Cardioprotective Effects of Ramipril and Losartan in Right Ventricular Pressure Overload in the Rabbit
Importance of Kinins and Influence on Angiotensin II Type 1 Receptor Signaling Pathway

Jean L. Rouleau, MD; Gaston Kapuku, MD; Stéphane Pelletier, MSc; Hugues Gosselin, DT; Albert Adam, PhD; Caroline Gagnon, BSc; Chantal Lambert, PhD; Sylvain Meloche, PhD

Background—The role of kinins in the cardioprotective effects of ACE inhibitors remains controversial.

Methods and Results—Right ventricular pressure overload in rabbits was produced by pulmonary artery banding for 21 days. Rabbits were untreated, or they received the ACE inhibitor ramipril with or without bradykinin B 1 and B 2 receptor blockers or the angiotensin (Ang) II type I (AT 1 ) receptor blocker losartan. Pulmonary artery banding caused right ventricular hypertrophy, depressed papillary muscle contractility, and loss of Ang II contractile effects because of a signaling defect downstream of AT 1 receptors. Paradoxically, AT 1 receptor density and G protein α subunits αq and αi1/2 increased. Inotropic responsiveness to the α-receptor agonist phenylephrine was normal. Ramipril preserved cardiac contractility, but this effect was attenuated by simultaneous use of kinin receptor blockers. Ramipril also maintained responsiveness to Ang II and prevented AT 1 receptor and G protein upregulation. The simultaneous use of a kinin receptor blocker attenuated but did not prevent upregulation in the AT 1 receptor and G protein. Losartan had no effect on baseline contractility, but it maintained cardiac inotropic responsiveness to Ang II, prevented upregulation of AT 1 receptors, but did not modify G protein upregulation.

Conclusions—Pressure overload of the right ventricle decreases contractility, uncouples AT 1 receptors to downstream signaling pathways, and changes the expression of components of the AT 1 receptor signaling pathway. Ramipril attenuates these effects via kinins. Interventions that prevent local increases in Ang II or block AT 1 receptors also prevent decreased responsiveness of the AT 1 receptor in this model. (Circulation, 2001;104:939-944.)

Key Words: angiotensin ■ kinins ■ ramipril ■ losartan ■ receptors

The cardioprotective effect of ACE inhibitors was originally thought to be nearly exclusively the result of inhibition of the conversion of angiotensin (Ang) I to Ang II. Other enzymes, however, are also capable of converting Ang I to Ang II, the most important of these being chymase.1,2 Indeed, in the heart and vasculature, chymase is an important mechanism for the conversion of Ang I to Ang II.1,2 More recently, inhibition of kinin metabolism by ACE inhibitors has also been shown to have important cardioprotective effects.3 Increasing kinin levels results in an increase in nitric oxide and vasodilatory prostaglandins, both of which have numerous actions that counter the effects of vasoconstrictor–growth-promoting substances and stimuli.3 These effects include vasodilatation, attenuation of cardiac and vascular growth, antiatherogenic and antithrombotic effects, and improved myocardial metabolic efficiency.3 Their cardioprotective effects appear to be both direct and indirect. The importance of the cardioprotective effects of bradykinin were most elegantly demonstrated in bradykinin B 1 receptor–knockout mice, which were found to have hypertension and both left ventricular dilatation and dysfunction.4

A new class of medication, the Ang II type I (AT 1 ) receptor blocker, has been developed to better block the vasoconstrictor–growth-promoting effects of Ang II directly at the receptor level.5 The AT 1 receptor is responsible for most of the known biological effects of Ang II.5 The aortic banding pressure overload model of left ventricular hypertrophy in rats by Weinberg et al6,7 suggests that in this setting, ACE inhibitors may have some advantages over AT 1 receptor blockers. The only clinical study comparing the 2 classes of drugs is the ELITE 2 study,8 which found a statistically insignificant trend in favor of the ACE inhibitor (12% reduction in mortality, P=0.16). Thus, the superiority of one class over the other still remains controversial and may

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depend on the setting in which it is studied. More studies comparing the cardioprotective effects of these 2 classes of medications would be helpful, particularly after the HOPE study, which demonstrated that the ACE inhibitor ramipril significantly reduced the mortality of patients with atherosclerosis.

