Attenuated Afferent Arteriolar Response to Acetylcholine in Goldblatt Hypertension

Joseph M. Ortenberg, Anthony K. Cook, Edward W. Inscho, and Pamela K. Carmines

We tested the hypothesis that endothelium-dependent afferent arteriolar vasodilation is impaired in the nonclipped kidney of two-kidney, one clip Goldblatt hypertensive rats relative to sham-operated controls. Five to six weeks after positioning of a 0.25-mm clip on the left renal artery, systolic pressure averaged 173±10 mm Hg in Goldblatt rats and 118±4 mm Hg in controls (p<0.01). The right kidney was harvested for videometric study of the microvasculature using the in vitro blood-perfused juxtamedullary nephron technique. Kidneys from Goldblatt and control rats were perfused at renal arterial pressures of 150 and 110 mm Hg, respectively. Afferent arteriolar inside diameter did not differ between control (20.3±0.7 μm) and Goldblatt (21.1±1.7 μm) kidneys. Determination of afferent responses to increasing concentrations of the endothelium-dependent vasodilator acetylcholine (1 nM to 10 μM) in the bathing solution unveiled a shift to the right in the dose–response relation in Goldblatt rats. Afferent arterioles from control kidneys dilated significantly when exposed to 1 nM acetylcholine, whereas a 1,000-fold higher concentration was required to dilate arterioles from Goldblatt rats. Sodium nitroprusside, an endothelium-independent vasodilator, increased afferent diameter to a similar extent in both groups. In a separate group of normal kidneys, vasodilator responses to 10 μM acetylcholine were completely blocked by 1,000 μM nitro-L-arginine, an inhibitor of nitric oxide synthesis. Thus, endothelium-dependent afferent vasodilation appears to be impaired in the nonclipped kidney of Goldblatt hypertensive rats. This phenomenon could contribute to the altered renal hemodynamic status characteristic of Goldblatt hypertension. (Hypertension 1992;19:785–789)

Key Words • microcirculation • vasodilation • acetylcholine • Goldblatt hypertension • nitroprusside

In recent years, increasing attention has focused on the role of the endothelium as a determinant of vascular function under physiological and pathophysiological conditions. In this regard, the development or maintenance of hypertension has been suggested to involve an inappropriately reduced endothelium-dependent dilator influence on the vasculature.1,2 Indeed, arterial rings and strips harvested from hypertensive animals exhibit markedly attenuated responses to endothelium-dependent vasodilators.3–6 Despite the growing body of evidence implicating impaired endothelium-dependent vasodilation in hypertension, little information is available regarding the involvement or integrity of this system in the renal macrovascular and microvascular dysfunction characteristic of most models of hypertension. The two-kidney, one clip (2K1C) model of renovascular hypertension requires involvement of the renin-angiotensin system for development of the hypertension;7 however, alterations in vascular structure and neurohumoral influences have also been implicated.8–10 Thus, the cause of the sustained systemic and renal hemodynamic dysfunction in 2K1C hypertension remains uncertain. The current study was designed to determine whether afferent arterioles in the nonclipped kidney exhibit a defect in endothelium-dependent vascular responsiveness that might contribute to the pathogenesis of 2K1C hypertension.

Methods

Eighteen male Sprague-Dawley rats, weighing approximately 150 g, were anesthetized with sodium pentobarbital (40 mg/kg i.p.). In seven rats, a 0.25-mm (i.d.) silver clip was placed around the left renal artery, and the animals were allowed to recover from anesthesia and surgery. Seven sham-operated rats (SHAM) served as controls. A 5–6-week period ensued between placement of the clip and the acute experiments. During this time, rats were allowed free access to food and water, and systolic arterial pressure was monitored by tail-cuff sphygmomanometry (Harvard Apparatus, South Natick, Mass.). Rats exhibiting renal infarction resulting from clip placement were excluded from the study.

