AT$_1$-receptor blockade enhances ischemic preconditioning in hypertrophied rat myocardium

KARYN L. BUTLER,$^{1,2}$ ALICE H. HUANG,$^2$ AND JUDITH K. GWATHMEY,$^3$
Departments of $^1$Surgery and $^2$Physiology, Morehouse School of Medicine, Atlanta, Georgia 30310; and $^3$Departments of Medicine and Physiology, Boston University School of Medicine, Boston, Massachusetts 02138

Preconditioning was originally described as a decrease in high-energy catabolism during brief periods of ischemia that limits myocardial infarct size after a subsequent prolonged ischemic challenge (22). This concept of cellular adaptation has been expanded to include protection against various consequences of ischemia-reperfusion (I/R) injury, including contractile dysfunction (1), arrhythmias (30), and intracellular acidosis (12). Further investigation has demonstrated that this improved ischemic tolerance extends across species (3, 16, 29), including humans (13, 25).

The basis of our understanding of the phenomenon of preconditioning arises from exhaustive research on normal myocardium. Protection of myocardial function in hypertensive patients with left ventricular hypertrophy and coronary artery disease may potentially alter morbidity and mortality from ischemic heart disease. It is not clear, however, that protection of normal myocardium under controlled laboratory conditions is reproducible in a clinical setting where patients typically have varying degrees of myocardial hypertrophy and cardiac dysfunction. The question then arises whether preconditioning is possible in hearts with left ventricular hypertrophy and whether such conditioning might be beneficial. Randall and colleagues (26) demonstrated that ischemic preconditioning significantly improved contractile function in rats with an enhanced renin-angiotensin system. Contradictory results were obtained by Moolman et al. (21) who could not demonstrate protected contractile function in genetically hypertensive rat hearts with three periods of 4 min of global ischemia interspersed with 6 min of reperfusion (21).

High salt intake has been shown to correlate with the incidence of left ventricular hypertrophy in patients with essential hypertension (2) and has been shown to induce hypertrophy in several strains of rats, including Sprague-Dawley, salt-sensitive Dahl, spontaneously hypertensive rats, and the "new" Harlan Sprague Dawley Dahl salt-sensitive rat (8, 24, 32, 36). It has been reported that salt-induced hypertrophy in the Dahl salt-sensitive rat exhibits similar abnormalities in excitation-contraction coupling events as reported by us and by others in nonsalt-induced models of hypertrophy (9, 11, 23). We therefore induced left ventricular hypertrophy by feeding a high-salt diet to Dahl salt-sensitive rats.

Questions arise as to whether the mechanism(s) operable in preconditioned normal myocardium are also operable in hypertrophied myocardium. In normal myocardium, stimulation of adrenergic and purinergic receptors initiates cleavage of phosphatidylinositol 4,5-diphosphate to diacylglycerol and inositol 1,4,5-trisphosphate with subsequent activation of protein kinase C.

Butler, Karyn L., Alice H. Huang, and Judith K. Gwathmey. AT$_1$-receptor blockade enhances ischemic preconditioning in hypertrophied rat myocardium. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2482–H2487, 1999.—The purpose of this study was to determine whether ischemic preconditioning protects contractile function in hypertrophied rat myocardium from ischemia-reperfusion (I/R) injury. Male salt-sensitive rats were fed a high-salt diet for 2 wk to induce myocardial hypertrophy. Nonhypertrophied hearts were obtained from age-matched Sprague-Dawley (SD) rats fed a regular diet. Heart weight-to-body weight ratios were higher in salt-sensitive rats than in SD rats (6.9 ± 0.2 vs. 4.7 ± 0.2 g/kg, P < 0.01). A second group of salt-sensitive and SD rats was administered losartan (10 mg·kg$^{-1}$·day$^{-1}$), an AT$_1$-receptor blocker, for 1 wk before the study. Isolated hearts were preconditioned with transient ischemia before global I/R. After I/R, preconditioned hypertrophied hearts exhibited greater recovery of left ventricular developed pressure compared with that of preconditioned normal hearts (73 ± 8 vs. 18 ± 8%, P < 0.01). Left ventricular developed pressure was further enhanced by losartan in both hypertrophied and normal myocardium (99 ± 5 vs. 73 ± 8%, P < 0.05 and 97 ± 15 vs. 18 ± 8%, P < 0.01). Hypertrophied rat myocardium can be protected from I/R-induced contractile dysfunction by ischemic preconditioning. Losartan improves the ischemic tolerance of normal and hypertrophied myocardium.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Losartan is a selective, competitive AT1-receptor blocker that enhances the AT1 blocker losartan. The purposes of this study were to determine the efficacy of ischemic preconditioning in hypertrophied rat myocardium and to determine whether protection, if induced, is enhanced by the AT1 blocker losartan.

