Angiotensin II-Induced Renal Vasoconstriction in Genetic Hypertension

EDWIN K. JACKSON, WILLIAM A. HERZER, SUBHASH J. VYAS, and CURTIS K. KOST Jn

Departments of Pharmacology (E.K.J., C.K.K.) and Medicine (E.K.J., W.A.H., S.J.V., C.K.K.), Center for Clinical Pharmacology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

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ABSTRACT

Previous studies demonstrate that renovascular responses to angiotensin II (Ang II) are enhanced in spontaneously hypertensive rats (SHRs); however, it is possible that this hyperresponsiveness is mediated by Ang II-induced release of substances from the adrenal gland. Previous studies also show that pertussis toxin normalizes renovascular responses to Ang II in SHRs; however, it is possible that this response is mediated by effects of pertussis toxin on endogenous Ang II levels and/or the sympathoadrenal axis. The purpose of this study was 2-fold: 1) to determine whether the renovascular response to Ang II in SHRs is enhanced even in adrenalectomized SHRs and 2) to determine whether pertussis toxin normalizes enhanced renovascular responses to Ang II when pertussis toxin-induced changes in the renin-angiotensin system and the sympathoadrenal axis are prevented. SHRs and Wistar Kyoto (WKY) rats were anesthetized and administered 20 ml/kg 0.9% saline, and an infusion of aldosterone and hydrocortisone was initiated. After bilateral adrenalectomy, left renal denervation, and pretreatment with captopril, animals received an intrarenal artery infusion of Ang II at 10 ng/kg/min for 5 min. Ang II-induced changes in renal vascular resistance were greater in SHRs compared with WKY rats (p = .010, n = 19/group). Pertussis toxin (10 μg/kg i.v. 3 days before the experiment) attenuated Ang II-induced changes in renal vascular resistance in SHR (p < .05), but not in WKY rats (strain × treatment interaction: p = .046). These results suggest that the enhanced renovascular response to Ang II in SHRs is mediated by a G, dependent pathway within the renal vasculature.

Intravenous, suprarenal aortic, and intrarenal artery infusions of angiotensin II (Ang II) exert a greater effect on renal vascular resistance (RVR) in spontaneously hypertensive rats (SHRs) compared with Wistar-Kyoto (WKY) rats (Li and Jackson, 1989; Kost and Jackson, 1993; Kost et al., 1994; Vyas and Jackson, 1995; Kost et al., 1998b). Moreover, the enhanced ability of Ang II infusions to increase RVR in SHRs exists in SHRs maintained normotensive by daily administration of captopril for 4 weeks of age until the animals were studied at 11 to 14 weeks of age (Li and Jackson, 1989; Kost and Jackson 1993) as well as in young (6 weeks of age) SHRs (Chatziantoniou et al., 1990; Vyas and Jackson, 1995). Thus, it appears that the enhanced renovascular responsiveness to Ang II in SHRs is not secondary to hypertension, but rather is an inborn trait that may contribute to the pathophysiology of high blood pressure in this genetic model of essential hypertension.

An important caveat of previous studies demonstrating an enhanced effect of Ang II on the renal vasculature of SHRs is that Ang II releases catecholamines (Robinson, 1967), mineralocorticoids (for review, see Giachetti et al., 1996), and endogenous ouabain-like factors (Laredo et al., 1997) from the adrenal gland. Thus, it is conceivable that release of multiple factors by the adrenal gland could modify the RVR responses to Ang II differentially in SHRs compared with WKY rats. This would be true even with intrarenal artery infusions of Ang II since part of the blood supply to the adrenal gland is supplied via a branch of the renal artery. Therefore, one objective of this study was to determine whether the SHR kidney is hyperresponsive to intrarenal infusions of Ang II since part of the blood supply to the adrenal gland is supplied via a branch of the renal artery. Therefore, one objective of this study was to determine whether the SHR kidney is hyperresponsive to intrarenal infusions of Ang II in animals lacking intact adrenal glands

