Effect of $N^G$-Monomethyl L-Arginine on Endothelium-Dependent Relaxation in Arterioles of One-Kidney, One Clip Hypertensive Rats

Tetsuya Nakamura and Russell L. Prewitt

Dose-response curves to topically applied acetylcholine, bradykinin, and nitroprusside were obtained by intravital microscopy in arcading arterioles of the spinotrapezius muscle of control ($n=9$) and one-kidney, one clip hypertensive (1K1C) rats ($n=11$) of 4 weeks' duration before and during superfusion with the specific inhibitor of nitric oxide formation $N^G$-monomethyl L-arginine (LNMMA) (10^{-4} M) and both LNMMA (10^{-4} M) and indomethacin (2.8×10^{-5} M). Resting arteriolar tone was higher in 1K1C rats than in controls, and vasodilation to acetylcholine and bradykinin, but not to nitroprusside, was reduced ($p<0.05$) in 1K1C rats compared with controls. LNMMA increased arteriolar tone ($p<0.05$) and inhibited the vasodilator responses to acetylcholine and bradykinin ($p<0.05$) in controls but not in 1K1C rats. LNMMA did not alter the response to nitroprusside in either group. Addition of indomethacin to LNMMA increased arteriolar tone and markedly reduced the response to bradykinin, but not to acetylcholine or nitroprusside, in both groups. These findings suggest that resting arteriolar tone is increased in 1K1C rats partially because of the decreased basal release or synthesis of nitric oxide. Responses to the endothelium-dependent vasodilators acetylcholine and bradykinin were attenuated in 1K1C rats, possibly because of changes in synthesis or release of nitric oxide for acetylcholine and of prostacyclin for bradykinin, because the response to the endothelium-independent vasodilator nitroprusside did not differ between the groups. (Hypertension 1991;17:875–880)
Drug Application

ACh, NP, Bdk, and LNMMA were dissolved in saline and frozen and stored at -20°C for no more than 6 weeks. Indomethacin was dissolved daily in saline–sodium carbonate (9.4x10^-3 M) to give a solution of 2.8x10^-3 M, and pH was adjusted to 7.4 with hydrochloric acid. All the drugs subsequently were diluted with Krebs-Henseleit solution to the desired concentrations. LNMMA (p-hydroxyazobenzene-sulfonate salt) was obtained from Calbiochem Corp., La Jolla, Calif. All other drugs were obtained from Sigma Chemical Co., St. Louis, Mo.

Experimental Protocol

After an equilibration period of 20 minutes, concentration–response curves to topically applied ACh, NP, and Bdk were obtained. Then, LNMMA was continuously added to the superfusion solution, and the response was observed for 15 minutes, after which a second set of dose–response curves was measured. The superfusion solution was switched to one containing both 10^-4 M LNMMA and 2.8x10^-5 M indomethacin, and the response was observed for 30 minutes. Subsequently, a third set of concentration–response curves of ACh, NP, and Bdk was obtained in the presence of both 10^-4 M LNMMA and 2.8x10^-5 M indomethacin. The concentration of each drug was increased in log increments after the arteriolar diameter had returned to the control level. Preliminary studies indicated that 2-minute intervals were sufficient for the arteriolar diameter to return to control levels. Responses reached maximal levels within 30 seconds after application of agents. The order of drugs was randomized, and applications of different drugs were separated for at least 10 minutes to ensure clearance of drug from the bath. At the end of the experiment, 0.1 ml adenosine (10^-3 M) was added to the bath to achieve maximal vascular smooth muscle relaxation, and maximal vessel diameter was measured. The duration of the whole experiment did not exceed 3.5 hours.

The response was expressed as a percentage of the putative maximal response to vasodilation: percent response to ACh, Bdk, or NP=(D_r-D_c)/(D_m-D_c)x100, where D_r is the control resting diameter, D_c is the diameter obtained with each concentration of the drugs, and D_m is the maximal diameter obtained with 10^-3 M adenosine. Arteriolar tone (percent) was expressed as follows: ((D_m-D_c)/D_m)x100, where D_m is the maximal diameter obtained by 10^-3 M adenosine, and D_c is the measured diameter.

Statistical Analysis

Values are given as mean±SEM. Multiple data were analyzed by analysis of variance followed by multiple comparisons made with Duncan's new multiple range test. Student's t-test was used when appropriate. Statistical significance was considered to be p<0.05.

Results

Body weight at the time of the experiment was 347±8 g in control rats and 340±10 g in KIC rats.
Table 1. Body Weight, Mean Arterial Pressure, and Heart Rate in Normotensive and One-Kidney, One Clip Goldblatt Hypertensive Rats

<table>
<thead>
<tr>
<th>Body wt (g)</th>
<th>MAP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>Normotensive (n=9)</td>
<td>347±8</td>
<td>123±3</td>
</tr>
<tr>
<td>1K1C (n=11)</td>
<td>340±10</td>
<td>182±6*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Mean arterial pressure (MAP) and heart rate are shown at the beginning and end of the experiments. 1K1C, one-kidney, one clip hypertensive rats.
*p<0.01 vs. normotensive.

