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Physiology

Defective G protein activation of the cAMP pathway in rat kidney during genetic hypertension

(blood pressure/renal circulation/vascular smooth muscle/angiotensin II/dopamine)

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ABSTRACT **The development of hypertension in the spontaneously hypertensive rat (SHR) is associated with renal dysfunction and vasoconstriction. The kidneys of young SHRs exhibit exaggerated reactivity to angiotensin II (Ang-II) and attenuated responses to vasodilators that normally activate the cAMP signal to buffer hormone-induced vasoconstriction. The present study investigates the mechanism(s) responsible for this abnormality in activation of the cAMP second-messenger pathway in hypertensive animals. Renal vascular reactivity was assessed in 7-week-old anesthetized SHRs and normotensive Wistar–Kyoto rats. The animals were pretreated with indomethacin to block prostanoid production throughout an experiment. Ang-II was injected into the renal artery either alone or mixed with the vasodilator fenoldopam, a dopamine-receptor agonist. These two opposing vasoactive agents were administered before and during intrarenal infusion of NaF or cholera toxin, two activators of G proteins that stimulate cAMP production. The results show that Ang-II reduced renal blood flow by 45% in both strains. In Wistar–Kyoto rats, fenoldopam reduced the Ang-II-induced decrease in renal blood flow from −45% to −30%. This protective effect of fenoldopam was increased further during infusion of NaF or cholera toxin (−18% or −19% decrease in renal blood flow). In SHRs, fenoldopam failed to attenuate Ang II-mediated vasoconstriction (−45% vs. −44%). In contrast, fenoldopam effectively blunted the Ang-II-induced vasoconstriction when it was given concurrently with NaF or cholera toxin (−27 or −31% decrease in renal blood flow). These findings provide evidence for defective interaction between receptor coupling and activation of guanine nucleotide stimulatory factor proteins in the renal microcirculation of 7-week-old SHRs. Such a deficiency could play an important role in renal dysfunction associated with the development of genetic hypertension.**

Rats developing genetic hypertension, such as the Okamoto–Aoki strain of spontaneously hypertensive rat (SHR), provide opportunities for insight into mechanisms involved in the pathogenesis of essential hypertension in humans. Cross-transplantation studies indicate that the kidneys play a pivotal role in the development of hypertension in genetically hypertensive rats (1–3). Alterations in renal vascular resistance, glomerular filtration rate, renal blood flow, and sodium and water retention have been described in 6- to 8-week-old SHRs compared with age-matched control Wistar–Kyoto rats (WKYs) (4–6). The abnormalities in renal hemodynamics and function become less pronounced as the hypertension advances to an established phase in 12-week-old SHRs (7, 8). Genetic cosegregation studies reveal a direct relationship between increased renal vascular resistance and arterial hypertension (9).

The mechanism(s) responsible for increased vascular resistance and reactivity have been the subject of intense investigation. Increased renal vascular resistance in adult animals is proportional to the increase in arterial pressure and may represent an appropriate autoregulatory response (7). In contrast, the reduced renal blood flow and glomerular filtration rate in young SHRs with minimally elevated arterial pressure are consistent with the participation of vasoconstrictor factor(s).

Although circulating and intrarenal levels of renin are considered normal in SHRs, several lines of evidence support the notion that the renin–angiotensin system exerts a stronger than normal influence on the renal circulation in young SHR(s). Acute and chronic inhibition of angiotensin-converting enzyme prevents the development of hypertension in young SHR(s) (10). In previous studies we observed that renal vascular responses to angiotensin II (Ang-II) are exaggerated in young SHRs compared with those in WKYs (10–13). This strain difference was not due to differences in the affinity and/or density of the Ang-II receptors found in the renal vasculature but rather was due to identified interactions with other vasoactive substances (12, 13).