Pretreatment of SHRs with pertussis toxin, a maneuver that blocks the function of G protein, normalizes the RVR response to infusions of Ang II (Jackson, 1994). These results suggest that the enhanced RVR response to Ang II in SHRs is mediated by a pathway involving G protein in the renal vasculature. However, the effects of pertussis toxin also could be due to activation of the renin-angiotensin system (RAS) because pertussis toxin is well known to increase renin release (Hackenthal et al., 1985). In this regard, an increase in renin release would result in higher basal levels of Ang II,
which may alter the response to exogenous Ang II. Moreover, because α2 adrenoceptor activation reduces stimulation of the sympathoadrenal axis (SAA) (for review, see Stornetta et al., 1995) and inhibits, via prejunctional receptors, renal sympathetic neurotransmission (Bohmann et al., 1994), inactivation by pertussis toxin of Gq proteins that mediate responses to α2 adrenoceptor stimulation (for review, see Ruffolo and Hieble, 1994) could increase renal sympathetic tone and adrenal catecholamine release. Thus, increased exposure of the renal vasculature to catecholamines in pertussis toxin-treated animals also confounds interpretation of the effects of pertussis toxin on RVR responses to Ang II. Therefore, a second objective of this study was to determine whether pertussis toxin normalizes Ang II-induced RVR responses in SHR in which pertussis toxin-induced activation of the RAS and SAA was prevented.

Materials and Methods

Animals. Adult male SHRs and WKY rats were purchased from Taconic Farms, Inc. (Germantown, NY) and maintained in the University of Pittsburgh Animal Facility. Rats were provided free access to Prolab Isopro RMH 3000 rodent diet (PMI Nutrition Intl., Richmond, IN) and tap water. Light cycle, relative humidity, and room temperature were 7:00 AM to 7:00 PM, 55% and 22°C, respectively. Animals were used for acute experiments at ~16 weeks of age. The Institutional Animal Care and Use Committee approved all procedures.

Pretreatment with Pertussis Toxin. Three days before the acute experiments, some animals were anesthetized briefly with methoxyflurane, and a small incision in the neck was made to expose the right jugular vein. Pertussis toxin (10 μg/kg; Sigma Chemical Co., St. Louis, MO) was injected into the jugular vein, the incision was sutured, and the animals were allowed to regain consciousness. Studies have previously shown that i.v. administered pertussis toxin completely blocks the heart rate effects (Jackson, 1994) and renal excretory effects (Kost et al., 1998a) of receptors coupled to Gq.

Surgical Preparation. Rats were anesthetized with Inactin (90 mg/kg i.p.) and placed on a Deltaphase Isothermal Pad (Braintree Scientific Inc., Braintree, MA). Body temperature was monitored with a digital rectal probe thermometer (Physitemp Instruments, Inc., Clifton, NJ) and maintained at 37°C by adjusting a heat lamp above the animal. After cannulation of the trachea to maintain airway patency, a polyethylene (PE)-50 catheter was inserted into the left jugular vein.

In pilot studies, we found that when pertussis toxin-treated rats were adrenalectomized and treated with an angiotensin-converting enzyme (ACE) inhibitor, their cardiovascular systems collapsed and the animals died within a few minutes. Because one objective of this study was to examine RVR responses to Ang II in pertussis toxin-treated animals in which the RAS and SAA were blocked, we experimented with various approaches that would allow pertussis toxin-treated animals to tolerate adrenalectomy plus ACE inhibition. In this regard, we found that if animals were volume loaded and administered aldosterone and hydrocortisone, they could survive the triple stress of pertussis toxin, adrenalectomy, and ACE inhibition. Therefore, in our experiments, all animals received a volume load (20 ml/kg of 0.9% saline i.v.) followed by a sustained i.v. infusion of aldosterone (20 ng/min) and hydrocortisone (20 μg/min) dissolved in 0.9% saline (50 μl/min).

A left carotid artery catheter (PE-50) was inserted and was connected to a digital blood pressure analyzer (Micro-Med, Louisville, KY) for continuous measurement of mean arterial blood pressure (MABP) and heart rate (HR). The digital blood pressure analyzer was set to time-average MABP and HR at 1-min intervals. Next, both adrenal glands were removed, and the left kidney was denervated by stripping away all visible renal nerves and by painting the renal artery with 10% phenol in ethanol. A transite-time blood flow probe (model 1RB; Transonic Systems, Inc., Ithaca, NY) was placed around the renal artery and connected to a transite-time flowmeter (model T206; Transonic Systems, Inc.) to monitor renal blood flow (RBF) continuously. Finally, a 32-gauge needle connected to a PE-10 catheter was carefully inserted (proximal to the flow probe) into the renal artery and an intrarenal infusion of 0.9% saline (50 μl/min) was initiated. After the surgery, animals were given a bolus injection of captopril (30 mg/kg) and allowed to stabilize for 1 h.

Protocol. Basal MABP, HR, and RBF were recorded for 1 min, and then Ang II was infused into the left renal artery at 10 ng/kg/min. Between 4 and 5 min into the infusion of Ang II, MABP, HR and RBF were again recorded. Each animal received only one brief infusion of a single dose of Ang II, thus avoiding any possibility of time-related changes in renal responsiveness.

