Impaired Prostaglandin E₂/Prostaglandin I₂ Receptor–Gₛ Protein Interactions in Isolated Renal Resistance Arterioles of Spontaneously Hypertensive Rats

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Abstract—The protective effect of vasodilator agents linked to the cAMP pathway is less effective for buffering the vasoconstrictor effect of angiotensin II in young animals with genetic hypertension. To determine the underlying cellular mechanism, experiments were performed on freshly isolated preglomerular resistance arterioles obtained from kidneys of 7-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Specific high-affinity saturable binding of ³H-prostaglandin (PG) E₂ revealed 1 receptor class in renal microvessels; PGE₂ receptor density was similar in SHR and WKY (106 versus 115 fmol/mg; P > 0.8), as was receptor affinity (3.6 versus 3.5 nmol/L; P > 0.7). Basal cAMP activity was similar in renal arterioles from SHR and WKY. A major finding was that PGE₂, PGI₂, and isoproterenol produced weaker stimulation of cAMP formation in arteriolar cells of SHR (P < 0.02). In contrast, GTP₉ₛ and forskolin stimulated cAMP generation to a similar degree in both rat strains, which suggests normal adenylyl cyclase activity in hypertension-prone SHR. Immunoblots revealed the presence of 3 classes of G proteins (Gₛ, Gᵢ, and Gₚ) in preglomerular arterioles. The relative amounts of discernible G-protein α-subunits in renal resistance vessels did not differ between SHR and WKY. These results extend previous in vivo studies of abnormal renal vascular reactivity in SHR and more directly localize defective coupling of the prostaglandin and β-adrenergic receptors to a stimulatory G protein and cAMP production in freshly isolated preglomerular arteriolar cells of young SHR. This dysfunction may be due to an abnormal interaction between prostaglandin receptors and Gₛ protein that leads to inefficient coupling of initiating steps in the cAMP–protein kinase A cascade during the development of hypertension. (Hypertension. 1999;34:1134-1141.)

Key Words: muscle, smooth, vascular n adenyl cyclase n renal circulation n arterioles n vasoconstriction n rats, inbred strains

An imbalance between vasodilator and vasoconstrictor systems may contribute to pathogenesis and maintenance of hypertension. In vivo hemodynamic studies indicate abnormal vasodilator activity in agents that stimulate the cAMP pathway.¹-⁴ This categorization holds for vasodilator substances such as prostaglandin (PG) E₂, PGI₂, and the dopamine D₁ receptor agonist fenoldopam. Dysfunction of this type is present in young spontaneously hypertensive rats (SHR) early in the development of hypertension and in adult animals with established hypertension.¹,²,⁴ The defect is not universal for all vasodilator substances; it appears to be localized to receptor–G protein coupling and activation of the cAMP pathway. For example, normal buffering is provided by agents such as acetylcholine or bradykinin that primarily produce vasodilation through endothelial activation of the nitric oxide–cGMP pathway.¹,² Stimulation of the cAMP signaling pathway results from sequential interactions among a cell-surface receptor, heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins), and adenylyl cyclase. Within this cascade, any defect in the efficiency of functional interactions between receptor and postreceptor signal transduction may be responsible for impaired cAMP generation.

