Endothelium-derived relaxing factor in regulation of basal cardiopulmonary and renal function

MARK A. PERRELLA, FREDRIC L. HILDEBRAND, JR., KENNETH B. MARGULIES, AND JOHN C. BURNETT, JR.
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Perrella, Mark A., Fredric L. Hildebrand, Jr., Kenneth B. Margulies, and John C. Burnett, Jr. Endothelium-derived relaxing factor in regulation of basal cardiopulmonary and renal function. Am. J. Physiol. 261 (Regulatory Integrative Comp. Physiol. 30): R323–R328, 1991.—The endothelium has emerged as an important modulator of vascular tone by producing both vasodilating and vasoconstricting substances. In vitro studies have demonstrated that endothelial cells produce endothelium-derived relaxing factor (EDRF), which promotes vasodilation via the stimulation of intracellular guanosine 3′,5′-cyclic monophosphate (cGMP). However, the role of EDRF in the basal regulation of cardiopulmonary and renal function is not well defined. The present study was therefore designed to assess the function of EDRF by studying two groups of normal anesthetized dogs, of which one received a competitive inhibitor to EDRF generation, Nω-monomethyl-L-arginine (L-NMMA), which is a competitive inhibitor of EDRF synthesis from L-arginine (29). These investigators reported that an acute intravenous bolus of L-NMMA resulted in a dose-dependent increase in arterial pressure, supporting the conclusion that EDRF may play a role in the regulation of blood pressure. Similar results by Aisaka et al. (1) have been obtained in guinea pigs. Tolins et al. (35) extended this concept in rats and demonstrated that an intravenous bolus of L-NMMA increases renal vascular resistance (RVR) and arterial pressure, suggesting that the release of EDRF may play a role in the basal regulation of arterial pressure and renal hemodynamics. Although these latter studies suggest an important role for EDRF in the regulation of renal blood flow (RBF), its role in the regulation of glomerular filtration rate (GFR) and sodium excretion in overall volume and arterial pressure homeostasis has not been assessed.

The hypothesis of the current study is that endogenous EDRF may serve as a homeostatic mediator in the basal regulation of integrated cardiopulmonary and renal hemodynamic function as well as in the regulation of sodium homeostasis. To test this hypothesis, hemodynamic function of the systemic, pulmonary, and renal circulations was measured as well as renal sodium excretion. Two groups of anesthetized dogs were studied receiving either a vehicle or L-NMMA. A recent study by Boulanger and Lüscher (5) suggested that in vitro EDRF inhibits the release of the potent endothelium-derived vasoconstrictor endothelin (ET) (39). Thus plasma ET was also assessed in the L-NMMA group to evaluate the action of EDRF inhibition on circulating ET.

METHODS

Surgical Preparation

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THE ENDOTHELIUM has emerged as an important modulator of vascular tone by producing both vasodilating and vasoconstricting substances (6, 9, 14, 38). Endothelium-derived relaxing factor (EDRF), discovered by Furchgott and Zawadski in 1980 (10), is a labile humoral factor that is synthesized and released from the vascular endothelium. Recent studies have suggested that the biological and physiochemical properties of nitric oxide are indistinguishable from those of EDRF (17, 18, 27) and that nitric oxide in part mediates the vasodilatation associated with endothelium-dependent vasodilators such as acetylcholine. However, other nitrosothiols have also been demonstrated to have properties analogous to EDRF (25). Although EDRF plays an important role in mediating vasodilation, the role of EDRF in the basal regulation of cardiopulmonary and renal hemodynamic function in vivo is unclear.

The physiological precursor for the formation of EDRF is L-arginine (12, 26, 28, 32). In a recent study, Rees et al. (30) inhibited EDRF generation in vivo in rabbits with the L-arginine analogue Nω-monomethyl-L-arginine (L-NMMA), which is a competitive inhibitor of EDRF synthesis from L-arginine (29). These investigators reported that an acute intravenous bolus of L-NMMA resulted in a dose-dependent increase in arterial pressure, supporting the conclusion that EDRF may play a role in the regulation of blood pressure. Similar results by Aisaka et al. (1) have been obtained in guinea pigs. Tolins et al. (35) extended this concept in rats and demonstrated that an intravenous bolus of L-NMMA increases renal vascular resistance (RVR) and arterial pressure, suggesting that the release of EDRF may play a role in the basal regulation of arterial pressure and renal hemodynamics. Although these latter studies suggest an important role for EDRF in the regulation of renal blood flow (RBF), its role in the regulation of glomerular filtration rate (GFR) and sodium excretion in overall volume and arterial pressure homeostasis has not been assessed.