Statistical Analysis. RVR responses to Ang II in SHRs versus WKY rats were compared with a two-factor ANOVA with repeated measures in which one factor was rat strain (two levels, SHRs versus WKY rats) and the other factor was treatment (two levels, intrarenal infusion of vehicle versus intrarenal infusion of Ang II). The effects of pertussis toxin in SHRs versus WKY rats on basal RBF, basal RVR, Ang II-induced percentage changes in RBF, and Ang II-induced percentage changes in RVR were determined with a two-factor ANOVA in which one factor was rat strain (two levels, SHRs and WKY rats) and the other factor was treatment (two levels, nonpertussis toxin-treated rats versus pertussis-treated rats). Percentage changes were made normally distributed by arcsin transformation before ANOVA. If and only if the interaction term in the two-factor ANOVA was significant, post hoc comparisons were calculated with a Fisher’s least-significant difference test. MABPs were compared with a two-sample Student’s t test. The criterion of significance was p < .05.

Results

Effects of Ang II on RVR in SHRs versus WKY Rats (Fig. 1). Infusions of Ang II into the renal artery significantly increased RVR in both SHRs and WKY rats; however, the RVR response to Ang II was significantly greater in SHRs (strain × Ang II interaction, p = .010).

Effects of Pertussis Toxin on Basal RBF and Basal RVR. Basal RBF was not significantly (p = .768) different between the strains (Fig. 2). Pertussis toxin modestly, but significantly (p = .009), decreased basal RBF in both SHRs and WKY rats; however, this decrease was not significantly different (p = .767) between the strains. Basal RVR was significantly (p = .019) greater in SHRs compared with WKY rats; however, pertussis toxin did not significantly (p = .783) affect basal RVR in either strain (Fig. 3).

Effects of Pertussis Toxin on Ang II-Induced Percentage Changes in RBF and RVR. Two-factor ANOVA...
Numerous studies demonstrate that chronic blockade of the renin-angiotensin system with pharmacological agents prevents the development of hypertension in young SHRs (Fer-
treated rats the RVR response to Ang II was normal in SHRs (Antonaccio et al., 1979; Bunkenburg et al., 1991), despite the fact that the renin-angiotensin system does not appear to be overly activated in SHRs (Koletsky et al., 1970; Sen et al., 1972; Shiono and Sokabe, 1976; Campbell et al., 1995). Furthermore, renal transplantation studies in SHRs clearly demonstrate that the mechanism responsible for genetic hypertension in SHRs resides in the kidney (Rettig et al., 1991; Rettig and Unger, 1991). An enhanced renal vascular response to Ang II in SHRs would explain why Ang II is critical to the development and maintenance of hypertension in SHRs and would explain why hypertension in SHRs tracks the kidney. It is important therefore to elucidate the renal biochemical defect that gives rise to enhanced renal sensitivity to Ang II.

Although the exact molecular defect remains elusive, some progress has been made in illuminating the mechanism of the enhanced renal sensitivity to Ang II in genetic hypertension. In this regard, several lines of evidence point to an abnormality involving dysregulation of renal cAMP metabolism (formation and/or catabolism). For instance, renal blood flow studies demonstrate that the ability of prostacyclin, as well as other adenylyl cyclase-activating agents, to attenuate Ang II-induced renal vasoconstriction is diminished in SHRs (Chatziantoniou and Arendshorst, 1992; Jackson and Herzer, 1993; Chatziantoniou et al., 1993; Jackson and Herzer, 1994; Chatziantoniou et al., 1995). Because the ability of a lipophilic cAMP analog to inhibit renal vascular responses to Ang II is not diminished in SHRs (Chatziantoniou et al., 1993), the signal transduction defect appears not to involve biochemical systems activated by cAMP, thus suggesting a defect in renal cAMP metabolism. In support of this hypothesis, the ability of Ang II to inhibit cAMP production is enhanced in isolated perfused SHR kidneys (Vyas et al., 1996).

AT₁ receptors are known to activate G proteins that are negatively coupled to adenylyl cyclase (Obayashi et al., 1997). Therefore, in a recent study, we tested the hypothesis that inhibitory G proteins participate in the enhanced renovascular response to Ang II in SHRs (Jackson, 1994). Specifically, we examined renovascular responses to Ang II in SHRs and WKY rats pretreated with pertussis toxin to ADP ribosylate and inactivate inhibitory G proteins. Interestingly, pertussis toxin completely normalized RVR responses to Ang II in SHRs. However, a major limitation of our previous study is that activation of the RAS and SAA by pertussis toxin was not prevented, and these pertussis toxin-induced changes could have been responsible for the normalization of RVR responses to Ang II in SHRs.

