The β2 Subunit Inhibits Stimulation of the α1/β1 Form of Soluble Guanylyl Cyclase by Nitric Oxide

Potential Relevance to Regulation of Blood Pressure

Gopa Gupta, Mohammad Azam, Lin Yang, and Robert S. Danziger
Department of Medicine, College of Physicians and Surgeons of Columbia University, New York, New York 10032

Abstract

Cytosolic guanylyl cyclases (GTP pyrophosphate-lyase [cyclizing; EC 4.6.1.2]), primary receptors for nitric oxide (NO) generated by NO synthases, are obligate heterodimers consisting of an α and a β subunit. The α1/β1 form of guanylyl cyclase has the greatest activity and is considered the universal form. An isomer of the β1 subunit, i.e., β2, has been detected in the liver and kidney, however, its role is not known. In this study, we investigated the function of β2. Immunoprecipitation experiments showed that the β2 subunit forms a heterodimer with the α1 subunit. NO-stimulated cGMP formation in COS 7 cells cotransfected with the α1 and β2 subunits was ∼ 1/3 of that when α1 and β1 subunits were cotransfected. The β2 subunit inhibited NO-stimulated activity of the α1/β1 form of guanylyl cyclase and NO-stimulated cGMP formation in cultured smooth muscle cells. Our results provide the first evidence that the β2 subunit can regulate NO sensitivity of the α1/β1 form of guanylyl cyclase. Northern analysis for guanylyl cyclase subunits was performed on RNA from kidneys of Dahl salt-sensitive rats, which have been shown to have decreased renal sensitivity to NO. Compared to the Dahl salt-resistant rat, message for β2 was increased, β1 was decreased, and α1 was unchanged. These results suggest a molecular basis for decreased renal guanylyl cyclase activity, i.e., an increase in the α1/β2 heterodimer, and decrease in the α1/β1 heterodimer. (*J. Clin. Invest.* 1997. 100:1488–1492.) Key words: guanylyl cyclase • cGMP hypertension • Dahl rat

Introduction

Cytosolic guanylyl cyclases (GTP pyrophosphate-lyase [cyclizing; EC 4.6.1.2]) are a primary receptor for nitric oxide (NO) generated by NO synthases. These are heme-containing heterodimers, i.e., consisting of one α and one β subunit (1, 2). NO activates the enzyme by complexing with the central iron in heme (3). Both an α and a β subunit are required for basal and NO-stimulated catalytic activity. The α1/β1 heterodimeric form of guanylyl cyclase is thought to be the universal form. The heterodimer has been purified from lung and brain while message for the α1 and β1 subunits have been detected by Northern analysis in lung, cerebellum, kidney, heart, liver, skeletal muscle, olfactory mucosa, and tongue (4–6).

Neither α or β subunits are catalytically active when expressed alone. Recently, it has been shown that dimerization of the COOH termini from both subunits is required for catalytic activity (7). Although each of the subunits show homologies over the entire sequence, the highest is at the carboxy terminus, which forms the catalytic domain and shares significant homology with that of membrane-bound guanylate cyclases and adenylate cyclases (6). The NH_2 termini are less homologous and are important in the binding and/or signal transduction of the stimulatory binding signal, i.e., NO, to the catalytic domain (7). The NH_2 termini of both the α1 and β1 subunits are required for full regulation of enzyme activity via the heme moiety (7, 8).

Recently, cDNA for isomers of the α1 and β1 subunits, i.e., α2, α2i, and β2 (9–11) have been identified by homology screening. All of the subunits are ∼ 70–80 kD. The α subunit isoforms are known to form heterodimers with the β1 subunit (9, 10). The α1/β1 heterodimer has the most basal and stimulated activity. The α2/β1 heterodimer has less basal and stimulated activity, but appears to be similarly regulated. The α2i/β1 heterodimer has no basal or stimulated catalytic activity (10). This finding has been attributed to an in-frame insert of 31 amino acids within the catalytic domain of the α2i subunit (10). The function of the β2 subunit remains enigmatic.

