Prostaglandin H2 and Thromboxane A2 Are Contractile Factors in Intrarenal Arteries of Spontaneously Hypertensive Rats

Dai Fu-Xiang, Joseph Skopec, Alice Diederich, and Dennis Diederich

Vascular resistance is increased in the kidneys of spontaneously hypertensive rats (SHR). Previous studies have demonstrated impaired vascular relaxations of mesenteric resistance arteries of SHR because of increased production of a cyclooxygenase-dependent endothelium-derived contracting factor. To test the hypothesis that altered endothelial function contributes to the enhanced constriction in kidneys of SHR, endothelium-mediated relaxations of renal resistance arteries from 5–6-week-old prehypertensive SHR and Wistar-Kyoto rats were compared in arteriographs. Acetylcholine induced endothelium-dependent contractions in SHR arteries, while potent endothelium-dependent relaxations were noted in renal arteries from Wistar-Kyoto rats. Inhibition of cyclooxygenase (indomethacin) or blockade of prostaglandin H2-thromboxane A2 receptors (SQ 29,548) blocked acetylcholine-induced contractions in SHR renal arteries; relaxations in SHR renal arteries after either treatment were similar to those observed in renal arteries from Wistar-Kyoto rats. Nω-Nitro-l-arginine inhibited acetylcholine-mediated relaxations in both SHR and Wistar-Kyoto arteries. Endothelium-independent relaxations induced by verapamil were comparable in SHR and Wistar-Kyoto arteries. Thus, the impaired response to acetylcholine in SHR renal resistance arteries may result from the release of endothelium-derived cyclooxygenase products (prostaglandin H2 or thromboxane A2), which oppose endothelium-derived nitric oxide–mediated relaxation.

KEY WORDS • endothelium • prostaglandin H2 • prostaglandin synthase • endothelium-derived relaxing factor • vascular resistance • acetylcholine • oxygen radicals • spontaneously hypertensive rats

Endothelial cells modulate vascular smooth muscle tone through synthesis and release of vasodilating and vasoconstricting substances.1–3 Imbalanced production of relaxing and contracting factors by the endothelium may play an important role in both the initiation and the maintenance of the abnormal vasoconstriction characteristically seen in humans with essential hypertension and in genetic models of hypertension in animals.4,5 Endothelium-dependent relaxations are impaired in the aorta and mesenteric resistance arteries of spontaneously hypertensive rats (SHR).6–11 In both vessels, the impaired relaxations result from enhanced production of a cyclooxygenase-dependent endothelium-derived contracting factor (EDCF) that antagonizes the relaxing properties of endothelium-derived nitric oxide.6–11 More than one EDCF may be produced in SHR arteries.12 Two recent reports suggest that the EDCF produced by the SHR aorta may be prostaglandin H2 (PGH2) or thromboxane A2 (TXA2).10,11

The objectives of the studies described in this report were twofold: 1) to determine if endothelium-mediated relaxations were impaired in resistance arteries isolated from kidneys of young SHR and 2) to characterize the nature of the factor(s) that promotes enhanced constriction in resistance arteries. EDCFs may function by inactivating the endothelium-derived relaxing factor nitric oxide,13 by direct activation of contractile responses in vascular smooth muscle cells, or by a combination of the two mechanisms.

Methods

Male, 5–6-week-old SHR and normotensive Wistar-Kyoto (WKY) rats were obtained from Harlan Laboratories, Boston, Mass. The rats were maintained four per cage at constant temperature (24 ± 1°C), with a 12-hour dark/light cycle and standard rat chow. All procedures followed were in accordance with institutional guidelines. Systolic blood pressure was measured in conscious, prewarmed, restrained rats by the tail-cuff method using photoplethysmography and a physiograph recorder (model 11 TC, Innovators in Instrumentation, Woodland Hills, Calif.).