In this study, we created right ventricular (RV) pressure overload by banding the pulmonary artery of rabbits and studied them 21 days later. Rabbits were untreated for control, or they received the ACE inhibitor ramipril or without bradykinin (B₁ and B₂) receptor blockers or the Ang II AT₁ receptor blocker losartan. B₁ and B₂ receptor blockers were given because with inflammation, such as may occur in this model, B₁ receptors may be expressed and exert effects similar to those of B₂ receptors. Cardiac hypertrophy and myocardial contractility were assayed. We then evaluated the changes in Ang AT₁ and AT₂ receptors and in G protein α subunit expression and the effects of these changes on the integrity of the cardiac angiotensin II signaling pathway by using an isolated papillary muscle bioassay.

Methods

New Zealand White male rabbits (Charles River, St-Constant, Québec) weighing ∼1.95 kg were used. The protocols used in this study were approved by the Institutional Animal Research Ethics Committee, and all animals were treated and prepared in accordance with the guidelines of the “Canadian Council on Animal Care Regulations.”

Creation of the Model and Therapeutic Interventions

RV heart failure was induced in 90 rabbits by pulmonary artery banding according to methods previously described by Alpert and Mulieri.10 Once the rabbits were sedated, a spiral model metal coil (inside diameter 2.8 to 3.2 mm; length 6.6 mm; 2.5 turns; 0.7-mm-diameter wire) was applied to the outside of the central end of the pulmonary artery. The rabbits were separated into 5 groups: (1) sham-operated controls; (2) banded, no therapy; (3) banded, ramipril (Avantis Pharmaceuticals) 37.5 mg/kg IP 1 hour after surgery, then 1 mg · kg⁻¹ · d⁻¹ in the drinking water; (4) banded, with the same ramipril regimen, except that the kinin B₁ and B₂ receptor blockers Lys-[Leu 8]desArgBK at 1 mg/kg and HOE 140 at 1 mg/kg (Avantis Pharmaceuticals, respectively), were also given by osmotic pump placed subcutaneously at the time of surgery; and (5) banded, losartan 0.25 mg/kg IP 1 hour after surgery, then 50 mg · kg⁻¹ · d⁻¹ in the drinking water.11

Experiments were conducted 21 days after surgery, after ramipril was stopped for 72 hours, the kinin receptor antagonists for 24 hours, and losartan for 48 hours to permit adequate washout of the medications before further studies.

Hemodynamic and Morphological Studies

Animals were anesthetized with halothane 0.5% to 1% and RV pressure measured by cannulating the jugular vein and advancing a 5F Millar catheter (Millar Instruments Inc) connected to a Gould 2400’s recorder (Gould Inc Instruments Division) to the RV. The animals were then euthanized, and 1 or 2 RV papillary muscles were removed and mounted in an isolated bath. The heart was then separated into the left ventricular free wall, the septum, the RV free wall, and the atria before being weighed and rapidly frozen in liquid nitrogen for measurements of Ang II AT₁ and AT₂ receptor density and G protein α subunit expression.

Papillary Muscle Studies

Papillary muscles were mounted in an isolated bath with Krebs-Henseleit solution at 29°C (in mmol/L: NaCl 118, KCl 4.7, MgSO₄·0.7H₂O 1.2, CaCl₂·H₂O 1.25, KH₂PO₄ 0.18, NaHCO₃ 24, glucose 5, and albumin 6.01×10⁻⁴) and bubbled with 95% O₂/5% CO₂. The base of the muscle was held by a Luice clamp, and the other end was tied to a lever with an electromagnetic feedback system previously described to allow control of force, length, and velocity. Only muscles with a cross section <1.2 mm² were accepted.