The β2 subunit is a 76.3-kD protein that most closely resembles the 70-kD β1 subunit of lung guanylyl cyclase (11); it is primarily expressed in the kidney and liver. An 86-amino acid carboxy-terminal region extends beyond the carboxy terminus of the β1 subunit. In this study, we show that the β2 subunit can inhibit activation of α1/β1 form of the enzyme by NO, and reduce NO-stimulated cGMP levels in smooth muscle cells. Furthermore, Northern analysis shows message for the β2 is increased, and for β1 is decreased in the kidney of Dahl salt-sensitive rat compared to the Dahl salt-resistant rat, suggesting a molecular basis for reduced renal sensitivity to NO in this model.

Methods

Materials. Lipofectin reagent for transient transfection and all cell culture media were purchased from Life Technologies (Gibco BRL, Gaithersburg, MD). The cell lines, i.e., COS 7 (African monkey kidney) and rat aortic smooth muscle cells (A7R5) were purchased from American Type Culture Collection (Rockville, MD). The expression vector pGFP-c1 as well as monoclonal and polyclonal antisera against GFP (green fluorescent protein) were purchased from Clontech (Palo Alto, CA). Poly A^+ RNA isolation kits were from Invitrogen Corp (San Diego, CA).

The rat α1 and β1 full-length cDNA, subcloned into the expres-

Address correspondence to Robert S. Danziger, M.D., Assistant Professor of Medicine, Department of Medicine, College of Physicians and Surgeons of Columbia University, 630 W. 168th Street, New York, NY 10032. Phone: 212-305-2632; FAX: 212-305-4825; E-mail: rsd6@columbia.edu

Received for publication 30 January 1997 and accepted in revised form 25 June 1997.

1. *Abbreviations used in this paper:* GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NO, nitric oxide.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/97/09/1488/05 $2.00
Volume 100, Number 6, September 1997, 1488–1492
http://www.jci.org

sion vector pcDNA-neo as 2.2-kb and 1.9-Kb fragments, respectively, were a generous gift from M. Nakane (Abbott Laboratories Diagnostic Division, Chicago, IL). Antibodies to the rat α1 (monoclonal H6) and β1 (polyclonal A794) subunits were obtained from F. Murad (Molecular Geriatrics, Lake Bluff, IL) and D. Garbers (Howard Hughes, Dallas, TX) respectively. Male Dahl salt-sensitive and salt-resistant rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN).

Construction of β2 plasmid. The full-length cDNA for the rat β2 subunit subcloned into the EcoR1 sites of Blue script(KS) was a gift from P. Yuen (Memphis, TN). For construction of the GFP-β2 fusion protein, cDNA of the β2 subunit was digested with Xmn1/BamH1, and the resulting 2.1-kb fragment was ligated at the Sma1/BamH1 sites of pGFP-c1 expression vector. The final construct, pGFPc1-β2, was confirmed by restriction analysis and dideoxy sequencing.

Transient transfections. The full-length cDNA of the α1 and β1 subunits inserted into the expression vector pcDNAneo, and GFP-modified β2 in expression vector pGFP-c1 under the CMV promotor, were transfected into COS 7 and A7R5 cells with Lipofectin as previously described (12). Cells were grown to 60% confluence and transfected in six-well plates. 72 h after transfection, cells were harvested, resuspended in buffer containing 10 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM EDTA, 10 μg/ml Aprotinin, 100 μM Leupeptin, 350 μM PMSF, and sonicated at 4°C. The soluble fraction was obtained by centrifugation at 20,000 *g* for 20 min at 4°C. Each set of transfections was performed in triplicate and repeated two times. Control transfections were also performed with the expression vectors.

Cell cGMP measurement. Cells grown in six-well plates were washed twice with ice-cold PBS and incubated for 10 min at 37°C with PBS containing 300 μM isobutylmethylxanthine with or without 10 μM SIN-1. The media was aspirated, and the cells were lysed with 0.1 M acetic acid (pH 3.5) and subsequently neutralized with 0.1 M sodium acetate. cGMP was measured in the supernatant by using a standard radioimmunoassay (Biomedical Technologies, Inc., Stoughton, MA). Levels of cGMP were normalized to protein content.