Vessel Preparation

Rats were anesthetized with ether. The abdominal aorta was cannulated to enable in situ perfusion of the aorta and renal and superior mesenteric arteries with
chilled, heparinized saline at a pressure of 75–80 mm Hg. After washout of visible blood elements, the kidneys were removed and placed in chilled Krebs-Ringer bicarbonate solution containing (mM): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 22, edetate calcium disodium 0.026, and guanine 11. Intrarenal arteries were exposed by a tangential longitudinal incision through the kidney starting at the hilum. Distal branches of the interlobar arteries with lumen diameter 100–250 μm were carefully dissected free and cannulated at each end with glass micropipettes (50–80-μm-diameter tips) secured in arteriograph chambers (Living Systems Instruments, Burlington, Vt.). The arteriograph was positioned on the stage of a microscope equipped with a video camera to project the vessel image onto a television monitor. A videoelectronlc dimension analyzer provided on-line digital display of vessel lumen diameter and wall thickness and DC signals of lumen diameter and luminal pressure, which were recorded. The vessels were allowed to equilibrate for 45 minutes by luminal perfusion with Krebs solution, maintained at 37°C and pH 7.40±0.05, at a pressure of 45–50 mm Hg and a flow rate of 60–80 μl/min. The maximum constriction to norepinephrine (1–2 μM) applied to the extraluminal Krebs bathing solution was determined to occur at a distending pressure of 45–50 mm Hg. Lumen pressure and perfusion rate were kept constant throughout all experiments. To remove endothelium, a branch of the interlobar artery was cannulated and perfused for 45 seconds with chilled 0.5% 3-(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) followed by Krebs solution. A 1.5–2-mm segment of the artery was then prepared for study in the arteriograph. Removal of the endothelium was confirmed by the absence of relaxation in response to acetylcholine.

**Study Protocol**

To study relaxations, the arteries were first partially constricted (40–50% decrease in lumen diameter) with norepinephrine (0.1–1 μM); after stabilization of the contraction, vasodilating agonists were added in a cumulative fashion (10⁻⁸–10⁻⁴ M). All drugs were added to the extraluminal bathing solution except SQ 29,548, which was added to luminal perfusate also. Relaxations, measured by increases in lumen diameter, are expressed as percent of the increased tone (constriction) induced by norepinephrine; thus, 100% represents zero relaxation, and 0% represents complete relaxation. Vessels were washed with Krebs buffer and allowed to equilibrate for 30–45 minutes between interventions.

Endothelium-dependent relaxations were assessed by the responses to acetylcholine or histamine, added in a cumulative fashion. When indicated, the arteries were preincubated with 1) indomethacin (5 μM, 30 minutes) to block cyclooxygenase-dependent synthesis of prostanoids; 2) L‐nitro-l-arginine (L-NA; 100 μM, 45 minutes) to inhibit endothelial production of nitric oxide from l-arginine; 3) SQ 29,548 (5 μM), an inhibitor of PGH₂-TXA₂ receptors; or 4) CGS 13080 (5 μM), a thromboxane synthetase inhibitor. Two vessels were examined from each animal to enable comparison of the responses to acetylcholine with and without inhibitor during a single exposure to acetylcholine. Endothelium-independent relaxations were assessed by concentration–response data to verapamil or sodium nitroprusside.

**Drugs**

Acetylcholine hydrochloride, sodium nitroprusside, l-norepinephrine, indomethacin, CHAPS, L-NA, and histamine hydrochloride were obtained from Sigma Chemical Co., St. Louis, Mo. CGS 13080 was a gift from CIBA-GEIGY, Summit, N.J., and SQ 29,548 was provided by Squibb Research Institute, Princeton, N.J. Indomethacin was dissolved in equimolar Na₂CO₃, and L-NA was dissolved in 0.01 mM HCl. Stock solutions of SQ 29,548 and CGS 13080 were prepared in ethanol and stored at −20°C.

**Statistical Analysis**

Data are presented as the mean±SEM; n refers to the number of rats studied. The concentration of an agonist required to produce 50% relaxation was calculated for each experiment and expressed as negative log molar (pD₂) value. Individual data points, the area under the concentration–response curves (10⁻⁸–10⁻⁴ M), and pD₂ values were analyzed. Statistical analysis of the data comparing responses in vessels obtained from SHR with WKY rats was carried out by one-way analysis of variance (ANOVA). If significant results were revealed by this analysis, then differences between group means were evaluated by Student’s t test for unpaired observations. Statistical significance was defined as p<0.05. The effects of inhibitors on endothelium-dependent responses were analyzed by comparing the response of the same artery to acetylcholine before and after incubation with the inhibitor. Statistical analysis of these data was carried out using Student’s t test for paired observations.

**Results**

**Blood Pressure and Vessel Dimensions**

Blood pressures measured at 5–6 weeks of age were 134±3 and 122±3 mm Hg for SHR and WKY rats, respectively (n=8, p<0.005). The intraluminal diameter of arteries studied was not significantly different for SHR and WKY rats (212±57 and 216±69 μm, respectively, p<0.08; n=14). Wall thickness was significantly greater in arteries from SHR than from WKY rats (32.7 and 31.3 μm, respectively, p<0.047).