The muscles were stimulated at 6 stimuli/min and stabilized at Lmax for 15 minutes. Isometric, isotonic, and unloaded (Vmax) contractions were recorded as previously described.12

A cardiac inotropic concentration-response relationship was performed with Ang II (Sigma Chemical Co) by increasing doses from 1×10⁻¹⁰ to 1×10⁻³ mol/L. Then, to assess maximum contractile capacity, extracellular calcium concentration was increased to 10 mmol/L. At each concentration, isometric, isotonic, and unloaded muscle contractions were recorded.

An additional 6 papillary muscles from rabbits with pulmonary artery banding but no treatment had a phenylephrine concentration-response relationship (10⁻⁴ to 10⁻³ mol/L) done, in the presence of propranolol 10 μmol/L, to assess responsiveness of phospholipase C to stimulation by another receptor system.

Ang II AT₁ and AT₂ Receptor Binding Assays

[Sar², Ile⁶]Ang II (sarile) was iodinated by a solid-phase method, and the moniodinated peptide was purified by high-performance liquid chromatography.13 Radioligand binding assays were conducted as previously described.13 Equilibrium binding constants are reported as dissociation constant (Kd), and receptor concentrations are expressed as fmol/mg protein.

Immunoblot Analysis of G Protein α Subunits

Cardiac membranes were isolated as described.14 Equal amounts of membrane proteins (50 μg) were resolved on 10% acrylamide gels and analyzed by immunoblotting using the following polyclonal antibodies: anti-α₁q W082-8 (1:1000) and anti-α₁/2 SG2.5 (1:5000). Protein bands were quantified by densitometric analysis, and the area of each band was evaluated with NIH Image software.

Statistics

Values are expressed as mean±SEM. Quantitative data were compared between groups by 1-way ANOVA and adjusted for inequalities of variances with a Brown-Forsythe’s test. Student’s t test or, when appropriate, a Tukey or Bonferroni test was used to compare individual differences between groups. Statistical significance was determined by a value of P<0.05.

Results

Pulmonary Artery Banding and Therapeutic Interventions

Survival

The survivals of the various pulmonary artery banded groups beyond the first 24 hours after operation were similar: untreated 94%, ramipril 83%, losartan 82%, and ramipril+kinin B₁ and B₂ receptor blockers 90%.

Hemodynamics and Measurements of Cardiac Hypertrophy

Heart rate was similar in all groups whether pulmonary artery banding had been performed or not (Table 1). RV systolic pressure and maximum rate of pressure rise (+dP/dt) and decline (−dP/dt) increased similarly in the pulmonary artery banded groups. RV end-diastolic pressure was unchanged.

The ratios of RV to body weight and heart to body weight were significantly increased in all banded groups (Table 2), although there was a tendency for this increase to be smaller in the treated groups.
calcium. Modify the normal response to Ang II and extracellular ramipril also resulted in significant prolongation of twitch. Interestingly, the use of the kinin receptor blockers with partially identical to those of the untreated banded group.

During isometric contractions in which values were essentially identical to those of the untreated banded group. Losartan also completely blocked the upregulation of AT1 receptors observed after pulmonary artery banding. No significant change in the affinity of losartan for the AT1 receptor was observed between the various experimental groups (Table 5). We also measured the expression of the AT2 receptor subtype in these membrane preparations. The AT2 receptor density was very low and represented <5% of the total 125I-labeled sarile binding activity. No detectable change in the abundance of AT2 receptors was observed between control rabbits or the various pulmonary artery banded rabbit groups (data not shown).

