Is the Pregnancy Hormone Relaxin Also a Vasodilator Peptide Secreted by the Heart?

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Background—It has been shown recently that the pregnancy and parturition hormone, relaxin, is secreted by the heart. This study examined the effects of relaxin in small human resistance arteries from the systemic and pulmonary circulations.

Methods and Results—Arteries were obtained from gluteal biopsies and resected lung tissue and studied with the use of wire myography. Cumulative concentration relaxation curves were constructed in systemic arteries with substance P, epoprostenol, atrial natriuretic peptide, and relaxin (concentration range $10^{-13}$ - $10^{-7}$M). The maximal responses were $88(\pm5)\%$, $67(\pm10)\%$, $52(\pm16)\%$, and $66(\pm16)\%$, respectively. Endothelium removal virtually abolished the action of relaxin. Relaxin had no vasodilator effect in pulmonary arteries.

Conclusions—Relaxin is a powerful dilator of systemic resistance arteries secreted by the heart that may contribute to cardiovascular regulation. (Circulation. 2002;106:292-295.)

Key Words: vasodilation • arteries • endothelium • peptides

Relaxin, a hormone belonging to the insulin family and recently shown to act through 2 orphan G-protein receptors, has an established role in the female reproductive tract.1–3 Known sources of relaxin are the ovary, decidua, placenta, and the prostate.1 Three molecular forms of relaxin, H1 through H3, encoded for by separate genes, are found in humans.1–3 H2 is the main circulating form, whereas H1 is produced in the placenta.1 The source of H3 is uncertain.3 Until lately, relaxin has only been thought to prepare the birth canal and mammary gland for parturition.1 New evidence suggests that relaxin may be more than a pregnancy hormone.

Recently, relaxin mRNA expression has also been shown in the human left ventricle, atrium, internal mammary artery, and saphenous vein.4 Cardiac mRNA expression and secretion of relaxin are increased in chronic heart failure.4 Circulating concentrations of relaxin are also markedly elevated in heart failure, in proportion to clinical severity.4 Consequently, as with other peptides, such as atrial natriuretic peptide (ANP) and brain natriuretic peptide, cardiac relaxin secretion may be a compensatory response in heart failure, and relaxin may be a circulating vasoactive hormone. This study evaluates the actions of relaxin in human systemic and pulmonary resistance arteries.

Methods

Patients

Thirteen patients with coronary heart disease but normal left ventricular systolic function were studied. Lung tissue was obtained from an additional 5 patients undergoing pneumonectomy for cancer. The study had Ethics Committee approval, and patients gave informed consent.

Materials

Human recombinant H2 relaxin was a gift from Connetics Corporation, Palo Alto, Calif. Experiments were performed in physiological salt solution with the following composition (mmol/L): NaCl 118.4, KCl 4.7, MgSO4·H2O 1.2, KH2PO4 1.2, NaHCO3 24.9, CaCl2 2.5, glucose 11.1, EDTA 0.023 (which gives a pH of 7.4 when gassed with a 5% CO2 : 95% O2 mixture). A Mulvany-Halpern 4-channel wire myograph (Danish Myotech) was used.

Systemic Resistance Artery Studies

Gluteal Biopsy Procedure and Artery Preparation

Gluteal biopsies were obtained under local anesthesia (1% lidocaine), as previously described.6 Resistance arteries (diameter <300 μm, length ~2 mm) were dissected and mounted in the myograph as previously described.5,6 The bath was gassed and heated for the duration of the experiment.

Experimental Protocol (With Intact Endothelium)

After 30 minutes of rest, each artery was set to the normalized internal diameter at which contraction is thought to be optimal.5,6 The vessels were left for another hour and then repeatedly exposed...
to a high potassium salt solution until reproducible maximal contractions were achieved. Vessels were preconstricted with $10^{-3}$ norepinephrine and $10^{-6}$ ACh was added to test for endothelial integrity. Cumulative concentration response curves (CCRCs) were then constructed in vessels preconstricted with $10^{-5}$ mol/L norepinephrine using substance P, epoprostenol, ANP, and relaxin (concentration range $10^{-15}$ to $10^{-7}$ mol/L).

