G protein mRNA expression in renal microvessels from spontaneously hypertensive and Wistar-Kyoto rats

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Mokkapati, Rupa, Subhash J. Vyas, and Edwin K. Jackson. G protein mRNA expression in renal microvessels from spontaneously hypertensive and Wistar-Kyoto rats. Am. J. Physiol. 273 (Renal Physiol. 42): F877–F882, 1997.—The exaggerated sensitivity of spontaneously hypertensive rat (SHR) renal microvasculature to angiotensin II (ANG II) may be due to an imbalance between the effectiveness of Gαs-utilizing vasodilator pathways and vasoconstrictor pathways activated by ANG II (mediated by Gαi1, Gαi2, Gαi3, and Gαq). Because the alteration appears to be distal to the hormone receptors and proximal to the effector adenyl cyclase, we hypothesized that SHR have altered amounts of signal-transducing G proteins. This was examined by quantifying the steady-state mRNA levels of specific Gα subunits in renal microvessels of 12- to 14-wk-old SHR and control Wistar-Kyoto (WKY) rats, using a quantitative-competitive polymerase chain reaction technique coupled to reverse transcription. No significant differences were detected in the absolute levels of Gαs (0.96 ± 0.35 vs. 0.74 ± 0.25 amol/50 ng RNA) or in the relative levels of Gαi1 (0.44 ± 0.05 vs. 0.48 ± 0.13), Gαi2 (40.9 ± 7.8 vs. 45.2 ± 8.9), or Gαi3 (0.79 ± 0.05 vs. 0.82 ± 0.15) normalized to the level of Gαq for WKY vs. SHR, respectively. The ratio of Gαq to Gαs tended to be higher in SHR, but this difference did not achieve statistical significance (0.41 ± 0.08 vs. 1.04 ± 0.32, P = 0.08). In conclusion, the steady-state levels of Gαq, Gαi1, Gαi2, Gαi3, and Gαs are similar in SHR and WKY renal microvasculature, suggesting that other components of the ANG II signal transduction mechanism are responsible for the enhanced renal vascular responsiveness in SHR.

The number and subtype composition of ANG II receptor subtypes is similar in the preglomerular vessels of the two strains (2). Moreover, pretreatment with pertussis toxin, which inactivates Gαi, normalizes enhanced renovascular responses to ANG II in SHR (14). Additionally, experiments using a cell-permeable analog of cAMP, a direct activator of adenyl cyclase (5), and direct activators of Gαq (4) suggest that renovascular responses are equivalent in SHR and WKY when the cAMP signal-transduction pathway is activated downstream of the ANG II receptor-G protein coupling step. These observations strongly suggest a role for G proteins in altered signal transduction in the renal microvasculature of SHR.

Accordingly, the goal of the present study was to test the hypothesis that SHR have altered expression of the G proteins involved in ANG II signal transduction and/or of Gαq, which is the common signal transducer for several vasodilators that have defective interaction with ANG II in SHR. In this regard, steady-state mRNA levels were measured, because quantification of low-abundance proteins using Western blotting presents difficulties related to specificity and sensitivity. Moreover, in the literature pertaining to SHR and/or ANG II effects in vascular smooth muscle cells, mRNA levels for G proteins correlate well with protein levels (11, 17, 29).

The ANG II type 1 receptor (AT1 receptor) is a seven-transmembrane domain receptor that is linked to G proteins. The various G proteins known to be associated with the ANG II receptor are the three subtypes of Gαq (Gαq1, Gαq2, and Gαq3) and Gαs. ANG II acts through a Gq1 subtype protein to inhibit production of cAMP by adenyl cyclase (15). This can oppose the effects of vasodilator agents that act via Gαs such as dopamine (5), prostaglandin I2 (3), and β2-adrenoceptor agonists (31). Linkage of the AT1 receptor to all three subtypes of Gq has been demonstrated in the case of recombinant Gαi1, Gαi2, and Gαi3 (9). Also, Pobiner et al. (26) have shown linkage of the AT1 receptor with Gαi3 in hepatocytes. Another Gαi isoform, Gαi2, is the most abundant Gα subunit in most systems, and the role of Gαi1, while unknown, may be inhibitory to adenyl cyclase activity and thereby mediate the vasoconstrictor effects of ANG II. Thus we measured the mRNA for all three isoforms of Gαq.