Immunoprecipitation and immunoblots. Aliquots of the cytosolic fraction of cells (prepared as described above) were briefly centrifuged and cleared by incubation with monoclonal antibody against GFP (1:500 dilution) and 50 μl protein A/G sepharose for 30 min. Fractions were then recentrifuged, and the supernatants were incubated with the immunoprecipitating antibody (monoclonal anti-GFP) and 50 μl of protein A/G sepharose for 12–16 h at 4°C. The complexes were washed twice with lysis buffer and subjected to SDS/PAGE under reducing conditions and Western blotting. The nitrocellulose membrane was blocked with 5% nonfat milk at 4°C for 12 h, and was then incubated with α1 antibody at room temperature for 60 min. The blot was then washed and incubated with secondary antibody (mouse anti–Ig-horseradish peroxidase) at room temperature for 40 min. Immunodetection was by enhanced chemiluminescence (Amersham Corp., Arlington Heights, IL). The blot was then stripped and probed with antibodies to β1 and β2 subunits, respectively.

SDS/PAGE and Western blotting. 3% stacking and 7.5% resolving gels were cast, samples were boiled at 95°C for 3 min in Lammelli buffer and briefly centrifuged, and the supernatants were loaded on the gel (2–4 μg protein/lane). Resolved proteins were blotted on nitrocellulose membranes, and were probed using specific antibodies against the expressed proteins.

Northern analysis. A total of 12 μg of poly A+ RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde, and was blotted onto nitrocellulose membrane with 20× SSC. RNA was fixed by baking in an 80°C vacuum oven for 2 h. Random prime-labeled cDNA probes were used. α1, β1, and β2 probes were PvuII/EcoRV (550 bp), Kpn1/Nae1 (650 bp), and EcoR1/EcoRV (330 bp) fragments, respectively, from the corresponding parent plasmids.

The membrane was prehybridized at 42°C in 5× SSC, 5× Denhardt's solution, 50% formamide, 0.25% SDS, and 100 μg/ml salmon sperm DNA for 3 h, and was hybridized for 16 h with the labeled probe. For β2 subunit probing, the membrane was washed twice at room temperature with 2× SSC and 0.1% SDS for 10 min, and once with 0.1× SSC and 0.1% SDS for 15 min. For α1 and β1 subunit probing, a second wash was performed with 0.5× SSC and 0.1% SDS at 65°C for 30 min. Blots were exposed for autoradiography for 10 d. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (13) was probed by rehybridizing the blot at 42°C in the presence of a random prime-labeled 1.2-kb fragment of rat GAPDH cDNA.

Statistics. All results are expressed as means±standard error. Differences were assessed by one-way ANOVA or the Student's paired *t* test, when appropriate. $P < 0.05$ was considered significant.

Results

To determine the interaction of the β2 subunit of soluble guanylyl cyclase with the α1 subunit and the α1/β1 heterodimeric form, we coexpressed subunits in COS 7 cells. The α1, β1, and β2 subunits are not catalytically active when expressed alone. To assess the nature of the activities and interactions of α1, β1, and β2 subunits of the cyclase, a number of cotransfection studies were performed. In one set of experiments, we coexpressed the β2 subunit with the α1 subunit. In another set of experiments, the β2 subunit was coexpressed with the α1 and β1 subunits to determine the effect of β2 on the activity of the α1/β1 form of guanylyl cyclase. To show that the amount of α1 and β1 did not change in these experiments, Western blots using antibodies directed against the α1 and β1 subunits were used. To determine whether the β2 subunit forms a heterodimer with the α1 subunit and the associations of the α1, β1, and β2 subunits, immunoprecipitation experiments were