**Isolated Papillary Muscle Studies**

Papillary muscles from untreated banded rabbits demonstrated marked depression of all tension-generating and shortening indices and lost responsiveness to Ang II (Tables 3 and 4). Muscles responded normally to addition of calcium or phenylephrine $10^{-5}$ mol/L, however, indicating that the signaling pathway from phospholipase C to the myofibril was still functional. With the addition of phenylephrine, tension increased from 17 to 51 mN/mm² and $+\text{d}P/\text{d}t$ from 39 to 206 mN $\cdot$ mm $^{-2}$ $\cdot$ s $^{-1}$, both $P<0.001$.

Ramipril best preserved cardiac contractility, particularly in isometric contractions in which contractility and responsiveness to Ang II were normal. The beneficial effects of ramipril during isometric contraction were less marked, but inotropic responsiveness to Ang II was maintained. Blockade of the kinin B1 and B2 receptors significantly attenuated the cardioprotective effects of ramipril. This was most obvious during isometric contractions in which values were essentially identical to those of the untreated banded group. Interestingly, the use of the kinin receptor blockers with ramipril also resulted in significant prolongation of twitch duration. Treatment with the kinin receptor blockers did not modify the normal response to Ang II and extracellular calcium.

The Ang II AT1 receptor blocker losartan had no obvious beneficial effect on contractile indices during baseline isometric or isometric contractions. It did, however, preserve contractile responsiveness to Ang II. Interestingly, treatment with losartan resulted in the greatest prolongation of twitch duration (time to half tension decline from initiation of twitch of 671 ms).

**Ang II AT1 and AT2 Receptor Binding Properties**

AT1 receptor density was significantly upregulated in the banded group compared with control animals (Table 5). Treatment with ramipril completely prevented this upregulation of the AT1 receptor, whereas the combined administration of kinin receptor antagonists and ramipril only partially prevented the increased expression of the receptor (not significantly different from the banded group). Losartan also completely blocked the upregulation of AT1 receptors observed after pulmonary artery banding. No significant change in the affinity of losartan for the AT1 receptor was observed between the various experimental groups (Table 5). We also measured the expression of the AT2 receptor subtype in these membrane preparations. The AT2 receptor density was very low and represented <5% of the total 125I-labeled sarile binding activity. No detectable change in the abundance of AT2 receptors was observed between control rabbits or the various pulmonary artery banded rabbit groups (data not shown).

**Analysis of G Protein $\alpha$ Subunit Expression**

Pulmonary artery banding significantly increased the expression of $\alpha q$ and $\alpha i1/2$ subunits (Figure). Treatment with ramipril completely reversed the increased expression of $\alpha i1/2$ proteins but had no significant effect on the expression of $\alpha q$. Interestingly, coadministration of kinin B1/B2 receptor antagonists with ramipril restored the high-level expression of $\alpha i1/2$ subunits. In contrast to ramipril, treatment with losartan failed to prevent the upregulation of $\alpha i1/2$ expression in the RV of banded rabbits.

**Discussion**

This study demonstrates that pressure overload of the RV in the rabbit results in marked RV hypertrophy and depression of myocardial contractility. It also results in the nearly total loss of responsiveness to the inotropic effects of Ang II by a mechanism occurring downstream of the AT1 receptor, despite an increase of AT1 receptor density and G protein $\alpha q$ and $\alpha i1/2$ subunit expression. Of the 2 therapeutic interventions evaluated, the one that best preserved cardiac contrac-