MATERIALS AND METHODS

Animals. Hearts were obtained from adult male Dahl salt-sensitive and Sprague-Dawley (SD) rats (weight range 300–400 g). Animal protocols conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, Revised 1996) and were approved by the Animal Care and Use Committee of Morehouse School of Medicine. Salt-sensitive rats were fed a high-salt diet (8.5% sodium chloride) and water ad libitum for 2 wk to induce myocardial hypertrophy. Nonhypertrophied hearts were obtained from age-matched SD rats fed a regular diet and water ad libitum. A second group of salt-sensitive and SD rats was administered the AT1 antagonist losartan (DuPont Merck, Wilmington, DE) by oral gavage (10 mg·kg⁻¹·day⁻¹) for 1 wk before experimentation. This dose has been reported to prevent an increase in systolic blood pressure in rats with reduced renal mass (15) and is significantly greater than that used clinically to treat hypertension in humans (1 mg·kg⁻¹·day⁻¹).

Isolated heart perfusion. Animals were anesthetized (pentobarbital sodium, 60 mg/kg) and heparinized (500 U) via intraperitoneal injection. The hearts were rapidly excised and arrested in ice, oxygenated (95% O₂–5% CO₂) buffer, mounted on a Langendorff apparatus, and perfused with Krebs-Henseleit buffer (mmol/l: 10 glucose, 118 NaCl, 2.5 CaCl₂, 4.7 KCl, and 25.0 NaHCO₃) in a nonrecirculating mode at a constant pressure of 85 mmHg. The perfusion pressure was selected based on earlier experiments in similar animals as the pressure that resulted in the best contractile performance and oxygen consumption (unpublished data). A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle through an incision in the left atrium. The pressure transducer was coupled to a Powerlab (AD Instruments, MA) data recording system. End-diastolic pressure was set at 6–8 mmHg, and the volume of the balloon was left unchanged during the experiment. Hearts were allowed to equilibrate for 20 min before any intervention and then randomized to ischemic preconditioning with 5 min of transient ischemia or no preconditioning (Fig. 1). Hearts were then subjected to 30 min of global, normothermic ischemia and 40 min of reperfusion (I/R). Global ischemia was achieved by interrupting the flow of buffer through a three-way stopcock placed above the aortic cannula. During global ischemia, hearts were immersed in a perfusate-filled organ bath maintained at 37°C, and asystole was documented with electrocardiogram tracings. Contractile function was assessed by determining left ventricular developed pressure (LVDP, mmHg, peak systolic end-diastolic pressure) and left ventricular end-diastolic pressure (LVEDP, mmHg) at the end of equilibration (baseline) and at the end of I/R. LVDP after reperfusion is expressed as percentage of baseline function. Coronary flow (normalized for heart weight, ml·min⁻¹·g⁻¹ of heart tissue) was determined by timed collection of coronary effluent.

Assessment of angiotensin receptor blockade. To test for blockade of angiotensin receptors, we challenged hearts from rats treated with losartan versus hearts from nontreated rats with 0.3 ng/ml of angiotensin II. Hearts were handled as described earlier. Coronary flow was determined before and after angiotensin II challenge. In losartan-treated hearts, coronary flow was decreased by 11.5 ± 0.5% vs. 20.5 ± 0.5% for non-losartan-treated hearts (P < 0.05) confirming blockade of the angiotensin receptors. Our results are in agreement with Yoshiyama et al. (40) who demonstrated AT1-receptor blockade in isolated perfused rat heart following oral administration of the AT1-receptor antagonist TCV-116.

