Guanine Nucleotide–Binding Proteins in Aortic Smooth Muscle From Hypertensive Rats

Pinlan Li, Ai-Ping Zou, Nabil J. Al-Kayed, Nancy J. Rusch, David R. Harder

Abstract
To explore the hypothesis that altered vascular muscle signal transduction may underlie some of the vascular changes observed in hypertensive models, we measured expression of GTP-binding protein (G protein) α-subunits, Gᵢ, Gₛ, and Gₛ, in aortic muscle of reduced renal mass and sham-operated rats and proximal and distal aortic segments from rats with interrenal aortic coarctation (IR-AC). G protein expression was measured by immunoblot analysis. When we probed aortic muscle membrane with Gₛ and Gₛ α-subunit antibodies, we identified 41- and 42-kD immunoreactive proteins, respectively. Three immunoreactive bands specific to Gₛ α-subunit antibody were resolved. Immunoreactive blot densities were compared. In aortic muscle membrane of reduced renal mass rats (blood pressure, 148±7 mm Hg), we found significantly reduced Gₛ and Gₛ blot densities compared with sham-operated controls (blood pressure, 99±12 mm Hg). There were no differences in Gᵢ blot densities between reduced renal mass and control rats. Gₛ and Gₛ blot densities were significantly lower in IR-AC proximal aortic segments (carotid pressure, 165±5 mm Hg) and distal aortic segments (femoral pressure, 121±4 mm Hg) than in aortas of sham-operated controls. In contrast, Gₛ expression was significantly increased in the high-pressure proximal aortic segments compared with low-pressure distal aortic segments from IR-AC rats. Thus, altered G protein expression occurs in aortic muscle from nongenetic rat models of hypertension. Given the differences between reduced renal mass and IR-AC models, it is clear that pressure is not the only variable regulating G protein expression and that hormonal and/or metabolic influences probably play a larger role.

Key Words • G protein • muscle, smooth, vascular • hypertension, experimental • rats

Methods
Experimental Animal Models
Adult male Sprague-Dawley rats (weight, 250±8 g; purchased from Sasco) were subjected to 75% reduction in renal mass by a two-stage operation to produce reduced renal mass (RRM) rats.³⁴ Normal control rats were subjected to the same surgical procedure except for removal of renal tissue (sham rats). After recovery for 1 week, both RRM and sham rats received a 4% NaCl diet for 2 weeks. Then rats were anesthetized with 40 mg/kg ketamine-HCl (Ketaset, Aveco) followed by 20 to 30 mg/kg sodium pentobarbital (Veterinary Labs, Inc.). Catheters were placed into the femoral artery for measurement of arterial pressure, and then thoracic aortas were removed and placed in physiological salt solution.

In other experiments interrenal aortic coarctation (IR-AC) rats were produced. Sprague-Dawley rats were anesthetized with telazol (40 mg/kg IM), and the aorta was partially ligated between the renal arteries with 2-0 silk.³⁶ The animals received penicillin (40 000 IU IM) and were allowed to recover. After 4 weeks rats were anesthetized intraperitoneally with 40 mg/kg ketamine-HCl followed by 20 to 30 mg/kg sodium pentobarbital. Mean arterial pressure (MAP) above and below the coarctation was measured by catheterization of common carotid and femoral arteries, respectively, and aortas proximal and distal to the coarctation site were removed.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Blotting
Plasma membrane microsomes of aortic smooth muscle were prepared according to described methods.⁷ Protein concentrations of the plasma membrane preparations were determined by described methods.⁸ Thirty micrograms of membrane protein was subjected to electrophoresis after heating to 100°C for 3 minutes. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed with a 12% gel for 140 minutes using a Mini-Protein II electrophoresis cell (Bio-Rad).⁹ The proteins were electrophoretically transferred overnight onto nitrocellulose with a Mini-Transblot apparatus (Bio-Rad) at 30 V in transfer buffer (25 mmol/L Tris, 192 mmol/L glycine, 20% [vol/vol] methanol) containing 0.0025% to 0.005% sodium dodecyl sulfate. Non-specific binding sites were blocked by immersing the membrane in Tris buffer saline with Tween 20 (TBS-T) (30 mmol/L Tris-HCl, pH 7.5, 0.2 mmol/L NaCl, 0.05% Tween 20) containing 5% bovine serum albumin (Sigma Chemical Co) with continuous shaking. The membranes were washed once for 15 minutes and twice for 5 minutes in fresh TBS-T and then incubated with 1:1000 specific rabbit anti-Gₛα, anti-Gₛα, and anti-Gₛα protein antibodies for 1 hour at 22°C, respectively.
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**915**

**Gi**

- 49.5 KD
- 32.5 KD

**Gq**

- 49.5 KD
- 32.5 KD

**Gs**

- 49.5 KD
- 32.5 KD

**SHAM** **RRM**

**Figure 1.** Immunoblot analysis shows α-subunits of G proteins of plasma membrane of aortas from reduced renal mass (RRM) and sham rats.