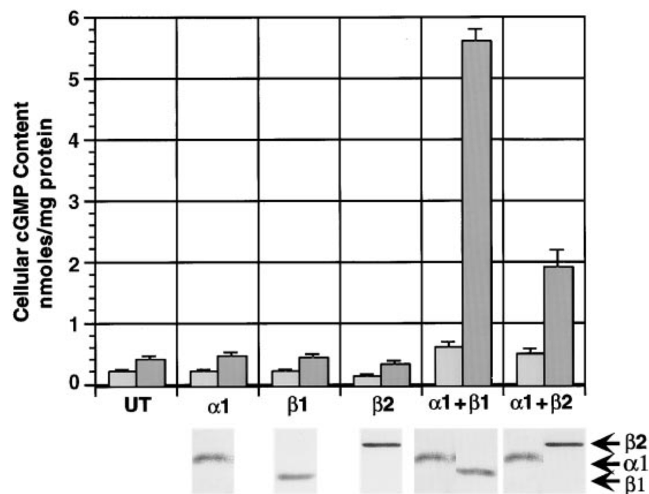


Figure 1. Effect of SIN-1 on cyclic GMP content of COS 7 cells transiently transfected with soluble guanylyl cyclase subunits. COS 7 cells (1×10^6) were transfected with plasmids containing cDNAs for α1, β1, and β2 subunits. Cytosolic cGMP levels were determined in the absence (basal) or presence (stimulated) of 10 μM SIN-1 (*top*) (see Methods). In two independent transfections with the expression vectors alone, basal and stimulated cGMP levels were not different from those of untransfected cells (UT) (not shown). Error bars represent standard error; $n = 3$. Cytosolic protein (4 μg/lane) was separated by SDS-PAGE. After transfer to nitrocellulose membranes, guanylyl cyclase subunits were detected using antibodies to the α1 and β1 subunits and the GFP-fusion protein (to detect β2) (*bottom*) (see Methods). *Light gray bars*, minus SIN-1; *dark gray bars*, plus SIN-1.

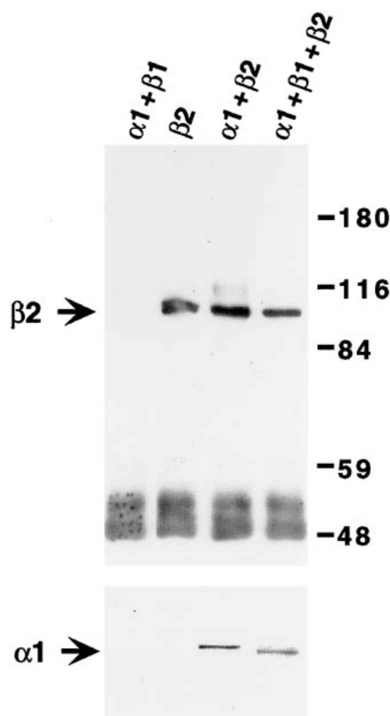


Figure 2. Detection of heterodimer formation by immunoprecipitation with GFP-specific antibody, and subsequent immunoblotting. Cytosolic fractions of COS 7 cells transfected with GFP-modified β2, and either α1 or α1 plus β2 were immunoprecipitated using an antibody to the GFP-fusion protein and analyzed by Western blotting as described in Methods. An anti-α1 antibody was used to detect α1. β2 was detected with an antibody to GFP. No signal was detected with anti-β1 antibody (not shown).

performed. In the last experiments, we assessed the effect of β2 on NO-stimulated cGMP formation in cultured rat aortic smooth muscle cells.

cGMP formation in the transfected COS 7 cells was determined under basal and NO-stimulated conditions. cGMP under basal conditions in COS 7 cells was 0.2 nmol/mg protein. Treatment with 10 μM SIN-1 for 15 min resulted in a twofold increase in cGMP. Neither basal nor stimulated cGMP formation was altered when cells were transfected with either α1, β1, or β2 subunits alone.

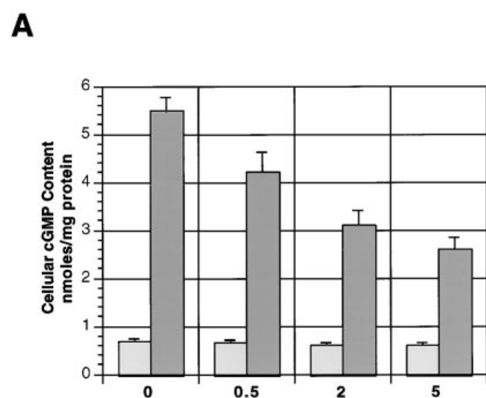
α1/β1 and α1/β2 activity. In COS 7 cells in which the α1 and β1 subunits were coexpressed, basal cGMP was increased fourfold, and SIN-1 resulted in a further sixfold increase in cGMP, i.e., to 5.5 nmol/mg protein. COS 7 cells in which both the α1 and β2 subunits were coexpressed had the same basal cGMP level as those transfected with the α1/β1 subunits. SIN-1, however, resulted in an increase in cGMP of approximately twofold, i.e., 1.9 nmol/mg protein, or ~ 1/3 of that seen in α1/β1 cotransfected cells (Fig. 1).