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**TABLE 1. RV Hemodynamics**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart Rate, bpm</th>
<th>RV Systolic Pressure, mm Hg</th>
<th>RV End-Diastolic Pressure, mm Hg</th>
<th>Right Atrial Pressure, mm Hg</th>
<th>$+\text{d}P/\text{d}t$, mm Hg/s</th>
<th>$-\text{d}P/\text{d}t$, mm Hg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls (n=10)</td>
<td>181±9</td>
<td>15±1</td>
<td>2±10</td>
<td>3±0</td>
<td>395±29</td>
<td>−385±18</td>
</tr>
<tr>
<td>Banded untreated (n=10)</td>
<td>199±9</td>
<td>34±2*</td>
<td>4±1</td>
<td>3±1</td>
<td>692±82*</td>
<td>−824±86*</td>
</tr>
<tr>
<td>Banded ramipril (n=8)</td>
<td>173±6</td>
<td>38±3*</td>
<td>4±1</td>
<td>3±1</td>
<td>846±78*</td>
<td>−886±86*</td>
</tr>
<tr>
<td>Banded ramipril + B1 + B2 blocker (n=9)</td>
<td>201±7</td>
<td>35±2*</td>
<td>4±1</td>
<td>2±0</td>
<td>954±58*</td>
<td>−796±65*</td>
</tr>
<tr>
<td>Losartan (n=9)</td>
<td>188±4</td>
<td>33±1*</td>
<td>2±0</td>
<td>2±0</td>
<td>761±77*</td>
<td>−797±63*</td>
</tr>
</tbody>
</table>

* $P<0.05$ vs normal controls.

**TABLE 2. Morphological Characteristics of the Hearts**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight, kg</th>
<th>RV-to-Body Weight Ratio, g/kg</th>
<th>Heart-to-Body Weight Ratio, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>2.61±0.12</td>
<td>0.35±0.01</td>
<td>1.87±0.03</td>
</tr>
<tr>
<td>Control banded</td>
<td>2.35±0.06</td>
<td>0.79±0.04*</td>
<td>2.60±0.10*</td>
</tr>
<tr>
<td>Ramipril banded</td>
<td>2.26±0.09</td>
<td>0.68±0.04*</td>
<td>2.31±0.11*</td>
</tr>
<tr>
<td>Ramipril + B1 + B2 blocker banded</td>
<td>2.10±0.06*</td>
<td>0.71±0.03*</td>
<td>2.46±0.09*</td>
</tr>
<tr>
<td>Losartan</td>
<td>2.32±0.05</td>
<td>0.74±0.02*</td>
<td>2.47±0.05*</td>
</tr>
</tbody>
</table>

B1 and B2 blockers specifically inhibit receptor for bradykinin (B1) and des-arg4 bradykinin (B2).

* $P<0.05$ vs normal controls.
tivity and best normalized AT1 receptor density and G protein expression was the ACE inhibitor ramipril. This difference compared with the AT1 receptor blocker losartan appeared to be largely due to its effect on kinins, because many of the beneficial effects of ramipril were eliminated by the simultaneous use of kinin receptor blockers. All therapeutic interventions preserved the contractile responsiveness to Ang II, suggesting that reduction in the production of Ang II or blockade of the AT1 receptor prevents the functional uncoupling of the receptor to downstream signaling events.

The importance of specific enzyme systems in the conversion of Ang I to Ang II varies according to the species and the tissue being studied.1,2 For this reason, in pilot studies (data not shown), we verified that in the rabbit, the conversion of Ang I to Ang II occurred at the cardiac level. We also determined that the combined use of an ACE inhibitor and a chymase inhibitor resulted in much greater inhibition of the contractile effects of Ang I than either alone, however, indicating that when one pathway is inhibited, the other compensates by increasing the conversion rate of Ang I to Ang II.

Cardioprotective Effect of the ACE Inhibitor Ramipril Compared With the Ang II AT1 Receptor Blocker Losartan: Importance of Kinins

RV pressure overload led to significant RV hypertrophy and papillary muscle dysfunction. Despite this marked reduction in contractility, no in vivo reduction in RV function was documented, perhaps because of the contribution of compensatory hypertrophy and in vivo activation of compensatory mechanisms such as neurohumoral activation.