The free primary antibodies were completely removed by washing the membrane with TBS-T as above. Subsequently, the membranes were incubated for 1 hour with 1:1000 horseradish peroxidase-labeled donkey anti-rabbit IgG. After the membranes were washed five times for 10 minutes to remove free secondary antibody, 10 mL of detection solution 1 and 2 (1:1) (Amersham) was added directly to the blots on the surface carrying the protein. After incubation for 1 minute at 22°C, excess detection solution was drained off, and the membranes were wrapped in plastic wrap. The densities of blot bands were determined with a microcomputer imaging system (MCID; Imaging Research, Inc). Anti-G\(\alpha\)Q\(\alpha\), anti-G\(\alpha\)α, and anti-G\(\alpha\)α protein antibodies were purchased from Du Pont.

**Statistical Analysis**

Data are presented as mean±SEM. Significance of differences within and between groups was evaluated with ANOVA for independent measures followed by Duncan's multiple range test. A value of \(P<.05\) was considered statistically significant.

**Results**

**Hypertensive Animal Models**

After 2 weeks on a high salt diet, RRM rats under anesthesia showed an average MAP of 148±7 mm Hg compared with 99±12 mm Hg in sham rats. MAP proximal to the coarctation site in IR-AC rats was 165±5 mm Hg in the common carotid artery, which was higher than pressure distal to the coarctation (122±4 mm Hg) in the femoral artery. Sham-operated controls for IR-AC rats showed a normal MAP of 122±6 mm Hg.

**Changes in α-Subunit Density in RRM**

**Aortic Membranes**

Enhanced chemiluminescence blots of nitrocellulose membrane probed with different α-subunit antibodies of G proteins are presented in Fig 1. Bands of 41 and 42 kD were identified, respectively, when membrane was probed with antibodies against G\(\alpha\)α and G\(\alpha\)Q\(\alpha\). Three bands of 37, 42, and 44 kD were detected when membrane was probed with antibody to G\(\alpha\)Q\(\alpha\). The densities of G\(\alpha\)α and G\(\alpha\)Q\(\alpha\) immunoreactive blots of aortic muscle membrane (AMM) from RRM rats were markedly lower than in sham rats, but G\(\alpha\)α blot densities were the same. Changes in G\(\alpha\) proteins of AMM between RRM and sham rats are summarized in Fig 2.

**Changes in α-Subunit Density in IR-AC**

**Aortic Membranes**

A comparison of G\(\alpha\)α blot densities between AMM of IR-AC and sham rats is shown in Fig 3. A 41-kD immunoreactive protein band was detected in AMM of IR-AC and sham rats when probed with anti-G\(\alpha\)α antibody (panel A). G\(\alpha\)α was lower in AMM of distal

**Results**

**Hypertensive Animal Models**

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**Changes in α-Subunit Density in RRM**

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**Aortic Membranes**

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compared with proximal segments of both IR-AC and sham rats, suggesting anatomic variation within the aorta (panel B). In addition, $G_\alpha$ was decreased in both proximal and distal aortic segments of IR-AC compared with sham rats. Because in situ pressure was similar between sham rats and distal aorta of IR-AC rats, this finding implied altered $G$ protein expression independent of pressure modulation.

Changes in $G_\alpha$ in AMM of IR-AC and sham rats are presented in Fig 4. Three bands specific to anti-$G_\alpha$ in IR-AC and sham rats were identified (panel A). $G_\alpha$ blot density was decreased in distal compared with proximal aortic segments of IR-AC rats, implying pressure regulation of $G_\alpha$ expression. In addition, however, blot densities of AMM from both proximal and distal aorta were lower in IR-AC than sham rats, paralleling the altered expression of $G_\alpha$ in this model and providing further evidence for a nonpressure modulator of $G$ protein expression (panel B).

Densities of $G_\alpha$ from AMM of IR-AC and sham rats are shown in Fig 5. A 42-kD protein was identified in proximal and distal aortas of IR-AC and sham rats (panel A). Sham AMM showed a similar $G_\alpha$ in proximal and distal aortic segments, but IR-AC rats expressed significantly higher $G_\alpha$ in proximal segments (panel B). Compared with the same aortic segments in sham rats, $G_\alpha$ in proximal aortas of IR-AC rats was significantly higher, whereas $G_\alpha$ in distal aortas of IR-AC rats was reduced (panel B). A summary of the direction of $G$ protein expression in AMM of all rats is given in Table 1.