α1/β2 subunit association. To determine whether the β2 subunit formed a heterodimer with the α1 subunit, the latter was coexpressed with the β2-GFP fusion protein. Immunoprecipitation was performed using a monoclonal antibody to the GFP protein. The immunoprecipitated proteins were analyzed by Western analysis with antibodies against the α1 and β1 subunits and the GFP protein (to detect the β2 subunit). Both the α1 and β2 proteins were present in the immunoprecipitated fraction (Fig. 2), indicating that the β2 subunit forms a heterodimer with the α1 subunit. Control experiments were performed to show (*a*) that the α1 subunit did not bind to the GFP protein, i.e., α1 did not immunoprecipitate from the lysate of cells transfected with the expression vector and the α1 subunit, and (*b*) that α1 and β1 do not bind nonspecifically to the Sepharose matrix.

We further investigated whether β2 could bind to the α1/β1 form of the enzyme to form a heterotrimer. The α1, β1, and modified β2 subunits were coexpressed in COS 7 cells. Immunoprecipitation was performed with a monoclonal antibody to the GFP protein. The α1 and β2 subunits were present in the immunoprecipitated fraction (Fig. 2). The β1 subunit was not detected, implying that a heterodimer was formed consisting of the α1 and β2 subunits, and that the β1 subunit did not bind to the complex, i.e., a heterotrimer was not formed.

Effect of β2 on the activity of the α1/β1 form of guanylyl cyclase. We investigated whether the β2 subunit could inhibit the activity of α1/β1 heterodimer. Coexpression of increasing amounts of the β2 subunit with constant amounts of the α1 and β1 subunits in COS 7 cells resulted in a decrease in SIN-1–stimulated cGMP without significant decreases in basal levels (Fig. 3 *A*). To rule out that inhibition was due to a decrease in the expression level of the α1 or β1 subunits, Western analysis was performed. The amount of α1 and β1 was unaffected by the expression of β2 (Fig. 3 *B*).

Effect of β2 on NO-stimulated cGMP formation in smooth muscle cells. In these experiments, cultured aortic smooth muscle cells were transiently transfected with the GFP-modified β2 in the same manner as COS 7 cells. SIN-1 stimulated



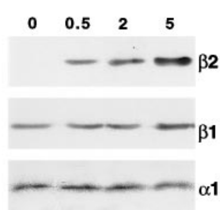


Figure 3. Dose response effect of the β2 subunit on the NO-stimulated cGMP responses of the α1/β1 form of soluble guanylyl cyclase. (*A*) COS 7 cells (2×10^6 per well) were cotransfected with 5 μg each of both the α1 and β1 plasmids and increasing concentrations of the β2 plasmid (0.5–5.0 μg). Cell cGMP content was determined in the presence and absence of 10 μM SIN-1 (see Methods). Endogenous cGMP levels ranged from 0.20–0.65 nmoles/mg protein. Error bars represent Mean±SE of triplicate determinations from one representative experiment. *Light gray bars*, minus SIN-1; *dark gray bars*, plus SIN-1. (*B*) Western analysis of cytosolic protein extracts from the same cells in which cGMP content was measured. Cytosolic proteins (4 μg/lane) were separated by SDS-PAGE, blotted onto nitrocellulose, and probed separately with antibodies to the α1, β1, and β2 subunits.

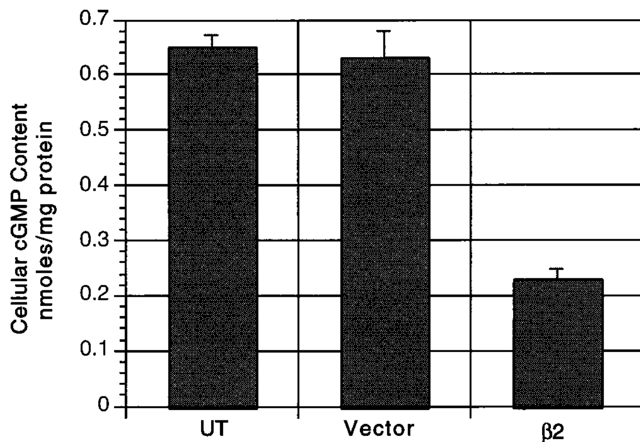


Figure 4. NO-stimulated cGMP content in rat aortic smooth muscle cells transiently transfected with the GFP-modified β2 plasmid. Cultured rat aortic smooth muscle cells (2×10^6 cells/well) were transfected with 5 μg of GFP-modified β2 plasmid. Smooth muscle cell cGMP levels were determined as described in the Methods, in the presence of 10 μM SIN-1 concentration. Control transfection with the expression vector pGFP-c1 did not change basal or SIN-1 stimulated cGMP levels. Basal levels of cGMP ranged from 0.35–0.42 nmoles/mg protein. Bars represent mean±standard error. UT, untransfected.

