Renal Vasodilatory Effect of Endothelial Stimulation in Patients With Chronic Congestive Heart Failure

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Objectives. This study sought to examine the vasodilatory response of the renal circulation to endothelial stimulation in patients with chronic heart failure.

Background. Renal blood flow is often reduced in patients with chronic congestive heart failure and may lead to deterioration of renal function. Stimulation of renal endothelium has been shown to cause renal vasodilation in animals and in isolated human renal artery. The vasoregulatory role of the renal endothelium in patients with heart failure has not been evaluated.

Methods. Renal vasodilatory effect of endothelial stimulation with acetylcholine was assessed and compared with that of endothelial-independent vasodilation with nitroglycerin. Both drugs were infused into the main renal artery. Renal artery cross-sectional area was measured with intravascular ultrasound and renal blood flow velocity with the aid of an intravascular Doppler technique.

Results. Both drugs caused a significant and comparable increase in renal artery cross-sectional area (maximal increase [mean ± SE] 14 ± 5% with acetylcholine, 15 ± 5% with nitroglycerin; both values <0.05 vs. baseline). Acetylcholine also caused a significant reduction in renal vascular resistance (maximal reduction 55 ± 6%) and increase in renal blood flow (maximal increase 136 ± 54%). In contrast, nitroglycerin administration showed no significant effect on renal vascular resistance and blood flow.

Conclusions. Stimulation of endothelium-derived nitric oxide with acetylcholine results in a significant vasodilatory effect on both conductance and resistance blood vessels and leads to a marked reduction in renal vascular resistance and enhancement of renal blood flow. Nitroglycerin, an exogenous nitric oxide donor, caused a selective vasodilator effect on conductance but not on resistance blood vessels and failed to increase renal blood flow. These data suggest the possibility that stimulation of endogenous nitric oxide production in the kidney could be used as a therapeutic target for enhancement of renal flow in patients with heart failure.

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The study included patients with chronic New York Heart Association class III or IV heart failure who underwent diagnostic cardiac catheterization for evaluation and possible treatment of heart failure. The etiology of heart failure was coronary artery disease in four patients and unknown in eight. All patients had severe depression of left ventricular systolic function as determined by contrast or radionuclide ventriculography or by echocardiography (three patients). Left ventricular ejection fraction as determined in nine patients ranged from 14% to 32% (mean 24 ± 2%). Mean hemodynamic values as measure during cardiac catheterization were as follows: heart rate 86 ± 5 beats/min, mean blood pressure 92 ± 4 mm Hg, mean right atrial pressure 8 ± 1 mm Hg, mean pulmonary artery pressure 31 ± 3 mm Hg, mean pulmonary artery wedge pressure 20 ± 2 mm Hg, cardiac index 3.3 ± 0.2 liters/min per m², stroke volume index 39 ± 4 ml/min per m², and systemic vascular resistance 1,158 ± 721 dynes·cm⁻². All patients signed a consent form approved by the research committee of the Los Angeles County/University of Southern California Medical Center.

Study protocol. Organic nitrates (six patients) were discontinued at least 24 h before the study to prevent attenuation of nitroglycerin effect during the study due to nitrate tolerance (18); other medications (diuretics in all patients, digoxin in 11 and angiotensin-converting enzyme inhibitors in 10) were withheld on the day of the study. Premedication before cardiac catheterization included intravenous metoclopramide (10 mg) and benadryl (50 mg). During cardiac catheterization and the study, patients received 1.0 to 2.0 mg of intravenous midazolam hydrochloride for sedation. Nitroglycerin, either sublingual, intravenous or intracoronary, was not allowed during cardiac catheterization and until the end of the study. After completion of diagnostic cardiac catheterization, a triple-lumen Swan-Ganz catheter was used for the performance of right heart catheterization (19) and Doppler blood flow velocimetry. Nitroglycerin effect during the study due to nitrate tolerance was confirmed with a small amount (~5 ml) of angiographic contrast medium (Hexibrix, Mallinkrodt Medical). A 0.018-in. (0.045 cm) Doppler guide wire (Flowire, Cardiometrics, Inc.) was then introduced into the guiding catheter through a valved sidearm connector, and its tip was positioned under direct visualization in the proximal main artery and maintained in a parallel position to flow. A commercially available 3.5F or 4.3F intravascular ultrasound catheter (Mansfield, Boston Scientific Corporation) was then placed through the guiding catheter and was positioned under fluoroscopic guidance next to the Doppler wire (Fig. 1). Images were obtained with a commercially available intracoronary ultrasound imaging system (SonoIntravascular, Hewlett-Packard) at 30 frames/s and recorded on 0.5-in. high quality super VHS videotape for subsequent off-line analysis. Arterial and venous pressure data was recorded from the taped images, and cross-sectional area was determined with specially designed software. The spectral Doppler renal blood flow velocities were recorded. The time-integral of Doppler spectral velocities was measured. The time-integral velocity was measured as the area under the outermost portion of the spectral velocity envelope. To correct for changes related to respiration and cardiac cycle, 15 to 30 beats were used for measurements of both renal artery cross-sectional area and Doppler velocity time integral, and average values are reported. Renal blood flow (in ml/min) was calculated as the product of the cross-sectional area of the renal artery and the velocity time integral using the following formula: Flow = Heart rate per minute x Velocity-time integral x Cross-sectional area. Renal blood flow index was calculated for each patient by dividing renal blood flow by body surface area. Renal vascular resistance was calculated as follows: 80 (Mean renal artery blood pressure - Mean renal vein pressure) Renal blood flow.