The increased vasoconstriction could be caused by reduced offsetting activity of a vasodilator system. Several renal vasodilators, such as prostaglandins E_2 and I_2 and the dopamine (DA1)-agonist fenoldopam, could not buffer the Ang-II-induced vasoconstriction in the kidneys of 6- to 8-week-old SHRs. The same vasodilator agents were, however, able to almost completely abolish the Ang-II effect in kidneys of age-matched WKYs (13–15). The abnormality seemed specific to activators of the cAMP messenger system. Receptor agonists leading to increased nitric oxide production and activation of the cGMP pathway were equally effective in normotensive and hypertensive strains (13, 14).

The present study examined the mechanisms responsible for the inability of vasodilator autacoids/paracrine substances to counteract the Ang-II-induced vasoconstriction in the renal vasculature of SHR(s) that are young and in the developmental phase of hypertension. Our results suggest that this abnormality is probably due to an impaired activation of a guanine nucleotide stimulatory protein (G_s) coupled to receptors of agents that normally activate the cAMP pathway. Such a defect may cause hypertension by acting on vascular smooth muscle cells and renal tubular epithelial cells.

MATERIALS AND METHODS

Experiments were done on 7-week-old rats of the normotensive WKY and the hypertensive SHR strains obtained from the Chapel Hill breeding colony. The animals were maintained on a standard rat chow diet and tap water ad libitum until the

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Abbreviations: Ang-II, angiotensin II; SHR, spontaneously hypertensive rat; WKY, Wistar–Kyoto rat; G_s, guanine nucleotide stimulatory protein.

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night before an experiment. Anesthesia was induced by an i.p. injection of pentobarbital sodium (65 mg/kg of body wt), and the animals were placed on a heating table to maintain body temperature at 37°C. Standard surgical procedures were used (5, 7, 11–14, 16). Briefly, after a tracheotomy a carotid artery was cannulated to obtain blood samples and monitor arterial pressure (Statham P23 Db transducer). A jugular vein was cannulated for the administration of maintenance infusions, a cyclooxygenase inhibitor, and supplemental doses of anesthetic. Euvolemia was maintained by infusion of iso-oncotic bovine serum albumin. Midline and subcostal incisions exposed the abdominal aorta and the left kidney. A noncannulating electromagnetic flow probe (1.5-mm circumference, Carolina Medical Electronics, King, NC) was placed around the left artery to measure renal blood flow. A tapered and curved PE-10 catheter was introduced into the left femoral artery and advanced through the aorta until its tip was positioned in the left renal artery. This procedure did not affect renal blood flow. The renal arterial catheter was used for the local administration of vasoactive agents. After completion of the surgery, the animals were allowed to stabilize for 1 hr before starting the measurements.

After the stabilization period, indomethacin (5 mg/kg of body wt) was administered i.v. to inhibit cyclooxygenase activity. This dose of indomethacin produces a 60–80% decrease in the rate of urinary prostaglandin E_2 excretion for at least 3 hr (11). During the experiment, heparinized isotonic saline was infused (5 μl/min) via the renal arterial catheter. A Cheminert sample injection valve was used to introduce a 10-μl bolus of test agent into the infusion line. One minute before an injection, the rate of saline infusion was increased to 120 μl/min to deliver the entire bolus of test agent to the kidney within 5 sec. After renal blood flow returned to its baseline level (usually <2 min), the saline infusion was returned to 5 μl/min.

The following drugs were used in this study: Ang-II, NaF, and indomethacin (Sigma), fenoldopam (SmithKline Beecham), and cholera toxin (Biomol, Plymouth Meeting, PA). Ang-II (2 ηg) was injected into the left renal artery either alone or in a mixture with fenoldopam (10 ηg). The G protein activators NaF and cholera toxin were infused at low doses (13 ηg/min and 1.3 μg/min, respectively); they did not affect basal renal blood flow or arterial pressure. The intrarenal administration of the G protein activators was started 2 min before Ang-II was injected either alone or with fenoldopam and was continued until the end of each 2-min recording. The time interval between Ang-II injections was 15 min. To avoid treatment interactions, only one G protein activator was given to an animal. Ang-II responsiveness was tested between infusions of NaF or cholera toxin. In all cases, Ang-II produced a similar decrease in renal blood flow. Thus, G protein activators produced specific changes in the responsiveness of the renal vasculature.