One possible hypothesis is that the defect is reduced density or affinity of cell surface receptors of the vasodilators in question. Although no biochemical information is available about characteristics of PGE₂ receptor density or affinity in rat renal resistance vessels, one report indicates that no strain difference exists in isolated glomeruli of 6-week-old SHR and Wistar-Kyoto rats (WKY).¹ An increase in PGE₂ receptor number has been reported for the renal medulla of 12-week-old SHR. The density of dopamine receptors appears to be normal in the renal cortex of 3- to 7-week-old SHR but is reduced in proximal convoluted tubules of 18- to 20-week-old SHR versus WKY.⁵,⁷ Analyses by use of immunoblot of amounts of protein suggest a decrease in the amount of Gₛ protein in freshly
isolated and cultured aortic vascular smooth muscle cells or isolated membranes of kidneys in adult hypertensive animals. Increased levels of Gi protein are reported for cardiac myocytes and aortic smooth muscle in 12-week-old SHR and in proximal convoluted tubules in 16- to 20-week-old SHR. However, other investigators find no strain difference in levels of G, or Gi proteins on the basis of Western immunoblots of various tissues, including isolated mesenteric arteries and membranes of myocardium and renal cortex. With regard to the renal vasculature, no differences in mRNA for various G proteins were found in isolated preglomerular vessels of 12- to 14-week-old SHR and WKY. Alternatively, a weaker-than-normal anticonstrictor action may be related to decreased activity of a Gi, protein or increased inhibitory activity of G, proteins. Both views are consistent with the observed improvement in anticonstrictor activity in renal smooth muscle cells when cAMP levels are increased by infusion of either forskolin or administration of a membrane-permeable cAMP analog into the renal artery of young SHR. In vivo studies suggest that the functional defect appears to be localized to impaired signal transduction at a step of receptor coupling to a Gi, protein or a step associated with G-protein activation of adenylate cyclase. The purpose of the present in vitro study was to investigate to a greater depth the cellular mechanisms responsible for the impaired protective mechanism provided by prostaglandins and fenoldopam to angiotensin (Ang) II effects observed in vivo in the renal vasculature of 6- to 8-week-old SHR. Studies were conducted on freshly isolated renal resistance arterioles to assess by PGE2 receptor number and function and postreceptor events that link adenylate cyclase to the cAMP signal transduction pathway. To this end, PGE2 receptor density and affinity were evaluated by radioligand binding during equilibrium conditions in fresh renal microvessels isolated from 7-week-old SHR and WKY. Various G protein families were assessed quantitatively by Western immunoblot. Signal transduction was analyzed by radiomunoassay of cAMP production in response to receptor agonists, G protein stimulation with GTP, and adenylate cyclase stimulation by forskolin.

Methods

Preparation of Isolated Renal Resistance Vessels

Renal preglomerular arterioles were isolated from kidneys of 7-week-old euvolemic WKY and SHR by use of previously established methodology. In our previous studies, mean arterial pressure averaged 140 to 150 mm Hg in 6- to 8-week-old SHR and 110 to 120 mm Hg in age-matched WKY. Briefly, the kidneys were infused with a magnetized iron oxide suspension (1% Fe3O4 in saline), and the preglomerular vessels were separated from the rest of the cortex with the aid of a magnet and sequential sieving. All animal protocols were performed in accordance with the University of North Carolina at Chapel Hill institutional guidelines (IACUC approval Nos. 96-07-0 and 99-030-0).

Receptor Binding Studies

For each experiment, tissue or cells were obtained from kidneys of 3 to 4 rats. Multiple aliquots of microvessels (40 μg of protein) were incubated at room temperature in a final volume of 0.25 mL of buffer containing 0.3% BSA (Sigma Chemical Co). Incubations lasted 45 minutes and included 10 concentrations (between 0.25 and 10 nmol/L) of 3H-PGE2 (Amersham, 154 μCi/mmol/L) subjected to continuous gentle shaking. The protein concentration, binding conditions, and time for equilibrium were determined in preliminary experiments; the results for preglomerular vascular tissues agreed in general with those previously reported for isolated glomeruli. Bound ligand was separated from the free ligand as previously described with a sucrose gradient. In competitive inhibition studies, PGE2, PGE1, and PGI2 were used to displace 3H-PGE2 (5 nmol/L). Analyses of the data using the LIGAND (Biosoft) program gave estimates of the maximum specific binding and dissociation constant.

Immunoblotting of α Subunits of G Protein

Solubilized protein (15 to 30 μg, given according to the linearity of protein loaded) was subjected to 10% SDS–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane in buffer (20% methanol, 48 mmol/L Tris, 39 mmol/L glycine, and 0.0375% SDS). Polyvinylidene difluoride membranes were incubated with the desired primary antibody (1:1000; rabbit G-protein antisera against Ga (DuPont, CalBiochem), Ga, Ga, and Ga, G, G, Ga, and Ga (CalBiochem), Ga, and Ga+G, (DuPont), and β-actin (1: 5000; Sigma) and immerses in either horseradish peroxidase–labeled goat anti-rabbit IgG (1:4000; Amersham Life Science) for G protein or horseradish peroxidase–labeled goat anti-mouse IgG for β-actin (1:4000; Amersham) according to the ECL Western blotting protocols (Amersham). Relative blot intensity was determined by scans made with Adobe Photoshop software.