Statistical analysis. All reported values are expressed as means ± SE. Differences at the 95% confidence level were considered significant using Student’s t-test assuming unequal variances. Multiple comparisons between groups were performed using ANOVA with post hoc analysis by Scheffe’s test.

RESULTS

Myocardial hypertrophy. Heart weight-to-body weight ratios were higher in the salt-sensitive rats (n = 6) after receiving a high-salt diet for 2 wk than the ratios of SD rats (n = 6) fed a regular diet (6.9 ± 0.2 vs. 4.4 ± 0.2 g/kg, P < 0.01), indicating myocardial hypertrophy. Heart weight-to-body weight ratios were significantly lower after 1 wk of treatment with losartan in salt-sensitive rats receiving a high-salt diet compared with nontreated salt-sensitive rats on a high-salt diet (5.9 ± 0.3 vs. 6.9 ± 0.2 g/kg, P < 0.05). Losartan-treated, salt-sensitive animals on a high-salt diet still had evidence of myocardial hypertrophy compared with rats (5.9 ± 0.3 vs. 4.4 ± 0.2 g/kg, P < 0.01) receiving a regular diet of rat chow (Fig. 2). There was no difference...
in heart weight-to-body weight ratios in hearts from SD rats treated with losartan. Preliminary experiments on hearts from salt-loaded SD rats demonstrated an increase in heart weight-to-body weight ratios similar to the ratios of Dahl salt-sensitive rats (6.5 ± 0.4 vs. 6.9 ± 0.2 g/kg, P = 0.22) (unpublished results).

Baseline contractile function. The stability of the isolated heart preparation was observed over 2 h of continuous perfusion. There was no significant difference in LVDP at the beginning of the observation period (88 mmHg) and after 2 h of continuous perfusion without intervention (-93 mmHg). There was no difference in baseline cardiodynamics in any of the experimental groups (Table 1).

Contractile function after ischemic challenge. After reperfusion, an ischemic challenge of 30 min resulted in significant contractile dysfunction in hypertrophied (LVDP, 18 ± 5%) and normal hearts (LVDP, 5 ± 2%); however, this dysfunction was more noticeable in normal hearts (P < 0.05). Pretreatment with losartan slightly improved postischemic recovery of LVDP in normal (27 ± 8% vs. 5 ± 2%, P < 0.05) and hypertrophied hearts (36 ± 8% vs. 18 ± 5%, P < 0.05). LVDP was not different between hypertrophied and normal hearts (90 ± 8 vs. 91 ± 12 mmHg, P = 0.47) after ischemic challenge and reperfusion. Losartan, however, reduced diastolic dysfunction (LVEDP, 63 ± 6 mmHg, P < 0.05) only in nonhypertrophied hearts after ischemic challenge and reperfusion.

Contractile function in preconditioned hearts after ischemic challenge and reperfusion. Preconditioned hypertrophied hearts exhibited significant (P < 0.01) improvement in the recovery of LVDP (73 ± 8%) compared with preconditioned normal hearts (18 ± 8%) after I/R. Recovery of LVDP following ischemic challenge and reperfusion was further enhanced in hearts from SD and salt-sensitive rats pretreated with losartan and then preconditioned. LVDP after reperfusion for preconditioned hypertrophied hearts treated with losartan was 99 ± 5% (P < 0.05 vs. non-losartan-treated hypertrophied hearts) and 97 ± 15% for normal hearts (P < 0.01 vs. non-losartan-treated normal hearts). Nonhypertrophied hearts demonstrated a greater improvement in LVDP with losartan treatment (Fig. 3). After ischemic preconditioning, diastolic dysfunction was significantly (P < 0.01) reduced in hypertrophied hearts with or without losartan compared with that of normal preconditioned hearts (LVEDP, 70 ± 5 mmHg) (Table 2). The diastolic dysfunction in normal preconditioned hearts was, however, reduced by losartan (LVEDP, 20 ± 4 vs. 70 ± 5 mmHg for hearts from non-losartan-treated preconditioned normal hearts, P < 0.01). In preconditioned hypertrophied hearts, treatment with losartan had no additional beneficial effect on LVEDP (Table 2). Preliminary experiments in salt-loaded SD rat hearts demonstrated that the recovery of contractile function was similar to salt-loaded Dahl salt-sensitive rat hearts (59 ± 7% vs. 73 ± 8%, P = 0.09).

