Amelioration with vessel dilator of acute tubular necrosis and renal failure established for 2 days

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Clark, Linda C., Hanan Farghaly, Sabiha R. Saba, and David L. Vesely. Amelioration with vessel dilator of acute tubular necrosis and renal failure established for 2 days. Am J Physiol Heart Circ Physiol 278: H1555–H1564, 2000.—Seventeen Sprague-Dawley rats had ischemic nonoliguric acute renal failure (ARF) induced by vascular clamping resulting in their preischemic blood urea nitrogen (BUN) and creatinine levels of $16 \pm 1$ and $0.56 \pm 0.05$ mg/dl to increase to $162 \pm 4$ and $8.17 \pm 0.5$ mg/dl, $P < 0.001$, respectively, at day 4 of posts ischemia. Vessel dilator, a 37-amino-acid cardiac peptide hormone ($0.3 \mu g \cdot kg^{-1} \cdot min^{-1}$ ip), decreased the BUN and creatinine levels to $53 \pm 17$ mg/dl and $0.98 \pm 0.12$ mg/dl ($P < 0.001$) in another seven animals where ARF had been established for 2 days. Water excretion doubled with ARF and was further augmented by vessel dilator. Transthoracic echocardiography revealed left ventricular dilation as a probable cause of the increase in vessel dilator in the circulation with ARF, and vessel dilator infusion reversed this dilation. At day 6 of ARF, mortality decreased to $14\%$ with vessel dilator from $88\%$ without vessel dilator. Acute tubular necrosis was $<5\%$ in the vessel dilator-treated rats compared with $25\%$ to $75\%$ in the placebo-treated ARF animals. We conclude that vessel dilator improves acute tubular necrosis and renal function in established ARF.

atrial natriuretic peptides; blood urea nitrogen; serum creatinine; diuresis; transthoracic echocardiography

A number of potential therapies of ischemic acute renal failure (ARF) have been examined with infusion of one cardiac peptide, i.e., atrial natriuretic factor (ANF), which had encouraging results in early studies of ARF in animals (5, 12, 16, 19). However, the administration of $0.2 \mu g$ of ANF·kg body wt$^{-1}$·min$^{-1}$ for 24 h to humans with ARF revealed that ANF did not cause significant improvement and did not reduce the need for dialysis or reduce mortality (2). ANF actually decreased survival in the nonoliguric ARF subjects, which was $75\%$ of the subjects (2). The usefulness of ANF for treatment is hampered by its short half-life of 2.5 min (1, 23) and by its very short duration of action (14, 25, 26). Of 504 ARF patients treated with ANF, $46\%$ developed hypotension (2), which would further limit its usefulness in ARF.

Vessel dilator, a 37-amino-acid peptide hormone synthesized within the heart by the same gene as ANF, is distinctly different from ANF. Vessel dilator, a linear peptide hormone, and ANF, a ring-structured peptide, have no structural similarity and no similarity, whatsoever in their amino acid sequences (23). Vessel dilator binds to a different receptor than ANF, and the mechanism of its natriuretic effects is completely different from ANF (4, 6, 23). Vessel dilator causes a natriuresis by enhancing the synthesis of prostaglandin $E_2$, which, in turn, inhibits renal $Na^+-K^+-ATPase$ (4, 6). ANF does not enhance the synthesis of prostaglandin $E_2$ and does not inhibit $Na^+-K^+-ATPase$ (4, 6).

Vessel dilator has at least equally potent natriuretic and diuretic effects in healthy animals (14) and humans (25, 26) as ANF and markedly better ($P < 0.001$) natriuretic and diuretic effects than ANF in one sodium and water-retaining state, i.e., congestive heart failure (24). There have not been any hypotensive episodes in either healthy individuals (25, 26) or persons with congestive heart failure treated with vessel dilator (24). The beneficial natriuretic and diuretic effects of vessel dilator are not blunted in congestive heart failure compared with healthy subjects, whereas the effects of ANF are markedly blunted (7, 24).

For the potential treatment of ARF with these two cardiac hormones, vessel dilator has several advantages over ANF. First, the effects of vessel dilator on the kidney last more than 6 h compared with 30 min or less for ANF (24). Second, vessel dilator enhances the synthesis of prostaglandin $E_2$ within the kidney (4, 6), and prostaglandins have protective effects in ARF (3, 8, 15, 21, 28). The present investigation was designed to determine whether vessel dilator has beneficial effects in renal failure [i.e., decreases serum blood urea nitrogen (BUN) and creatinine] and/or improves renal histology when renal failure secondary to ischemic acute tubular necrosis has been established for 2 days.

MATERIALS AND METHODS

Surgical procedure. Ischemic renal failure was induced in 24 male Sprague-Dawley rats (Zivic-Miller), weighing 200–270 g with 50 min of ischemia. Seven of the ARF rats received vessel dilator. There were 17 ARF rats in the control group. Each of these rats was handled identically with the control rats as well as the experimental group of rats receiving an osmotic pump placement 2 days after receiving a unilateral nephrectomy as described below. The only difference was that the control group received 0.9% saline only in their osmotic pumps, whereas the experimental group had vessel dilator...