Renal venous and arterial pressures were measured directly with the aid of the venous and arterial catheters, fluid-filled pressure tubing and standard transducers. Infusion of drugs was done through the arterial catheter directly into the renal artery.

Drugs. Acetylcholine (Miochol, Iolab Pharmaceuticals) was mixed in 5% dextrose in water to achieve a blood concentration of 10⁻⁸, 10⁻⁷ and 10⁻⁶ mol/liter, assuming an

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**Figure 1.** A, Verification of position of guiding catheter in the proximal part of the main renal artery by a small amount of contrast medium. B, Positions of the various equipment used for measurements: 1 = guiding catheter positioned in the proximal main renal artery; 2 = Doppler flow wire; 3 = tip of intravascular ultrasound catheter; 4 = fluid-filled catheter in the ipsilateral renal vein.
Table 1. Effects of Acetylcholine and Nitroglycerin on Systemic and Renal Hemodynamic Variables in 12 Patients With Chronic Heart Failure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acetylcholine (mean ± SE)</th>
<th>Nitroglycerin (mean ± SE)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (mean ± SE)</td>
<td>10^{-9} mol/liter</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>89 ± 5</td>
<td>68 ± 5</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>91 ± 4</td>
<td>88 ± 4</td>
</tr>
<tr>
<td>RVP (mm Hg)</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>0.27 ± 0.03</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>VTI (cm)</td>
<td>22 ± 3</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>RBF (ml/min per m²)</td>
<td>289 ± 25</td>
<td>497 ± 58</td>
</tr>
</tbody>
</table>

CSA = main renal artery cross-sectional area; HR = heart rate; RAP = mean renal artery blood pressure; RBF = renal blood flow; RVP = mean renal vein pressure; RVR = renal vascular resistance; VTI = velocity-time integral.

average renal blood flow in patients with chronic heart failure of 600 ml/min (4-6). Nitroglycerin (Baxter Healthcare Corporation) was infused at a rate calculated to achieve blood concentrations of 10^{-7}, 10^{-6} and 10^{-5} mol/liter. Both drugs were drawn from hospital formulary stacks and prepared <60 min before their use. The drugs were infused at a rate of 2 ml/min controlled by a volumetric pump for approximately 3 to 5 min for each concentration into the renal artery. Similar concentrations of both acetylcholine and nitroglycerin were used in previous studies evaluating endothelial function of coronary and peripheral arterial circulation (19,20).

Baseline hemodynamic variables, intravascular ultrasound and Doppler flow velocity were measured after 3 to 5 min of intraarterial infusion of 5% dextrose in water. After baseline measurements, patients were randomized to receive either acetylcholine (six patients) or nitroglycerin infusion (six patients) as the first drug. Hemodynamic measurements and intravascular ultrasound and Doppler flow velocity recordings were repeated during the last minute of infusion of each drug concentration. Ten to 15 minutes was then allowed for washout of the first drug and was followed by a second baseline measurement and the administration of the second drug using the identical protocol.