Coronary flow. Baseline coronary flow (ml·min⁻¹·g of heart tissue⁻¹) was lower in hypertrophied hearts compared with the flow of normal hearts (2.3 ± 0.2 vs. 4.9 ± 0.3 ml·min⁻¹·g⁻¹, P < 0.01). Losartan-treated hypertrophied hearts had higher baseline coronary flow compared with non-losartan-treated hypertrophied hearts (3.7 ± 0.3 vs. 2.3 ± 0.2 ml·min⁻¹·g⁻¹, P < 0.05). There was no difference in coronary flow at end reperfusion in any of the experimental groups (1.96 ± 0.5 ml·min⁻¹·g⁻¹).

Table 1. Baseline cardiodynamics

<table>
<thead>
<tr>
<th></th>
<th>Nonhypertrophied Hearts</th>
<th>Hypertrophied Hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control + Lo</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>92 ± 7</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>296 ± 20</td>
<td>246 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 for nonhypertrophied hearts and 12 for hypertrophied hearts. LVDP, left ventricular developed pressure; HR, heart rate; Lo, Losartan.
An important finding in this study is that hypertrophied myocardium can be protected from I/R-induced contractile dysfunction by preconditioning with transient ischemia, and this protection can be further enhanced by treatment with losartan, an AT₁-receptor blocker. Our results demonstrate that after 30 min of global ischemia, the impairment in mechanical performance was more prominent in nonhypertrophied myocardium. Treatment with losartan attenuated I/R-induced contractile dysfunction in both hypertrophied and nonhypertrophied hearts.

Preconditioning with transient ischemia significantly reduced contractile dysfunction in hypertrophied hearts after I/R but afforded normal myocardium little protection. This is consistent with reports in the literature and our experience that efficacy of cardioprotection varies with the type of stimulus and the severity of the ischemic challenge. We have been able to protect contractile function in normal hearts following a modest (30 min) and severe (40 min) ischemic challenge with cyclic ischemia (four cycles of I/R before prolonged ischemic challenge, data not shown) but have not consistently demonstrated cardioprotection with transient ischemia. We have, however, demonstrated consistent cardioprotection against an ischemic challenge of 20 min with transient ischemia (unpublished data). It is important in this study to use a preconditioning stimulus that is only marginally effective without losartan in order to demonstrate improved protection by losartan. Our results demonstrate that treatment with losartan induced cardioprotection in normal hearts and further enhanced contractile function in hypertrophied hearts.

The effectiveness of losartan in enhancing ischemic preconditioning suggests a role for angiotensin II in I/R-induced contractile dysfunction. Meissner et al. (18) demonstrated that angiotensin II induced a decline in the mechanical performance and calcium availability in rat myocardium. These effects were greater in hypertrophied myocardium than in normal myocardium (18). An increase in angiotensin II receptor expression has been reported at the site of ventricular infarction in normal rat myocardium (34). Furthermore, it has been demonstrated that AT₁ receptor expression and mRNA expression increases in hearts subjected to 25 min of ischemia followed by 30 min of reperfusion (38, 39).

It is now established that there exists a local cardiac renin-angiotensin system within cardiac tissue. The tissue-specific cardiac renin-angiotensin system has been found to have higher activity in myocardium from hypertrophied hearts. Inhibition of angiotensin receptors has been shown to decrease hypertrophy and improve diastolic function. Lopez and colleagues (17) found in Wistar rats with pressure-overload hypertrophy induced as a result of aortic banding that AT₁ inhibition with losartan (10⁻⁵ M), but not AT₂-receptor antagonists, prevented an increase in coronary vascular resistance, when challenged with angiotensin II. Similar to their observations, we found that hypertrophied hearts had lower coronary flow per gram of tissue. Losartan-treated hypertrophied hearts had higher baseline coronary flow compared with that of non-losartan-treated hypertrophied hearts. Lopez et al. (17) also reported that angiotensin II increased LVEDP in hypertrophied hearts and significantly decreased diastolic relaxation; these changes were prevented by AT₁ but not AT₂ inhibition. We similarly found that after ischemic challenge in hearts from losartan-treated animals, there was a significant improvement in diastolic function. Lopez et al. (17) concluded that the AT₁ receptor mediates the effects of angiotensin II on coronary tone and diastolic function. These studies and our data with losartan suggest that the local cardiac renin-angiotensin system is active and may reduce the tolerance of rat myocardium to ischemia. Additionally, the role of the cardiac renin-angiotensin system may be more significant in hypertrophied myocardium. Ischemic preconditioning was beneficial in hearts from SD rats only when they were treated with losartan; this suggests that endogenous angiotensin II may have a deleterious effect on mechanical function following ischemia in the rat.