(10 μM, 10 min, 37°C) cGMP levels were reduced by ~ 33% compared to untransfected cells, i.e., 0.26 nmol/mg protein versus 0.65 nmol/mg protein ($P < 0.05$) (Fig. 4).

Northern analysis for guanylyl cyclase subunits in the Dahl salt-sensitive rat. Finally, we compared guanylyl cyclase subunit message in the kidney of Dahl salt-sensitive and salt-resistant rats by Northern analysis. Message for the β2 subunit was significantly more abundant in kidney from the Dahl salt-sensitive rats while the β1 subunit message was more highly expressed in the kidney from the Dahl salt-resistant rat (Fig. 5). Approximately equal levels of α1 message were detected in both Dahl salt-sensitive and salt-resistant rats.

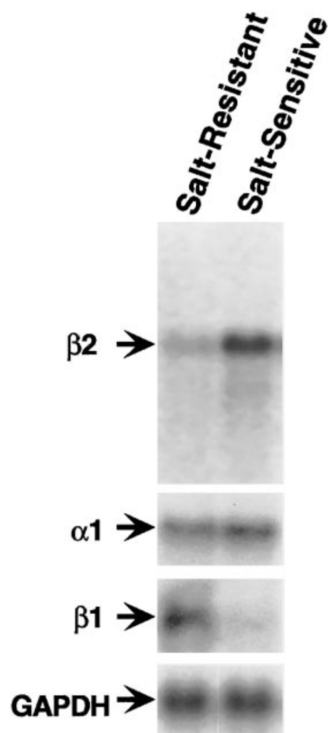


Figure 5. Northern analysis for soluble guanylyl cyclase subunits in Dahl salt-sensitive and salt-resistant rat kidneys. 12 μg poly A+ RNA was prepared from Dahl salt-sensitive and salt-resistant rat kidney and electrophoresed, blotted, fixed to nitrocellulose membrane, and hybridized to random prime labeled probes for α1, β1, and β2 (see Methods). GAPDH was used to confirm equal RNA loading in both lanes.

Discussion

This study indicates that the β2 subunit complexes with the α1 subunit to form a heterodimer with reduced sensitivity to NO, and can inhibit the NO-stimulated cGMP accumulation in cells containing the α1/β1 form of guanylyl cyclase by exchanging with the β1 subunit. The ability of β2 to inhibit guanylyl cyclase activity may be important in the pathogenesis of hypertension in the Dahl rat.

That the β2 subunit forms a heterodimer with the α1 subunit is consistent with the prevailing view that α1 complexes to a β subunit to form a heterodimer (14), and generalizes the hypothesis that both α and β subunits are interchangeable. There is no evidence for the formation of heterotrimers, i.e., α1, β1, and β2, suggesting that β2 can exchange with the β1 subunit. Our data also indicates that, like other heterodimeric forms of guanylyl cyclase, the α1/β2 heterodimer contains a heme group, since its activity is regulated by NO. The histidine 105 of the β1 subunit has been shown to be a likely residue forming a linkage to the central iron of the heme moiety, since substitution of the histidine yielded an NO-insensitive enzyme lacking the prosthetic heme group (15). Interestingly, the β2 subunit does not have a histidine between residues 86 and 129, suggesting that the heme–ligand complex interaction with the subunits is different in heterodimers involving the β2 subunit. We found less NO-stimulated cGMP formation in COS cells transfected with the α1/β2 heterodimer versus the α1/β1, indicating that the α1/β2 heterodimer is less NO-sensitive. Whether this result is due to reduced complexation of NO to the heme iron or to activation of the enzyme by the NO–heme complex cannot be determined from these experiments. Reduced activity of heterodimers containing the β2 subunit, however, is consistent with deletion mutant experiments that have shown that the 64 NH_2-terminal amino acids of the β1 subunit are necessary for regulation by NO, since these are not present in the β2 subunit (7).