The data-acquisition system consisted of an IBM PC-compatible computer and a 12-bit analog-to-digital converter (11, 12). Each recording was started when Ang-II was introduced into the renal artery infusion line. The outputs of the transducers monitoring arterial pressure and renal blood flow were sampled at a rate of 100 samples per sec for a period of 2 min. Data were averaged to obtain sec-by-sec estimates of renal blood flow and arterial pressure, and these averages were used to estimate renal vascular resistance each sec. The renal blood flow, arterial pressure, and renal vascular resistance values were normalized and expressed as a percentage of baseline values. The baseline was calculated separately for each injection using the mean values of the corresponding variables observed during the time between the introduction of Ang-II into the infusion line and the onset of the renal vascular response. Plots of normalized arterial pressure, renal blood flow, and renal vascular resistance as a function of time were prepared by using the SIGMAPLOT software package. Nonlinear least-squares estimation was used to fit a smooth curve to each blood flow and vascular resistance recording as described (11). The best-fit curve was used to calculate the maximum response and kinetic parameters, such as the time required to reach the maximum response and the half-times for constriction and recovery.

Statistical analyses were done by using the Multivariate Generalized Linear Hypothesis module of the SYSTAT software package. Statistical analyses of the maximum changes in renal blood flow gave similar results, regardless of whether these changes were expressed as a percentage of the baseline or as absolute values. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline renal hemodynamic data are summarized in Table 1. Seven-week-old SHRs were moderately hypertensive compared with age-matched WKY controls. In SHRs, basal renal flow was reduced and renal vascular resistance was increased in agreement with previous results (5, 11). Indomethacin affected neither arterial pressure nor renal blood flow in WKYs and SHRs, in accordance with earlier results (11).

Injection of Ang-II into the renal artery produced a transient decrease in renal blood flow and an increase in renal vascular resistance, without affecting systemic arterial pressure. Because arterial pressure was constant, local changes in renal blood flow were associated with reciprocal changes in renal vascular resistance (data not shown). Representative examples of the renal vascular response to Ang-II are shown in Fig. 1. The maximum vasoconstriction caused by 2 ηg of Ang-II was similar in WKYs and SHRs. Renal blood flow was reduced by 44 ± 2 vs. 45 ± 2% ($P > 0.6$), in close agreement with previous observations (11, 12).

Fenoldopam, a dopamine DA1-receptor agonist, was coadministered with Ang-II intrarenally to determine whether receptor-mediated stimulation of intracellular cAMP can buffer the vasoconstrictor effect of Ang-II. The data in Fig. 1*A* show that fenoldopam attenuated 50% of the transient renal vasoconstriction produced by Ang-II in WKY kidneys. The maximum decrease in renal blood flow observed at 29 sec after injection was reduced from −52% to −31% of basal renal blood flow. The buffering effect of fenoldopam could be blocked by coadministration of the receptor-antagonist SCH-23390 in a dose-related manner, indicating selective activation of vascular DA1 receptors. On the other hand, fenoldopam was ineffective in SHR kidneys (Fig. 1*B*). The maximum response to the mixture of Ang-II and fenoldopam did not differ from that of Ang-II alone in the genetically hypertensive strain.

Table 1. Baseline renal hemodynamic variables in euvolemic 7-week-old WKYs and SHRs

	WKY	SHR	*P**
Age, week	7 ± 1	7 ± 1	NS
Body wt, g	196 ± 6	201 ± 1	NS
Arterial pressure, mmHg	124 ± 2	148 ± 3	<0.001
Renal blood flow, $ml \cdot min^{-1} \cdot (g\ kidney\ wt)^{-1}$	7.4 ± 0.4	6.5 ± 0.2	<0.05
Renal vascular resistance, $mmHg \cdot ml^{-1} \cdot min \cdot (g\ kidney\ wt)^{-1}$	17.2 ± 0.9	23.5 ± 1.2	<0.001
Hematocrit, $ml \cdot dl^{-1}$	46 ± 1	47 ± 1	NS
Rats, no.	14	14	

Values are means ± SEM. 1 mmHg = 133 Pa.
*P value of unpaired t test; NS, not statistically significant ($P > 0.05$).

