Intrarenal infusion of acetylcholine in meclofenamate-treated dogs significantly increased renal blood flow, diuresis, and natriuresis. Intrarenal infusions of either \(N^G\)-monomethyl-L-arginine (inhibitor of endothelium-derived relaxing factor formation), or L-arginine (precursor of endothelium-derived relaxing factor formation) did not modify basal levels of those parameters. However, the infusion of \(N^G\)-monomethyl-L-arginine inhibited the acetylcholine-induced increases in renal blood flow and diuresis without affecting natriuresis, which increased significantly. The infusion of L-arginine failed to further enhance hemodynamic and excretory effects elicited by acetylcholine. The concomitant infusion of L-arginine and \(N^G\)-monomethyl-L-arginine did not change renal blood flow, urine flow, or sodium excretion rate. L-Arginine administration prevented the inhibitory effect of \(N^G\)-monomethyl-L-arginine on acetylcholine-induced renal vasodilatation and diuresis. Glomerular filtration rate and mean arterial pressure did not change throughout the experiment. The results indicate that the vasodilatory and diuretic responses to intrarenal acetylcholine in meclofenamate-treated dogs are largely dependent on endothelium-derived relaxing factor. (Hypertension 1990;15:659–663)

It is well known that the in vitro vascular relaxation induced by acetylcholine (Ach) results from the release of a diffusible relaxing substance, termed endothelium-derived relaxing factor.\(^1\) Experimental evidence obtained in these in vitro preparations demonstrates that the endothelium-derived relaxing factor is the free radical nitric oxide.\(^2,3\) Nitric oxide is synthesized from the amino acid L-arginine (L-Arg),\(^4\) and this synthesis is competitively inhibited by \(N^G\)-monomethyl-L-arginine (LNMMA).\(^5\) The role of nitric oxide in blood pressure regulation has been studied using LNMMA and L-Arg in guinea pigs and rabbits.\(^6,7\) However, the importance of these actions in vivo is incompletely understood. Ach has been shown to induce the release of endothelium-derived relaxing factor in vitro\(^1,2\) and when infused intrarenally produces vasodilation accompanied by diuresis and natriuresis.\(^8\)

The objective of the present study was to determine whether the vasodilatory and excretory effects of Ach in the dog kidney are mediated by nitric oxide during prostaglandin synthesis inhibition. To address this purpose, we evaluated 1) if the renal vasodilatory and excretory effects of Ach were inhibited in the presence of LNMMA and 2) if these inhibitory effects of LNMMA were abolished by the simultaneous administration of L-Arg.

**Methods**

The experiment was performed in 16 mongrel dogs of either sex (15–20 kg) anesthetized with sodium pentobarbital (25 mg/kg i.v.). Previously, the dogs had been maintained on a normal diet (sodium 0.42%, potassium 0.71%, calcium 1.6%) and tap water ad libitum. The dogs were ventilated mechanically with a respirator. A femoral artery was cannulated and mean arterial pressure was monitored with a pressure transducer (Statham P23ID, Gould Inc., Hato Rey, Puerto Rico) and recorded on a polygraph (Gould Inc., Cleveland, Ohio). A femoral vein was cannulated for the administration of 2% inulin solution (1 ml/min) and additional anesthetic. The left kidney was exposed through a flank incision, and care was taken not to damage the renal nerves. Renal blood flow (RBF) was measured with a noncannulating electromagnetic flow probe (Carolina Medical Electronics Inc., King, North Carolina) placed around the renal artery. Distal to the flow probe a curved...
needle (23-gauge) attached to polyethylene tubing was inserted in the renal artery for infusion of saline or drugs (1 ml/min). The left ureter was cannulated for urine flow (UV) evaluation and sampling. Completion of the surgical procedures and a 60-minute period of equilibration, the dogs received an intravenous bolus infusion of meclofenamate (5 mg/kg) to eliminate the effect of Ach-stimulated prostacyclin production. This was followed by a 30-minute intrarenal infusion of Ach (25 ng/kg/min, Sigma Chemical Co., St. Louis, Missouri) at the end of which 30 additional minutes were allowed for recovery. After this step, the dogs were assigned to one of the following experimental protocols.

**Group 1**

Six dogs received an intrarenal infusion of LNMMA (Sulfonate salt, Calbiochem, La Jolla, California) (97 μg/kg/min) for the remainder of the study. Thirty minutes after the infusion of LNMMA was started, Ach (25 ng/kg/min) was again infused for 30 minutes.