ANG II also acts through Gαq to activate phospholi- pase C, thereby generating inositol 1,4,5-trisphosphate and diacylglycerol (12). One consequence of activating this cascade is the induction of smooth muscle contraction. With this in mind, the levels of Gαq mRNA were measured. Furthermore, since SHR may have defective interaction between Gαq-coupled vasodilatory and ANG

SPONTANEously hypertensive rats (SHR), compared with the control Wistar-Kyoto (WKY) strain, possess an intrinsically greater sensitivity of the renal vasculature to angiotensin II (ANG II) (10, 18–21, 30). This abnormality in renal vascular regulation appears to be due at least in part to an imbalance between the effectiveness of Gαs-utilizing signal-transduction pathways mediating vasodilatation and ANG II-mediated pathways causing vasoconstriction. This imbalance has been demonstrated not only for regulation of vascular resistance (3–5, 30) but also with regard to synthesis of adenosine 3′,5′-cyclic monophosphate (cAMP) (31). The preglomerular arterioles are key resistance vessels of the kidney and mediate renovascular responses to various stimuli, including ANG II. Importantly, Kost et al. (19) showed that medial hypertrophy in response to ANG II is more pronounced in SHR preglomerular vessels (e.g., interlobular and arcuate arteries), suggesting that hyperresponsiveness to ANG II of preglomerular microvessels mediates the enhanced renovascular responses of SHR.
ill-coupled vasoconstrictor pathways, the levels of mRNA for \( \gamma _{i-2} \) were quantified as well.

To accomplish the goal of quantifying mRNA for five different \( \gamma _{i} \) subunits in the same microvessel sample, we developed a quantitative polymerase chain reaction (PCR) assay for individual \( \gamma _{i} \) protein \( \alpha _{i} \)-subunit mRNAs (25). The assay utilizes an internal competitor that is identical to the target sequence of interest except for a 20-base cassette in its midportion. Thus the primer recognition areas are identical for both target and competitor, whereas the altered midportion allows differentiation of PCR products of the target from that of the competitor. Quantification of PCR products is accomplished by means of a colorimetric plate assay, the enzyme-linked oligonucleotide-sorbent assay (ELOSA), which is similar in format to an enzyme-linked immunosorbent assay (ELISA), with the exception that a biotin-linked oligo is used (in place of the ELISA primary antibody) for the specific detection of complementary PCR products. We previously demonstrated that this assay can resolve twofold differences in initial analyte amount from 2,000 \( \times 10^{-21} \) mol to 7 \( \times 10^{-21} \) mol with extreme specificity conferred at two levels: first, by specific PCR primers, and second, by specific ELOSA capture probes. Moreover, the assay is highly precise (11.9% intra-assay and 14.7% inter-assay coefficients of variation), because of the use of an internal standard very similar to the analyte.

**METHODS**

Male SHR and WKY rats 12–14 wk of age from Taconic Farms (Germantown, NY) were housed at the University of Pittsburgh Animal Facility with controlled temperature, relative humidity, and light cycle (22°C, 55%, and 7 AM to 7 PM). Animal care was in accordance with institutional guidelines. The animals were maintained on Wayne Rodent Blox 8604 (Madison, WI). Studies had prior approval of the Institutional Animal Care and Use Committee.

Renal preglomerular microvessels were isolated using the method described by Dubey et al. (8), with modification. Briefly, rats were anesthetized with pentobarbital sodium, and the abdomen was opened to expose the aorta and superior mesenteric artery. The aorta was cannulated with polyethylene tubing (Becton Dickinson, Parsippany, NJ), the superior mesenteric artery and proximal aorta were ligated, the inferior vena cava was cut, and the kidneys were perfused with 20 ml of phosphate-buffered saline (PBS) to flush out blood. Twenty milliliters of a 1% suspension of iron oxide particles (Aldrich Chemical, Milwaukee, WI) in PBS was infused, and the kidneys were obtained. The medulla was removed, and the cortex was pressed through a wire mesh, thus separating microvessels from cortical tissue. Larger vessels, if seen, were removed using a pair of fine-pointed scissors. The preparation was washed repeatedly in ice-cold PBS while using a magnet to retain the iron-laden vessels. Next, the samples were digested with collagenase at 37°C for 15–30 min to free vessels from surrounding structures and connective tissue. After this, they were passed through a 20-gauge needle to shear off glomeruli, then washed repeatedly until the wash fluid was clear. Samples were then frozen at \(-70^\circ \text{C}\) until RNA extraction was performed.