(NS) (Table 1). Mean arterial pressure was significantly (p<0.01) elevated in 1K1C rats at 182±6 mm Hg compared with normotensive control rats at 123±3 mm Hg. Heart rate did not differ between the groups, at 384±12 beats/min for 1K1C rats and 390±6 beats/min for controls. Except in one normotensive and two hypertensive animals that were excluded from the study, mean arterial pressure and heart rate did not change during the experiment.

The top panel of Figure 1 shows the resting arteriolar diameters for the concentration-response curves of ACh, NP, and Bdk, as well as the maximal relaxant diameter obtained with 10−3 M adenosine in normotensive and hypertensive rats. In either group, successive resting arteriolar diameters before the application of each vasoactive drug did not change significantly under the same bath conditions. The average resting arteriolar diameter in the normotensive group was 51±5 μm (n=9) during the control period and decreased significantly (p<0.05) to 38±4 μm during the superfusion of 10−4 M LNMMMA and to 35±4 μm during the superfusion of both 10−4 M LNMMMA and 2.8×10−3 M indomethacin. The average resting arteriolar diameter in the 1K1C group was 42±5 μm (n=11) during the control period and did not change significantly on superfusion of LNMMMA (38±5 μm). Average arteriolar diameter in hypertensive rats decreased significantly (p<0.05) to 35±4 μm on superfusion of both LNMMMA and indomethacin compared with the control period. Maximal arteriolar diameters were 79±6 μm in normotensive rats and 84±7 μm in 1K1C rats.

Resting arteriolar tone (Figure 1, bottom panel) during the control period was significantly higher in hypertensives (50±4%) than in normotensives (36±4%). Superfusion of LNMMMA raised arteriolar tone significantly (p<0.05) in normotensive (52±4%) but not in 1K1C rats (56±4%). Superfusion of both LNMMMA and indomethacin increased arteriolar tone significantly (p<0.05) from control levels to 56±4% and 60±4% in normotensive and 1K1C rats, respectively. There was no significant difference in arteriolar tone between the two groups.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Top panel: Resting arteriolar diameters for concentration-response curves of acetylcholine, nitroprusside, and bradykinin during control period and superfusion of 10−3 M monomethyl L-arginine (L-NMMA) and both L-NMMA and indomethacin (INDO), as well as maximal relaxant arteriolar diameter with adenosine in normotensive (NT) control and one-kidney, one clip Goldblatt hypertensive (1K1C) rats. Successive data points under each condition represent the three resting arteriolar diameters preceding concentration-response curves of acetylcholine, nitroprusside, and bradykinin. The order of application of the three drugs was randomized during the same bath condition. Bottom panel: Arteriolar tone, expressed as a percent of the maximally relaxed diameter, during superfusion of L-NMMA and both L-NMMA and INDO in NT and 1K1C rats. Values are mean±SEM. *p<0.05 vs. NT control; +p<0.05 vs. hypertensive control.
Figure 2. Concentration–response curves of acetylcholine, bradykinin, and nitroprusside during control period and during superfusion of L-NMMA or both L-NMMA and indomethacin in normotensive (NT) control and one-kidney, one-clip Goldblatt hypertensive (1K1C) rats. Values are mean±SEM.

Discussion

Successful topical applications of drugs did not change basal smooth muscle tone over the duration of the experiments, as indicated by the unchanged resting arteriolar diameters between dose–response curves (Figure 1). Arteriolar tone, estimated from the difference between resting and maximally relaxed diameters, was significantly higher in 1K1C than in normotensive rats during the control period. Increased vasoconstriction has been reported in arterioles of cremaster and gracilis muscles in 1K1C rats of 4–6 weeks. Superfusion of L-NMMA reduced arteriolar diameter and increased arteriolar tone in the normotensive group, but not in 1K1C rats. The onset of action of L-NMMA was nearly immediate. These findings with L-NMMA suggest that endogenous biosynthesis and basal release of NO modulate resting arteriolar diameter and tone in normotensive rats; however, in 1K1C rats, basal synthesis or release of NO may be reduced, and reduction of NO release may raise vascular tone. In hypertension, all aspects of vasoconstriction seem to be enhanced, especially during the early stage of experimental hypertension when structural changes have not yet occurred. Arterioles of hypertensive animals have increased sensitivity and reactivity to norepinephrine and other vasoactive agents, suggesting that vascular hyperresponsiveness may contribute to enhanced vasoconstriction. The basal release of endogenous NO could act as a functional antagonist to vasoconstrictors, and the reduction of endothelial NO release may increase the sensitivity of vascular smooth muscle to vasoconstrictive agents, leading to a rise in arteriolar tone. In fact, it has been reported that endothelial removal enhances the contractile response to a number of vasoconstrictors.