**Group 2**

Five dogs received an intrarenal infusion of L-Arg (1 mg/kg/min) for the remainder of the study. Thirty minutes after the infusion of L-Arg was started, Ach (25 ng/kg/min) was again infused for 30 minutes at the end of which 30 additional minutes were allowed for recovery. After this step, LNMMA (97 μg/kg/min) infusion was started and continued until the end of the experiment, and 30 minutes later Ach (25 ng/kg/min) was again infused for another 30-minute period.

**Group 3**

In the remaining five dogs (time control group), saline was infused instead of LNMMA or L-Arg after the same experimental protocol as in Group 1.

In the course of the experiment, urine samples were collected during the last 15 minutes of each infusion period. Blood samples from the femoral artery were drawn at the middle of each 15-minute clearance period. Plasma and urine inulin concentrations were measured according to the anthrone method. Sodium and potassium concentrations in plasma and in urine were measured with an electrolyte analyzer (Beckman E2A, Brea, California). Values are expressed as mean±SEM. Data were analyzed with randomized block analysis of variance and Newman-Keuls multiple range test; *p*<0.05 was considered significant.

**Results**

**Group 1**

The intrarenal infusion of Ach in the presence of meclofenamate induced a significant increase in RBF, UV, and sodium excretion rate (U Na V) (Figures 1, 2, and 3). These hemodynamic and excretory changes returned to values not different from the control period after the Ach infusion was terminated.

**Group 2**

The intrarenal infusion of L-Arg or L-Arg and LNMMA in meclofenamate-treated dogs did not alter RBF, glomerular filtration rate (GFR), UV, or U Na V. The administration of Ach during the infusion of LNMMA failed to change RBF and UV, whereas U Na V increased significantly.
RBF, UV, and $U_{Na}V$. The changes induced by Ach in the presence of L-Arg or L-Arg and LNMMA were comparable with those produced by Ach alone.

**Group 3**

In time control dogs, RBF, UV, and $U_{Na}V$ significantly increased with the first and second administration of Ach (Table 1).

GFR did not change significantly throughout the study in any of the groups (Figures 1 and 4). Mean
arterial pressure progressively decreased in group 2 from 127±3 mm Hg during the first control period to 109±12 mm Hg during the last experimental period. This change did not appear to be functionally important because hemodynamic or excretory parameters in the contralateral kidney remained unaltered, as occurred in the contralateral kidneys of the other two groups. Mean arterial pressure did not change significantly in groups 1 and 3, ranging from 116±3 to 123±1 mm Hg and from 114±5 to 110±8 mm Hg, respectively.

**Discussion**

The present study demonstrated that intrarenal infusion of LNMMA in meclofenamate-treated dogs was able to inhibit the renal vasodilatory effect of Ach and that this inhibitory effect of LNMMA was abolished during the simultaneous administration of L-Arg.

The effect of LNMMA on systemic vascular tone in vivo has been reported to produce dose-dependent increases in systemic blood pressure in guinea pigs and in rabbits. However, in our study the intrarenal infusion of LNMMA had no effect on mean arterial pressure. This difference in response may be attributed to the lower dose of LNMMA used in our study, the different route of administration, or the differences in animal species. The absence of systemic effects during local administration of LNMMA has also been reported by Vallance et al. These investigators observed that LNMMA (1-4 μmol/min) caused a dose-dependent decrease in the basal forearm blood flow of healthy volunteers with no change in the noninfused arm. Our results also show that LNMMA administration had no effect on basal RBF and excretory function, but it inhibited the Ach-induced vasodilation and diuresis. This might indicate that LNMMA is more effective on blocking Ach-stimulated nitric oxide than on basal nitric oxide production. Similarly, Amezcua et al. observed in the Langendorf-perfused rabbit heart that lower doses of LNMMA were required to inhibit the Ach-induced fall in coronary perfusion pressure than those required to increase basal coronary perfusion pressure. The inhibitory effect of LNMMA in the Ach-induced renal vasodilatation is further supported by the results of Rees et al. who showed in rabbits that LNMMA (3-100 mg/kg i.v.) caused a significant inhibition of Ach-induced hypotension. These investigators also reported that the release of nitric oxide induced by Ach in aorta obtained from rabbits treated with LNMMA was significantly lower than that obtained from nontreated animals. As indicated above, LNMMA was able to blunt the diuretic effect induced by intrarenal Ach, although the natriuretic response was not affected. These results suggest that during prostaglandin synthesis inhibition, the diuretic effect of Ach appears to be nitric oxide–dependent, whereas the Ach-induced natriuresis seems to be independent of nitric oxide formation. However, our results do not allow us to further speculate on the specific mechanism underlying the dissociation between the diuretic and natriuretic responses to Ach infusion. It should be noted that the inhibitory effect of LNMMA on the renal vasodilatory and diuretic action of Ach was observed in dogs pretreated with a prostaglandin synthesis inhibitor. This suggests that the present Ach-induced effects result from the formation of a relaxing factor not related to prostaglandin. Furthermore, the inhibitory action of LNMMA on the response to Ach infusion should not be attributed to the administration of prostaglandin synthesis inhibitor or to the deterioration of the preparation, as in the time control group there was no significant difference between the two responses to Ach infusion in the presence of prostaglandin synthesis inhibitor.