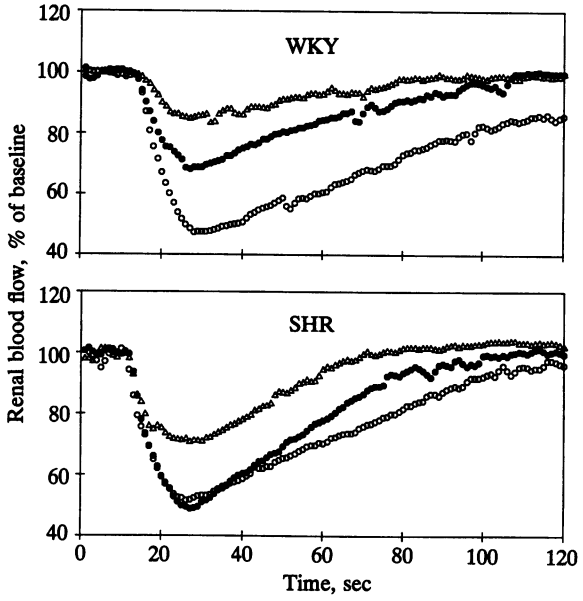

FIG. 1. Representative examples of temporal variations in renal blood flow produced by injection of Ang-II (2 ηg) into the renal artery. Ang-II was administered alone (○) and when mixed with the DA1 dopamine agonist fenoldopam (10 ηg) (● and △). The agents were injected before and during infusion of NaF (● and △, respectively) into the renal artery. (*A*) WKY data. (*B*) SHR data.

Statistical analysis using the best-fitting curve model described earlier in *Materials and Methods* confirmed that fenoldopam significantly reduced the maximum vasoconstriction produced by Ang-II in seven WKYs (−30 ± 2% vs. −45 ± 3% basal renal blood flow, $P < 0.001$), but it had no effect in seven SHRs (−44 ± 2% vs. −45 ± 2%, $P > 0.7$) (Fig. 2). The kinetics describing the transient response to Ang-II when administered alone and coadministered with fenoldopam did not differ between SHR and WKY (Table 2). Interestingly, fenoldopam shortened the half-time of recovery from the Ang-II-induced vasoconstriction similarly in WKYs (from 75 ± 3 to 64 ± 4 sec, $P < 0.05$) and SHRs (from 71 ± 3 to 64 ± 2 sec, $P < 0.05$), even though a strain difference was observed in the magnitude of the maximum response (Fig. 2) (11, 13).

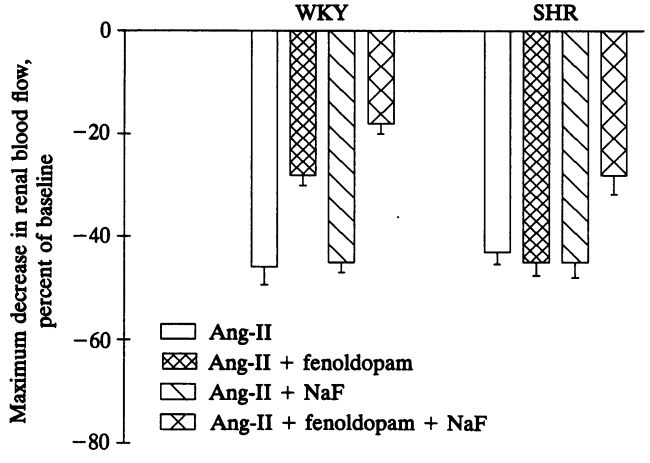

FIG. 2. Group averages for the maximum decrease in renal blood flow produced by intrarenal injection of Ang-II in WKYs (*A*) and SHRs (*B*). Ang-II was given alone and mixed with the dopamine DA1-agonist fenoldopam before or during NaF administration. Values are means ± SEM for seven WKYs and seven SHRs. $P < 0.05$ for WKYs vs. SHRs; $P < 0.05$ for control vs. fenoldopam or vs. fenoldopam plus NaF within strain.

