Distinct Endothelial Impairment in Coronary Microvessels from Hypertensive Dahl Rats

Kathryn M. Gauntner-Rein, Nancy J. Rusch

Abstract—Hypertension has been linked to an impaired dilator function of the coronary microvascular endothelium in vivo. However, the profile and mechanism of this dysfunction remain obscure. Thus, this study compared diameter responses to acetylcholine (ACH), bradykinin (BKN), and substance P (SP) between coronary microvessels (d = 106 ± 4 μm) dissected from left ventricles of normotensive and hypertensive Dahl rats (Dahl-NT and Dahl-HT, respectively). Vessels were cannulated and pressurized on glass pipettes at 80 mm Hg, and internal diameters were monitored by videomicroscopy. Coronary microvessels from Dahl-NT and Dahl-HT showed similar dilator responses to ACH (100 μmol/L to 10 μmol/L), with maximal diameter increases of 63 ± 25 μm and 6 ± 7 μm, respectively (n=31, 17). However, only vessels from Dahl-NT showed dilator responses to SP (10 fmol/L to 1 nmol/L) and BKN (100 fmol/L to 10 nmol/L). All dilator responses persisted after N-nitro-L-arginine (10 μmol/L) or indomethacin (10 μmol/L), but were blunted after inhibition of cytochrome P450 by 10 μmol/L octadecynolc acid (n=6–8). These results suggest that (1) coronary microvessels from Dahl-HT show a unique pattern of endothelial impairment, whereby ACH-induced relaxations persist at a time when dilator responses to SP and BKN are severely blunted, and (2) a cytochrome P450 product, rather than nitric oxide or prostacyclin, may partly mediate the vasodilator responses to ACH, SP, and BKN (Hypertension. 1998;31[part 2]:328-334.)

Key Words: endothelium ★ hypertension ★ coronary arteries ★ vascular smooth muscle ★ cytochrome P450 ★ nitric oxide ★ cyclooxygenase

Hypertension is associated with increased cardiovascular morbidity and mortality, even when large epicardial coronary arteries are angiographically normal.1-5 Hence, coronary vascular dysfunction is proposed to reside at the microcirculatory level in some forms of hypertension.6-8 In this regard, Egashira et al5 reported that epicardial coronary arteries from normotensive and hypertensive patients showed similar dilations during intracoronary infusion of substance P, a potent endothelium-dependent dilator peptide. However, substance P-induced increases in coronary blood flow were blunted fourfold in the hypertensive subjects, implicating the small coronary resistance vessels as the site of endothelial dilator dysfunction. Additionally, Antony et al6 observed that hypertensive patients with angiographically normal coronary arteries showed a blunting of endothelium-dependent, flow-mediated dilation, which impaired the coronary blood flow response to increased myocardial demand. This failure of the microvascular endothelium to provide adequate dilation of the coronary microcirculation has been postulated to facilitate myocardial ischemia in patients with hypertension.1-5 Surprisingly, although rat models often are used to identify the mechanisms of hypertension-related vascular pathologies, the effect of hypertension on endothelial dilator function has not been studied in rat coronary microvessels. Furthermore, little is known about the relative roles of nitric oxide, cyclooxygenase products, or cytochrome P450 products in mediating the endothelium-dependent responses of these regulatory vessels. Thus, the goals of this study were (1) to compare endothelium-mediated dilator responses to acetylcholine, substance P, and bradykinin between isolated coronary microvessels from normotensive and hypertensive salt-sensitive Dahl rats and (2) to begin to define the endothelial-derived factors which mediate the vasoactive responses induced by these same substances in isolated coronary microvessels from the Dahl rat model.