We also show that the β2 subunit inhibits stimulation of cGMP formation in COS cells transfected with the α1/β1 form of guanylyl cyclase and endogenous NO-sensitive guanylyl cyclase activity in smooth muscle cells. This study suggests that the mechanism is an exchange of β subunits and formation of the α1/β2 form of the enzyme. We have previously shown that adrenal smooth muscle cells contain message for the α1 and β1 subunits (16), however, whether aortic smooth muscle cells contain the same subunits is not known. Interactions of the β2 subunit with other subunits, i.e., α2 and α2i, have not been characterized.

Our results suggest that β2 may play a role in normal physiology and pathophysiology by regulating NO-sensitivity. These results extend the proposal that differential expression of α isoforms (α1, α2, and α2i) modulates NO-sensitive guanylyl cyclase activity (10, 17) by suggesting that differential regulation of the β subunits (β1 and β2) may also regulate NO-sensitive guanylyl cyclase activity in cells. Whether expression of the β2 subunit is constitutive or regulated is not known. In a study by Ujiie et al. (5) in which the differential expression of mRNA for the subunits was investigated in the rat kidney, the

β2 subunit was detected only 29% of the time in the interlobular artery/afferent arteriole as opposed to the detection of message for α1, α2, and β1 subunits 100% of the time. This result may provide evidence not only that α1, β1, and β2 subunits may be present in the same cell, but also that the expression of β2 is variable, and therefore, regulated in cells containing α1 and β1 subunits.

The Dahl salt-sensitive rat develops hypertension when fed a high-salt diet (18, 19). Increased renal vasculature resistance and reduced salt excretion have been determined in the Dahl salt-sensitive rat, and have been postulated to play a central role in the pathogenesis of hypertension (20, 21). NO reduces renal vascular resistance, and increases sodium excretion (22, 23). It has recently been shown that nitroprusside causes less of an increase in cGMP excretion in Dahl salt-sensitive versus salt-resistant rats (24), suggesting that there is a reduction in the sensitivity of renal guanylyl cyclase to NO. Our results may provide a molecular explanation for reduced stimulation of cGMP by NO in the Dahl salt-sensitive rat. Message for β2 is increased, and β1 is decreased in the kidney of the Dahl salt-sensitive compared to the Dahl salt-resistant rat, suggesting that reduced sensitivity to NO may be linked to an increase in the less NO-sensitive α1/β2 form, and/or decrease in the more active α1/β1 form of guanylyl cyclase in the kidney. The association between β1 and β2 expression remains to be determined. It is possible that expression of the β subunits is interdependent, i.e., increased β2 expression reduces β1 expression, or decreased β2 expression leads to increased β1 expression, or that they are independent of each other, suggesting two primary changes.

Acknowledgments

We appreciate the continuing support and help of Dr. Paul J. Cannon as well as the review of the manuscript by Dr. James Drewett.

References

1. Garbers, D.L., and D.G. Lowe. 1994. Guanylyl Cyclase Receptors. *J. Biol. Chem.* 269:30741–30744.

2. Drewett, J.G., and D.L. Garbers. 1994. The family of guanylyl cyclase receptors and their ligands. *Endocr. Rev.* 15(2):135–162.

3. Ignarro, L.T., P.M. Horwitz, and K.S. Wood. 1986. Activation of soluble guanylate cyclase by NO-hemoproteins involves NO-heme exchange. *J. Biol. Chem.* 261:4997–5002.

4. Koesling, D., C. Harteneck, P. Humbert, A. Bosserhoff, R. Frank, G. Schultz, and E. Bohme. 1990. The primary structure of the 70 kDa subunit of bovine soluble guanylate cyclase from bovine lung: homology between the two subunits of the enzyme. *FEBS Lett.* 266:128–132.

5. Ujiie, K., J.G. Drewett, P.S.T. Yuen, and R.A. Star. 1993. Differential expression of mRNA for guanylyl cyclase-linked endothelium-derived relaxing factor receptor subunits in rat kidney. *J. Clin. Invest.* 91:730–734.

6. Koesling, D., C. Harteneck, P. Humbert, A. Bosserhoff, R. Frank, G. Schultz, and E. Bohme. 1990. The primary structure of the larger subunit of soluble guanylyl cyclase from bovine lung. Homology between the two subunits of the enzyme. *FEBS Lett.* 266:128–132.

7. Wedel, B., C. Harteneck, J. Foerster, A. Friebe, G. Schultz, and D. Koesling. 1995. Functional domains of soluble guanylyl cyclase. *J. Biol. Chem.* 270:24871–24875.