The ACE inhibitor ramipril had the most important cardioprotective effect of the 3 therapeutic interventions evaluated. Although ramipril tended to reduce RV hypertrophy, this did not reach statistical significance, perhaps because of the fixed afterload of pulmonary artery banding as reflected by similar RV systolic pressures in all groups, or the relatively short follow-up period. The lack of effect of losartan on RV hypertrophy in this model was reported by Koide et al.14 Ramipril nearly completely prevented the decrease of contractility, however, this effect being more marked for isometric contractions than isotonic contractions. The more marked effect on isometric contractions may result from changes in expression of myosin isozymes to a slower, more energy-efficient one in this model.10 As a result, contraction would be slowed and prolonged, such that tension-generating abnormalities would be minimized while abnormalities in velocity of shortening would be maximized.

The protective effects of ramipril on cardiac contractility were significantly reduced by the simultaneous infusion of kinin B1 and B2 receptor blockers. The mechanisms remain speculative and probably multifactorial; however, this may result in part from nitric oxide–mediated improvement in cardiac metabolism.15 With left ventricular hypertrophy, subendocardial blood flow reserve is reduced16 such that improved cardiac metabolism and coronary flow would be expected to improve cardiac energetic balance and be cardio-

### TABLE 3. Isometric Papillary Muscle Contractile Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=12)</th>
<th>Banded Control (n=12)</th>
<th>Banded Ramipril (n=11)</th>
<th>Banded Ramipril + B1 + B2 Blocker (n=8)</th>
<th>Banded Losartan (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tension, mN/mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>26±2</td>
<td>16±2</td>
<td>26±6</td>
<td>14±2*</td>
<td>17±3*</td>
</tr>
<tr>
<td>Ang II</td>
<td>47±8</td>
<td>16±2</td>
<td>35±9†</td>
<td>23±4*</td>
<td>22±4*</td>
</tr>
<tr>
<td>High calcium</td>
<td>69±7</td>
<td>30±3</td>
<td>56±12†</td>
<td>42±7*</td>
<td>46±6†</td>
</tr>
<tr>
<td>+ dT/dt, mN·mm⁻¹·s⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>104±10</td>
<td>35±5</td>
<td>89±26†</td>
<td>36±7*</td>
<td>39±7*</td>
</tr>
<tr>
<td>Ang II</td>
<td>285±65</td>
<td>36±5</td>
<td>156±55†</td>
<td>79±17†</td>
<td>64±13†</td>
</tr>
<tr>
<td>High calcium</td>
<td>578±86</td>
<td>142±15</td>
<td>350±88†</td>
<td>231±44†</td>
<td>235±38†</td>
</tr>
<tr>
<td>− dT/dt, mN·mm⁻¹·s⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>69±7</td>
<td>32±5</td>
<td>76±23</td>
<td>34±8*</td>
<td>42±11*</td>
</tr>
<tr>
<td>Ang II</td>
<td>135±14</td>
<td>35±5</td>
<td>105±28†</td>
<td>73±15†</td>
<td>57±15†</td>
</tr>
<tr>
<td>High calcium</td>
<td>166±10</td>
<td>103±9</td>
<td>174±38</td>
<td>124±17</td>
<td>140±19</td>
</tr>
<tr>
<td>TTPT, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>249±7</td>
<td>292±27</td>
<td>329±24*</td>
<td>369±30*</td>
<td>426±34*†</td>
</tr>
<tr>
<td>Ang II</td>
<td>231±6</td>
<td>226±21</td>
<td>254±16</td>
<td>308±9†‡</td>
<td>342±30†‡</td>
</tr>
<tr>
<td>High calcium</td>
<td>193±7</td>
<td>238±6</td>
<td>239±8*</td>
<td>273±9†‡</td>
<td>299±13*†‡</td>
</tr>
<tr>
<td>RT₁/₂, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>449±16</td>
<td>486±43</td>
<td>561±42</td>
<td>617±37†</td>
<td>671±41†</td>
</tr>
<tr>
<td>Ang II</td>
<td>470±31</td>
<td>374±31</td>
<td>445±33</td>
<td>511±22†</td>
<td>553±43†</td>
</tr>
<tr>
<td>High calcium</td>
<td>480±21</td>
<td>414±20</td>
<td>450±34</td>
<td>499±15†</td>
<td>517±31†</td>
</tr>
</tbody>
</table>

TTPT indicates time to peak tension; RT₁/₂, time to half tension decline from initiation of twitch. Ang II at 10⁻⁵ mol/L concentration; high calcium = 10 mmol/L concentration.