**Procedure for Removal of Endothelium and Experimental Protocol in Deendothelialized Vessels**

Endothelium was mechanically removed from another set of vessels by gently rubbing the luminal side of the arterial wall with a human hair (stored in ethanol and rinsed with physiological salt solution before use).6 Endothelial removal was confirmed by the lack of relaxation to ACh.6 A CCRC was then constructed with relaxin $10^{-15}$ to $10^{-7}$ mol/L.

**Pulmonary Resistance Artery Studies**

**Artery Preparation**

Lung tissue was placed in cold Krebs-buffer solution. Pulmonary resistance arteries (diameter <300 µm, length ≈2 mm) were dissected and mounted in the myograph as described previously.7

**Experimental Protocol**

Tension was applied to vessels to give transmural pressures equivalent to 12 to 16 mm Hg to simulate in vivo pressures. Vessels were allowed to equilibrate and endothelial integrity was tested, as described above. CCRC were then constructed with relaxin $10^{-15}$ to $10^{-7}$ mol/L, after preconstricting each vessel with U46619—endothelin analog—a thromboxane A2 mimetic (norepinephrine does not induce sustained constriction of human pulmonary vessels).

**Analysis of Data**

Responses (mean±SEM) are expressed as % relaxation from maximally preconstricted levels.

**Comparisons Between Relaxin and Other Vasodilators**

Statistical comparison of maximum responses (within the concentration range tested) was performed with the use of unpaired Student’s t test and Dunnett’s post hoc test for multiple comparisons.3–7 Comparison of curves was by one-way ANOVA for repeated measures.3–7

**Results**

**Subjects**

Tables 1 and 2 show the characteristics of the patients studied.

**Studies in Small Systemic Resistance Arteries With Intact Endothelium**

**Resistance Artery Diameter**

The mean internal diameter of the systemic resistance arteries was $283±21$ µm. ACh caused an $88±4\%$ reduction in norepinephrine-induced tone, verifying the existence of an intact endothelium.

**Response to Vasodilators**

Figure 1 shows the vasodilator activity of substance P (n=8), epoprostenol (prostaglandin I$_2$) (n=6), ANP (n=6), and relaxin (n=8).

Substance P, as noted previously, was a powerful vasodilator. Relaxin had comparable activity to epoprostenol. ANP was the weakest of the vasodilators studied. The maximal responses were $88(±5)\%$, $66(±16)\%$, $67(±10)\%$, and $52(±16)\%$, respectively.

A 10% of maximal vasodilator response was achieved with $1×10^{-13}$ mol/L substance P, $2×10^{-13}$ mol/L epoprostenol, $1×10^{-12}$ mol/L relaxin, and $3×10^{-10}$ mol/L ANP ($P<0.05$ for relaxin versus ANP).

**Response to Relaxin in Systemic Resistance Arteries After Removal of Endothelium**

Figure 2 shows that endothelial rubbing virtually abolished the action of relaxin (n=5, P<0.05).

**TABLE 1. Characteristics of Patients Providing Small Systemic Resistance Arteries**

<table>
<thead>
<tr>
<th>Number of patients</th>
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</thead>
<tbody>
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<td>Sex, M/F</td>
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<tr>
<td>Age, y (range)</td>
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<tr>
<td>Previous MI</td>
<td>4</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>6</td>
</tr>
<tr>
<td>Current smoker</td>
<td>3</td>
</tr>
<tr>
<td>Drug therapy</td>
<td></td>
</tr>
<tr>
<td>$\beta$-Blocker</td>
<td>11</td>
</tr>
<tr>
<td>Aspirin</td>
<td>13</td>
</tr>
<tr>
<td>HMG CoA reductase inhibitor</td>
<td>13</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>6</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>2</td>
</tr>
<tr>
<td>Diuretic</td>
<td>4</td>
</tr>
<tr>
<td>Digoxin</td>
<td>13</td>
</tr>
<tr>
<td>Nitrate</td>
<td>5</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>2</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>105±5</td>
</tr>
</tbody>
</table>

M/F indicates male/female; MI, myocardial infarction; CABG, coronary artery bypass grafting; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; and ACE, angiotensin-converting enzyme.