Our results also demonstrated that the intrarenal infusion of L-Arg prevented the inhibitory effect of LNMMA on the renal Ach-induced vasodilatation. Rees et al. reported that the administration of L-Arg (300 μg/kg) in anesthetized rabbits abolished the inhibition by LNMMA of Ach-induced hypotension, whereas Aisaka et al. reported that the administration of L-Arg (30 mg/kg i.v.) in guinea pigs prevented the pressor effect of LNMMA. Recent studies by Tolins et al. in the rat, demonstrated that the systemic and renal hemodynamic effects of intravenous Ach can be prevented by LNMMA. These findings in vivo support the hypothesis that LNMMA inhibits the formation of nitric oxide by acting competitively with endogenous L-Arg. Consequently, the inhibition of LNMMA is not observed in the presence of excess L-Arg. This notion has been previously fostered by in vitro studies showing that L-Arg reverses the contractions produced by LNMMA in

<table>
<thead>
<tr>
<th>Variable</th>
<th>S1</th>
<th>Ach1</th>
<th>S2</th>
<th>S3</th>
<th>Ach2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF (ml/min)</td>
<td>115±20</td>
<td>154±21*</td>
<td>124±18</td>
<td>128±20</td>
<td>165±25*</td>
</tr>
<tr>
<td>UV (ml/min)</td>
<td>0.17±0.06</td>
<td>0.59±0.15*</td>
<td>0.27±0.16</td>
<td>0.28±0.18</td>
<td>0.94±0.36*</td>
</tr>
<tr>
<td>UoV (μequiv/min)</td>
<td>37.7±11</td>
<td>118.8±31.6*</td>
<td>52.3±18.8</td>
<td>46.4±18.8</td>
<td>158.3±48.2*</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of five dogs during intrarenal infusions of saline (S1, S2, S3) and acetylcholine (Ach1, Ach2). RBF, renal blood flow; UV, urine flow; UoV, sodium excretion rate.

*p<0.05 compared with the previous period.
rings from rabbit aorta and bovine pulmonary artery. Furthermore, the intrarenal infusion of L-Arg also prevented the inhibitory effect of L-NMMA on the diuresis elicited by Ach administration. This suggests that the diuretic effect produced by intrarenal Ach infusion may be secondary to an endothelium-dependent renal vasodilatation.

In the present study, the intrarenal infusion of L-Arg or L-Arg and L-NMMA did not alter RBF. Furthermore, several investigators found no effect of L-Arg on arterial pressure in guinea pigs and rabbits. However, in vitro studies of the relaxing properties of L-Arg have shown varied results. Rees et al reported a small but significant relaxation of rabbit aorta rings treated with L-Arg (10–100 μM), but Gold et al reported relaxation of bovine arterial rings only after exposure of the rings to a calcium ionophore (1 μM, A23187). Bhardwaj and Moore reported that L-Arg produced a biphasic effect (vasoconstriction followed by vasodilatation) in isolated perfused rat kidney. Furthermore, these authors also showed that L-Arg does not potentiate the renal vasodilatation induced by Ach. This observation is consistent with our results as the intrarenal infusion of L-Arg did not further enhance the vasodilatation produced by Ach.

In summary, in meclofenamate-treated dogs, 1) the intrarenal infusion of L-NMMA or L-Arg did not modify basal RBF, UV, or U₉₄; 2) the intrarenal infusion of Ach produces an increase in RBF, UV, and U₉₄ that is not modified by the simultaneous infusion of L-Arg; 3) the concomitant administration of L-NMMA inhibited the vasodilatory and diuretic effects of Ach without altering the natriuretic effect; and 4) the intrarenal infusion of L-Arg prevents the inhibitory effect of L-NMMA on both vasodilatory and diuretic effects induced by Ach.

It is concluded that, during prostaglandin inhibition, the vasodilatory and diuretic effects of Ach in the dog kidney appear to be mediated by the formation of endothelium-derived relaxing factor.

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References


Key Words • acetylcholine • endothelium • hemodynamics • renal function • arginine
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