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dissolved in 0.9% saline within their osmotic pumps. This research protocol was approved by the Institutional Animal Care and Use Committee of the University of South Florida and the James A. Haley Veterans Administration Hospital and followed the “Guiding Principles for Research Involving Animals and Human Beings.” All rats had free access to water and standard rat chow (Harlan Teklad, Madison, WI), which contained 1% phosphate. The rats were anesthetized with Equithesin (sodium pentobarbital/choiral hydrate; 2 mg/kg ip). The abdominal region was shaved, and each animal was placed on a heating pad to maintain constant temperature. The abdominal area was then prepared with Betadine, and sterile drapes were applied. After we performed a midline celiotomy, the intestines were displaced upward exposing the right kidney. After blunt dissection of the right kidney from its bed, the right renal artery and vein were identified and ligated with 1-0 silk suture, and the right kidney was harvested. The left kidney was identified, the left renal artery was bluntly dissected, and a nontraumatic vascular clamp (Roboz microaneurysm clamp, Roboz Surgical Instrument, Washington, DC) was applied across the artery for 50 min. (Pilot studies were performed using 30, 40, 45, and 60 min of ischemia, but the 30-min time period did not lead to severe, reproducible sustained increments in serum creatinine, and with the 60-min time period of ischemia, 40% died within 2 days and 90% died within 8 days). The 40, 45, and 50 min of ischemia produced similar results as detailed in RESULTS. Ischemia was visually confirmed by blanching of the kidney. Physiologic saline (50 ml/kg) at room temperature was instilled into the abdominal cavity during the entire procedure. After the clamp was released, the abdominal wall was closed with 3-0 Dexon 11 sutures. The animals were then allowed to recover. Forty-eight hours after the initial ischemia, the animals were reanesthetized, the abdominal wall was reopened, and an osmotic pump (model 1003D, Alzet, Alza, Palo Alto, CA) was inserted into the peritoneal cavity. These 3-day microosmotic pumps contained either 0.9% saline (control) or vessel dilator infused in 0.9% saline continuously at a concentration of 0.3 µg·kg body wt−1·min−1. (These pumps deliver their contents at the above constant rate of infusion for 72 h into the peritoneal cavity without having to be attached to vasculature). Thus each of the ARF animals had either vessel dilator (n = 7) or saline (n = 17) infused for 72 h after ARF had been established for 2 days. As an additional control for this investigation, there were six sham animals that had the same midline celiotomy and removal of their right kidney, but these animals did not have ischemia induced. The end point of these investigations was 8 days after induction of ischemic renal failure, but a number of the animals did not survive the full 8 days.

Renal function. BUN and serum creatinine levels were measured using colorimetric diagnostic kits from Sigma Diagnostics (St. Louis, MO); BUN was measured after deproteinization (10 min in boiling water bath) utilizing a spectrophotometer (Milton Roy Spectronic 1001, Rochester, NY) with results monitored at 535 nm. Serum creatinine was measured in this same spectrophotometer, but the wavelength monitored was 500 nm. Multiple assays were initially performed with rat blood to ensure that small changes in serum creatinine and BUN could be reliably assessed. Blood for these measurements was collected at baseline and every 2 days after induction of ARF for a total of 8 days via a tail snip; with 0.5 to 1 ml of blood collected, this volume was replaced orally with water.

Measurement of vessel dilator. The blood samples and flushings of the osmotic pumps with 4 ml of 0.9% sodium chloride were collected into chilled 5-ml EDTA tubes to prevent proteolytic breakdown of any peptides that might be present. Each sample was extracted with 100% ethanol (1:2 dilution) (26, 30). Vessel dilator was measured by a radioimmunoassay devised to amino acids 31 to 67 of its 126-amino acid prohormone as described in detail previously by our laboratory (24–26, 30). 125I-labeled vessel dilator (10,000 counts/min) and vessel dilator used for infusion were synthesized by Peninsula Laboratories (Belmont, CA). The intra-assay coefficient of variation for the vessel dilator radioimmunoassay was 5.3%, and the interassay coefficient of variation was 8%. The vessel dilator antibody has 100% cross-reactivity with vessel dilator in human plasma but only 14% cross-reactivity to vessel dilator in rat plasma. (This antibody was devised to the human amino acid sequence of vessel dilator.) The vessel dilator values in RESULTS are the actual measured values, but because of the 14% cross-reactivity, these values are approximately one-seventh of the concentration of vessel dilator present in the circulation of rats. Serial dilution of pooled plasma has revealed excellent parallelism of standards and unknowns in this assay (30).