Methods

Experimental Animal Model

Male Dahl salt-sensitive rats were obtained from either Harlan Sprague Dawley, Inc (Indianapolis, Indiana), or from an inbred colony at the Medical College of Wisconsin, which has been maintained by brother-sister mating since 1991. All rats were fed a 0.4% NaCl (low-salt) diet from weaning until 8 weeks of age. At this time, litter mates either continued on a 0.4% NaCl (low-salt) diet, or were placed on an 8% NaCl (high-salt) diet. All rats had free access to drinking water. At 9 to 10 weeks of age, the animals were weighed, anesthetized (ketamine 50 mg/kg IM, xacto 50 mg/kg IP), and blood pressure was recorded by direct catheterization of the femoral artery. Subsequently, hearts were immediately removed, rinsed in physiological salt solution (PSS), weighed, and placed in a dissecting dish filled with the same PSS of the following composition (mmol/L): 119 NaCl, 4.7 KCl, 1.6 CaCl₂, 1.17 MgSO₄, 5.5 glucose, 24 NaHCO₃, 1.18 NaH₂PO₄, and 0.026 EDTA.
Preparation of Cannulated Arteries and Diameter Measurements

Second and third order branches of the left anterior descending or septal coronary artery were identified in the left ventricular myocardium under a dissecting microscope and were carefully dissected free for immediate cannulation. The arteries were cannulated on glass micropipettes, and secured with 10-0 nylon suture in a heated (37°C) lactic acid chamber. Side branches were tied with a single strand of thread tied from 2-0 silk suture. Perfusion and superfusion solutions were equilibrated with a 21% O₂, 5% CO₂, balance N₂ gas mixture to maintain a pH of 7.4 and pO₂ of 140 mm Hg as previously described. The inflow pipette was connected to a gravity feed reservoir which was elevated to maintain an intraluminal pressure of 80 mm Hg. Arteries were equilibrated with continual superfusion and perfusion of drug-free PSS for at least 30 minutes before the initiation of experimental protocols. Intraluminal diameter was measured by a video microscopy system calibrated for micron resolution. In some experiments, vessel diameter also was captured with a video camera system (Mitsubishi P67U, Mitsubishi Electronics) to permit photographic illustration of arterial diameter responses.

Experimental Protocols

Cumulative concentration-response curves to acetylcholine (ACH), substance P (SP), and bradykinin (BK) were performed on arteries that had developed spontaneous tone. Drug aliquots were added to the bath superfusate, and maximal diameter response to each drug concentration was recorded. Arteries were allowed to re-equilibrate in drug-free superfusate between concentration-response curves, and baseline diameters were closely monitored to ensure complete washout and reversibility of drug responses. Drug concentration-response protocols were performed as solitary experiments in a single preparation or were performed on the same cannulated vessel only after demonstration of drug reversibility. Arteries for the same protocol were obtained from different rat hearts. Indomethacin, N-nitro-L-arginine, and octadecynoic acid (ODYA) were added to the perfusate and superfusate solutions for 30 minutes before the addition of ACH, SP, and BK. Only one blocking drug was tested in each artery. At the end of each experiment, the presence of Ca²⁺-dependent active tone, which is regarded as an indication of vascular smooth muscle viability, was examined by measuring the dilator response of arteries to perfusion and superfusion with Ca²⁺-free solution. Arteries that did not dilate in response to Ca²⁺-free solution were omitted from the study (n=3). In a subset of experiments, the endothelium was removed by intraluminal perfusion of a 2 to 3 ml air-bolus for 2 minutes, while maintaining intraluminal pressure at 40 to 60 mm Hg. Subsequently, arteries were reprefused with PSS at 80 mm Hg, and allowed to equilibrate for 30 minutes. Four arteries showed a loss of resting tone after the denudation procedure, and these vessels also were removed from further study.

Drugs

All drugs were purchased from Sigma Chemical Company, with the exception of octadecynoic acid (ODYA) which was obtained from Cayman Chemical. Drugs were reconstituted as concentrated stock solutions for direct dilution into PSS. Acetylcholine (ACH), substance P (SP), bradykinin (BKN), indomethacin, and N-nitro-L-arginine were dissolved as 1 mmol/L aqueous stock solutions in PSS. ODYA and nifedipine were reconstituted as 10 mmol/L and 1 mmol/L stocks in 95% ethanol, respectively, for direct dilution into PSS. Addition of ethanol per se did not affect the responses of coronary resistance arteries from normotensive Dahl SS rats to endothelium-mediated vasodilator drugs (n=2 to 3). Addition of the drugs also did not significantly affect the pH of the PSS, and resulted in ≤0.01% dilution of PSS constituents.