Preparation of cDNA. RNA was isolated using a modified guanidinium phenol-chloroform Tri-Reagent extraction method (6) with the addition of polysaccharide gel carrier (Microgel; Molecular Research Center, Cincinnati, OH) followed by deoxyribonuclease (DNase) digestion using ribonuclease-free DNase (Stratagene, La Jolla, CA). Contamination with genomic DNA was tested using 40 cycles of PCR with \( \gamma _{i-2} \) primers, and DNase digestion was repeated if necessary.

To reduce intersample variations in the efficiency of reverse transcription (RT), RT was carried out in at least three replicates for each sample, and the products were pooled before use in quantitative competitive-PCR. RT of total RNA was performed as described (25) using random hexamers and reverse transcriptase enzyme Superscript (GIBCO-BRL, Grand Island, NY). The procedure included an RT control to which no RNA was added; this reaction product was later subjected to PCR to verify the purity of RT reagents.

Quantitative competitive PCR assay. We developed an assay that utilizes, as an internal standard, a synthetic oligonucleotide that competes with the wild-type analyte during PCR amplification (25). For clarity, the segment of the wild-type template that is amplified by PCR is denoted the target template (TT), and the competing internal standard is termed the competitor template (CT). The CT was designed to be identical to the TT at both ends, to maximize the possibility that it would be amplified with the same efficiency and specificity as the TT and be affected in the same way by variations in reaction conditions. Differentiation of CT from TT in the PCR products is accomplished by altering a 20-base-length region in the midportion of the CT. This region carries a scrambled version of the sequence in the corresponding portion of the TT.

PCR products are quantified using a colorimetric 96-well microplate assay similar to the ELISA. PCR products are bound to streptavidin-coated plates by hybridization with biotin-labeled oligos, which serve the same function as the primary antibody in ELISAs. These oligos are complementary to the midportion of the CTs and TTs, allowing specificity of detection. PCR products bound to the plates are then incubated with an antibody that recognizes an antigen, fluorescein, that is tagged to the primers and thereby incorporated in the PCR products. The antibody is linked to an enzyme (horseradish peroxidase) that produces a colored compound from a substrate that is added in the final step. This assay is termed an ELOSA.

Competitive PCR was performed by amplifying the sample with several known dilutions of CT in parallel reactions. The amplified CT and TT were measured at the end of PCR by ELOSA. The results were quantified as ratio of CT signal to TT signal, or CT/TT. This ratio was plotted as a function of the amount of CT added, and a linear regression equation for the relation was derived using a least-squares fit. In theory, if the assay...
detection system is linear with respect to the amount of CT added, then, since the amount of TT is constant within a sample, the relation between CT/TT vs. amount of added CT should be linear with a y-intercept at zero, i.e., $CT/TT = \text{slope} \times \text{CT} = \text{CT}/\text{TT} \times \text{slope}$. Moreover, if the amplification and detection procedures are equally efficient for CT vs. TT, then, when the amount of added CT is equal to the amount of TT in the sample, the CT/TT ratio should be equal to unity (i.e., $CT/TT = 1$). Thus, when $CT/TT = 1$, then $CT = TT = 1$ with the relation held even when the intercept is nonzero, because of trace contamination of sample, the CT/TT ratio should be equal to unity (i.e., $CT/TT = 1$). Thus, when $CT/TT = 1$, then $CT = TT = 1$.

The temperature was then reduced to 86°C, and 2.5 U of DNA polymerase were added (AmpliTag, Perkin-Elmer, Foster City, CA). Primers and probes for PCR amplification (Table 1) were selected from the published cDNA sequence for rat $G_{a}$ (13), $G_{a1}$, $G_{a12}$, $G_{a13}$ (16), and $G_{a}$ (28) using MacVector 4.1 software (Kodak International Biotechnologies). CT and 5′ primers were obtained from Midland Certified Reagent (Midland, TX). The 5′ end labels of biotin and fluorescein were added to the capture probes and 3′ primers, respectively (Ransom Hill Biosciences, Ramona, CA; and Promega, Madison, WI), during oligo synthesis.