8. Foerster, J., C. Harteneck, J. Malkewitz, G. Schultz, and D. Koesling. 1996. A functional heme-binding site of soluble guanylyl cyclase requires intact N-termini of α_1 and β_1 subunits. *Eur. J. Biochem.* 240:380–386.

9. Harteneck, C., B. Wedel, D. Koesling, J. Malkewitz, E. Bohme, and G. Schultz. 1991. Molecular cloning and expression of a new alpha-subunit of soluble guanylyl cyclase. Interchangeability of the alpha-subunits of the enzyme. *FEBS Lett.* 292:217–222.

10. Behrends, S., C. Harteneck, G. Schultz, and D. Koesling. 1995. A variant of the alpha 2 subunit of soluble guanylyl cyclase contains an insert homologous to a region within adenylyl cyclases and functions as a dominant negative protein. *J. Biol. Chem.* 270:21109–21113.

11. Yuen, P.S., L.R. Potter, and D.L. Garbers. 1990. A new form of guanylyl cyclase is preferentially expressed in rat kidney. *Biochemistry.* 29:10872–10878.

12. Felgner, P.L., T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G.M. Ringold, and M. Danielson. 1987. Lipofectin: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA.* 84:7413–7416.

13. Fort, P., L. Marty, M. Piechaczyk, S. Sabrouty, C. Dani, P. Jeanteur, and J.M. Blanchard. 1985. Various adult rat tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res.* 13:1431–1442.

14. Kamisaki, Y., S. Saheki, M. Nakane, J.A. Palmieri, T. Kuno, B.Y. Chang, S.A. Waldman, and F. Murad. 1986. Soluble guanylate cyclase from rat lung exists as a heterodimer. *J. Biol. Chem.* 261:7236–7241.

15. Wedel, B., P. Humbert, C. Harteneck, J. Foerster, J. Malkewitz, E. Bohme, G. Schultz, and D. Koesling. 1994. Mutation of His-105 in the beta 1 subunit yields a nitric oxide-insensitive form of soluble guanylyl cyclase. *Proc. Natl. Acad. Sci. USA.* 91:2592–2596.

16. Yu, F., and R.S. Danziger. 1995. Molecular characterization of the NO-receptor and regulation of cGMP synthesis in smooth muscle cells. *Circulation.* 92:517.

17. Star, R.A., R.S. Danziger, L. Hogarth, J.G. Drewett, P. Yuen, I. Pang, and K. Ujiie. 1994. Heterologous desensitization of a guanylyl cyclase-linked nitric oxide receptor in rat medullary interstitial cells. *J. Pharm. Exp. Ther.* 270(2):761–767.

18. Dahl, L.K., M. Heine, and L. Tassinari. 1962. Effects of chronic salt ingestion: evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. Exp. Med.* 115:1173–1190.

19. Dahl, L.K., K.D. Knudsen, M. Heine, and G.J. Leith. 1968. Effects of chronic excess salt ingestion: modification of experimental hypertension in rat by variations in the diet. *Circ. Res.* 22:11–18.

20. Simchon, S., W.M. Manger, R.D. Carlin, L.L. Peeters, J.R. Rodriguez, D. Batista, T. Brown, N.B. Merchant, K.M. Jan, D.E. Brinbergm, and S. Chien. 1989. Salt-induced hypertension in Dahl salt-sensitive rats: hemodynamics and renal responses. *Hypertension (Dallas).* 13:612–621.

21. Simchon, S., W.M. Manger, and T.W. Brown. 1991. Dual hemodynamic mechanisms for salt-induced hypertension in salt-sensitive Dahl rats. *Hypertension (Dallas).* 17:1063–1071.

22. Raij, L. 1993. Nitric oxide and the kidney. *Circulation.* 87(Suppl.)5: V26–V29.

23. Bachmann, S. and P. Mundel. 1994. Nitric oxide in the kidney: synthesis, localization, and function [review]. *Am. J. Kidney Dis.* 24:112–129.

24. Simchon, S., W. Manger, G. Blumberg, J. Brensilver, and S. Cortell. 1996. Impaired renal vasodilation and urinary cGMP excretion in Dahl salt-sensitive rats. *Hypertension (Dallas).* 27:653–657.