**Endothelium-Dependent Responses**

Acetylcholine (10⁻⁸–10⁻⁴ M) produced concentration-dependent relaxations of renal resistance arteries from WKY rats; maximum relaxations reached 90±4% at 10⁻³ M (Figure 1). Relaxations induced by acetylcholine in SHR renal arteries were significantly impaired. Lower concentrations (10⁻⁹ to 5×10⁻⁶ M) of acetylcholine produced relaxations that reached a maximum of 43%; at concentrations above 10⁻⁶ M, relaxations were reversed to a contractile response. Prior removal of the endothelium blocked all responses to acetylcholine in both SHR and WKY arteries (n=4, data not shown). Acetylcholine (10⁻⁸–10⁻⁴ M) did not produce contractions in quiescent renal arteries from either group of rats (data not shown).

The contractile response to acetylcholine observed in SHR renal arteries was inhibited by prior incubation of
the arteries with indomethacin (5 μM) (Figure 1) or with meclofenamate (5 μM, n=4, data not shown). After inhibition of cyclooxygenase activity, acetylcholine produced essentially identical relaxations in SHR and WKY renal arteries (pD₂ values were 7.3±0.4 versus 7.2±0.3, respectively). Pretreatment of SHR arteries with the PGH₁-TEX₃ receptor antagonist SQ 29,548 (5 μM) also blocked the contractile response to acetylcholine and improved relaxations (pD₂ 6.8±0.4 versus 6.1±0.4, for treated and untreated arteries, respectively). CGS 13080 pretreatment blocked acetylcholine-induced contractions in SHR arteries, but maximum relaxations at 10⁻⁵ M acetylcholine were less than those observed with indomethacin or SQ 29,548 (50% versus 82−86%, n=5).

Preincubation with L-NA (100 μM) along with indomethacin (5 μM) markedly blunted acetylcholine-mediated relaxations in SHR renal arteries (pD₂ 5.6, n=5). The inhibitory effects of L-NA on acetylcholine-mediated relaxations were considerably less in WKY renal arteries (pD₂ 6.3±0.2, n=5).

Because histamine has been reported to produce endothelium-dependent relaxations mediated by nitric oxide, 14,15 we compared the responses of SHR and WKY arteries to this agonist. Relaxations produced by histamine (10⁻⁵−10⁻⁴ M) were significantly less in SHR than in WKY renal arteries (maximum relaxations 67±4% versus 91±4%, respectively; n=6) although the pD₂ values were not significantly different (5.2±0.4 versus 5.6±0.1, respectively). Relaxations induced by histamine were abolished in both WKY and SHR arteries by prior removal of the endothelium.

**Endothelium-Independent Responses**

Relaxations induced by verapamil (10⁻⁵−10⁻⁴ M) were similar in SHR and WKY renal arteries; maximum relaxations were 99% in each group, and the pD₂ values were 6.3±0.2 (n=13) and 6.6±0.1 (n=11), respectively. SHR and WKY renal arteries were equally sensitive to incremental concentrations of norepinephrine (pD₂ 6.5±0.1 and 6.4±0.1, respectively; n=14).

**Discussion**

The present study demonstrates that acetylcholine induces endothelium-dependent contractions in isolated renal resistance arteries of prehypertensive SHR, while in renal arteries from WKY rats, the muscarinic agonist induces potent endothelium-dependent relaxations. Because of the young age and minimal (12 mm Hg) elevations in blood pressure observed, it is unlikely that the impaired relaxations in SHR arteries result from acquired endothelial dysfunction produced by hypertension, as has been reported for conduit arteries of rats with experimentally induced hypertension. 16 Rather, a primary alteration in the cyclooxygenase pathway of SHR endothelial cells may serve as a more plausible explanation for the impaired endothelium-dependent relaxations observed in SHR arteries. Several lines of evidence support this hypothesis.