Our data demonstrate that preconditioning protects contractile function in hearts with left ventricular hypertrophy. Randall et al. (26) showed a similar beneficial effect of ischemic preconditioning on mechanical performance in transgenic hypertensive rats. In contrast to our findings, Randall’s group also showed that AT₁ inhibition “partially reversed the beneficial effects of preconditioning on diastolic dysfunction.” We found no difference in LVEDP between losartan-treated and nontreated hypertrophied rat hearts after transient ischemic preconditioning. The data reported here suggest that pretreatment with the AT₁ blocker losartan before ischemic preconditioning provides enhanced protection for systolic pressure development in hypertrophied myocardium and protection of both end-

### Table 2. Postischemia-reperfusion cardiodynamics

<table>
<thead>
<tr>
<th></th>
<th>Nonhypertrophied Hearts</th>
<th>Hypertrophied Hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control + Lo</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>LVDP, %</td>
<td>5 ± 2</td>
<td>27 ± 8</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>91 ± 12</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>152 ± 61</td>
<td>220 ± 15</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It is now established that there exists a local cardiac renin-angiotensin system within cardiac tissue. The tissue-specific cardiac renin-angiotensin system has been found to have higher activity in myocardium from hypertrophied hearts. Inhibition of angiotensin receptors has been shown to decrease hypertrophy and improve diastolic function. Lopez and colleagues (17) found in Wistar rats with pressure-overload hypertrophy induced as a result of aortic banding that AT₁ inhibition with losartan (10⁻⁵ M), but not AT₂-receptor antagonists, prevented an increase in coronary vascular resistance, when challenged with angiotensin II. Similar to their observations, we found that hypertrophied hearts had lower coronary flow per gram of tissue. Losartan-treated hypertrophied hearts had higher baseline coronary flow compared with that of non-losartan-treated hypertrophied hearts. Lopez et al. (17) also reported that angiotensin II increased LVEDP in hypertrophied hearts and significantly decreased diastolic relaxation; these changes were prevented by AT₁ but not AT₂ inhibition. We similarly found that after ischemic challenge in hearts from losartan-treated animals, there was a significant improvement in diastolic function. Lopez et al. (17) concluded that the AT₁ receptor mediates the effects of angiotensin II on coronary tone and diastolic function. These studies and our data with losartan suggest that the local cardiac renin-angiotensin system is active and may reduce the tolerance of rat myocardium to ischemia. Additionally, the role of the cardiac renin-angiotensin system may be more significant in hypertrophied myocardium. Ischemic preconditioning was beneficial in hearts from SD rats only when they were treated with losartan; this suggests that endogenous angiotensin II may have a deleterious effect on mechanical function following ischemia in the rat.