Purity of vessel dilator. Vessel dilator utilized in these studies was synthesized by Peninsula Laboratories. Before its use in these studies, samples of this commercially synthesized peptide were subjected to high-performance liquid chromatography to determine purity by use of a Novapak C18 (5 µm) cartridge column. The flow rate for the high-performance liquid chromatography study was 1 ml/min with 0.1% trifluoroacetae solvent in pump A and 60% acetonitrile in 0.1% trifluoroacetae in pump B, with a gradient of 0–60% acetonitrile achieved in 40 min. This evaluation verified purity and authenticity compared with the known high-performance liquid chromatography elution profile of the vessel dilator (30). After vessel dilator was determined to be pure, the vessel dilator was dissolved in 0.9% saline solution for the infusion studies.

Histopathological scoring of severity of ARF. To determine whether there are beneficial effect(s) of vessel dilator on renal histology, we utilized the pathological scoring method of Klausner et al. (11). For this histopathological evaluation, the kidneys were cut coronally and embedded in paraffin. Four-micrometer sections were prepared. The sections then were stained with hematoxylin and eosin, examined in a blinded fashion by a renal pathologist (S. R. Saba), and scored with a semiqualitative scale designed to evaluate changes in the kidney 8 days after ischemic renal failure. Harvesting of the kidneys for this evaluation was accomplished utilizing 4 mg/kg ip of Equithesin (pentobarbital sodium/choiral hydrate). One whole deep coronal section was examined under the microscope and graded according to the extent of tubular necrosis, based on the percentage of involvement of the kidney. Higher scores represent more severe damage (maximum score = 4): 0 = normal kidney; 1 = minimal necrosis, <5% involvement; 2 = mild necrosis, 5–25% involvement; 3 = moderate tubular necrosis, 25–75% involvement; and 4 = severe, >75% involvement. Any other pathology in addition to tubular necrosis that was noted is outlined in Table 1.

Nephrocalcinoses was the most consistent finding in addition to tubular necrosis (Table 1). The BUN and creatinine values at the time of harvesting of the control kidneys in Table 1 are the rats' preischemic and maximal values because they had no further BUN and creatinine values after their kidneys were removed.

Echocardiography. At baseline, 2, 4, and 6 days of renal failure, all animals underwent transthoracic echocardiogra-
value for %FS was obtained by the following formula:

\[
\text{SE} = \frac{LVDd - LVDs}{LVDs} \times 100
\]

Statistical analysis. The data obtained in this investigation are given as means ± SE. Differences in measurements between subjects or groups of subjects were evaluated by the unpaired t-test. Measurements of BUN and creatinine obtained in the same rat over time were evaluated by the second factor (first factor: time; second factor: drug or no drug) ANOVA. To be considered statistically significant, we required a probability value of \( P < 0.05 \) (95% confidence limits).

**RESULTS**

Renal function of ARF rats. There were 24 ARF rats in this investigation. Seventeen rats that had ischemic renal failure (induced by removal of the right kidney and 50-min clamping of the left kidney arterial supply) and that did not receive vessel dilator all developed ARF, with their baseline BUN level of 16 ± 1 mg/dl increasing to 144 ± 6 mg/dl (\( P < 0.001 \)) at 2 days postclamping (Fig. 1). Each of these control renal failure rats continued in renal failure after placebo treatment was begun on day 2 with their BUN levels at day 4 being 162 ± 4 mg/dl. At day 6 postischemia, 88% of the animals died in ARF. The BUN levels of the animals that survived at 6 and 8 days were 182 ± 7 and 128 ± 6 mg/dl, respectively. The serum creatinine

![Table 1. Comparison of histology and renal failure in vessel dilator-treated and untreated acute renal failure rats](http://ajpheart.physiology.org/)

<table>
<thead>
<tr>
<th>ARF</th>
<th>Histology</th>
<th>BUN, mg/dl</th>
<th>Creatinine, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATN</td>
<td>Pre Max</td>
<td>Kidney harvest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney harvest</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre Max</td>
<td>Kidney harvest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>19 190</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>19 192</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>14 134</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>14 162</td>
<td>0.78</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>14 170</td>
<td>0.56</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>14 194</td>
<td>0.62</td>
</tr>
<tr>
<td>Average</td>
<td>3.3 ± 0.6</td>
<td>16 ± 2 174 ± 21</td>
<td>0.64 ± 0.07 9.17 ± 2.16</td>
</tr>
<tr>
<td>ARF + VD</td>
<td>1</td>
<td>19 118</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>10 to 1</td>
<td>14 92</td>
<td>0.55</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>15 92</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>12 82</td>
<td>0.58</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>19 111</td>
<td>0.59</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>19 112</td>
<td>0.53</td>
</tr>
<tr>
<td>Average</td>
<td>0.5 ± 0.02</td>
<td>16 ± 3 101 ± 13</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>Control kidneys (Averages)</td>
<td>0</td>
<td>15 ± 1</td>
<td>0.60 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 ± 1</td>
<td>0.60 ± 0.7</td>
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<td></td>
<td></td>
<td>0.60 ± 0.7</td>
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</tr>
</tbody>
</table>