Table 2. Summary of kinetic parameters describing the transient renal vascular response to Ang-II alone and in a mixture with fenoldopam before and during intrarenal infusion of NaF or cholera toxin

	Half-time, sec		
	WKY	SHR	Rats, no.
Constriction half-time, sec			
Ang-II	16 ± 1	16 ± 1	14
Ang-II + fenoldopam	17 ± 1	17 ± 1	14
Ang-II + fenoldopam + NaF	16 ± 2	17 ± 1	7
Ang-II + fenoldopam + CTX	17 ± 1	17 ± 1	7
Recovery half-time, sec			
Ang-II	75 ± 3	71 ± 3	14
Ang-II + fenoldopam	64 ± 4*	64 ± 2*	14
Ang-II + fenoldopam + NaF	64 ± 3*	64 ± 3*	7
Ang-II + fenoldopam + CTX	78 ± 4	71 ± 3	7

Values are means ± SEM. CTX, cholera toxin.
*$P < 0.05$ vs. Ang-II. None of the values differed between SHRs and WKYs.

Fenoldopam had no effect on the time to maximum vasoconstriction (29 ± 1 vs. 29 ± 1 sec) in both strains.

Further studies were done to gain insight into the mechanism(s) responsible for this abnormality in vascular reactivity. We investigated whether the strain difference in the ability of fenoldopam to buffer the Ang-II-induced renal vasoconstriction could be related to the efficiency of coupling of DA1 receptors to G proteins. For this reason, the renal vasculature was exposed to NaF, a general activator of G proteins (17–19). In the absence of a vasodilator agent, NaF did not affect the decrease in renal blood flow induced by Ang-II in WKYs (−46 ± 4% vs. −45 ± 2%, $P > 0.7$) or in SHRs (−45 ± 2% vs. −45 ± 3%, $P > 0.6$). An important finding was that a NaF effect was observed when fenoldopam was injected in combination with Ang-II. The vasoconstriction produced by Ang-II was

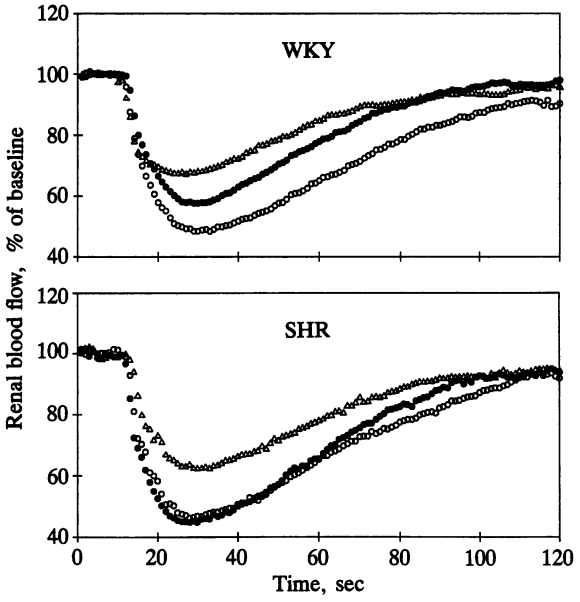

FIG. 3. Representative examples of temporal variations in renal blood flow produced by injection of Ang-II (2 ηg) into the renal artery. Angiotensin was administered alone (○) and mixed with the dopamine DA1-agonist fenoldopam (10 ηg) (● and △). The agents were injected before and during infusion of cholera toxin (● and △, respectively) into the renal artery of a WKY (*A*) or a SHR (*B*).