Data analysis. An analysis of variance for repeated measurements was used to determine a statistical difference between the absolute values as well as percent differences from baseline. In case of a difference, the Newman-Keuls test was applied. Data were analyzed for the entire group of 12 patients. To detect any carry over effect related to the crossover design of the study (21), the same analysis was performed for the two subgroups of six patients who received either acetylcholine or nitroglycerin as their first drug. All results are expressed as mean value ± SE; a p value ≤0.05 was considered statistically significant.

Results

Data group analysis for all 12 patients studied. Renal artery cross-sectional area. The main renal artery cross-sectional area was 0.37 ± 0.03 cm² at baseline before administration of acetylcholine and 0.35 ± 0.03 cm² before nitroglycerin (Table 1); the difference was not statistically significant. Both acetylcholine and nitroglycerin resulted in a dose-dependent, small but statistically significant, increase in renal artery cross-sectional area (Fig. 2 and 3). Maximal increase in this variable was 14 ± 5% for acetylcholine and 15 ± 5% for nitroglycerin. The difference between these two interventions was not statistically significant.

Velocity-time integral. There was no significant difference between baseline values of the velocity-time integral before administration of both drugs (22 ± 3 vs. 24 ± 3 cm). A significant and dose-dependent increase in velocity-time integral was found during acetylcholine infusion (Table 1, Fig. 3). The velocity-time integral increased 61 ± 10% during infusion of 10^{-8} mol/liter acetylcholine (p < 0.01 vs. baseline) and continued to increase during 10^{-7} and 10^{-6} mol/liter (99 ± 15% and 120 ± 18%, p < 0.05 vs. 10^{-8} mol/liter for both). In contrast, the effect of nitroglycerin on the velocity-time integral was small (Table 1, Fig. 4) and did not achieve statistical significance.

Renal blood flow. As shown in Table 1, there was no significant difference between baseline values of renal blood flow.
Figure 3. Renal artery cross-sectional area (top) and Doppler flow velocity (m/s) (bottom) at baseline and during infusion of graded doses of acetylcholine into the renal artery in one of the study patients. Reported values of cross-sectional area are average of 15 to 30 beats.

Flow as measured before administration of both acetylcholine and nitroglycerin (289 ± 25 vs. 311 ± 23 ml/min per m²). A significant and dose-related increase in calculated renal blood flow was found during acetylcholine infusion (Fig. 5). Renal blood flow increased 67 ± 12% during 10⁻⁸ mol/liter acetylcholine (p < 0.05 vs. baseline), 122 ± 17% during 10⁻⁷ mol/liter acetylcholine and 145 ± 19% during 10⁻⁶ mol/liter acetylcholine (both p < 0.05 vs. 10⁻⁸ mol/liter). In contrast to the significant effect of acetylcholine on renal blood flow, intraarterial infusion of nitroglycerin at all three doses (Fig. 5) demonstrated only a small and statistically insignificant change in renal blood flow (13 ± 6%, 16 ± 8% and 16 ± 6%, respectively).

Heart rate and blood pressures. No significant changes were noted in values of heart rate and renal arterial and venous blood pressures during the intrarenal infusion of both acetylcholine and nitroglycerin (Table 1).

Renal vascular resistance. Calculated baseline values of renal vascular resistance were comparable (11,171 ± 1,295 dynes·cm⁻⁵ for acetylcholine, 10,275 ± 931 dynes·cm⁻⁵ for nitroglycerin) (Table 1) during acetylcholine infusion. Renal vascular resistance was reduced 39 ± 5% during 10⁻⁸ mol/liter acetylcholine, 52 ± 7% during 10⁻⁷ mol/liter acetylcholine and 55 ± 6% during 10⁻⁶ mol/liter acetylcholine (all p < 0.05 vs. baseline) (Fig. 6). In contrast, intrarenal infusion of graded doses of nitroglycerin resulted in a small but statistically insignificant decrease (6 ± 6%, 4 ± 10% and 12 ± 6%, respectively).