Average values are means ± SE; \( n = 6 \) rats for each group. The pathological scoring (max score 4) is outlined in MATERIALS AND METHODS. ATN, acute tubular necrosis; BUN, blood urea nitrogen; Pre, before renal failure was induced; Max, maximum concentration of BUN and creatinine concentrations at the time the kidney was removed for histological examination; ARF, acute renal failure; VD, vessel dilator treatment after acute renal failure had been present for 2 days. Control kidneys are kidneys removed before ischemia was induced in the opposite kidney of the same animal. These kidneys were normal histologically.
levels in these ARF animals followed a similar pattern (Fig. 2). Thus their serum creatinine levels increased from a preclamp value of $0.56 \pm 0.05 \text{mg/dl}$ to $6.18 \pm 0.39 \text{mg/dl}$ ($P < 0.001$) at day 2 postclamp and increased further to $8.17 \pm 0.51 \text{mg/dl}$ at day 4 postclamp. By day 6, 88% of the ARF animals died, with the serum creatinine levels in the surviving ARF animals being $4.16 \pm 1.30 \text{mg/dl}$ at day 6 and $4.19 \pm 1.40 \text{mg/dl}$ at day 8 postischemia. The sham animals ($n = 6$) that did not have ischemia had no significant change in BUN or creatinine levels over the 8-day experimental period.

The mortality percentage in the 17 ARF animals treated with saline only at day 2 postclamp was 0%, whereas at day 4 postclamp 29% of the animals had died in ARF. After 6 days of ARF, there was an 88% mortality with no further mortality through day 8. The weight of the animals with ARF declined from a preclamp weight of 226 ± 4 to 209 ± 5 g at day 2 postclamp and declined further to 194 ± 9 g at day 4 postclamp. At 6 days postrenal ischemia, the weight of the animals in renal failure was 160 ± 3 g, whereas at 8 days postclamp their weight averaged 165 ± 12 g, which was a 27% decrease in weight from their preclamp weight. The weight of six sham Sprague-Dawley rats increased 38 ± 6 g during this 8-day period, which was a 17% increase in weight.

Improvement of renal function in vessel dilator-treated ARF rats. The animals that received vessel dilator after 2 days of renal failure had similar preclamp BUN levels of $16 \pm 1 \text{mg/dl}$, which increased to $123 \pm 16 \text{mg/dl}$ after 2 days of renal failure. Two days after vessel dilator treatment was begun (i.e., day 4 of Fig. 1), the BUN levels of these animals averaged $137 \pm 33 \text{mg/dl}$ and then dramatically decreased at day 6 (i.e., 4 days after beginning vessel dilator) to $70 \pm 18 \text{mg/dl}$ with a further decrease to $53 \pm 17 \text{mg/dl}$ 6 days after placement of the vessel dilator pump (i.e., on day 8 of Fig. 1). The BUN levels of the ARF group treated with vessel dilator were 62% less ($P < 0.01$) at day 6 postischemia compared with the BUN levels of the untreated ARF animals.

The serum creatinine levels of the animals with renal failure that received vessel dilator were $0.54 \pm 0.03 \text{mg/dl}$ preclamp and $4.81 \pm 0.67 \text{mg/dl}$ ($P < 0.001$) 2 days postischemic clamp. The serum creatinine levels ($5.17 \pm 1.37 \text{mg/dl}$) remained elevated 2 days after placement of the vessel dilator pump (i.e., at day 4 postischemia) and then markedly decreased ($P < 0.001$) at day 4 ($1.72 \pm 0.38 \text{mg/dl}$) and day 6 ($0.98 \pm 0.12 \text{mg/dl}$) after the pump placement (i.e., at days 6 and 8 of Fig. 2). The serum creatinine levels of the vessel dilator-treated ARF group were 59 and 67% less (both at $P < 0.01$) on days 6 and 8 postischemia compared with the untreated group of ARF animals. One of the vessel dilator-treated ARF animals was evaluated 10 days postischemia, and this animal’s creatinine level decreased to 0.61 mg/dl, which was not significantly different from its preischemic serum creatinine level of 0.58 mg/dl. One of the seven ARF animals treated with vessel dilator died on day 6 of this investigation (i.e., 14% mortality during this investigation). The decrease in mortality in the vessel dilator-treated ARF rats (i.e., 14%) was significant at $P < 0.01$ compared with the placebo-treated ARF rats (i.e., 88%) when evaluated by a Kaplan-Meir plot (Fig. 3).