Data Analysis

Data are expressed as mean±standard error of the mean. Diameter values represent the measurement of internal diameter in microns. A replication factor of at least 6 to 8 was performed for each protocol to permit statistical analysis of acquired measurements. Significance of differences between rat preparations and of differences between control and interventional diameter responses was determined by Student's t-test or ANOVA with repeated measures, followed by a subsequent Duncan's test. Significance was accepted at P<.05.

Results

Rat Model

Coronary vessels were obtained from 39 Dahl-NT and 34 Dahl-HT rat hearts. The mean arterial pressure of anesthetized Dahl-NT rats after 10 to 14 days on a low-salt (0.4% NaCl) diet averaged 119±2 mm Hg, as measured by femoral catheterization. The cannulated pressure of age-matched Dahl-HT rats fed a high-salt (8% NaCl) diet for a parallel number of days was significantly higher, averaging 160±3 mm Hg. Body weights were similar between Dahl-NT rats (324±9 grams) and Dahl-HT rats (332±7 grams), whereas the wet weight of hearts from Dahl-HT rats was significantly higher than hearts from Dahl-NT rats, averaging 1.45±0.04 grams and 1.23±0.03 grams, respectively.

Comparison of Resting and Passive Diameters

Fig 1 shows that coronary microvessels from Dahl-NT and Dahl-HT rats showed similar resting diameters of 104±4 μm and 110±5 μm in control PSS, respectively (n=39, 34). After perfusion and superfusion with Ca²⁺-free PSS at the end of the experiments, the diameter of these vessels increased to 189±6 μm and 182±7 μm, respectively, demonstrating high and comparable levels of spontaneous and Ca²⁺-dependent active tone.
Comparison of vasodilator responses to ACH, SP and BKN

Fig. 2 shows that ACH (100 nmol/L to 10 μmol/L; half-log units) was equally potent and effective in increasing the diameters of coronary microvessels from Dahl-NT and Dahl-HT rats (n = 31, 19). The maximal resting diameter increase induced by 10 μmol/L ACH were 63±5 μm and 63±7 μm, respectively. After endothelium removal by an air bolus, the resting diameters were not significantly different from the endothelium-intact vessels, and incremental concentrations of ACH did not significantly dilate arteries from either rat group (n = 6-7; not shown).

In contrast, Fig. 3A shows that incremental concentrations of SP (10 fmol/L to 1 nmol/L; half-log units) progressively diluted coronary microvessels from Dahl-NT rats by a maximum of 22±2 μm, whereas the arteries from Dahl-HT rats did not dilate significantly (n = 30, 23). Similarly, Fig. 3B demonstrates that BKN (100 fmol/L to 10 nmol/L; half-log units) dilated coronary microvessels from Dahl-NT rats by a maximum of 17±2 μm, whereas the vessels from the hypertensive animals did not show significant change (n = 30, 26). These contrasting vasoactive responses to SP between vessels from Dahl-NT and Dahl-HT rats are depicted photographically in Fig. 4 (left and right, respectively). In these experiments, the microvessel from the Dahl-NT rat showed a pronounced diameter increase of 55 μm (from 105 μm to 160 μm) in response to 10 pmol/L SP, and it subsequently dilated to 210 μm in Ca2+-free solution. In contrast, the same concentration of SP only dilated the vessel from the Dahl-HT rat by 3 μm (from 113 μm to 116 μm), although it showed a comparable level of active tone as revealed by the large diameter increase in Ca2+-free PSS. After endothelium removal by an air bolus, the SP and BKN-induced dilations were absent in coronary microvessels of Dahl-NT rats, and the impaired diameter responses of arteries from Dahl-HT rats to these same vasodilator peptides were unchanged (n = 7-8; not shown). Diameters of the arteries were not altered by endothelial denudation.