PCR conditions were optimized for each primer pair so that optimal yield of a single band product of the appropriate size was obtained. These conditions were determined for a conventional thermal block PCR cycler (Perkin-Elmer, Norwalk, CT) and a rapid air thermal cycler (model 1605; Idaho Technology, Idaho Falls, ID). Both types of cyclers yielded similar sensitivity, specificity, accuracy, and reproducibility of results; however, most of the reported data were obtained from the air thermal cycler. Optimized PCR conditions are shown in Table 1.

For the conventional cycler, a final concentration of 50 mM KCl, 10 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride (pH 8.3), 2.5 mM MgCl₂, and 0.2 mM each of dATP, dGTP, dCTP, and dTTP were used. The unlabelled 5′ primer and the fluorescein-labeled 3′ primer (400 ng each), 10 μl of dimethyl sulfoxide, the requisite amount of cDNA, and varying amounts of CT and CT seq. were added and the mixture heated to 94°C for 5 min. The temperature was then reduced to 86°C, and 2.5 U of DNA polymerase were added (AmpliTag, Perkin-Elmer, Foster City, CA). Optimized cycling conditions were determined empirically using a PCR optimizer kit (Invitrogen, San Diego, CA). PCR cycles consisted of

<table>
<thead>
<tr>
<th>Isoform of $G_{a}$</th>
<th>Sequence of amplified CT and TT</th>
<th>Capture Probe CT</th>
<th>Capture Probe: TT</th>
<th>5′ Primer</th>
<th>3′ Primer</th>
<th>PCR Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_{a1}$ (487–579)</td>
<td>GCAAGCTAGAGTG CACAG CTAGA ACCAA GAAAG</td>
<td>AGATG</td>
<td>CAGAA</td>
<td>GACA</td>
<td>ACGCTT</td>
<td>55°C 1 mM MgCl₂</td>
</tr>
<tr>
<td>CT seq. $G_{a1}$</td>
<td>GCAAGCTAGAGTG CACAG CTAGA ACCAA GAAAG</td>
<td>AGATG</td>
<td>CAGAA</td>
<td>GACA</td>
<td>ACGCTT</td>
<td>55°C 1 mM MgCl₂</td>
</tr>
<tr>
<td>$G_{a2}$ (485–580)</td>
<td>GCGATCCATCGCTACGACGATCT</td>
<td>AGATG</td>
<td>CAGAA</td>
<td>GACA</td>
<td>ACGCTT</td>
<td>55°C 1 mM MgCl₂</td>
</tr>
<tr>
<td>CT seq. $G_{a2}$</td>
<td>GCGATCCATCGCTACGACGATCT</td>
<td>AGATG</td>
<td>CAGAA</td>
<td>GACA</td>
<td>ACGCTT</td>
<td>55°C 1 mM MgCl₂</td>
</tr>
<tr>
<td>$G_{a3}$ (486–579)</td>
<td>GACAGACGACGGGAATATCAGTTATCT AGCTC GTGTA CAGAC TCTAAG</td>
<td>CAGA-3′</td>
<td>AAGG-3′</td>
<td>TCCC-3′</td>
<td>TGGGTT</td>
<td>50°C</td>
</tr>
<tr>
<td>CT seq. $G_{a3}$</td>
<td>GACAGACGACGGGAATATCAGTTATCT AGCTC GTGTA CAGAC TCTAAG</td>
<td>CAGA-3′</td>
<td>AAGG-3′</td>
<td>TCCC-3′</td>
<td>TGGGTT</td>
<td>50°C</td>
</tr>
<tr>
<td>$G_{a4}$ (438–543)</td>
<td>ATCGGATTAGTTCGCTACGGACGACGGTA</td>
<td>AGATG</td>
<td>CAGAA</td>
<td>GACA</td>
<td>ACGCTT</td>
<td>55°C 3 mM MgCl₂</td>
</tr>
<tr>
<td>CT seq. $G_{a4}$</td>
<td>ATCGGATTAGTTCGCTACGGACGACGGTA</td>
<td>AGATG</td>
<td>CAGAA</td>
<td>GACA</td>
<td>ACGCTT</td>
<td>55°C 3 mM MgCl₂</td>
</tr>
<tr>
<td>$G_{a5}$ (516–594)</td>
<td>GATCGATCTAGGATGACAGT-3</td>
<td>GATCG</td>
<td>ATGGT</td>
<td>TGCATC</td>
<td>AAGCA</td>
<td>55°C 3 mM MgCl₂</td>
</tr>
<tr>
<td>CT seq. $G_{a5}$</td>
<td>GATCGATCTAGGATGACAGT-3</td>
<td>GATCG</td>
<td>ATGGT</td>
<td>TGCATC</td>
<td>AAGCA</td>
<td>55°C 3 mM MgCl₂</td>
</tr>
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</table>