All acute experiments used the in vitro blood-perfused juxtamedullary nephron technique to provide access to the renal microvasculature.11–13 SHAM, 2K1C, or untreated rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and the renal artery of the right (nonclipped) kidney was cannulated via the superior mesenteric artery. The kidney was perfused with Tyrode’s solution (25°C) containing 52 g/l dialyzed bovine serum albumin and a complement of L-amino acids as previously detailed.11 The perfused kidney was removed and sectioned longitudinally, with the dorsal two thirds

From the Department of Physiology, Tulane University School of Medicine, New Orleans, La.

Supported by grants HL-26371 and DK-39202 from the National Institutes of Health. P.K.C. is an Established Investigator of the American Heart Association.

Address for correspondence: Pamela K. Carmines, PhD, Department of Physiology #SL39, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112.
with intact papilla being retained. The lateral fornices were partially sectioned to permit reflection of the papilla and to expose the underlying pelvic cavity. The pelvic mucosa, connective tissue, and large veins were removed, thus exposing the cortical and medullary surfaces that delimit the pelvic cavity. The cortical surface in this region is endowed with juxtamedullary nephrons and their associated microvasculature.12

Tight ligatures were placed around the distal ends of the main arterial branches supplying the superficial vascularization of interest.12,14 This maneuver maintains perfusion pressure and blood flow to the inner cortical regions located proximal to the ligations. The Tyrode's perfusate was replaced with blood collected from a normotensive donor rat and was processed as previously described.11 Resting diameter and reactivity of 2K1C afferent arterioles are not appreciably altered by perfusion with blood obtained from normotensive rather than 2K1C rats.15 The blood perfusate was filtered through a 5-μm nylon mesh, stirred continuously in a pressurized reservoir, and equilibrated with 95% O2-5% CO2. Renal arterial perfusion pressure was monitored with a Statham P23Db pressure transducer linked to a polygraph (Grass Instrument Co., Quincy, Mass.). In vivo conditions were approximated by maintenance of renal perfusion pressure at either 150 (2K1C) or 110 (SHAM or untreated rats) mm Hg. The tissue surface was bathed with Tyrode's solution (37°C) containing 10 g/l bovine serum albumin. The microvasculature was visualized by videomicroscopy as previously described.11,13

In most experiments, a single afferent arteriole with a definite connection to a glomerulus was selected for study. In one experiment, two arterioles that could be visualized in the same image field were studied simultaneously. The site for diameter measurement was selected based on the clear visibility of both vascular walls; regions near the branch point of the arteriole from its parent artery or its insertion into the glomerulus were excluded.

After a 15-minute equilibration period, one of two experimental protocols was initiated. The first series of experiments characterized the effect of increasing concentrations of acetylcholine on afferent arteriolar diameter in kidneys from 2K1C and SHAM rats. Afferent arteriolar caliber was monitored continuously throughout the experiment, which consisted of consecutive 3-minute treatment periods. After the initial control period, the vessels were exposed to increasing bath concentrations of acetylcholine over the range of 1.0 nM to 10.0 μM. Finally, in the continued presence of 10 μM acetylcholine, sodium nitroprusside (380 mM) was added to the bathing solution for 5 minutes to determine if any additional vasodilation could be induced.

The second experimental series assessed whether the afferent vasodilator response to acetylcholine is dependent on nitric oxide synthesis in the in vitro blood-perfused juxtamedullary nephron. Kidneys from normotensive rats were perfused as described above, and afferent diameter was monitored throughout the protocol, consisting of consecutive 5-minute treatment periods. After the initial control period, 10 μM acetylcholine was added to the bathing solution to establish acetylcholine responsiveness. After recovery from acetylcholine treatment, 1,000 μM nitro-L-arginine was added to the bath to inhibit nitric oxide formation.14 In the continued presence of nitro-L-arginine, the acetylcholine treatment was repeated.