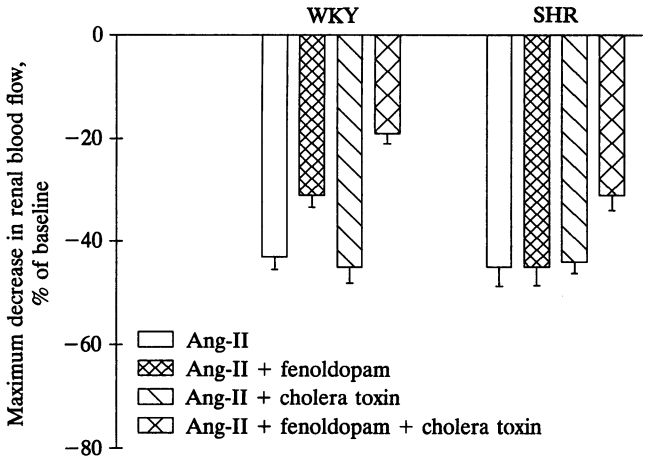

FIG. 4. Group averages for the maximum decrease in renal blood flow produced by intrarenal injection of Ang-II and Ang-II plus the dopamine DA1-agonist fenoldopam before or during concurrent administration of cholera toxin. (*A*) WKY data. (*B*) SHR data. Values are means ± SEMs for seven WKYs and seven SHRs. $P < 0.05$ for WKY vs. SHR; $P < 0.05$ for control vs. fenoldopam or fenoldopam plus cholera toxin within strain.

more effectively buffered by NaF plus fenoldopam as compared with fenoldopam administration alone. This synergism occurred in both rat strains. In seven WKYs the mean maximum decrease in renal blood flow was reduced from −28 ± 2% to −18 ± 2% ($P < 0.001$) (Figs. 1 and 2). A major observation was that the combination of NaF and fenoldopam significantly blunted the Ang-II induced vasoconstriction in seven SHRs (−27 ± 3% vs. 45 ± 3%, $P < 0.001$ (Figs. 1 and 2). This result contrasts with the earlier data for the hypertensive strain showing that fenoldopam was ineffective without NaF.

To determine whether or not a specific family of G proteins was involved in the buffering effect, other animals were treated with cholera toxin, a selective activator of G_s proteins (17–19). Representative examples of the renal vascular response to Ang-II injected alone and in mixture with fenoldopam before and during cholera toxin infusion are shown in Fig. 3. In WKYs, fenoldopam blunted the Ang-II-induced vasoconstriction (−31 ± 2% vs. −44 ± 3%, $P < 0.001$) (Figs. 3*A* and 4). Cholera toxin infusion enhanced the buffering effect of fenoldopam (−19 ± 3% vs. −31 ± 2%, $P < 0.001$) (Figs. 3*A* and 4). In SHRs, coadministration of fenoldopam with Ang-II did not alter the renal vasoconstriction produced by Ang-II (−44 ± 3% vs. −45 ± 4%) (Fig. 4). In marked contrast, simultaneous exposure to cholera toxin unmasked a buffering effect of fenoldopam in the hypertensive strain. As is shown in Fig. 3*B*, the transient decrease in renal blood flow was clearly blunted during infusion of cholera toxin as compared with the administration of Ang-II plus fenoldopam without cholera toxin. The average maximum vasoconstriction was significantly reduced when the G protein stimulator was administered (−31 ± 2% vs. −45 ± 4%, $P < 0.01$) (Figs. 3*B* and 4).

The amplifying effect of NaF and cholera toxin appeared specific for an interaction between the DA1 receptor and Gs protein. The agents administered into the renal artery by themselves had no demonstrable effect on basal renal blood flow, and neither affected the renal vasoconstriction produced by Ang-II in either strain of rat. In the absence of fenoldopam, Ang-II-induced decreases in renal blood flow were similar before and during administration of NaF or cholera toxin.