Determination of cAMP Content

Preglomerular arterioles were prepared as described above, and cAMP generation was determined by use of standard methodology. Briefly, 10 μL of cell suspension (30 μg of protein per milliliter in 50 mmol/L HEPES, 1 mmol/L DTT, 0.25 mmol/L sucrose, 1 mmol/L EDTA, and 0.01% bacitracin) was incubated in 1 mmol/L isobutyl methylxanthine, 4 mmol/L MgCl2, 2 mmol/L GTP, 1 mmol/L ATP, and an ATP regenerating system (15 mg/mL of phosphocreatine and 0.8 mg/mL creatine phosphokinase) and appropriate drugs were added to a final volume of 200 μL. In indicated studies, various amounts of GTPyS were used instead of GTP. After 30 minutes of incubation at 37°C, the reaction was stopped by adding 6% trichloroacetic acid. The samples were put on ice and sonicated for 1 minute. The cell lysate was then extracted 4 times with 1 mL of water-saturated ether and evaporated. An aliquot was acetylated, and cAMP was measured by radioimmunoassay (Biomedical Technologies Inc.). Statistical analyses were performed using unpaired t test. Multiple comparisons were evaluated using ANOVA. Post hoc tests were evaluated by Student-Newman-Keuls test. Results with P<0.05 were considered statistically significant. All values reported are mean±SE.

Results

Studies were performed to determine whether the inefficient buffering effect of prostaglandins in vivo can be localized to a primary strain difference in receptor density or affinity. Radioligand binding studies were conducted under equilibrium conditions to characterize PGE2 receptors in preglomerular arterioles of young SHR and WKY. Scatchard analysis indicated a density for 3H-PGE2 binding sites of 106±5 fmol/mg for the 7-week-old SHR and 115±11 for age-matched WKY (P<0.8). The Hill coefficient for PGE2 binding averaged 0.93 and 0.96 in WKY and SHR, respectively, which indicates 1 class of high-affinity receptor in both groups of animals. PGE2 receptor affinity averaged 3.5±0.6 and 3.6±0.5 nmol/L and did not differ between strains (P>0.7).

In other receptor-binding studies, unlabeled authentic PGE2, PGE1, and PGI2 were added to assess ability to
compete with and displace \(^3\text{H}\)-PGE\(_2\) (5 nmol/L) binding to vascular receptors. The results for the 2 strains were almost identical and thus were pooled for SHR and WKY. These 3 agents displaced PGE\(_2\) from binding sites in a concentration-dependent manner (Figure 1). PGE\(_1\) and PGE\(_2\) exhibited similar inhibitory potency, with IC\(_{50}\) values of 0.9 and \(1.0 \times 10^{-7}\) mol/L, respectively. In contrast, PGI\(_2\) was less potent than the PGE analogs. PGI\(_2\) in excess of \(5 \times 10^{-6}\) mol/L inhibited \(60\%\) of the PGE\(_2\) binding.

Other experiments were conducted to evaluate possible differences in expression of G-protein subunits in renal resistance vessels obtained from young normotensive and hypertensive rats. Immunoblotting techniques were used to determine the existence and relative amounts of G proteins. Western blots demonstrated the presence of 3 classes of G-protein families in preglomerular microvessels. Figure 2 (top) shows that the immunolabeling signal is directly proportional to the amount of protein loaded (5 to 60 µg). The signal begins to plateau when protein is >40 µg. As shown in Figure 2, 2 forms of G\(_{\text{sa}}\) (20 µg protein per lane) were identified: 45 kD (short) and 52 kD (long). Average values for the relative amounts of G\(_{\text{sa}}\) subunits normalized to β-actin are not significantly different between vascular smooth muscle cells of SHR and age-matched WKY. Moreover, neither short nor long forms of the G\(_{\text{sa}}\) subunit differed between animal groups (Figure 2, bottom). Western immunoblots documented the presence of G\(_{\text{sa}}\)+G\(_{\text{ia}}\), G\(_{\text{sa}}\)+G\(_{\text{ib}}\), and G\(_{\text{i}}\) in preglomerular arterioles. Relative amounts of these G proteins normalized to β-actin are shown in Figure 3. No major strain differences were seen in the density of any of the detected G-protein subunits. Interestingly, G\(_{\text{ia}}\) was not detected in preglomerular arterioles from either strain.