Afferent arteriolar inside diameters were measured from videotaped images with a calibrated digital image measuring system (Instrumentation for Physiology & Medicine, Inc., San Diego, Calif.). This system yields diameter measurements that are reproducible to within 1.0 μm. Vessel diameter was measured at 12-second intervals throughout each protocol. Plateau vessel diameter was calculated by averaging data obtained during the final 2 minutes of each experimental period. Within-group statistical comparisons were made with analysis of variance for repeated measures, in concert with the least significant difference test for individual comparisons. Between-group comparisons were made with the unpaired t test or, in cases of heterogeneous variances, the Mann-Whitney U test. A value of p<0.05 was considered significant. Data are reported as mean±SEM.

**Results**

At the time of the acute experiment, systolic arterial pressure averaged 118±4 mm Hg in the SHAM group and 173±10 mm Hg in 2K1C rats (p<0.01). During the control period of the acetylcholine dose–response experiments, afferent arteriolar diameter in SHAM rats averaged 20.3±0.7 μm (n=8 vessels from seven kidneys) compared with an average diameter of 21.2±1.7 μm (n=11) in 2K1C animals. These values did not differ significantly. Topical administration of increasing concentrations of acetylcholine resulted in a dose-dependent increase in afferent arteriolar caliber in both SHAM and 2K1C groups (Figure 1). In the SHAM group, afferent diameter increased significantly in response to 1.0 nM acetylcholine (p<0.05) and continued to increase up to the maximum acetylcholine concen-
Acetylcholine elicits vasodilation through an endothelium-dependent mechanism, primarily through formation of endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide or a related nitrosothiol compound. Prostacyclin formation has also been implicated in eliciting acetylcholine-induced renal vasodilation, although this issue remains unsettled. Our data indicate that acetylcholine-induced dilation of rat juxtamedullary afferent arterioles can be blocked by the specific nitric oxide synthesis inhibitor nitro-L-arginine. Furthermore, nitro-L-arginine reverses existing acetylcholine-induced afferent vasodilation in a dose-dependent manner and does not interfere with the dilatory response to sodium nitroprusside at the concentrations used in the present study (unpublished observations from our laboratory). This indicates that acetylcholine-induced vasodilation in afferent arterioles from normotensive rats is primarily dependent on EDRF formation when studied using the in vitro blood-perfused juxtamedullary nephron technique. It is possible that the mechanism of acetylcholine-induced vasodilation in 2K1C arterioles is EDRF independent (e.g., through prostacyclin production). If this occurs, our data overestimate EDRF-dependent vasodilation in 2K1C afferent arterioles. Nevertheless, because both EDRF and prostacyclin originate primarily in endothelial cells, our data support the contention that afferent arterioles exhibit a suppressed endothelium-dependent vasodilator response in 2K1C hypertension.

Like other compounds that act through EDRF formation, interaction of acetylcholine with receptors on the endothelial cell membrane stimulates the formation of nitric oxide from L-arginine. Nitric oxide leaves the endothelial cell, enters nearby smooth muscle cells, activates soluble guanylate cyclase, and increases cyclic GMP levels, resulting in vascular smooth muscle relaxation. Any defect in this progression of events could compromise vascular responsiveness to acetylcholine in hypertension. It is possible that the density of muscarinic receptors on afferent arteriolar endothelial cells is reduced in 2K1C animals; however, no evidence in the literature supports this contention. Alternatively, a higher density of muscarinic receptors on vascular smooth muscle cells of afferent arterioles from hypertensive animals could exert a competing vasoconstrictor response and offset the acetylcholine-induced vasodilation mediated by EDRF; however, this explanation seems unlikely, because no difference existed between...
the resting afferent diameters for the SHAM and 2K1C groups and also because acetylcholine administration never significantly reduced afferent arteriolar diameter in the 2K1C group. A more plausible explanation for reduced acetylcholine responsiveness would point to a defect in the formation or release of nitric oxide, an impediment to its subsequent diffusion to the vascular smooth muscle cells, or an impaired soluble guanylate cyclase mechanism.