DISCUSSION

The present study provides important information about the mechanism(s) by which Ang-II enhances vasoconstriction in kidneys of rats developing hypertension of genetic origin. Transient changes in the renal vasculature were monitored after bolus administration of Ang-II into the renal artery of 7-week-old SHRs with age-matched WKYs serving as normotensive controls. The applied technique and data analysis permitted estimation of local responses of the renal vasculature to vasoactive agents without systemic complications. As a result, a comprehensive evaluation of intrarenal mechanisms governing the renal microcirculation *in vivo* was feasible (11).

Recent studies by our laboratory and other investigators (11, 12, 20) have established that kidneys of young SHRs exhibit exaggerated reactivity to Ang-II compared with normotensive WKY controls. The fact that treatment of the rats with indomethacin, a cyclooxygenase inhibitor, increased the Ang-II-induced vasoconstriction in WKYs and abolished the strain difference in vascular reactivity suggests that the action of endogenous prostaglandins affords protection in WKY, but not in SHR, kidneys (11).

In support of this hypothesis, intrarenal infusion of authentic vasodilator prostaglandins E_2 and I_2 or of their respective receptor agonists viprostol and iloprost failed to protect kidneys of the SHR from the Ang-II-induced vasoconstriction, whereas the same prostanoids effectively blunted ≈50% of the effect of Ang-II in WKY kidneys (14). Similar to prostaglandins, the dopamine DA1-receptor agonist fenoldopam significantly buffered the Ang-II-induced vasoconstriction in WKY, but not in SHR, kidneys (16). On the contrary, acetylcholine and bradykinin, representing another class of renal vasodilators, effectively attenuated the vasoconstrictor effect of Ang-II in a dose-dependent fashion in the kidneys of both SHRs and WKYs (16). The actions of fenoldopam and prostaglandins are initiated by binding to specific receptors on the surface of vascular smooth muscle cells. The receptor–ligand complex stimulates the cAMP pathway through activation of GTP-binding stimulatory G_s proteins. On the other hand, acetylcholine and bradykinin primarily act *in vivo* through endothelial-derived nitric oxide to activate the cGMP pathway. Thus, the defect seemed to be localized to the cAMP signaling pathway that would normally respond to several different receptor agonists.

Our previous studies evaluated the role of cAMP production as a mechanism responsible for the failure of fenoldopam and prostaglandins to buffer the Ang-II effect in SHRs. *In vivo* activation of the intracellular cAMP pathway in the renal resistance vessels was tested by using forskolin (adenylyl cyclase activation independently of receptor binding) or by administering dibutyryl-cAMP (cell-membrane permeable form of cAMP) into the renal artery (16). Both cAMP-elevating agents buffered the Ang-II-induced vasoconstriction. A similar degree of protection was provided in WKY and SHR kidneys. These observations indicate that renal vasodilators acting through receptor coupling to activate the cAMP pathway fail to buffer the Ang-II-induced vasoconstriction in SHRs. The defective event in this abnormal transmission of the signal(s) appears to be localized to a step proximal to activation of adenylyl cyclase.

The intracellular events preceding adenylyl cyclase activation are ligand–receptor coupling and receptor–G_s protein interaction. Several lines of evidence argue against the possibility of a strain difference in the first alternative. By design, the amount of the ligand administered was the same in SHRs and WKYs in all of our experiments. In addition, radioligand binding studies revealed no difference in the characteristics (affinity and/or density) of prostaglandin E_2 and prostaglandin I_2 receptors in isolated glomeruli or preglomerular resistance vessels between SHRs and WKYs (ref. 14, unpublished observations). Prostaglandin E_2 receptor availability was similar in renal medullary membranes of SHR and WKY, although the amount of cAMP generated by prostaglandin E_2 was 3-fold lower in SHRs (21, 22). Furthermore, the dopamine

DA1 receptor found in the renal proximal convoluted tubule displayed similar affinity, density, and molecular mass in SHRs and WKYs, although the activation of cAMP was attenuated in SHRs (23).