A separate series of studies evaluated further the mechanism responsible for defective function of receptor coupled to cAMP activation, as suggested from in vivo hemodynamic studies. The activity of adenylate cyclase was determined in the isolated preglomerular arterioles from 7-week-old SHR and WKY before and during receptor stimulation. A small, nonsignificant difference occurred in basal adenylate cyclase activity (12.4±2.3 pmol per 30 minutes per 30 µg/mL for WKY versus 15.8±0.6 for SHR; \(P>0.2\)). To establish concentration-response relations, PGE\(_2\) and PGI\(_2\) were used to stimulate receptors and activate adenylate cyclase. As Figure 4 shows, cAMP production increased in response to specific receptor stimulation by PGE\(_2\) and PGI\(_2\) in a concentration-dependent manner. Each set of concentration-response data was analyzed by ANOVA to assess dose-related effects and a strain difference in response to agonist. Major findings were that stimulation was significantly reduced in SHR with both vasodilator prostaglandins (\(P<0.02\)). This strain difference was observed when the data were analyzed in terms of absolute value as well as percentage change. As PGE\(_2\) concentrations increased from \(10^{-8}\) to \(10^{-5}\) mol/L, cAMP production in SHR increased from 83% to 166% of basal values. The maximum SHR increase was less than the 194% observed in WKY. The results for PGI\(_2\) also demonstrate a marked strain difference in cAMP production. None of the tested doses in SHR came close to approaching the 196% stimulation observed with \(10^{-8}\) mol/L PGI\(_2\) in WKY. The highest tested concentration of PGI\(_2\) produced a 141% increase in cAMP generation in SHR.
To determine whether such impaired receptor-mediated cAMP stimulation is specific to the prostaglandins, the \( \beta \)-adrenoceptor agonist isoproterenol was used to stimulate a different receptor class that is commonly coupled to cAMP formation. Isoproterenol elicited dose-dependent increases in cAMP production in WKY (Figure 4). The effect of \( \beta \)-adrenoceptor activation was consistently attenuated in SHR \( (P < 0.04) \). Maximum stimulation was 160% in SHR and 227% in WKY. Another index of the attenuated response in SHR is the dose required to produce a similar degree of stimulation. A \( 10^{-8} \) mol/L dose of isoproterenol caused a 161% increase in cAMP formation in WKY vessels versus 160% stimulation by a much greater concentration \( (10^{-4} \text{ mol/L}) \) in SHR. Thus, ligand activation of 3 distinct classes of receptors elicited less-than-normal stimulation of cAMP in freshly isolated preglomerular arterioles from young SHR.

Function of G proteins was evaluated by measuring the effects of GTP on adenylate cyclase activity and cAMP production in isolated renal resistance vessels. Nonhydrolyzable GTP\(_{\gamma}\) had similar biphasic effects on adenylate cyclase activity in vascular tissue of SHR and WKY (Figure 5). As concentration of GTP\(_{\gamma}\) increased from \( 10^{-7} \) to \( 10^{-5} \) mol/L, marked stimulation of cAMP formation was seen, which suggests mediation by activation of a G\(_s\) protein. GTP\(_{\gamma}\) (at a concentration of \( 10^{-4} \) mol/L) had the greatest stimulatory effect on cAMP accumulation. However, concentrations of GTP\(_{\gamma}\) in excess of \( 10^{-4} \) mol/L progressively reduced cAMP accumulation from peak levels, which probably reflects activation of G\(_i\) proteins.

Additional studies were performed to determine the action of forskolin on adenylate cyclase activity. Forskolin was used to stimulate the catalytic unit of adenylate cyclase directly; this bypassed cell surface receptors and their interaction with G proteins. The results indicate that forskolin caused concentration-dependent increases in cAMP generation. No strain difference existed in the ability of forskolin to stimulate adenylate cyclase in preglomerular arterioles \( (P > 0.3) \) (Figure 5).