Influence of vessel dilator on the weight of ARF rats. The ARF animals that received vessel dilator at day 2 of renal failure had a similar decrease in weight from a baseline of $220 \pm 3 \text{g}$ before clamping to $195 \pm 3 \text{g}$ at day 2 and $184 \pm 5 \text{g}$ at day 4 postischemic renal clamp, which was similar to the untreated ARF rats (8% decrease in weight in treated vs. 9% in untreated). The weight of the animals that received vessel dilator then stabilized ($182 \pm 9 \text{g}$) 4 days after vessel dilator was begun, with the weights of five of six animals having further increased to $202 \pm 15 \text{g}$ at day 8 postischemia ($188 \pm 15 \text{g}$ for the entire group). The weights of the animals that received vessel dilator were significantly ($P < 0.01$) higher at day 8 of renal failure (92% of their...
Vessel dilator increases urine excretion in ARF rats. The addition of vessel dilator decreased urine volume to 20.0 ± 3.4 ml/24 h by day 2 of its infusion (P < 0.05).

Circulating concentration of vessel dilator before renal failure induction and during renal failure with and without treatment. The circulating concentration of vessel dilator increased fourfold (149 ± 54 vs. 35 ± 15 pg/ml, baseline; P < 0.05) within 30 min of renal ischemia and was 10-fold higher (382 ± 130 pg/ml; P < 0.01) 1 h after the ischemic event. At 24 h postrenal ischemia, the circulating concentration of the vessel dilator had increased ~27-fold (P < 0.001) and plateaued at this level at 48 h postischemia. The circulating concentration was ~17-fold (P < 0.001) increased at 72 h postischemia (Fig. 4). Infusion of vessel dilator at the time of renal ischemia did not significantly increase the circulating concentration of vessel dilator during the first hour of postischemia above its renal failure-induced increase. By 4 h of infusion the circulating concentration of vessel dilator had doubled (791 ± 107 pg/ml; P < 0.05) in the ARF animals that received vessel dilator versus the ARF animals that did not receive vessel dilator (417 ± 37 pg/ml, Fig. 4). During the remaining 72 h of infusion, the concentration of the vessel dilator in the circulation was two- to threefold higher (P < 0.05) in the animals with renal failure that received vessel dilator versus the animals with renal failure that did not receive vessel dilator (Fig. 4). The concentration of vessel dilator excreted into the urine increased in ARF from a baseline of 474 ± 61 pg/ml/24 h to 1,424 ± 111 pg/ml/24 h.

Vessel dilator improves renal histology. The ARF animals that did not receive vessel dilator had moderate (i.e., 25–75% of all tubules involved) to severe (i.e., >75% of all tubules necrotic) acute tubular necrosis by day 8 after their ischemic event (Fig. 5B). As shown in Fig. 5, the tubules of this animal (animal 3 in Table 1) are almost completely destroyed. The destruction of the tubules included both the proximal and distal tubules with the proximal tubules being more severely affected (Fig. 5B). The ARF animals also had evidence of nephrocalcinosis. The glomeruli of the ARF animals was spared compared with tubules with glomeruli appearing to be normal (Fig. 5B). The histology in the ARF animals correlated very closely with their renal function as observed in Table 1 where the histology of individual ARF animals and their respective renal function are delineated. At the time these kidneys were harvested from the animals that did not receive vessel dilator their BUN levels averaged 174 ± 21 mg/dl with creatinine levels of 8.93 ± 2.6 mg/dl (Table 1).

The addition of vessel dilator after renal failure had been present for 2 days resulted in a marked improvement in the renal histology with scores ranging from 0 (i.e., no tubular necrosis) to 1 (i.e., <5% of the tubules involved) (Table 1). When the kidneys were examined at day 8 of renal failure, the brush borders of the proximal tubules of the ARF animals treated with vessel dilator were present (Fig. 5C), which was similar to the proximal tubules of healthy animals (Fig. 5A). The presence of brush borders in the vessel dilator-treated animals (Fig. 5C) was distinctly different from the ARF animals not treated with vessel dilator where the brush borders of the tubules have been destroyed (Fig. 5B). The glomeruli of vessel dilator-treated ARF animals also appear normal (Fig. 5C). At the time the