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METHODS

Surgical Preparation

Experiments were conducted on two groups of normal anesthetized dogs (n = 6 dogs/group) weighing 16–21 kg. The dogs were fasted overnight 16 h before the acute
experiment and were allowed to drink water ad libitum until the time of the experiment. Dogs were anesthetized with pentobarbital sodium (30 mg/kg iv), and supplemental doses were given as necessary to maintain anesthesia. The dogs were then intubated and artificially ventilated with room air at a tidal of 15 ml/kg and a rate to maintain physiologically normal blood gases (Harvard respirator, Harvard Apparatus, Millis, MA).

Surgical preparation in the acute experiment was as follows. The right external jugular vein was cannulated with a flow-directed balloon-tip thermomilization catheter (95A-1317F, American Edwards Laboratory, Anasco, PR) and advanced into the pulmonary artery for measurement of cardiac filling pressures and determination of cardiac output (CO). The right femoral artery was cannulated for measurement of arterial pressure and sampling of arterial blood, and femoral veins were cannulated for infusion of inulin, L-NMMA, or supplemental anesthetic. The left kidney was exposed via a flank incision, and the ureter was cannulated for timed urine collections. An electromagnetic flow probe (Carolina Medical Electronics, King, NC) was placed on the left renal artery for on-line monitoring of RBF.

Experimental Protocol

On completion of the surgical preparation, an inulin-saline solution was infused via a femoral vein catheter at 1 ml/min to achieve a plasma inulin concentration of ~50 mg/dl. The dogs were then allowed to stabilize for 60 min without intervention. At the end of the equilibration period, a 20-min baseline clearance was obtained. Each clearance period consisted of a 20-min urine collection, measurement of hemodynamic parameters (midpoint of each clearance period), and withdrawal of 25 ml of arterial blood for hormone analysis and electrolyte determination.

After the preparation, equilibration period, and a baseline 20-min clearance period, the study was initiated. The remainder of the protocol differed between the two experimental groups. L-NMMA group. L-NMMA, a competitive inhibitor of EDRF generation, was infused for 70 min at a continuous rate (50 μg·kg⁻¹·min⁻¹ iv). After a 30-min lead-in, two 20 min clearances were performed, and their results were averaged to assess the effect of L-NMMA. At the end of the L-NMMA infusion, L-arginine was administered (300 mg/kg iv), and hemodynamics were assessed immediately.

Vehicle group. A vehicle (normal saline iv) was infused at the same rate and duration as L-NMMA in the previous group. Clearances were performed as in the L-NMMA group to serve as a cardiopulmonary and renal time control.

Analyses

Blood for plasma sodium and inulin measurements was placed in heparinized tubes on ice, centrifuged at 2,500 revolutions/min and 3°C, and refrigerated pending analysis. Plasma and urinary sodium concentrations were measured by use of ion-selective electrodes (Beckman Instruments, Brea, LA). Plasma and urinary inulin concentrations were determined by the anthrone method (8). Blood for hormone assay was placed in EDTA tubes and immediately placed on ice. After centrifugation at 2,500 revolutions/min and 3°C, the plasma was decanted and stored at −20°C until analysis. Plasma ET and guanosine 3',5'-cyclic monophosphate (cGMP) were measured by specific radioimmunoassay techniques (15, 34). Urinary cGMP was also measured by radioimmunoassay. Hemodynamic data included mean arterial pressure (MAP), right atrial pressure (RAP), pulmonary arterial wedge pressure (PAWP), RBF, and CO. CO was measured by the thermodilution technique and determined via the average of four measurements. Calculated parameters included 1) systemic vascular resistance (SVR = MAP - RAP/C0), 2) pulmonary vascular resistance (PVR = PAP - PAWP/C0), 3) RVR (RVR = MAP - RAP/RBF), and 4) GFR as determined by inulin clearance (GFR = U_inulin X V_ureine/P_inulin).