**Discussion**

Functional studies highlight an imbalance between vasodilator and vasoconstrictor systems in hypertension. Several vasodilator systems exhibit a defect in effectiveness with which they attenuate responses elicited by vasoconstrictor agents such as Ang II. As a result, young genetically hypertensive rats present with decreased renal blood flow and increased renal resistance. These abnormalities alone or in concert with enhanced tubular reabsorption may contribute to the genesis of hypertension. It is of particular interest to elucidate the mechanisms responsible for weaker-than-normal protection from the actions of potent vasoconstrictor substances. In vivo studies strongly suggest defective activation of the cAMP pathway as a mechanism rather than near normal or exaggerated activity of nitric oxide activation of...
the cGMP system in the renal vasculature of 7-week-old SHR and WKY.\textsuperscript{1–3}

The present study extends our previous findings that one mechanism that causes an impaired buffering effect of prostaglandins against the action of Ang II concerns an abnormal interaction between the PGE\textsubscript{2} or PGI\textsubscript{2} receptor and a G protein linked to stimulation of cAMP formation.\textsuperscript{1–4} Our results indicate that vasodilators such as PGE\textsubscript{2}, PGI\textsubscript{2}, and isoproterenol are inefficient stimulators of cAMP production in renal resistance vessels from young SHR, given the greater responses seen in age-matched WKY. The abnormality in G\textsubscript{i}-protein function tends to be generalized in the renal vasculature of SHR; 3 receptor classes exhibit similar dysfunction in coupling.

The decreased ability of vasodilator agents to stimulate adenylate cyclase may be attributable to abnormal interaction of ligand with receptor. Altered \(\beta\)-adrenergic receptor and PGE\textsubscript{2} receptor numbers or affinities in association with diminished adenylate cyclase responses have been reported for fresh myocardial and renal membranes and for cultured aortic smooth muscle cells from SHR and Milan hypertensive rats.\textsuperscript{8,13,21} To examine this issue in preglomerular arterioles of 7-week-old SHR, we characterized PGE\textsubscript{2} receptors during equilibrium binding conditions. Our results demonstrate no marked strain difference in PGE\textsubscript{2} receptor density or affinity between vessels of SHR and WKY. Thus, PGE\textsubscript{2} receptor binding characteristics per se cannot account for the deficiency in activation of adenylate cyclase in SHR. This finding is consistent with our earlier data of similar PGE\textsubscript{2} receptor density and affinity in glomeruli of young SHR and WKY.\textsuperscript{1} The density of the PGE\textsubscript{2} receptor (110 fmol/mg) observed in preglomerular arterioles is in the range of 75 to 80 fmol/mg that has been reported for glomeruli.\textsuperscript{1,22,23} We conclude that the major defect in cAMP production in preglomerular arterioles of young SHR is more related to postreceptor events or receptor coupling to G\textsubscript{i} proteins that normally activate adenylate cyclase and protein kinase A than to strain differences in PGE\textsubscript{2} receptor number and affinity. Accordingly, other investigators seem to eliminate receptor density as causative factor in cases for dopamine or \(\beta\)-adrenergic receptor activation of adenylate cyclase activation in epithelial cells of renal proximal tubule and femoral arterial smooth muscles cells of young SHR. On the other hand, decreased density is reported for these cells in animals with established hypertension.\textsuperscript{5,7,24,25}