Identification of Possible Endothelium-Derived Vasodilator Factors

Before the mechanisms which impair endothelial dilator function in coronary microvessels of hypertensive rats can be explored, the pathway for normal dilation must be defined. In this respect, nitric oxide, prostacyclin, and cytochrome P450 dilator products are generally considered the primary mediators of endothelium-dependent dilation. However, in the rat coronary circulation, endothelium-dependent dilations are not mediated by prostacyclin,11,12 and, hence, our further studies to characterize endothelium-dependent dilator responses focused on the involvement of NO and cytochrome P450 metabolites in endothelium-dependent responses. In this regard, Fig. 5A (top) shows that the nitric oxide synthase inhibitor, N-nitro-L-arginine (L-NNA, 10 μmol/L),13 did not significantly inhibit the dilator responses of coronary microvessels from Dahl-NT rats to ACH, SP or BKN (n = 6-8). Rather, L-NNA appeared to augment the SP and BKN concentration-response curves, but this effect did not reach statistical significance. Additionally, increasing the concentration of L-NNA by 10-fold (100 μmol/L) did not alter dilator responses (n = 3-4; data not shown).

In contrast, ODYA (10 μmol/L), a suicide substrate inhibitor of cytochrome P450,14 inhibited the concentration-dependent dilator responses of coronary microvessels from Dahl-NT rats to ACH, SP, and BKN. The maximal dilations to these endothelium-dependent dilator substances were reduced 51±12%, 49±16%, and 59±14% by ODYA, respectively (n = 7-8). Although this effect of ODYA implicated an endo-
Figure 5. A, top, Concentration-relaxation curves to acetylcholine (ACH), substance P (SP) and bradykinin (BKN) in coronary microvessels from Dahl-NT rats. Responses were measured in control PSS, and after vessel incubation with L-NNA (10 μmol/L) or ODYA (10 μmol/L). Dilator responses were not altered by L-NNA, but were attenuated by ODYA (n=7-8). Initial resting diameters averaged 106±3 μm. B, lower panel, Similar concentration-response curves in vessels from Dahl-HT rats also indicated a lack of effect of L-NNA (n=6), whereas ODYA (10 μmol/L) attenuated the dilator response to ACH and unmasked a constriction response to higher concentrations of BKN. Initial resting diameters averaged 103±3 μm. In both panels, values represent mean±SEM. *Diameter response was significantly different in ODYA compared to control PSS. Control curves, which did not differ significantly between the L-NNA and ODYA sample groups, are displayed as averaged values.

Figure 6. A, top, Concentration-relaxation curves to acetylcholine (ACH), substance P (SP), and bradykinin (BKN) in coronary microvessels from Dahl-NT rats. Indomethacin (INDO, 10 μmol/L) did not change the relaxation responses. Resting diameters averaged 102±4 μm (n=6). R, lower panel, Similar concentration-response curves in vessels from Dahl-HT rats Indomethacin (μmol/L) did not alter ACH-induced dilations, and did not restore dilator responses to SP or BKN. Resting diameters averaged 104±4 μm (n=6-7). Values represent mean±SEM.

Effects of Indomethacin on ACH, SP, and BKN-Induced Diameter Responses

Because constrictor products of the cyclooxygenase pathway may blunt endothelium-dependent dilations in systemic arteries, we further examined if cyclooxygenase-derived products were involved in modulating the vasoactive responses observed in this study. However, Fig 6A shows that indomethacin (INDO, 10 μmol/L), an inhibitor of cyclooxygenase, did not alter the progressive dilator responses to ACH, SP, or BKN in coronary microvessels from Dahl-NT rats (n=6-7). Furthermore, Fig 6B shows that indomethacin (10 μmol/L) also did not significantly affect concentration-dependent dilations to ACH in vessels from Dahl-HT rats and did not restore vasodilator responses to SP or BKN in vessels from the hypertensive animals (n=6-7).

