic action of ACE inhibitors in vitro, in accordance with findings in chronic hypertrophy in vivo. Inhibition of endogenous ACE–mediated Ang II production is unlikely to be involved in the present model of acute hypertrophy, because exogenous Ang II was infused as the hypertrophic stimulus.

The antihypertrophic action of exogenous bradykinin in the whole heart was accompanied by powerful stimulation of LV cyclic GMP (Figure 5), reflecting our earlier reports in cultured cells. Moreover, the soluble guanylyl cyclase inhibitor ODQ abolished the bradykinin effect in cultured cells (Figure 1), highlighting the importance of cyclic GMP. The endogenous kallikrein-kinin system is likely to contribute to the regulation of cardiac hypertrophy in vivo, possibly involving activation of NO/cyclic GMP signaling. Specific inhibition of cyclic GMP phosphodiesterase activity has also been shown to prevent hypertrophy of neonatal cardiomyocytes. Thus, cyclic GMP is a key intracellular signal by which bradykinin counteracts hypertrophic stimuli in the heart. This action may involve inhibition of mitogen-activated protein kinase cascades or of endothelin-1 expression by cardiac fibroblasts. In addition, regulation of cardiac Ca2+ current through cyclic GMP–gated cation channels may prevent hypertrophic signaling via calcineurin. A recent report suggested that bradykinin may also stimulate cardiac P2 purinoceptors via ATP, but whether this contributes to the antihypertrophic action of bradykinin in the heart is not known.

In our hands, the antihypertrophic action of ramiprilat was attenuated to a similar degree by both the soluble guanylyl cyclase inhibitor ODQ and the B2-kinin receptor antagonist HOE 140 (Figure 2), suggesting that elevation of cyclic GMP is a common pathway in the rat heart used by bradykinin, ramiprilat, and possibly, SNP. However, both ramiprilat and SNP failed to induce significant increases in LV cyclic GMP (Figure 4), despite exerting an acute antihypertrophic effect comparable to bradykinin (Figures 2 and 3). Although higher concentrations, or the addition of a cyclic GMP phosphodiesterase inhibitor such as zaprinast, might have caused greater elevation of cyclic GMP, amounts of SNP used were higher than previously reported as effective in isolated hearts. It is possible that only small increases in cyclic GMP in the myocyte cytosol are required to suppress hypertrophic sig-
naling in the rat heart. Alternatively, additional mechanisms not involving cyclic GMP may contribute. NO has been reported to exert cyclic GMP–independent actions, including oxygen radical scavenging, increased intracellular Ca
eurological functions, 


Acknowledgments

Part of this work was supported by the National Health and Medical Research Council of Australia, the High Blood Pressure Research Foundation of Australia, and the Diabetes Australia Research Trust. Ms Rosenkranz was supported by an Australian Postgraduate Award.

References


Acute Antihypertrophic Actions of Bradykinin in the Rat Heart: Importance of Cyclic GMP
Anke C. Rosenkranz, Sally G. Hood, Robyn L. Woods, Gregory J. Dusting and Rebecca H. Ritchie

Hypertension. 2002;40:498-503; originally published online August 26, 2002;
doi: 10.1161/01.HYP.0000032854.74042.CF
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/40/4/498

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/