Superfusion of L-NMMA inhibited the vasodilator response to ACh and Bdk, but not to NP in the normotensive control, indicating that ACh and Bdk act through the formation of endogenous NO in vivo and that vasodilatation to NP is independent of endothelium-derived NO in skeletal muscle arterioles. NO formed within the vascular smooth muscle cur-
recently is considered to be the mediator expressing the relaxant action of NP. These results are in agreement with those of Tolins et al, whose results indicated that NO mediates the hemodynamic effects of ACh in vivo.

The vasodilator response to ACh was substantially inhibited but not abolished by LNMMA. These results are consistent with those obtained in vascular rings in vitro, where more LNMMA was required to inhibit ACh-induced relaxation than was required to increase basal tone. At this stage, it is difficult to know whether sufficiently high concentrations of LNMMA in the microenvironment of the vascular endothelial cell were achieved at these doses to compete effectively with endogenous levels of L-arginine, or if ACh is mediated by NO arising from a pool of arginine inaccessible to LNMMA, or by an EDRF distinct from NO, such as endothelium-derived hyperpolarizing factor. Because LNMMA suppressed the response to lower doses of ACh more effectively than to higher, the concentration of ACh may alter the manner in which ACh stimulates NO release.

Superfusion of either indomethacin or LNMMA can separate the roles of NO and prostacyclin in endothelium-dependent vasodilation. Prostacyclin is a potent vasodilator, and endothelial cells are a major source of prostacyclin within the vessel wall. Therefore, prostacyclin released from endothelial cells can act as a dilator of smooth muscle, and stimulation of prostacyclin release from the endothelium causes endothelium-dependent relaxation. Koller et al reported that light-dye treatment of rat cremasteric arterioles could damage the endothelium and produce a selective inhibition of dilator response to ACh and Bdk but not to NO. Moreover, because cyclooxygenase inhibition reduced the vasodilation to Bdk, they suggest that both ACh and Bdk are endothelium-dependent vasodilators and that Bdk seems to stimulate both endothelial production of prostaglandins and release of other endothelium-derived factors. In the present study, addition of indomethacin to LNMMA in the superfusion solution did not change the response to ACh but reduced the response to Bdk markedly. These results suggest that vasodilation by Bdk could be attributable to NO and prostacyclin, with the latter having the major role. Although ACh releases prostacyclin in aortic rings, indomethacin did not block the dilator response to ACh in these vessels nor in vessels from normotensive animals in other studies. The unchanged response to ACh after indomethacin also suggests that contracting factors produced by cyclooxygenase in response to ACh in spontaneously hypertensive rats are not involved in 1K1C hypertension.

The vasodilator response to ACh during the control period and to Bdk during superfusion of LNMMA was significantly lower in 1K1C rats than in normotensive controls. There was no significant difference between the groups in their vasodilator responses to ACh during superfusion of LNMMA or their responses to Bdk during superfusion of both LNMMA and indomethacin. These results indicate that synthesis or release of endothelium-derived NO and vasodilator prostaglandins, stimulated by ACh and Bdk, respectively, are reduced in 1K1C rats and that the endothelial dysfunction may occur in a nonspecific manner. Theoretically, the relaxations could be depressed because of decreased release or synthesis of mediators from the endothelium or decreased responsiveness of the vascular smooth muscle to the mediators. Because the response to NP did not differ between normotensive and 1K1C rats, it is unlikely that the reduction of endothelium-dependent relaxation in 1K1C rats results from changes in vascular smooth muscle reactivity.

Chronic hypertension in all animal models studied reportedly is associated with morphological changes in the endothelium of aorta or large arteries. A dynamic remodeling of rat skeletal muscle arterioles has been demonstrated during the development of hypertension, with marked structural reduction in arteriolar diameters and density in rats with 1K1C hypertension. Recently, Hansen-Smith et al reported atrophy and degeneration of endothelial cells in skeletal muscle arterioles from rats with chronic reduced renal mass hypertension, and they suggested that the observed structural changes occur during the process of microvascular remodeling. A study using rats with aortic coarctation showed that pressure- or flow-dependent mechanisms were responsible for this remodeling, and it is likely that endothelial function may be affected by changes in either microvascular pressure or blood flow (shear stress). Furthermore, the renin-angiotensin system, mineralocorticoid hormones, and catecholamines, as well as high salt intake, have been implicated in hypertensive vascular injury, suggesting that neurohumoral factors also may contribute to the endothelial dysfunction in hypertension.

In conclusion, arteriolar responses to the endothelium-dependent vasodilators ACh and Bdk were attenuated in 1K1C rats, possibly because of changes in synthesis or release of endothelium-derived NO and prostacyclin, because the response to the endothelium-independent vasodilator NP was unchanged. Indomethacin attenuated the vasodilation by Bdk, which implicates a partial role of prostacyclin for its action in vivo. Decreased basal release of endothelium-derived vasodilators may contribute to increased vascular tone in this model of renal hypertension.

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References

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