*P<0.05 vs normal controls, †P<0.05 vs untreated banded, ‡P<0.05 vs ramipril banded.
measure the integrity of the Ang II signaling pathway. In the presence of the positive inotropic effects of Ang II, but they did not describe the functional ability of these receptors. One study in the present study, we found significant upregulation of AT1 receptor expression despite functional uncoupling of the AT1 receptor. Although the pattern of uncoupling of Ang II to its effector organ response differs somewhat from that of the protective. The effects of ramipril without its kinin component were comparable to those of losartan, suggesting that the major cardioprotective effect of ramipril in this model is due to its effect on kinins.

**Functional Uncoupling of the Ang II AT1 Receptor: Effects of ACE Inhibition and AT1 Receptor Blockade**

There was complete functional uncoupling of the AT1 receptor from its contractile effects despite an increase in AT1 receptor density. In previous studies, AT1 receptor density and affinity were evaluated in various models of ventricular dysfunction and overload, but little information exists as to the functional ability of these receptors. One study in the pacing-overdrive model of heart failure demonstrated a loss of the positive inotropic effects of Ang II, but they did not measure the integrity of the Ang II signaling pathway. In the present study, we found significant upregulation of AT1 receptor expression despite functional uncoupling of the AT1 receptor, indicating a functional defect beyond the receptor. Although the pattern of uncoupling of Ang II to its effector organ response differs somewhat from that of the β-adrenergic system, the end result is protection against chronic overactivation of an endogenous neurohumoral system.

Whether protection of the integrity of the AT1 signaling pathway with the various interventions that we tested is beneficial or potentially detrimental if the drug is discontinued, as occurs with β-blockers, remains to be determined, but a risk of rebound overactivation clearly exists.

We did not find any modification in AT2 receptor expression. This finding is consistent with previous studies in heart failure and cardiac hypertrophy, in which AT2 receptor density has been found to be unchanged. The expression of the G protein subunit αq, which is primarily responsible for coupling the AT1 receptor to phospholipase C-β and Ca2+ mobilization in cardiomyocytes, increased by 65% in banded rabbits, excluding the possibility that the loss of Ang II responsiveness was due to a decreased expression of Gαq. Also, the contractile effects of phenylephrine, another potent agonist of the Gαq-phospholipase C-β2 signaling pathway, were preserved, if not exaggerated, indicating that the signaling pathway from phospholipase C to the myofibril was intact.

Interestingly, all pharmacological interventions tested re-established the cardiac responsiveness to Ang II, suggesting that the functional uncoupling of the AT1 receptor in the untreated banded group results from the local increase in Ang II levels and the chronic stimulation of the receptor. The most
plausible explanation to reconcile all these observations is that the increased level of agonist in cardiac tissue uncouples the \( \text{AT}_1 \) receptor from the \( G \) protein, resulting in functional desensitization of the receptor.

The expression of \( G \) protein \( a \) subunits increases in heart failure and in various models of cardiac hypertrophy. 23–25 We found that expression of the \( G_{\alpha i1} \) proteins was upregulated and that treatment with ramipril, but not with losartan or the combination of ramipril with kinin receptor blockers, completely prevented this increase. These results suggest that the increase in \( G \) protein \( a1/2 \) subunits may be causally linked to the contractile dysfunction observed in these animals but not related to the desensitization of the \( \text{AT}_1 \) receptor.

Acknowledgments

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