Our results clearly support the hypothesis of a defective interaction between receptor and G_s protein activation in the renal vasculature of SHRs. Fenoldopam alone did not counteract the Ang-II-induced vasoconstriction in SHR kidneys. Only when the G proteins of the renal vasculature were first activated was fenoldopam able to buffer the Ang-II-induced vasoconstriction in SHRs. It is noteworthy that NaF, an activator of G proteins in general, had a similar protective effect to that of cholera toxin, a selective activator of the G_s family of proteins. This finding indicates that the defective interaction is specific to receptors coupled to G_s proteins.

Raymond (24) has recently reviewed hereditary defects in signaling through hormone receptor–G protein complexes. To our knowledge, the present study is the only report of a defective interaction of receptors linked to activation of intracellular cAMP pathway with their respective G_s proteins in the renal vasculature of rats developing hypertension. A similar type of abnormality is apparently expressed in renal proximal tubular cells as well. Felder and coworkers (23, 25) have observed abnormal coupling of a DA1 receptor to cAMP generation in proximal convoluted tubules microdissected from young SHRs. Their biochemical studies of radioligand binding and adenylyl cyclase activity suggest a primary hereditary defect in the interaction of membrane receptors to G protein activity. Persistent defective coupling in SHRs was observed while receptor-mediated cAMP generation in WKYs became stronger with age. Apparent receptor number and affinity were constant in 3-, 8-, and 20-week-old WKYs, as was basal, forskolin, and guanyl nucleotide-stimulated adenylate cyclase activity and D_{1A} receptor mRNA. Interestingly, the tubular defect was observed in a proximal, but not a collecting duct, segment. Such a defect in receptor stimulation of cAMP production could promote hypertension by a dual mechanism. Attenuated generation of cAMP within the renal vasculature could result in increased renal vascular resistance, and a similar defect in cAMP generation in renal tubules could lead to increased sodium retention. Both of these renal abnormalities are associated with the initiation and development of hypertension (4, 5).

Further support for our findings and conclusions derives from a study comparing the function of G proteins in membranes of mesenteric arteries isolated from SHRs and WKYs. Agents that activate the cAMP pathway through receptor coupling to G proteins caused a smaller stimulatory response of cAMP generation in SHRs (26). However, when forskolin was used to stimulate adenylyl cyclase independent of activation of cell-surface receptors, the cAMP levels were increased to an equal extent in SHR and WKY membranes. In addition, no strain difference was found in the G protein levels of this tissue, as evidenced by immunoblotting.

In our studies, fenoldopam facilitated the recovery from transient vasoconstriction after the activation of Ang-II receptors. The half-time of recovery after injection of the mixture of fenoldopam and Ang-II was similar in SHRs and WKYs, even though the magnitude of the maximum response was 2-fold larger in SHRs. A similar observation was made in earlier studies (12, 14) when several doses of fenoldopam and prostaglandins were tested. To explain these results we postulated a dual mechanism of action of renal vasodilators against the Ang-II-induced vasoconstriction. One part of this mechanism is considered dependent on cAMP activation and thereby impacts on the magnitude of the maximum renal vascular response. The other part is related to the rate of recovery and may be related to the rate of dephosphorylation of myosin. SHRs appear to be defective in the first, but not the second, mechanism (12, 14). Our present results provide evidence to support this hypothesis. The combination of G protein activation and fenoldopam did not provide any additional change of the recovery rate, although the magnitude of the response to Ang-II was buffered.

In conclusion, vasodilators that activate the cAMP intracellular signaling pathway are ineffective in counteracting the vasoconstrictor effect of Ang-II in the renal vasculature of young SHRs. The cause of this defect is probably related to a deficient interaction between the cell-surface receptors of these agents and their respective G_s proteins. Such a generalized defect of the renal vasculature could be a major contributor to the development of genetic hypertension.

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