Discussion

This is one of the first studies to examine endothelial dilator function in isolated coronary microvessels from a rat model of hypertension, and several new findings have emerged. First, it appears that coronary microvessels from Dahl-HT rats show a unique pattern of endothelial impairment compared to large systemic vessels. In particular, the blunted dilator response to ACH observed in systemic arteries of hypertensive animals and humans was not observed in this study of coronary microvessels from Dahl-HT rats. Second, we found little evidence that the impaired dilator responses to SP and BKN in coronary microvessels from Dahl-HT rats were related to the release of a cyclooxygenase-derived constrictor factor, although this mechanism has been proposed to contribute to endothelial dilator defects in systemic arteries. Finally, our...
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findings implicate a cytochrome P450 product, rather than nitric oxide, as an important mediator of the dilator responses to ACH, SP, and BKN in coronary microvessels from the Dahl rat strain

**A Distinct Pattern of Endothelial Dilator Dysfunction in CRA from Dahl-HT Rats**

Our study has uncovered initial evidence of a distinct pattern of endothelial dysfunction in coronary microvessels exposed to short-term hypertension in Dahl rats. In these rat intramural coronary arteries, which possess a high level of myogenic tone and may assist in regulating coronary blood flow, normal dilator responses to ACH persisted at a time when the dilator responses to the native coronary peptides, SP and BKN, were markedly blunted. This profile is distinctly different from the pattern of endothelial dysfunction observed in the aorta, cerebral, and renal arteries of several rat models of hypertension, including the aorta of Dahl salt-sensitive rats, in which a blunted dilator response to ACH has been the hallmark finding. In contrast, the finding of impaired dilator responses to SP and BKN in our coronary microvessels concurs with observations of recent clinical studies, which have demonstrated that SP-induced dilations are blunted in the coronary microcirculation of human subjects with essential hypertension. However, our findings may relate to earlier reports that ACH-induced, nitric-oxide mediated dilations persist in rings of main coronary arteries from spontaneously hypertensive rats, and that they also persist in main coronary arteries from the same rat strain cannulated for vessel perfusion. Furthermore, a diminished dilator response to BKN concurrent with intact ACH-induced dilations also has been observed in the mesenteric vasculature of genetically hypertensive rats and in the cutaneous circulation of the forearms of humans with essential hypertension. Hence, to date, it appears that the sparing of ACH-induced dilation during hypertension may be a novel feature of several vascular beds. Interestingly, a normal dilator response to ACH also has been reported in coronary arteries of humans with essential hypertension, although this finding has not been consistent between all investigators.

In contrast, the finding of impaired dilator responses to SP and BKN in our coronary microvessels concurs with observations of recent clinical studies, which have demonstrated that SP-induced dilations are blunted in the coronary microcirculation of human subjects with essential hypertension. Hence, our report of a similar abnormality in a coronary microvessel preparation may prove helpful in providing an in vitro model in which to obtain mechanistic information on the nature of this abnormality. Notably, our data show that SP and BKN potently dilate isolated rat coronary microvessels, and, similarly, intracoronary infusion of these peptides increases coronary blood flow in vivo. However, in isolated rings of large coronary arteries from some species, including the rat, SP shows little vasoactive effect, whereas BKN elicits vascular contraction. The different vasoactive effects of SP and BKN on large versus small coronary arteries suggest that the in vitro study of vascular pathologies related to these peptides may be best characterized in microvessel preparations.

**What is the Site of Endothelial Dilator Dysfunction in Coronary Microvessels?**

It is unclear which pathophysiological processes would permit, in the same coronary microvessel, the persistence of an intact ACH-induced dilation at a time when SP and BKN-induced dilations are impaired by the hypertensive disease. Our finding that ODYA, an inhibitor of the cytochrome P450 pathway, blunted the dilator responses to ACH, SP, and BKN in coronary microvessels of Dahl-NT rats to a similar extent, implies that all three of these substances may exert at least part of their dilator action through this common pathway. Furthermore, it appears that this pathway is still intact in coronary microvessels from Dahl-HT rats, because these vessels, which demonstrated full dilator responses to ACH, also were sensitive to block by ODYA. Hence, the defective site in the endothelial cells associated with impaired SP and BKN-induced dilation appears to be located proximal to mediator release, and, apparently, it is related to membrane or intracellular events specific to the processing of the SP and BKN receptor signal.