Statistics

For each experimental group, data from baseline and all clearance periods were measured and expressed as means ± SE. All data were assessed by Student’s paired t test for comparisons of absolute changes within each group and Student’s unpaired t tests for percent changes between groups. Statistical significance was accepted for P < 0.05.

RESULTS

The cardiopulmonary and renal data for the two experimental groups are summarized in Tables 1 and 2. The hemodynamic responses to L-NMMA or vehicle in the two groups are depicted by percent change from baseline in Fig. 1.

Cardiopulmonary and Renal Hemodynamic Function

In the L-NMMA group, no increase in MAP was observed despite a modest decrease in heart rate (138 ± 2 to 127 ± 4 beats/min, P < 0.05), and CO decreased from baseline. SVR, PVR, and RVR all significantly increased during L-NMMA (Table 1). In the vehicle group, no significant changes occurred in MAP, CO, SVR, PVR, or RVR. No differences were observed in baseline hemodynamic parameters between the two

<table>
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<tr>
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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>L-NMMA</td>
<td>Baseline</td>
<td>Vehicle</td>
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<tr>
<td>MAP, mmHg</td>
<td>119±1</td>
<td>124±3</td>
<td>133±6</td>
<td>133±7</td>
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<tr>
<td>CO, l/min</td>
<td>3.4±0.3</td>
<td>2.6±0.2*</td>
<td>4.2±0.2</td>
<td>3.8±0.3</td>
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<tr>
<td>SVR, mmHg·l⁻¹·min⁻¹</td>
<td>35.1±2.7</td>
<td>49.1±4.4*</td>
<td>31.6±2.2</td>
<td>36.4±3.6</td>
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<tr>
<td>PVR, mmHg·l⁻¹·min⁻¹</td>
<td>2.95±0.13</td>
<td>4.17±0.33*</td>
<td>2.19±0.18</td>
<td>2.42±0.20</td>
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<tr>
<td>RVR, mmHg·l⁻¹·min⁻¹</td>
<td>0.65±0.04</td>
<td>0.78±0.07*</td>
<td>0.67±0.12</td>
<td>0.68±0.12</td>
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</tbody>
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Values are means ± SE; n = 6 dogs/group. EDRF, endothelium-derived relaxing factor; l-NMMA, N⁵-monomethyl-L-arginine; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; RVR, renal vascular resistance. * P < 0.05 change from baseline.
CARDIOPULMONARY AND RENAL RESPONSE TO EDRF INHIBITION

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TABLE 2. Renal excretory and hemodynamic function in presence and absence of EDRF inhibition

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>GFR, ml/min</td>
<td>37.8±4.4</td>
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<tr>
<td>RBF, ml/min</td>
<td>194±13</td>
</tr>
<tr>
<td>V, ml/min</td>
<td>0.37±0.06</td>
</tr>
<tr>
<td>U_{Na}V, μEq/min</td>
<td>62.1±13.1</td>
</tr>
<tr>
<td>FENa, %</td>
<td>1.13±0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 dogs/group. GFR, glomerular filtration rate; RBF, renal blood flow; V, urine flow; U_{Na}V, absolute urinary sodium excretion; FENa, fractional urinary sodium excretion. * P < 0.05 change from baseline.

groups. In contrast, the changes in SVR, PVR, and RVR during L-NMMA were markedly greater than in the vehicle group.

Renal Hemodynamic and Excretory Function

In the L-NMMA group, a significant decrease in RBF was observed. No changes in GFR, urine flow (V), or urinary absolute (U_{Na}V) or fractional (FENa) sodium excretion were evident during L-NMMA (Table 2). No alterations in renal hemodynamic or excretory parameters occurred in the vehicle group.