PCR, polymerase chain reaction. TT, target template; CT and CT seq., competitor template and competitor template sequence, respectively.
94°C for 1 min, annealing temperature (see Table 1) for 1 min, and 72°C for 3 min.

Reactions in the rapid cycler were performed in glass capillary tubes in a total volume of 10–12 μl. Each reaction received 40–48 ng of the 5’ and 3’ primers, 1–1.2 μl of 10× PCR reaction buffer [Tris 500 mM (pH 8.3), bovine serum albumin (BSA) 2.5 mg/ml, and 10, 20, or 30 mM MgCl2], 1–1.2 μl enzyme diluent (10 mM Tris and 2.5 mg/ml BSA), a final concentration of 100 mM of each dNTP, and 0.4 U of DNA polymerase. Reaction conditions were optimized using the startup kit provided by the manufacturer. Samples were denatured for 15 s to 2 min, followed by rapid cycling through 94°C, the annealing temperature (see Table 1), and 72°C, with a final extension at 72°C for 2 min.

Each set of reactions included the following three controls: 1) a negative control that had no wild-type template or CT, 2) a TT-positive control that had TT but no CT, and 3) another positive control that had CT but no TT.

In each measurement, the microvessel cDNA was amplified with five different concentrations of CT. Briefly, reactions were performed using 12.5 to 100 ng of RNA (depending on the abundance of the specific target) equivalent RT product. Figure 1, which depicts assay results for all five Gα isoforms in the same sample, illustrates this procedure.

SHR and WKY samples were batched together at all stages of processing, starting with the day of surgery until final assay by ELOSA. Absolute quantities as measured by ELOSA were then expressed as a ratio to the level of Gαi. Not only does this use Gαi as a housekeeping message to normalize for variability in RNA quality and RT efficiency, but it measures possible imbalances between Gαi1, Gαi2, and Gαi3 (mediating ANG II effects) and Gαq (mediator of several vasodilators that appear to be subnormally effective in buffering ANG II–mediated effects in SHR).

Data analysis and statistical tests. Quantifications of specific isoforms of Gαi TT in each sample were performed at least in duplicate. These values were averaged, and the ratio of the specific TT to that of Gαi in the same sample was calculated. The average of this ratio for each Gα isoform was determined for each strain. Statistical comparisons between SHR and WKY samples were made using Student’s t-test for unpaired samples.

RESULTS

Quantification of G protein mRNA for Gαi1, Gαi2, Gαi3, and Gαo was performed in renal microvessels of 12- to 14-wk-old SHR and WKY rats. Levels of Gαi1 and Gαi2 message were measured in 22 samples each (9 WKY and 13 SHR). Gαi1 was measurable in 19 samples (7 WKY and 12 SHR), and Gαi2 and Gαi3 were measurable in 8 each (3 WKY and 5 SHR). All five subunits were quantifiable in eight samples. These data are shown in Fig. 2.

Gαi2 was by far the most abundant of the Gαi subunits measured, and Gαq was the least. The abundance rank order for the various isoforms was Gαi2 >> Gαi3 >> Gαi2 >> Gαi1 >> Gαo. When data were expressed as 10–18 mol (1 amol) per 50 ng of RNA equivalent cDNA, the amount of Gαi2 (25.3 ± 6.3 amol) was about 100-fold greater than that of Gαi3 (0.95 ± 0.08 amol), Gαi3 (0.83 ± 0.19 amol), or Gαi1 (0.51 ± 0.04 amol), and the amount of Gαq was the lowest of all the isoforms (3.45 ± 0.5 × 10–2 mol, or 3.45 ± 0.5 zmol).