Fig. 4. Increase in circulating concentration of vessel dilator with acute renal ischemia and further 2- to 3-fold increase with infusion of vessel dilator. Circulating concentration of vessel dilator increased 27-fold at 24 and 48 h postrenal ischemia and was 17-fold increased at 72 h postischemia in untreated ARF (○) animals, which was significant at P < 0.001 when evaluated by ANOVA. Infusion of vessel dilator at time of renal ischemia did not significantly increase circulating concentration of vessel dilator (●) during first hour, but by 4 h and during remaining 72 h of infusion vessel dilator's concentration in circulation was 2- to 3-fold higher than its concentration in untreated ARF, which was significant at P < 0.05 (*) when evaluated by unpaired t-test (n = 6 for both groups).
kidneys were harvested, the renal failure of the animals treated with vessel dilator correlated with the dramatic improvement in renal histology with the average BUN and creatinine levels being 34 ± 10 and 0.78 ± 0.14 mg/dl, respectively (Table 1). It should be pointed out that the animals treated with vessel dilator did have severe renal failure (see maximal BUN and creatinine levels in Table 1) before vessel dilator was
Table 2. Echocardiographic parameters of hemodynamic function in control and untreated ARF rats as well as vessel dilator-treated ARF rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ARF</th>
<th>ARF + VD</th>
</tr>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>417 ± 10</td>
<td>306 ± 35</td>
<td>337 ± 17</td>
</tr>
<tr>
<td>PWTd, mm</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>PWTs, mm</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>ODD, mm</td>
<td>9.0 ± 0.1</td>
<td>9.7 ± 0.2</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>6.0 ± 0.1</td>
<td>7.0 ± 0.5</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>LVds, mm</td>
<td>1.8 ± 0.1</td>
<td>2.7 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>FS%</td>
<td>70 ± 1</td>
<td>62 ± 6</td>
<td>64 ± 4</td>
</tr>
<tr>
<td>EF%</td>
<td>60 ± 1</td>
<td>52 ± 2*</td>
<td>58 ± 2t</td>
</tr>
</tbody>
</table>

Data are means ± SE of the above individual measurements in 24 control (i.e., preischemia) animals. Echocardiographic measurements were done at day 4 of acute renal failure (ARF, n = 4) and ARF plus vessel dilator (VD) for 2 days groups (i.e., 2 days of ARF plus another 2 days of ARF with vessel dilator infusion, n = 5) while these measurements were performed after 6 days of renal failure in the ARF plus VD for 4 days group (n = 3). *Significance at P < 0.05 when each variable was compared in the ARF rats vs. these measurements in the same rats before renal ischemic insult by ANOVA. †Significance at P < 0.05 when the vessel dilator-treated ARF rats (i.e., columns 3 and 4) were compared with ARF rats that had not received any treatment (i.e., column 2) by unpaired t-test. PWTd, posterior wall thickness during diastole; PWTs, posterior wall thickness during systole; IVS, interventricular septum thickness in diastole; ODD, outer ventricular diameter during diastole; LVDd, left ventricular diameter during diastole; LVds, left ventricular diameter during systole; FS, fractional shortening, in percent; EF, left ventricular ejection fraction.

began on the second day of renal failure. The vessel dilator-treated animals that had some tubular necrosis (i.e., grade 1, <5% involvement) also had some nephrocalcinosis as in the untreated ARF animals, whereas the vessel dilator-treated ARF animals whose histology appeared to normalize (i.e., grade 0) did not have any nephrocalcinosis (Table 1).

Echocardiographic parameters of hemodynamic function in treated and untreated ARF rats. Heart rate of the rats in ARF significantly (P < 0.05) decreased by day 4 of ARF from their baseline heart rates before renal ischemia (Table 2). The infusion of vessel dilator did not have a significant effect on heart rate (Table 2). The left ventricular end-systolic and end-diastolic diameters were significantly (P < 0.05) increased in the ARF rats at day 4 of their renal failure compared with their prerenal ischemia diameters (Table 2). Infusion of vessel dilator significantly (P < 0.05) decreased both the left ventricular end-systolic and end-diastolic diameters (Table 2). The posterior wall thickness during diastole and systole of the animals with renal failure was not different from before their renal ischemia (Table 2). Infusion of vessel dilator had no effect on the posterior wall thickness of the animals in renal failure. The outer ventricular diameter increased with renal failure, and the infusion of vessel dilator, in turn, decreased the outer ventricular diameter to that of healthy animals (Table 2). The interventricular septum thickness did not change with renal failure or with the vessel dilator infusion (Table 2). Fractional shortening (%) was decreased in the ARF rats consistent with depressed left ventricular systolic function, but this did not reach a level of statistical significance (Table 2). The ejection fraction of the ARF rats decreased significantly (P < 0.05) at day 4 of renal failure (Table 2). Infusion of vessel dilator improved the ejection fraction to the extent that the ejection fraction of the vessel dilator-treated ARF rats was not any different from that of healthy rats (Table 1).
diagram after 2 days of renal failure and a third echocardiogram after 2 days of treatment with vessel dilator.