Ligands for 3 distinct receptors were used to evaluate receptor-mediated activation of adenylate cyclase. Our values for basal cAMP production in rat renal vascular tissue are in close agreement with published data for isolated rabbit preglomerular vessels and tissue from rat renal cortex and medulla.\textsuperscript{21,26} We found weaker stimulatory effects of PGE\textsubscript{2}, PGI\textsubscript{2}, and isoproterenol on formation of cAMP in the renal arterioles of 7-week-old SHR. The magnitude of the cAMP response to receptor agonists was roughly 55% greater than control in SHR vessels, whereas stimulated values in WKY were 205% of basal levels. Previous studies had maximum stimulation in the range of 175% to 300% for PGE\textsubscript{2}, PGI\textsubscript{2}, and isoproterenol in rabbit renal vascular and rat renal cortical medulla tissues.\textsuperscript{21,26} Consistent with our results, attenuated responses of adenylate cyclase to several vasodilator agents have been reported for the proximal convoluted tubule, renal cortex, renal medulla, and whole-kidney homogenates.\textsuperscript{5,6,17,21,25} Similar findings are reported for isolated mesenteric artery, aorta, and myocardial membranes.\textsuperscript{12,13,16,17} In these studies, PGE\textsubscript{2} and PGI\textsubscript{2}, dopamine, and isoproterenol were found to exhibit less-effective stimulation of cAMP generation mediated by G\textsubscript{s} proteins in young and adult animals with genetic hypertension.\textsuperscript{5,6,21,25,27} On the other hand, some reports suggest that vasoconstrictors such as Ang II display exaggerated inhibition of adenylate cyclase activity in 12-week-old SHR.\textsuperscript{4,12} In preliminary studies, we find that Ang II has no effect on either resting or elevated levels of adenylate cyclase stimulated by PGE\textsubscript{2} or \(\beta\)-adrenergic receptors in freshly isolated preglomerular arterioles.

The present study suggests that impaired cAMP formation after vasodilator receptor stimulation is probably not due to changes in the expression of G-protein levels per se. New information is presented for 3 major classes of G-protein
families (G_s, G_i, and G_{q/11}) in freshly isolated preglomerular arterioles of 7-week-old rats. No strain difference was noted between amounts of any of the G proteins normalized to β-actin in arterioles from SHR and WKY. Also, no G_s proteins were detected by Western blot analysis. This observation is consistent with the apparent absence of this class of G protein from isolated rat glomeruli, proximal convoluted tubules, and cultured aortic smooth muscle cells.7–8,15,28

A deficient cAMP response could potentially arise from changes in functional interactions involved in the coupling between heterotrimeric GTP–binding proteins and adenylyl cyclase. Possibilities include diminished activity or expression of G_s protein or increases in G_i protein. Previous studies evaluated G-protein expression at different age ranges and various tissue sources. Reduced amounts of G_m long form protein are reported for the cells during development of hypertension, and such differences may account for abnormal coupling of a vasodilator receptor to the cAMP pathway.10 Lower-than-normal amounts of G_i have also been found in cultured aortic smooth muscle cells from Milan hypertensive rats.9 On the other hand, overexpression of inhibitory G protein, accompanied by overexpression of G_m mRNA, has been observed in freshly isolated aortic, myocardial, and kidney membranes from SHR and other hypertensive models.11,13–15 Increased inhibitory G_i protein levels would be expected to enhance vasoconstrictor action as a result of suppressed cAMP production.

Note that the G-protein alterations reported in most studies are evident in older animals (>12 weeks of age) and characterized by a phase of established hypertension. These strain differences most likely reflect secondary responses to long-standing elevation in arterial pressure, although humoral factors may play a role. For example, aortic coarctation leads to reductions in G_i and G_s proteins not only in aorta exposed to high arterial pressure but also in aorta downstream of the constriction at sites exposed to lower arterial pressure.9 G_m and its mRNA are greater than normal in isolated aortic and myocardial membranes in SHR as young as 2 weeks of age, which indicates a primary genetic defect.12 However, no consistent or common feature of altered G-protein levels occurs in various tissues in the SHR model of genetic hypertension. Indeed, some investigators believe that no changes exist in G protein expression in artery, heart, and renal cortex in 10- to 11-week-old SHR.10,16,17,29 In agreement with these reports, our results demonstrate that relative amounts of G_m, G_s, and G_{q/11} are similar in preglomerular arterioles of 7-week-old SHR and WKY. These data are consistent with a recent report that suggests that mRNA for most G proteins in renal artery is similar in adult SHR and WKY.15 Thus, clearly, the absolute amounts of the 2 forms of G_s proteins and other G proteins are not responsible for the defect in cAMP formation in SHR vessels.