Hormonal Response

No change was evident in plasma cGMP (7.8 ± 1.7 to 7.5 ± 2.4 pmol/ml, NS) or urinary cGMP excretion (U_{cGMP}V; 590 ± 123 to 523 ± 124 pmol/min, NS) during L-NMMA. Infusion of L-NMMA was associated with a modest increase in plasma ET (7.9 ± 1.3 to 10.2 ± 1.8 pg/ml, P < 0.05). These hormonal parameters were not altered by the vehicle infusion (Fig. 2).

L-Arginine Response

In a subset of the L-NMMA group (n = 4), dogs were given L-arginine to reverse the L-NMMA effects. L-Arginine significantly decreased MAP (126 ± 2 to 114 ±

FIG. 1. Cardiopulmonary and renal hemodynamic response to endothelium-derived relaxing factor (EDRF) inhibition. Percent change (%Δ) of mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), and renal vascular resistance (RVR) in presence of N^6-monomethyl-L-arginine (L-NMMA; 50 μg·kg^{-1}·min^{-1} iv) or vehicle (normal saline iv). * P < 0.05, L-NMMA vs. vehicle. NS, not significant.

FIG. 2. Hormonal response to EDRF inhibition. Absolute changes in plasma (PL cGMP) or urinary (U_{cGMP}V) guanosine 3',5'-cyclic monophosphate and plasma endothelin (PL ET) in presence of L-NMMA (50 μg·kg^{-1}·min^{-1} iv) or vehicle (normal saline iv). * P < 0.05, L-NMMA vs. vehicle.
4 mmHg, \( P < 0.05 \), whereas CO tended to decrease, although not significantly (2.64 \pm 0.29 to 2.56 \pm 0.36 \text{l/min, NS}). SVR, PVR, and RVR (Fig. 3) all returned to levels not different from baseline (SVR 49.2 \pm 6.7 to 46.5 \pm 7.3 \text{mmHg}\cdot\text{min}^{-1}\cdot\text{l}^{-1}, \text{PVR} 4.28 \pm 0.51 to 3.71 \pm 0.18 \text{mmHg}\cdot\text{min}^{-1}\cdot\text{l}^{-1}\), and RVR 0.80 \pm 0.11 to 0.62 \pm 0.06 \text{mmHg}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}.

**DISCUSSION**

The present study demonstrates in vivo the cardiopulmonary and renal responses to the inhibition of basal EDRF generation in normal anesthetized dogs. A continuous low-dose infusion of L-NMMA, a competitive inhibitor to EDRF generation, produced no significant increase in MAP. Previous studies have shown a pressor response to EDRF inhibition, although the L-NMMA in these previous studies was administered as a bolus and in higher doses (1, 30). In the current study, SVR, PVR, and RVR all increased from baseline with L-NMMA and demonstrated an exaggerated increase in vascular resistance compared with the vehicle group. The administration of L-NMMA produced a decrease in RBF from baseline, and this change was significant compared with the vehicle group. Despite such significant renal vasoconstriction, inhibition of basal EDRF did not decrease urinary sodium or water excretion in the overall regulation of volume and arterial pressure homeostasis. These actions of L-NMMA were not associated with alterations in plasma cGMP or \( U_{\text{cGMP} V} \). Associated with the inhibition of EDRF generation, a modest increase in plasma ET was observed.

Previous studies have demonstrated in vivo a dose-dependent increase in arterial pressure when L-NMMA is administered as an intravenous bolus (1, 30). These previous studies suggest that EDRF plays a role in the regulation of arterial pressure. L-NMMA has also been shown in vivo to inhibit the hemodynamic effects of acetylcholine, an endothelium-dependent vasodilator that acts through the release of EDRF-nitric oxide (35). Studies have assessed the role of EDRF in isolated vascular beds (2-4, 11, 13, 19, 36). These studies have shown that EDRF plays a role in regulating vascular tone in coronary, renal, mesenteric, and peripheral resistance blood vessels. However, the cardiopulmonary and renal hemodynamic changes that occur in vivo after inhibition of basal EDRF synthesis have not been elucidated.