No differences were detected between SHR and WKY with regard to the levels of Gαi1 assayed after RT from equal amounts of RNA (0.96 ± 0.35 for WKY vs. 0.74 ± 0.25 amol for SHR; P = 0.617) (Fig. 2, inset).
Figure 2 also shows that the ratios of the levels of $G_{\alpha_{1}}$, $G_{\alpha_{1.2}}$, $G_{\alpha_{1.3}}$, and $G_{\alpha_{q}}$ normalized to the level of $G_{\alpha_{s}}$ showed no significant differences between SHR and WKY (0.44 ± 0.05 vs. 0.48 ± 0.13 for $G_{\alpha_{1}}$, 40.9 ± 7.8 vs. 45.2 ± 8.9 for $G_{\alpha_{1.2}}$, and 0.79 ± 0.05 vs. 0.82 ± 0.15 for $G_{\alpha_{1.3}}$, respectively). Study of the $G_{\alpha_{q}}$-to-$G_{\alpha_{s}}$ ratio suggested, however, that $G_{\alpha_{q}}$ may be more abundant in SHR (WKY 0.41 ± 0.08 vs. SHR 1.04 ± 0.32, $P = 0.08$).

**DISCUSSION**

The hypothesis that the altered renovascular sensitivity of SHR to ANG II and/or $G_{\alpha_{s}}$-mediated vasodilators is associated with alterations at the $G$ protein level was studied in terms of quantifying steady-state mRNA levels for specific isoforms of $G_{\alpha}$ in freshly isolated renal microvessels.

Commercially available antibodies for $G$ protein $\alpha$-subunits present problems with sensitivity, specificity, and quantification issues. These difficulties are exacerbated in the case of low-abundance proteins like $G_{\alpha_{1}}$, $G_{\alpha_{1.2}}$, and $G_{\alpha_{q}}$. On the other hand, the use of PCR followed by a specific DNA capture probe allowed us to quantify individual $G_{\alpha}$ subunit messages with a high degree of sensitivity and specificity (25). To our knowledge, this is the first report of $G$ protein mRNA quantifications in renal preglomerular microvessels of SHR and WKY rats. The assay revealed no significant strain differences in the levels of $G_{\alpha_{s}}$ or of individual $G_{\alpha}$ subunits normalized to $G_{\alpha_{s}}$ levels. The level of $G_{\alpha_{q}}$ normalized to $G_{\alpha_{s}}$, however, tended to be higher in SHR.

The isolation procedure used for microvessels yielded vessels predominantly in the size range of 25–200 µm. The size and arborization pattern indicate that these samples are composed of afferent arterioles as well as interlobular and arcuate arteries. This range of vessel distribution was preferred in view of the study by Kost et al. (19) showing that arcuate and interlobular arteries are major sites of increased sensitivity to ANG II in SHR.

Another important consideration in the rationale for this study was whether increased RNA levels necessarily imply an increased level of protein. A review of the literature on SHR and $G$ proteins suggests that there is usually a good correlation. Anand-Srivastava (1) showed enhanced levels of $G_{\alpha_{i}}$ and unaltered $G_{\alpha_{s}}$ in SHR heart and aorta and in a later report demonstrated a corresponding increase in $G_{\alpha_{i}}$ mRNA, with no change in $G_{\alpha_{s}}$, in the same tissues (29). Gurich et al. (11) showed by Northern analysis and ADP ribosylation studies that equivalent amounts of $G_{\alpha_{i}}$ mRNA and protein were present in both strains. Kai et al. (17) showed in cultured aortic vascular smooth muscle cells from Sprague-Dawley rats that prolonged incubation with ANG II leads to a reduction of protein levels of $G_{\alpha_{i}}$ and $G_{\alpha_{1.3}}$ as well as a decrease in $G_{\alpha_{q}}$ mRNA levels. Thus mRNA levels should provide an indication of the level of the corresponding $G$ proteins.

Inasmuch as the mechanism of vascular hyperreactivity to ANG II in SHR involves either an exaggerated decrease in agonist-induced cAMP in response to ANG II (31) or a subnormal response to $G_{\alpha_{s}}$-mediated vasodilators (3–5) that act by increasing cAMP, we expected to see any or some of the following: 1) a lower level of $G_{\alpha_{s}}$ mRNA in SHR, 2) a higher level of any $G_{\alpha}$ in SHR, or 3) a higher level of $G_{\alpha_{s}}$ in SHR. In the present study, we normalized the levels of $G_{\alpha_{i}}$ and $G_{\alpha_{q}}$ to the level of $G_{\alpha_{s}}$. Because we were exploring the imbalance between ANG II-mediated and $G_{\alpha_{s}}$-mediated pathways, we reasoned that this ratio would be a sensitive indicator of such an imbalance.