Subgroup analysis. To detect any carry over effect due to the crossover design of the study, a separate analysis was performed for the two subgroups of six patients, each receiving either acetylcholine or nitroglycerin as the first drug. A comparison of baseline systemic and renal hemodynamic values in the two subgroups of six patients receiving either acetylcholine or nitroglycerin as their first drug demonstrated similar values of heart rate, mean renal arterial blood pressure, Doppler velocity-time integral, renal blood flow and renal vascular resistance. However, mean value of renal artery cross-sectional

Figure 4. Renal artery cross-sectional area (top) and Doppler flow velocity (m/s) (bottom) at baseline and during infusion of graded doses of nitroglycerin into the renal artery in one of the study patients. Reported values of cross-sectional area are average of 15 to 30 beats.
area was larger in the nitroglycerin group (0.40 ± 0.05 vs. 0.28 ± 0.04 cm², p < 0.05).

Heart rate showed no significant change during the infusion of both drugs at all concentrations (Tables 2 and 3). Mean renal arterial blood pressure showed a small but statistically significant decline during administration of nitroglycerin at concentrations of 10⁻⁷ mol/liter and remained unchanged during acetylcholine administration. Both drugs resulted in a dose-related increase in mean renal artery cross-sectional area. Although increase in absolute values of cross-sectional area did not reach statistical significance during acetylcholine administration, a maximal increase in this variable was similar for both drugs (20 ± 10% for 10⁻⁶ mol/liter acetylcholine, 19 ± 10% for 10⁻⁵ mol/liter nitroglycerin, p = NS). Renal vascular resistance demonstrated a significant decrease during acetylcholine administration that was associated with a marked and statistically significant increase in Doppler velocity-time integral and calculated renal blood flow. In contrast, these three variables showed no significant change during nitroglycerin infusion.

Discussion

The present study provides to the best of our knowledge a first evaluation of the renal circulatory response to endothelial stimulation in patients with chronic congestive heart failure. The results of the study demonstrate a marked vasodilatory effect of acetylcholine, resulting in a significant increase in cross-sectional area of the main renal artery and a substantial reduction in renal vascular resistance, accompanied by a marked augmentation of renal blood flow. These findings support previously published reports demonstrating acetylcholine-mediated renal vasodilatory effects in vitro and in vivo animal experiments (10–16,22–25) and in isolated human renal artery (17).

The vasodilatory effect of acetylcholine on the renal circulation is likely to be mediated by endothelial stimulation of endogenous nitric oxide production. This mechanism is supported by a similar relaxing effect of acetylcholine on human and animal renal arteries with but not in those without endothelium (17,26) and previously reported inhibition of renal hemodynamic effects of acetylcholine with N⁵-monomethyl-L-arginine, a specific nitric oxide inhibitor, in animal experiments (23,24,27,28). Endothelial-derived nitric oxide stimulates the enzyme guanylate cyclase, which leads to accumulation of cyclic guanosine monophosphate in the vascular smooth muscle, which results in vasorelaxation (30). Tolins et al. (29) evaluated the role of nitric oxide in regulation of renal hemodynamic responses in the anesthetized rat in vivo and demonstrated a dose-related relation between the hemodynamic effect of acetylcholine and rates of cyclic guanosine monophosphate excretion, providing further evidence for acetylcholine-induced endothelial-derived nitric oxide synthesis as the mechanism of renal vasodilation.

Table 2. Effect of Acetylcholine Given as First Drug in Six Patients

<table>
<thead>
<tr>
<th>Drug Concentration</th>
<th>HR (beats/min)</th>
<th>RAP (mm Hg)</th>
<th>CSA (mm²)</th>
<th>VTI (cm)</th>
<th>RBF (ml/min per m²)</th>
<th>RVR (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>89 ± 6</td>
<td>90 ± 5</td>
<td>0.28 ± 0.04</td>
<td>26 ± 5</td>
<td>278 ± 43</td>
<td>10,567 ± 1,926</td>
</tr>
<tr>
<td>ACH 10⁻⁶ mol/liter</td>
<td>91 ± 7</td>
<td>89 ± 7</td>
<td>0.29 ± 0.04</td>
<td>38 ± 4*</td>
<td>480 ± 56*</td>
<td>6,617 ± 1,027*</td>
</tr>
<tr>
<td>ACH 10⁻⁵ mol/liter</td>
<td>93 ± 7</td>
<td>88 ± 7</td>
<td>0.30 ± 0.04</td>
<td>48 ± 6*</td>
<td>648 ± 62*</td>
<td>4,835 ± 485*</td>
</tr>
<tr>
<td>ACH 10⁻⁴ mol/liter</td>
<td>93 ± 7</td>
<td>88 ± 8</td>
<td>0.32 ± 0.03</td>
<td>47 ± 6*</td>
<td>654 ± 85*</td>
<td>4,355 ± 243*</td>
</tr>
</tbody>
</table>