**DISCUSSION**

Progressive renal injury with necrosis of the proximal tubules and tubular occlusion with casts have been demonstrated to occur in the rat after ischemia secondary to arterial clamping (16). Usually this model has the potential for reversible renal failure when the ischemia is limited to 45–60 min, whereas longer periods of ischemia universally result in irreversible injury (16). In the present investigation it was found that with 30 min of ischemia (n = 10), over 50% of the ARF animals spontaneously recovered from ARF over a period of 8 days. Vessel dilator did cause significant (P < 0.01) improvement in renal function in the 30-min ischemia ARF model (data not shown). However, because of the spontaneous recovery when only 30 min of ischemia was utilized, it was difficult to discern whether the recovery of renal function with the addition of vessel dilator was due to vessel dilator itself or if it was a spontaneous recovery not associated with treatment. Sixty minutes of ischemia, on the other hand, was associated with a large early mortality of 40% at 2 days of ARF (i.e., before placement of osmotic pumps) and 90% mortality when the ARF animals were followed for 8 days posts ischemia (n = 10; data not shown). Seven animals with 50 min of ischemia had 0% mortality after 2 days of ARF, 29% mortality after 4 days of ARF, and 88% mortality at day 8 (Fig. 3). Although there was some improvement in renal function in the 50-min ischemic model of the ARF in three of the animals at 8 days posts ischemia, the BUN did not decrease below 65 mg/dl in any of these animals. One of these 50-min ischemia ARF animals did have a serum creatinine level decrease to 0.86 mg/dl, which was not significantly different from its preischemic serum creatinine. (This animal’s creatinine had reached a maximum of 3.08 mg/dl on day 2 posts ischemia.) The 50 min of ischemia thus produces severe renal failure with no early mortality (i.e., 2 days) but high (88%) mortality after 8 days of ARF. (Evaluation of 40 and 45 min of ischemia revealed results similar to the 50 min of ischemia.) The present arterial clamping model of ARF is a model of nonoliguric renal failure, because urine output doubled at day 4 of renal failure compared with urine output of the same animals before their renal ischemic event.

The addition of vessel dilator 2 days after ARF had been established resulted in amelioration of the renal failure as evidenced by the marked decrease in BUN and serum creatinine levels in the vessel dilator-treated animals. It should be noted that the serum creatinine and BUN levels of the ARF animals did not decrease immediately or within the first 2 days of vessel dilator treatment. By 4 days after beginning vessel dilator treatment, there was, however, a marked decrease in the BUN and serum creatinine levels, suggesting an improvement in renal function. The ARF animals that received vessel dilator lost weight initially similar to the control ARF animals. Simultaneous with their improvement in BUN and creatinine, the weights of the vessel dilator-treated ARF animals began to increase. This increase in weight in the vessel dilator-treated animals suggests overall improvement in the health of the vessel dilator-treated rats because the ARF rats that did not receive vessel dilator continued to lose weight.

There was a decrease in mortality with vessel dilator treatment. One of the ARF animals treated with vessel dilator died at day 6 of this investigation. Thus the mortality decreased to 14% with vessel dilator from 88% without vessel dilator treatment at day 6 of ARF. The 88% mortality indicates that this is a model of severe renal failure and suggests that vessel dilator is effective when severe renal failure has been established for several days.

In the present investigation of renal failure the endogenous circulating concentration of vessel dilator increased in the circulation similar to what has been noted previously in humans (29). The endogenous increase in vessel dilator was associated with an increase in water excretion in the present investigation. Water excretion doubled in the ARF animals that did not receive exogenously administered vessel dilator. Thus the endogenous increase in vessel dilator secondary to renal failure helps improve the water retention accompanying renal failure by causing a diuresis. This endogenous increase in vessel dilator, however, does not appear to protect ARF subjects from the high (40–60%) mortality currently observed even with hemodialysis treatment (10). The diuresis secondary to the endogenous increase of vessel dilator in ARF suggests that endogenous vessel dilator is biologically active in ARF.

Increasing the circulating concentration of vessel dilator further two- to threefold (Fig. 4) via exogenous administration of vessel dilator, however, did cause improvement in the ARF similar to its beneficial effects in the treatment of congestive heart failure (24). In congestive heart failure analogous to ARF, vessel dilator increases in the circulation proportional to the severity of sodium and water retention (30). The exogenous addition of vessel dilator to humans with severe congestive heart failure enhances sodium and water excretion as well as has beneficial hemodynamic effects (24). The exogenous addition of vessel dilator also enhances water excretion in nonoliguric renal failure animals as demonstrated in the present investigation. Vessel dilator in this investigation was given via an implanted peritoneal pump that was not directly attached to the venous or arterial system. The increase in the measured concentration of vessel dilator in the circulation, therefore, indicates that vessel dilator in the abdominal cavity does reach the circulation via the peritoneum.