Endothelial superoxide dismutase appears to be necessary for release of biologically active EDRF, an observation that suggests that any imbalance in the activity of this enzyme and production of superoxide anions might predispose to impaired endothelium-dependent relaxations in hypertension. An additional consideration rests on the fact that nitric oxide is an extremely labile autacoid whose half-life is only a few seconds. Any impediment in the ability of nitric oxide to reach the effector system in smooth muscle cells would reduce the concentration of EDRF available to stimulate guanylate cyclase and thereby diminish the vasodilator effect. Hypertension is associated with numerous transformations in vascular wall structure. Alterations such as thickening of the intimal basement membrane or increased fibrosis of the intimal-medial junction could increase the required diffusion distance for nitric oxide, thus reducing its effectiveness as a vasodilator stimulus. Such a phenomenon should be reflected by an increase in the acetylcholine response time in the present study, developing as a consequence of impaired diffusion of either exogenous acetylcholine (to the endothelium) or EDRF (to the tunica media); however, the time courses of SHAM and 2K1C responses to acetylcholine did not differ (data not shown). Therefore, an impaired diffusion rate of either endogenous nitric oxide or exogenous acetylcholine does not appear to explain the present results.

It is unlikely that the attenuated responsiveness to acetylcholine in the 2K1C group is the result of a structural limit on the vasodilator ability of the vessels, because sodium nitroprusside further vasodilated arterioles from both 2K1C and SHAM kidneys in the presence of 10 μM acetylcholine. In addition, sodium nitroprusside exerts its vasodilator effect by stimulating soluble guanylate cyclase in a manner similar to the action of nitric oxide. Thus, these experiments fail to provide evidence for a defect in soluble guanylate cyclase activation in afferent arterioles of 2K1C rats, as has been suggested to occur in aorta and carotid arteries from other hypertensive models. Nevertheless, such a defect might be evident in response to less potent or submaximal stimuli.

The impaired response to acetylcholine in 2K1C afferent arterioles may relate to the preexisting activation status of the microvasculature. EDRF interacts with the renin-angiotensin system in setting afferent arteriolar caliber, and the status of the intrarenal renin-angiotensin system in the nonclipped kidney is somewhat uncertain at this time. Differences in resting tone might have contributed to the discrepant vasodilator responsiveness observed in the two experimental groups. In the present study, SHAM kidneys were perfused at 110 mm Hg and 2K1C kidneys at 150 mm Hg. We have previously found that autoregulatory behavior is intact in SHAM arterioles, which were studied at relatively low pressure and thus were somewhat dilated in the present study. In contrast, autoregulatory behavior is absent in afferent arterioles from the nonclipped kidney of 2K1C rats studied under our experimental conditions. Thus, when studied at a relatively high pressure (similar to that seen in vivo), 2K1C afferent arteriolar diameter is increased relative to that seen at lower pressures or that observed when normal arterioles are studied at high pressures. Thus, it is not surprising that control afferent diameters were similar in SHAM and 2K1C groups perfused at different renal arterial pressures. Furthermore, we have also observed that acetylcholine responses remain impaired in 2K1C relative to SHAM arterioles when both groups are perfused at the same pressure or when vascular tone is diminished by perfusion with a cell-free Tyrode's perfusate. Thus, differences in resting tone are not likely to be responsible for the phenomenon, although we cannot rule out the possibility that higher intravascular pressure or shear stress could alter endothelial function in the 2K1C tissue. Finally, responsiveness to acetylcholine in 2K1C arterioles may be attenuated as the result of high intrinsic EDRF levels or acetylcholine-induced formation of an endothelium-derived contractile factor. Further studies are required to assess these possibilities. Nevertheless, our findings indicate that the defect in the endothelium-dependent vascular control previously reported for large arteries is also manifest in the renal microvasculature in Goldblatt hypertension and could contribute to the elevated renal vascular resistance and altered renal hemodynamics seen in this model.

Acknowledgment

We thank Luis Gabriel Navar for helpful discussions during the execution of this study.

References

Attenuated afferent arteriolar response to acetylcholine in Goldblatt hypertension.
J M Ortenberg, A K Cook, E W Inscho and P K Carmines

Hypertension. 1992;19:785-789
doi: 10.1161/01.HYP.19.6.785

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/19/6_Pt_2/785

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/