### Discussion

Reports discussing $G$ protein function of vascular smooth muscle in hypertension are limited. Vasoactive agents involved in the control of blood pressure such as catecholamines, angiotensin II, bradykinin, vasopressin, atrial natriuretic peptide, and endothelins are linked to $G$ protein activation.\(^\text{10,11}\) Recent studies showing altered adenylate cyclase activity in hypertension in the absence of altered $\beta$-adrenergic receptor expression suggest that high blood pressure may modulate other components of the signaling cascade.\(^\text{12,13}\) Also, enhanced phosphoinositide hydrolysis with normal ligand-receptor binding has been associated with supersensitivity of vascular muscle to $\alpha$-receptor agonists in several models of hypertension.\(^\text{14-16}\) However, cultured arterial muscle from spontaneously hypertensive rats may not exhibit altered expression of $G_\alpha$ or $G_\beta\gamma$\(^2\) suggesting that a clear link between $G$ protein expression and high blood pressure has not been established. Table 2 summarizes some of the transduction processes involving $G$ proteins, which theoretically could alter vascular muscle reactivity. Table 2 is not a complete list of $G$ protein involvement in signal transduction\(^\text{10}\) but does provide a theoretical framework from which to view our findings and suggests that $G_\alpha^\text{IR-AC vs Sham}$
the measurement of qualitative changes in arterial G protein expression is only the first step in unraveling the complex contribution of these proteins to the regulation of vascular reactivity.

There may be some correlation between the altered G protein expression reported here and previous changes in arterial reactivity in hypertension. For example, AMM exposed to high pressure (RRM rats and proximal segments of IR-AC rats) showed lower Gα subunit levels compared with sham rats. Because stimulation of Gα increases adenylate cyclase activity to mediate β-adrenergic vasodilation,1 this downregulation of Gα may help to explain the loss of β-adrenergic inhibition of arterial tone in RRM rats, which may promote arterial constriction.19 Furthermore, we have recently noted that pressurization of isolated renal arteries increases inositol trisphosphate levels, a process probably dependent on Gα. The finding in the present study of elevated Gα levels in aortas exposed to high pressure in IR-AC rats raises the possibility that enhanced Gα expression may reflect the increased requirement for this protein for phospholipase C pathway signaling in hypertension.18 Finally, Gα proteins were reduced in AMM of RRM rats and proximal segments of IR-AC rats. Their coupling to inhibition of voltage-sensitive Ca2+ channels and stimulation of K+ channels10-17 suggests that lowered Gα expression may increase Ca2+ influx and reduce K+ efflux in hypertension, thereby elevating arterial tone.

Although the definition of the relation between G protein expression and vascular reactivity changes in hypertension is still evolving, our results suggest that a complex interaction between anatomic site, pressure, and other modulating factors determined the final pattern of G protein expression. For example, Gα and Gα were reduced in high-pressure proximal aortas of IR-AC rats compared with proximal aortas of sham rats. Similarly, Gα and Gα levels were reduced in RRM aortas exposed to high pressure. However, Gα and Gα were also lower in the distal segment of IR-AC rats than in the distal segment of sham rats, despite apparently similar anatomic sites and final blood pressure levels. Such data suggest that pressure is not the only regulating variable and that other modulating factors, perhaps hormonal or neural, also may alter Gα and Gα expression.18,20,21 Similarly, Gα expression was unchanged in hypertensive RRM rats but was enhanced in high-pressure proximal segments of IR-AC rats, again suggesting that high pressure per se does not determine the final level of G protein expression in arterial muscle.

From these studies it is clear that alterations in G protein expression occur in AMM of experimental rat models of hypertension. At least one prior study has shown that Gα and Gα expression are not different in cultured arterial muscle from spontaneously hypertensive rats compared with normotensive controls.2 However, it is difficult to compare results between cultured and noncultured arterial cells given the length of time that cultured cells are removed from the hypertensive environment and the problems of cell dedifferentiation. Rather, our data suggest that one should consider changes in G protein expression when contemplating the source of altered signal transduction and vascular reactivity in arteries freshly isolated from rats with experimental hypertension.

**Acknowledgments**

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**References**


**TABLE 2. Summary of the Transduction Processes Involving G Proteins**

<table>
<thead>
<tr>
<th>Transduction Protein</th>
<th>Process</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gα</td>
<td>Activation of adenylate cyclase</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Activation of voltage-sensitive Ca2+ channel</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Inhibition of Na+ channels</td>
<td>5</td>
</tr>
<tr>
<td>Gα</td>
<td>Inhibition of adenylate cyclase</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Stimulation of K+ channels</td>
<td>10, 17</td>
</tr>
<tr>
<td></td>
<td>Activation of phospholipase A2</td>
<td>10</td>
</tr>
<tr>
<td>Gα</td>
<td>Phospholipase C (generation of IP3, DAG)</td>
<td>18</td>
</tr>
</tbody>
</table>

IP3 indicates inositol 1,4,5-trisphosphate; DAG, diacylglycerol.
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