*p < 0.05 versus baseline. +p < 0.05 versus acetylcholine (ACH) 10⁻⁶ mol/liter. Data presented are mean value ± SE. Other abbreviations as in Table 1.
Nitroglycerin is an organic nitrate with potent vasodilatory activity. The therapeutic effect of this drug occurs after intracellular conversion to nitric oxide, which activates the enzyme guanylate cyclase, which leads to the production of cyclic guanosine monophosphate and vasodilation (29–32). Despite the shared properties of endothelium-derived nitric oxide and organic nitrates, the effects of acetylcholine and nitroglycerin on the renal circulation were different in the present study. Whereas the intrarenal administration of nitroglycerin resulted in a selective vasodilatory effect on the main renal artery, it had only limited and statistically insignificant effect on renal vascular resistance and renal blood flow, indicating a selective effect on large conductance vessels but lack of effect on small resistance blood vessels in the kidney. In contrast, acetylcholine increased renal blood flow and renal vascular resistance, demonstrating its vasodilatory effect on both large conductance and small resistance renal blood vessels. The effect of acetylcholine on resistance blood vessels in the kidney has also been reported (33) in animal studies that showed a potent dilation of large, but only minimal effects on small, coronary microvessels. Lack of available sulfhydryl groups was suggested as the cause for the heterogenous response of coronary microvascular response to nitroglycerin (35).

The reason for the difference in the effect of acetylcholine, a stimulator of endogenous nitric oxide production, and nitroglycerin, an exogenous nitric oxide donor, is not clear. However, failure of systemic administration of nitroglycerin to augment renal blood flow measured by other methods has previously been demonstrated in both animals and humans with heart failure (1,5,33). In addition, a similar selective effect of nitroglycerin was demonstrated by Selke et al. (34), who showed a potent dilation of large, but only minimal effects on small, coronary microvessels. Lack of available sulfhydryl groups was suggested as the cause for the heterogenous response of coronary microvascular response to nitroglycerin (35).

Several investigators have reported impaired endothelium-dependent vasodilator function in patients with heart failure (18,20,36). Assessment of the coronary as well as the peripheral circulatory response to acetylcholine administration was shown to be diminished. The results of the present study clearly indicate a strong endothelial response in the renal circulation in patients with heart failure and should provide a basis for further evaluation of the therapeutic implications of renal endothelial stimulation for improvement of renal function in patients with chronic congestive heart failure.

Study limitations. The present study was conducted in a randomized, crossover manner. Because of patient considerations, a washout period in between infusion of the two study drugs was limited to 10 to 15 min. Although a similar washout period has been used in previous studies (21), a carryover effect with at least partial persistence of the therapeutic effect of the first drug during the administration of the second cannot be ruled out (Fig. 3 and 4). For this reason, an additional analysis of the effect of each of the study drugs in six of the patients who received it first was performed. The results of this analysis demonstrated similar results to those obtained in all 12 patients and confirmed the validity of the study findings. In addition, all patients in the present study were treated with standard heart failure therapy, which could have affected endothelial function.

Summary. Our study demonstrates a strong renal vasodilatory effect of acetylcholine, which results in a substantial decrease in renal vascular resistance and augmentation of renal blood flow. This effect is likely to be due to the stimulatory effect of acetylcholine on endothelial production of endogenous nitric oxide. Intrarenal administration of nitroglycerin, an exogenous nitric oxide donor, could not reproduce the acetylcholine effect. This drug caused a similar dilation of the main renal artery but failed to significantly affect either renal vascular resistance or renal blood flow. These findings indicate a selective vasodilatory effect of nitroglycerin, an exogenous nitric oxide donor, on renal conductance but not on resistance blood vessels. In contrast, stimulation of endothelium-derived nitric oxide with acetylcholine results in a significant vasodilation of both conductance and resistance vessels. The results of the present study therefore raise the possibility of renal endothelial stimulation and endogenous nitric oxide production as a potential therapeutic target for enhancement of renal flow in patients with chronic congestive heart failure.

We thank the nurses, technicians and cardiology fellows of the Cardiac Catheterization Laboratory of the Los Angeles County/University of Southern California Medical Center for their assistance in the performance of this study.

References