**TABLE 2. Characteristics of Patients Providing Small Pulmonary Resistance Arteries**

<table>
<thead>
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<tbody>
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<tr>
<td>Age, y (range)</td>
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<tr>
<td>Previous MI</td>
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<tr>
<td>Previous CABG</td>
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<tr>
<td>Current smoker</td>
<td>1</td>
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<tr>
<td>Drug therapy</td>
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<tr>
<td>$\beta$-Blocker</td>
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<tr>
<td>Aspirin</td>
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<tr>
<td>HMG CoA reductase inhibitor</td>
<td>0</td>
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<tr>
<td>ACE inhibitor</td>
<td>2</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>1</td>
</tr>
<tr>
<td>Diuretic</td>
<td>1</td>
</tr>
<tr>
<td>Digoxin</td>
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<tr>
<td>Nitrate</td>
<td>0</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>106±3</td>
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</tbody>
</table>

Abbreviations as in Table 1.
Response to Relaxin in Small Pulmonary Resistance Arteries

The mean internal diameter of the pulmonary vessels (n=5) was 208.8±10.6 μm. ACh caused a 76±21% reduction in agonist-induced tone, yet relaxin had no effect (data not shown).

Discussion

Our findings show that relaxin is a more potent arterial vasodilator than ANP, also secreted by the heart. It had been suggested that relaxin might exert its systemic vasodilator effects by stimulating the secretion of ANP. However, our results show that relaxin is a vasodilator in its own right. Furthermore, relaxin is vasoactive at concentrations comparable to those found in chronic heart failure. Mean plasma relaxin concentrations in patients with severe chronic heart failure average 2.5 to 3.34±111 mol/L. At 10⁻¹¹ mol/L, relaxin caused 26% vasodilation of resistance arteries (versus 0.68% with the same concentration of ANP).

The potency of relaxin is impressive. It is equipotent to epoprostenol, a substance regarded as a powerful vasodilator and used therapeutically in cardiovascular disease. Of course, epoprostenol is a particularly effective pulmonary vasodilator, whereas relaxin seems to be devoid of this action. We also found that relaxin-induced vasodilation is endothelium dependent. Removal of the endothelium almost abolished its effect. This is in keeping with observations in other species that relaxin may exert its vasodilator action through nitric oxide. This suggests that relaxin does not act as a vasodilator in the placental-fetal circulation. We found that relaxin is inert in preconstricted human pulmonary resistance arteries, in contrast to systemic vessels. This may reflect differing relaxin-receptor distribution in the circulation, as the nitric oxide vasodilator pathway was functionally intact in these pulmonary vessels.

Relaxin could have other potentially favorable vascular and nonvascular actions in cardiovascular disease. For example, it increases vascular endothelial growth factor, antagonizes the vasoconstrictor action of other peptides, such as angiotensin II, and inhibits collagen synthesis/increases collagen breakdown. The last action, key to the pelvic remodeling effect of relaxin, could also be important in cardiac and vascular remodeling. In addition, relaxin has also been reported to protect against experimental ischemia-reperfusion injury.

Though the true vasoregulatory role of relaxin can only be assessed with the use of an antagonist, it does seem to be more potent than ANP, a hormone that circulates at a similar concentration and the inhibition of which leads to vasoconstriction and other potentially adverse cardiovascular effects.

In summary, we have shown that relaxin, a hormone now known to be secreted by the heart, is a potent vasodilator of small systemic resistance arteries at pathophysiological concentrations, and that this action is endothelium dependent. Relaxin is not, however, a pulmonary vasodilator. The pleiotropic actions of relaxin suggest that its potential role in cardiovascular regulation merits further investigation.

Acknowledgments

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References

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