Another possible explanation for the vasodilator receptor–mediated abnormality in the cAMP pathway is reduced function of G protein and signal transfer from receptor to activation of adenylyl cyclase in genetic hypertensive rats. GTP plays an important role in activating G proteins to facilitate interaction with adenylyl cyclase. We used GTPγS, a nonhydrolyzable GTP analog, to activate G proteins and evaluate their coupling in preglomerular arterioles. In the absence of G_s proteins, G_i or G_{q/11} proteins probably are activated by moderate levels of GTPγS to promote stimulation and inhibition of cAMP, respectively. In keeping with this prediction, low GTPγS concentrations stimulate cAMP production markedly, reflecting a predominant action on the G_s–α-subunit. Higher concentrations of GTPγS reversed the maximum stimulation of cAMP, presumably through progressive activation of G_s protein. Importantly, the pattern and efficiency of these biphasic actions of GTPγS were similar in control and hypertensive rats. Other investigators have reported 2.5- to 6-fold increases of adenylyl cyclase activity in response to Gpp(NH)p in isolated rabbit preglomerular arterioles and rat proximal convoluted tubules.5,27 These findings suggest that function of both stimulatory and inhibitory G protein are intact and equally responsive to fixed levels of GTP in the renal vasculature.

Our study extends to the renal microvasculature earlier findings that GTP and its nonhydrolyzable analogs GTPγS or Gpp(NH)p and NaF efficiently stimulate cAMP generation in dissected proximal convoluted tubules, renal cortical and medullary membranes and cultured mesenteric arteriolar smooth muscle cells from young SHR.6,21,25 However, other studies have reported that GTP and its nonhydrolyzable analogs as well as NaF have a reduced ability to activate adenylyl cyclase in cultured or isolated aortic smooth muscle cells and myocardial membranes from adult SHR between 11 and 12 weeks of age.5,12,13,16,17 These conflicting results suggest tissue-dependent or age-dependent alterations in G-protein function that may be involved in different phases of the progression of hypertension. The fact that GTPγS produced similar stimulation of adenylyl cyclase in the renal resistance arterioles of young SHR and WKY in the present study suggests no major strain difference in activated G_s protein per se. A more likely possibility is reduced functional activity of receptor and G_s–protein coupling.

We also investigated postreceptor and post–G-protein components related to the adenylyl cyclase pathway. Forskolin was used to activate the catalytic site of adenylyl cyclase, independent of receptor stimulation and receptor–G protein interactions. We found that forskolin stimulated cAMP generation in vascular tissue from both strains. The magnitude of the stimulation by forskolin was almost identical in young SHR and WKY. Earlier reports indicate normal activity of adenylyl cyclase and response to forskolin stimulation in femoral artery strips, dissected proximal convoluted tubules, and membranes from whole-kidney homogenates from SHR.6,13,17,21,25 Other groups have reported unchanged or increased forskolin effect on cAMP formation in various cell types, including heart, aorta, and mesenteric artery.6,12 but Mn^{2+} abolished these variations.

In conclusion, we investigated 3 components (receptor, G_s protein, and adenylyl cyclase) associated with the cAMP signaling pathway in the renal microcirculation in genetic hypertension. Our findings provide important new information concerning which factor is responsible for attenuated activity of adenylyl cyclase to vasodilator stimulation in SHR. Signal transduction appears to be involved early in the process, specifically between G_i proteins and 3 identified
receptor classes. Several factors seem unlikely because of similarities in receptor number, G-protein function, or expression and activity of adenylate cyclase. The recent studies of Sanada et al. may provide an explanation for the defect in receptor–G protein coupling in renal resistance arterioles. These investigators discovered a novel serine kinase (FJ1) in proximal convoluted tubules and the renal cortex of young rats and humans with genetic hypertension. This new protein appears to phosphorylate cell surface dopamine D1 receptors that normally lead to increases in cAMP. In turn, receptor phosphorylation interferes with the efficiency of receptor coupling with G protein, thus decreasing D1 receptor stimulation of cAMP production. Our studies provide strong evidence that such a defect exists in the coupling of G proteins to EP, IP (prostacyclin), and β-adrenergic receptors and may be more generally located to include renal proximal tubular cells and the proximal convoluted tubule. The functional defect is evident during the development phase of hypertension. Abnormal renal vasoconstriction and increased tubular salt reabsorption may act in concert to cause genetic hypertension.

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References


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