In our previous study, we did not eliminate activation of the RAS and SAA by pertussis toxin because we were unable to block these systems in pertussis toxin-treated animals without causing death. However, in our present study, by combining volume loading with a constant rate infusion of aldosterone and hydrocortisone, we were able to stabilize pertussis toxin-treated animals sufficiently to conduct the experiments. Even with this approach, however, MABPs in pertussis toxin-treated animals were very low. Importantly, although low, MABPs were similar in pertussis-toxin treated SHRs and WKY rats so that the hypotension should not be a confounding variable. We observed that in pertussis toxin-treated rats the RVR response to Ang II was normal in SHRs compared with WKY rats. These results strongly suggest that the ability of pertussis toxin to normalize RVR responses to Ang II in SHRs is not secondary to activation of the RAS or the SAA. Therefore, we conclude that the ability of pertussis toxin to normalize enhanced RVR responses to Ang II in SHRs is due to the direct effects of pertussis toxin on the renal vasculature.

To maintain hemodynamic stability in adrenalectomized rats subjected to acute surgery, it is necessary to administer exogenous steroids. Although steroids are well known to increase AT₁ receptor number in vascular smooth muscle cells, it is unlikely that the results of this study are confounded by this phenomenon for three reasons. First, steroid-induced up-regulation of AT₁ receptors does not occur for the first 12 h of treatment and requires 24 h to achieve a maximum increase in AT₁ receptor number (Provencher et al., 1995). In this study, we infused steroids for only 90 min; therefore, there was insufficient time for steroid-induced expression of AT₁ receptors. Second, even when infused into rats for 6 days, aldosterone does not up-regulate AT₁ receptors when the infusion rate is ≤3.3 μg/h, and only increases AT₁ receptor number by 30% when the infusion rate is 6.6 μg/h (Schiffrin et al., 1985). In this study, we used a dose of 1.2 μg/h, which is below the threshold for increasing AT₁ receptor number. Third, even if angiotensin receptors were up-regulated by steroids in our study, this does not explain the fact that pertussis toxin-sensitive G proteins are involved in signal transduction in the renal microcirculation of SHRs but not WKY rats.

Another concern of this study is that pertussis toxin-induced hypotension during the 3 days preceding RBF and RVR measurements could have caused a sustained increase in circulating Ang II levels and sympathetic tone that may have modified the renovascular responses even after the renin-angiotensin system and sympathetic nervous system were acutely removed before the measurements were taken. In a previous study (Kost et al., 1999), we monitored for several weeks by radiotelemetry the MABP in conscious pertussis toxin-treated SHRs and WKY rats. In this telemetry study, pertussis toxin was administered exactly as in our study (10 μg/kg i.v.), and we found that in the conscious state pertussis toxin chronically reduced blood pressure in SHRs, but not in WKY rats. However, the reduction in blood pressure in SHRs was modest, ~15 mm Hg, and the pertussis toxin-treated SHRs were never hypotensive (arterial blood pressure was 146 ± 1 mm Hg).

What is the role of pertussis toxin-sensitive G proteins in Ang II-induced renal vasoconstriction, and how might this signal transduction system be altered in the renal microcirculation of SHRs? Fig. 6 illustrates our working hypothesis. It is well known that AT₁ receptors are coupled to Gq and Gi and that the α subunits of Gq and Gi activate and inhibit phospholipase C and adenylyl cyclase, respectively. Recent progress in signal transduction research has elucidated the concept of coincident signaling (for review, see Jordan and Iyengar, 1998). Coincident signaling refers to the convergence of two signaling pathways on the same detector protein, resulting in a unique response such as synergistic activation or attenuation of activation of the detector system. Coincident signaling involving α₁ and βγ subunits released from Gi is implicated in the regulation of two detector enzymes [i.e., phospholipase C (Selbie and Hill, 1998) and ad-
Ang II-Induced Renal Vasoconstriction in SHRs

Ang II binds to G-protein-coupled receptors (GPCRs) to activate signaling pathways, leading to renal vasoconstriction. In spontaneously hypertensive rats (SHRs), renal vasoconstriction to Ang II is enhanced compared to WKY rats. This enhanced response is mediated by a G$_i$-dependent pathway within the renal vasculature.

References


Send reprint requests to: Edwin K. Jackson, Ph.D., Center for Clinical Pharmacology, University of Pittsburgh Medical Center, 623 Scaife Hall, 200 Lothrop St., Pittsburgh, PA 15213-2582. E-mail: edj1@pitt.edu