In the current study, EDRF synthesis was inhibited in vivo by a continuous steady state infusion of L-NMMA, and the contribution of EDRF to the basal regulation of systemic, pulmonary, and renal hemodynamic function was assessed. MAP did not increase significantly in response to a low-dose infusion of L-NMMA. CO decreased with the L-NMMA infusion, although this decrease in CO from baseline was not different from a vehicle infusion. The most significant changes occurred in vascular resistance. Increases in vascular resistance with EDRF inhibition occurred not only systemically but also in regional pulmonary and renal vascular beds. The administration of L-arginine, the physiological precursor to EDRF, caused a decrease in MAP and returned vascular resistance to levels not different from baseline. Thus the role of endogenous EDRF in the basal regulation of the circulation may be related more to the regulation of regional vascular tone than to the regulation of arterial pressure. In addition, the basal production of EDRF may contribute to the low vascular tone of the pulmonary circulation under the basal condition.

In vitro studies have shown that EDRF promotes vasorelaxation by stimulating soluble guanylate cyclase and thus increasing intracellular cGMP in vascular smooth muscle cells (23, 24). Tolins and colleagues (35) have also suggested that \( U_{\text{cGMP} V} \) may be a biological marker for EDRF activity in vivo. In this study by Tolins et al. (35) and in a recent study by Lahera et al. (21), the renal effects of the endothelium-dependent vasodilator acetylcholine were prevented by L-NMMA. These previous studies are consistent with EDRF acting as the mediator of the renal hemodynamic and diuretic effects of acetylcholine. In the present study, L-NMMA produced a decrease in RBF that was not observed in the vehicle group. Despite this decrease in RBF, no alterations in GFR, \( V \), \( U_{\text{Na} V} \), or \( F \text{Ed}_{\text{Na}} \) were observed with EDRF inhibition. Associated with this renal response to L-NMMA, \( U_{\text{cGMP} V} \) did not change significantly, suggesting that \( U_{\text{cGMP} V} \) may be a relatively insensitive marker for basal EDRF activity perhaps because of the poor

![Graph](http://api.regu.physiology.org/) by 10.2203.2247 on July 6, 2017
cellular egression of cGMP after soluble guanylate cyclase stimulation (33). However, with higher doses of L-NMMA or in a state of increased EDRF generation (33), U_{GMP}V may better assess EDRF activity. The current study thus supports the conclusion that basal EDRF is an important endogenous regulator of renal hemodynamic function, but it is not important in the tubular regulation of sodium excretion.

Boulander and Lüscher (5) have recently demonstrated in vitro that EDRF may inhibit the release of ET. In the present study, the infusion of L-NMMA was associated with a modest increase in plasma ET. This may suggest that the cardiopulmonary and renal responses noted with the L-NMMA infusion may be attributed not only to EDRF inhibition but to an imbalance between basal endothelium-derived relaxing and contracting factors.

The current study may have pathophysiological relevance. Evidence suggests that abnormalities in EDRF generation may contribute to the pathophysiology of essential hypertension, atherosclerosis, and congestive heart failure (1, 14, 16, 30, 37). These disease states are also associated with increased plasma ET concentrations (7, 20, 22, 31). One may speculate that in these pathophysiological conditions, alterations in EDRF as well as endogenous vasoconstrictors such as ET contribute to increases in vascular tone. Thus an imbalance of these endothelium-derived vasoactive substances may modulate SVR and RVR.

In summary, this study demonstrates the role of EDRF in regulating basal cardiopulmonary and renal function by using a competitive inhibitor to EDRF generation, L-NMMA. A low-dose steady-state infusion of L-NMMA produced no change in MAP but significant alterations in vascular resistance. SVR, PVR, and RVR all increased compared with baseline, and the increases in resistances with L-NMMA were exaggerated compared with a vehicle infusion. Although the L-NMMA infusion produced a decrease in RBF, no significant changes were noted in GFR, V, U_{Na}V, F_{Eina}, or U_{GMP}V. Associated with the inhibition of EDRF, plasma ET increased modestly, suggesting that an imbalance of endothelium-derived relaxing and contracting factors may have contributed to the observed physiological responses. The current study, by utilizing an in vivo integrative approach, supports an important role of EDRF in regulating cardiopulmonary and renal physiological function in the normal anesthetized dog.

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