Our finding that there are similar levels of $G_{\alpha_{s}}$ in 12- to 14-wk-old SHR and WKY is at variance with the results of Ruan and Arendshorst (27), who found a slight increase in the quantity of $G_{\alpha_{s}}$ protein in 6- to 8-wk-old SHR preglomerular vessels. Possible reasons for the discrepancy are differences in the ages of rats in the two studies and the fact that our samples included arcuate arteries in addition to the interlobular and afferent arterioles studied by Ruan and Arendshorst (27). It is important to note that studies of renal $G$ protein expression at different ages in SHR and WKY (24) showed no alteration of any $G$ protein at 3 wk, whereas $G_{\alpha_{s, long}}$ and $G_{\alpha_{s}}$ were reduced in 28-wk-old SHR, with no alteration of $G_{\alpha_{s,short}}$ or $G_{\alpha_{i}}$. Our findings are consistent with those of Gurich et al. (11), who demonstrated equivalent amounts of $G_{\alpha_{s}}$ protein in renal cortex by cholera toxin labeling and of mRNA by Northern blotting. Measurements of $G_{\alpha_{s}}$ protein in extrarenal tissues have in most cases shown no differences between SHR and WKY (1, 7, 22, 23).

We found that the levels of mRNA for $G_{\alpha_{1.1}}$, $G_{\alpha_{1.2}}$, and $G_{\alpha_{1.3}}$, analyzed in terms of their ratio to the level of $G_{\alpha_{s}}$ in the same sample, were similar in both strains. Similarly, Ruan and Arendshorst (27) found no differences in $G_{\alpha_{1.1}}$, $G_{\alpha_{1.2}}$, or $G_{\alpha_{1.3}}$ protein in renal afferent and interlobular arterioles. These match the findings of Michel et al. (24) in renal membranes at various ages and McElellan et al. (22) in renal cortical plasma membranes. In membranes from freshly isolated mesenteric vessels (7), the levels of $G_{\alpha_{1.2}}$ and $G_{\alpha_{1.3}}$ were unaltered. Myocardial membranes from SHR have been shown to have increased $G_{\alpha_{i}}$ by pertussis toxin and immunolabeling (1) and Northern analysis (29). However, other studies have failed to demonstrate such increases in myocardial (22, 23) or renal plasma membranes (22).

With regard to $G_{\alpha_{q}}$, our finding was an increased level of mRNA expression in SHR (WKY 0.41 ± 0.08 vs. SHR 1.04 ± 0.32), which, however, was not statistically significant ($P = 0.08$). Ruan and Arendshorst (27) have reported no strain-related differences in $G_{\alpha_{q}}$ protein in preglomerular vessels from 6- to 8-wk-old rats. Renal membranes of 28-wk-old but not 3-wk-old SHR have been reported to have reduced levels of $G_{\alpha_{q}}$ (24). Quantification of $G_{\alpha_{q}}$ mRNA in cells derived from SHR and WKY mesenteric arteries or $G_{\alpha_{q}}$ in myocardium (23) showed no strain-specific differences.

In conclusion, the findings of the present study demonstrate that $G_{\alpha_{s}}$, $G_{\alpha_{i}}$, and $G_{\alpha_{q}}$ mRNA levels in the renal microvasculature do not differ in SHR vs. WKY.
rently in progress. Isolated SHR and WKY renal microvessels are cur-
specific differences in functional responses in freshly
regulatory proteins. Studies characterizing strain-
specific alterations in steady-state levels of these GTP-binding
agents such as ANG II may not be directly related to
enhanced renovascular sensitivity of SHR to pressor
rats. Furthermore, these data suggest that the enhanced
renovascular sensitivity of SHR to pressor
agents such as ANG II may not be directly related to
alterations in steady-state levels of these GTP-binding
regulatory proteins. Studies characterizing strain-
specific differences in functional responses in freshly
isolated SHR and WKY renal microvessels are cur-
rently in progress.

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