The addition of vessel dilator was associated with a remarkable improvement in renal histology in the ARF animals (Fig. 5). The amount of acute tubular necrosis decreased to <5% in all animals treated with vessel dilator (and in some cases there was no evidence of
acute tubular necrosis) versus >25% in all of the untreated ARF animals. In one-third of the untreated ARF animals >75% of all the tubules were necrotic (Table 1). This improvement in renal histology correlated directly with renal failure, i.e., BUN and creatinine, at the time of harvesting of the respective kidneys (Table 1). These findings would suggest that the improvement in renal failure attributed to vessel dilator is at least partially due to the ability of vessel dilator to decrease the amount of damage to the renal tubules and/or help in their regeneration after acute ischemic injury. With respect to the mechanism by which vessel dilator helps regenerate injured tubules, it is important to note that in the renal histology there was nephrocalcinosis present in addition to tubular damage in the untreated ARF rats. In the vessel dilator-treated rats, the animals with apparent complete recovery of their tubules (i.e., no necrotic tubules) had no nephrocalcinosis, whereas the treated kidneys with 5% or less tubular damage (i.e., grade 1) did have nephrocalcinosis present. These findings offer several insights into ARF in rats. First, one needs very little tubular damage (i.e., <5%) to have nephrocalcinosis, which develops very early in ARF. Autoradiography studies to localize $^{45}$Ca reveal that calcium doubles in necrotic proximal tubules 1 day after renal injury and is maximal (6-fold increased) at 3 days postinjury (27). This rapid development of nephrocalcinosis appears specific to rats because nephrocalcinosis is usually not seen within 3 days of developing ARF in humans. It has been suggested that part of the reason is that rats are often fed a high phosphorous diet (1.4%), with phosphorous leading to calcium-phosphate deposition within the kidney, resulting in nephrocalcinosis (13). In the present study the amount of phosphate in the normal rat chow diet was 1%, which may have contributed to the nephrocalcinosis.

Other atrial natriuretic peptides investigated to treat ARF have each resulted in severe hypotension and bradycardia (2, 19). For example, ANF resulted in 46% of renal failure patients becoming hypotensive (2). Urodilatin, a four amino acid extension of ANF that is formed mainly in the distal tubule of the kidney (18), has been suggested as a possible treatment of renal failure (20), and this peptide has also been associated with severe hypotension and bradycardia when given as a potential treatment of congestive heart failure (9). Vessel dilator, on the other hand, has never caused a hypotensive episode when given to either healthy animals or humans (14, 25, 26) or when given to humans with sodium and water retention (24).

Stretch of the atria is the main stimulus to the increased release of vessel dilator and ANF (from the same prohormone) in healthy animals and humans (23). In one salt and water-retaining animal model, i.e., the aortocaval fistula model of volume overload congestive heart failure, there is marked dilation of ventricles measured by echocardiography and upregulation of the gene that expresses vessel dilator (17). The present echocardiographic findings in the ARF animals suggest a similar mechanism for the increase in vessel dilator in the circulation with ischemic renal failure. With induction of acute ischemic renal failure, the left ventricular end-systolic and end-diastolic chambers and outer ventricular diameter increased significantly (Table 2). An increase in the ventricular diameter of the heart has been associated with increased expression of the ANP prohormone gene within the ventricle of the heart, resulting in an increased concentration of vessel dilator in the circulation (17). The present echocardiographic-documented dilation of the left ventricle with ARF thus helps to explain the endogenously measured increase of vessel dilator within the circulation after renal ischemia (Fig. 4). Of interest, infusion of vessel dilator reversed this ARF-induced dilation of the left ventricle when examined by echocardiography.

The increase of vessel dilator in the circulation with induction of ARF could also be partially contributed to by increased synthesis of vessel dilator by the kidney secondary to ischemic stress. The kidney is known to synthesize vessel dilator, which occurs mainly in the distal tubules (18). One could envision ischemia within the kidney-enhancing renal ANP prohormone gene expression, resulting in increased vessel dilator concentrations within the kidney for potential release into the circulation (22, 23). It has been demonstrated that if one removes the right kidney and then infarcts two-thirds of the left kidney, that ANP prohormone gene expression increases fivefold in this kidney at 4 days postinfarction (22). This knowledge suggests that the increase of vessel dilator in the circulation at 4 days of ARF found in the present investigation may have been also partially secondary to increased ANP prohormone gene expression within the kidney.

In summary, vessel dilator improves renal function (BUN and creatinine) and decreases mortality from 88 to 14% when given intraperitoneally 2 days after established ischemic ARF. Acute tubular necrosis is <5% in vessel dilator-treated rats compared with 25% to >75% (one-third of animals) in placebo-treated ARF animals. Transthoracic echocardiography revealed left ventricular dilation was one contributing mechanism to the increase of vessel dilator in the circulation with ARF. Vessel dilator infusion reversed this left ventricular dilation. This investigation indicates that vessel dilator improves acute tubular necrosis and renal function in established ARF.

In perspective, the ability of vessel dilator to ameliorate ARF and preserve renal tubules even when given 2 days after renal failure has been established is clinically important, because physicians are often not present at the time of renal injury. The present investigation demonstrates that vessel dilator has beneficial effects in ARF when given via a pump intraperitoneally, leading one to speculate that in future clinical therapeutic trials it may possibly be given via intraperitoneal dialysate. The multiple mechanisms of vessel dilator for the improvement of ARF (i.e., diuretic agent, prostaglandin $E_2$ synthesis enhancer, Na$^+$-K$^+$-ATPase inhibitor, and vasodilator) suggest that vessel dilator may be a useful addition to the treatment of ARF, especially in
light of its demonstrated beneficial effects on tubules exposed to ischemia.

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