In this respect, activation of BKN and SP receptor-linked G proteins has been identified as a critical step in endothelial cell signaling, which results in phospholipase activation and release of endothelial cycloxyrogenase P450 dilator products. Specific G proteins, insensitive to pertussis toxin, may be coupled to peptide receptors, whereas other G protein molecules may transduce receptor signals from other dilator agonists. Modulation of G protein function in endothelial cells is postulated to occur in atherosclerotic disease and may also occur during the endothelial remodeling, which occurs after balloon catheterization, suggesting that these proteins are readily modulated by cardiovascular pathologies. Thus, a further definition of the events, which signal the release of dilator factors from the endothelium, may help pinpoint the molecular sites of dysfunction in the endothelial cells during hypertension and may provide clues to enable the prevention or reversal of coronary endothelial abnormalities.

**No Evidence for an Endothelium-Derived Constrictor Factor in Coronary Microvessels.**

In the aorta and in the renal and cerebral arteries of the SHR, the blunted dilator response to acetylcholine is due to the production of a cyclooxygenase-derived constrictor factor, which may be prostaglandin H$_2$ (PGH$_2$). In these vessels, pharmacological block of cyclooxygenase by indomethacin restores ACH-induced dilator responses, and indomethacin also improves endothelium-mediated dilation in the human forearm. However, in our study, indomethacin had no significant effect on the dilator responses to ACH, SP, and BKN in coronary microvessels of Dahl-NT rats, and it also did not alter the dilator response to ACH, which persisted in the vessels from the Dahl-HT rat. Furthermore, cyclooxygenase inhibition also did not restore the dilator responses to SP and BKN to the coronary microvessels of the Dahl-HT rat. Hence, our results provide little direct evidence that the release of a cyclooxygenase-derived constrictor factor from the coronary endothelium contributes to the blunting of SP- and BKN-induced dilation in coronary microvessels from hypertensive Dahl rats. However, the possibility remains that the inhibition by indomethacin of the cyclooxygenase pathway for arachidonic acid metabolism may have shunted arachidonic acid to alternative metabolic pathways, thereby enhancing the synthesis of other constrictor factors and preventing the restoration of...
full dilator responses to SP and BKN during cyclooxygenase block

A Cytochrome P450 Product May Mediate Endothelium-Dependent Dilations

Several dilator factors are released by the coronary endothelium, including nitric oxide, prostacyclin, and at least one cytochrome P450 product postulated to be a hyperpolarizing factor. Of these substances, pharmacological studies have implicated nitric oxide as the primary mediator of endothelium-dependent dilations to ACH and BKN in large porcine and bovine coronary arteries, although products of the cytochrome P450 pathway also may contribute to dilator responses. Similarly, nitric oxide also has been implicated as the primary endothelium-derived mediator of dilation in large rat coronary arteries, where it appears to fully account for the relaxation response to ACH in strain gauge recordings. In contrast, results from this study in rat coronary microvessels, which were studied by similar pharmacological approaches, found no evidence for nitric oxide as a mediator of the dilator responses to ACH, SP or BKN. Rather, our results suggest that a product of the cytochrome P450 pathway mediates in part the dilator responses to these substances, with a residual dilator component resistant to drug block. Although the endothelium-derived P450 products which mediate dilation of isolated coronary microvessels have not been defined, an epoxygenase dilator product, with properties of a potassium channel opener and hence, a hyperpolarizing substance, has been described in large coronary arteries. Our results provide initial evidence for the role of a similar product in mediating endothelium-dependent dilations in the rat coronary microcirculation, and emphasize the potential importance of the P450 pathway in the regulation of coronary microvascular tone. However, the existence of multiple interacting vasoconstrictor pathways in these vessels should not be excluded, particularly in view of the finding that ODYA did not completely abolish the agonist-induced dilations induced by ACH, SP and BKN in coronary microvessels of Dahl-NT rats.