Our data demonstrate that preconditioning protects contractile function in hearts with left ventricular hypertrophy. Randall et al. (26) showed a similar beneficial effect of ischemic preconditioning on mechanical performance in transgenic hypertensive rats. In contrast to our findings, Randall’s group also showed that AT₁ inhibition “partially reversed the beneficial effects of preconditioning on diastolic dysfunction.” We found no difference in LVEDP between losartan-treated and nontreated hypertrophied rat hearts after transient ischemic preconditioning. The data reported here suggest that pretreatment with the AT₁ blocker losartan before ischemic preconditioning provides enhanced protection for systolic pressure development in hypertrophied myocardium and protection of both end-
diastolic and systolic pressure for nonhypertrophied myocardium. The difference in these results may be associated with differences in the doses and/or duration of AT₁ inhibition or the degree of baseline dysfunction between the two models of hypertrophy. Randall et al. (26) perfused hearts with 3 µM of losartan throughout the experiment, but controls without losartan were not reported. Despite the differences in the model of hypertrophy, transgenic versus salt induced, the heart weight-to-body weight ratios in Randall’s study and ours were similar. Baseline and postischemic LVPD, however, were lower in Randall’s hypertrophied group. This may reflect decompensation and transition to heart failure rather than the compensated hypertrophied state studied here. It is somewhat surprising that hypertrophied myocardium responded more favorably to ischemic preconditioning and, overall, appeared to have a higher degree of ischemic tolerance compared with normal hearts. A limitation of our study is that we performed I/R experiments after only 2 wk of a high-salt diet. There may be a direct correlation between the efficacy of preconditioning and the degree of ventricular hypertrophy that may not be evident after 2 wk of a high-salt diet. Our results support Homcy’s (10) suggestion that hypertrophy may encompass a beneficial myocyte response at one end of the spectrum and a dysfunctional response at the other end.

Our data demonstrate that baseline coronary flow per gram of cardiac tissue was lower in hypertrophied hearts (compared with normal hearts) but was significantly increased by pretreatment with the AT₁ blocker losartan. The decreased baseline flow in our study is similar to the decreased coronary flow seen in patients with left ventricular hypertrophy (14). In the absence of losartan, hypertrophied hearts may have prominent arterial vasoconstriction; blockade of the AT₁ receptor would reduce arterial vascular tone and improve coronary flow. An increase in preischemic coronary flow could result in better oxygenation of myocardium, decreased myocardial oxygen extraction, and may explain the improved contractile performance seen after reperfusion.

The present study demonstrates that the salt-induced increase in ventricular mass is partially reversed by losartan, which is consistent with a role for angiotensin II receptors in myocardial pathophysiology. In rat and human hearts, there are two angiotensin II receptor subtypes designated as AT₁ and AT₂ (27). The predominant subtype in adult myocardium is AT₁. Previous work has demonstrated that the AT₁ subtype is responsible for the effects of angiotensin II, and the AT₂ subtype is primarily related to growth and development (7). In addition to the vascular effects of angiotensin II, there is evidence to suggest that the AT₁ subtype is important in mediating ventricular remodeling following ischemia (33). These data and our observations of reduction in ventricular mass with AT₁ receptor inhibition suggest that AT₁ receptors may be important in modulating ventricular hypertrophy in salt-sensitive animals. The regression of hypertrophy in the salt-sensitive rats given losartan may be due in part to a reduction in loading conditions and systemic blood pressure. It is not known whether further reduction in ventricular mass would have occurred with extended therapy.

Although Yoshiyama and colleagues (40) demonstrated that AT₁ blockade with TCV-116 was effective against myocardial I/R injury in the rat heart, this is the first study, to our knowledge, to demonstrate enhanced ischemic preconditioning in hypertrophied rat hearts treated with the AT₁ blocker losartan. Myocardial hypertrophy is associated with many cardiovascular disorders but most commonly results from chronic hypertension. Hypertrophied hearts may have both systolic and diastolic dysfunction, and this impaired contractile function may significantly affect outcome when the heart is subjected to stresses such as ischemia or infarction. The potential clinical significance of this study is that controlling hypertension with AT₁ receptor blockade may allow patients to better tolerate subsequent myocardial ischemic events. As clinicians increasingly incorporate the use of losartan into their armamentarium of antihypertensive therapeutics, it will become important to consider the role of AT₁ receptors in cardioprotection.

This work was supported in part by National Institutes of Health Grant G12-RR03034 and by a Scientist Development Grant from the American Heart Association (to K. L. Butler). Address for reprint requests: K. L. Butler, Departments of Surgery and Physiology, Morehouse School of Medicine, 720 Westview Dr., SW, Atlanta, GA 30310 (E-mail: butlerk@msm.edu).

Received 3 August 1999; accepted in final form 30 August 1999.

REFERENCES