Inhibition of cyclooxygenase blocked contractions induced by acetylcholine in SHR arteries and improved relaxations to the level obtained in WKY renal arteries. Removal of the endothelium abolished both relaxations and contractions induced by acetylcholine in renal resistance arteries. Thus, acetylcholine stimulates the production of a cyclooxygenase-dependent EDCF in SHR renal resistance arteries that reverses endothelium-dependent relaxations. Similar findings have been noted in the aorta and mesenteric resistance arteries of SHR. 6−11 The absence of a contractile response to acetylcholine in quiescent SHR arteries in the present study contrasts with the findings observed in quiescent aorta rings of adult SHR, in which cyclooxygenase- and endothelium-dependent contractions were induced by high concentrations of the muscarinic agonist. 9 Release of membrane-bound arachidonic acid, the presumed precursor of the SHR contractile factor(s), by Ca²⁺-induced phospholipase A₂ activation 17,18 may be limiting in quiescent SHR resistance arteries.

The relaxations induced by acetylcholine and histamine in renal resistance arteries are at least in part mediated by endothelium-derived nitric oxide. After inhibition of nitric oxide synthesis with L-NA, 19 acetylcholine-induced relaxations are impaired in both SHR and WKY renal arteries. The inhibition of acetylcholine-induced relaxations by L-NA (in the presence of indomethacin) in SHR arteries was significantly greater than that observed in WKY renal arteries, suggesting that the nitric oxide generating system was activated to a greater extent in SHR arteries. This finding and the observation that acetylcholine-mediated relaxations of SHR and WKY renal arteries are essentially identical after inhibition of cyclooxygenase mitigate against a decreased production and/or response to endothelium-
derived nitric oxide in SHR arteries. Rather, the findings in this study support the hypothesis that SHR renal endothelial cells produce a contracting factor(s) that opposes the relaxing properties of nitric oxide. The impaired relaxation in SHR vessels in response to histamine could result either from impaired production of nitric oxide or from opposing effects from an EDCF. Further studies will be required to elucidate the mechanism.

The nature of the putative cyclooxygenase-dependent constricting substance released by acetylcholine from the endothelium of SHR renal resistance arteries has been partially clarified using specific inhibitors of cyclooxygenase products. Blockade of PGH₂-TXA₂ receptors with SQ 29,548⁵⁰ inhibited acetylcholine-induced contractions in SHR renal arteries. Acetylcholine-mediated relaxations of SHR renal resistance arteries pretreated with SQ 29,548 are comparable to those observed in WKY arteries. There are two possible explanations for these findings: the contractile factor released by acetylcholine from SHR renal endothelial cells may be PGH₂ or TXA₂, or the SHR arteries may be more sensitive than renal arteries of WKY rats to agonists for the PGH₂-TXA₂ receptor. An inhibitor of thromboxane synthetase (CGS 13080) also blocked the contractile response induced by acetylcholine in SHR arteries, but this agent was less effective in reversing the antagonism of nitric oxide–mediated relaxation than either indomethacin or SQ 29,548. Thus, it is not possible in the present studies to distinguish between PGH₂ and TXA₂ as the EDCF in SHR renal resistance arteries. The EDCF released by acetylcholine in SHR mesenteric resistance arteries does not appear to be TXA₂.⁶

Kato and colleagues¹¹ recently presented evidence to suggest that PGH₂ but not TXA₂ may serve as an EDCF in the aorta of both SHR and WKY rats. They report that contractile responses induced by 10⁻⁴ and 10⁻⁵ M acetylcholine in the aorta of 30–32-week-old rats were prevented by pretreatment with either indomethacin or a PGH₂-TXA₂ receptor antagonist, but not by a TXA₂ synthetase inhibitor. Koga and coworkers¹⁰ reported findings that aging and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of either SHR and WKY rats. Endothelium-dependent contractions were not observed in the aorta of either strain when studied at 4–6 weeks of age but were noted in 12–25-month-old WKY rats and 3–6-month-old SHR. Additional agents are capable of releasing cyclooxygenase-dependent EDCF(s) from the rat aorta. Dominiczak et al¹⁲ report endothelium-dependent contractions induced by ATP in both quiescent and precontracted rings of aorta from 5–9-month-old WKY and stroke-prone SHR.

In summary, endothelium-mediated relaxations are impaired in renal resistance arteries of prehypertensive SHR. Endothelium-dependent relaxations are inhibited by an EDCF that opposes nitric oxide–mediated relaxations. Inhibition of cyclooxygenase or blockade of PGH₂-TXA₂ receptors corrects the endothelial dysfunction noted in SHR renal resistance arteries. Alterations in the cyclooxygenase pathway of SHR endothelial cells leads to endothelial dysfunction that may well be instrumental in the development of hypertension in this genetic model.

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