Limitations of the Study

Several aspects of our study should be acknowledged as possible limitations. First, our vessels were obtained from salt-sensitive Dahl rats, some of which were fed a high-salt diet to induce a short-term, volume-expanded model of hypertension. Notably, this model permits the comparison of endothelial function between normotensive and hypertensive animals with the same genotype. The short duration of hypertension also minimizes vascular structural changes. Although some degree of vascular remodeling cannot be discounted in this study, the similar passive diameter values we observed between microvessels from Dahl-NT and Dahl-HT rats provided no overt evidence for structural changes. However, the genetic, pressure, and endocrine profiles of the Dahl rat may not resemble other rat models of hypertension and, hence, the functional profile of the endothelial cells also may not be readily comparable.

Second, our Dahl rats were fed a high-salt diet to produce a volume-expanded model of high blood pressure, and, hence, salt-feeding per se may have influenced endothelial function in our study. Studies performed by Gau and colleagues indicated little effect of a high-salt diet on endothelium-dependent dilations, including those to ACH. However, recent work by Liu et al suggests that isolated skeletal microvessels, obtained from normotensive rats fed a 4% NaCl diet for 4 to 8 weeks, show attenuated endothelium-dependent dilator responses to ACH and the prostanoid analogue iloprost. Studies by Bregenholtz further suggest an attenuation of endothelium-dependent, flow-mediated dilation in the rat skeletal muscle microcirculation in vivo, after feeding of a 7% NaCl diet to normotensive rats for 2 weeks. Hence, an effect of salt-feeding on the endothelium-dependent dilator responses of this study cannot be discounted, and additional studies examining endothelial function in coronary microvessels from other rat models of hypertension are warranted.

Possible Physiological Relevance of the Findings

The physiological relevance of our findings rely on a functional role for ACH, SP, and BKN in the coronary circulation in vivo. In this respect, ACH represents a cholinergic neurotransmitter, which is localized in parasympathetic nerve endings at the outer medial layer of small coronary arteries. Its release by vagal nerve activity is associated with stimulation of muscarinic receptors on coronary endothelial cells, and with coronary vasodilation. The clinical role of parasympathetic, neurally-mediated coronary dilation is untested, but it is postulated to reinforce the metabolic regulation of coronary blood flow. Hence, the persistence of ACH-induced dilator responses in coronary microvessels during hypertension may act to extend continued protection to some regions of the myocardium. In contrast, recent attention has focused on SP and BKN, which are native peptides of the coronary endothelium, and are 40- to 100-fold more potent than adenosine in triggering endothelium-dependent dilation in small coronary arteries. Recent studies suggest that release of BKN contributes to the resting level of coronary blood flow in human hearts and partially mediates flow-dependent dilation in response to increased myocardial demand in control subjects. Substance P also is postulated to contribute to the regulation of coronary blood flow by establishing an endothelium-dependent dilator influence and concentrations of SP in the fentomolar range may elicit profound dilation. Hence, impaired dilator responses to BKN and SP during hypertension, as observed in the coronary microvessels of Dahl-HT rats in this study, would be postulated to eliminate a potentially important dilator influence from the coronary vasculature and thereby favor construction of the coronary microcirculation. Recent reports that hypertensive patients with angiographically normal epicardial coronary arteries show a loss of dilation to SP, as well as a blunting of flow-dependent, endothelium-mediated dilation, raise the possibility that endothelial dysfunction at the coronary microvascular level may facilitate myocardial ischemia in patients with hypertension. Designing therapies to restore endothelium-dependent dilation to the coronary microcirculation during hypertension will require details about the normal endothelium-dependent pathway for dilation in these small vessels, and such therapies will benefit from information on the interaction of these pathways with...
other mechanisms that regulate arterial tone in the coronary microvasculature.

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