Role of Solute Excretion in Prevention of Norepinephrine-Induced Acute Renal Failure

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Infusion of 0.75 μg/kgbw/min norepinephrine (NE), for 40 minutes, into one renal artery in anesthetized dogs, induced acute renal failure (ARF). Subsequently there was nearly complete reversal of function within 8 weeks. Isotonic saline volume expansion, or renal vasodilation plus diuresis by acetylcholine (into renal artery: 20 μg/min) did not protect against this type of ARF. Volume expansion with either 5 or 20 percent mannitol partly prevented the fall of GFR 3 hours after NE, this protection being correlated with the magnitude of the osmolar clearance at the time of the insult. IV furosemide (10 mg/kg + 10 mg/kg/h; fluid losses replaced) afforded an even better protection. Proximal tubular necrosis in the "protected" kidneys was as severe as in non-protected kidneys. Glomerular cell morphology (scanning electron microscopy) was not altered by the 40-minute NE infusions. Functional "protection" appeared to depend on solute diuresis at the time of insult.

INTRODUCTION

Analyzing measures that protect experimental animals from acute renal failure (ARF) has been a useful device to study the pathogenesis of ARF. In using preventive measures for this purpose, it is assumed that the protective maneuver blocks or alters a pathogenic process that unopposed would lead to ARF. This approach, then, may offer insights regarding the pathogenesis of a particular ARF model. Acute extracellular fluid (ECF) volume expansion [1], renal vasodilators [2], induction of ECF hypertonicity [3], and solute diuresis [4,5] have all been found useful in protecting from experimental ARF. However, the markedly different models of ARF used in these studies make it difficult to obtain a comprehensive picture of the individual importance of each experimental maneuver in explaining the pathogenic lesion of ARF.

The objectives of the present studies were twofold: (1) to develop a reproducible, reversible experimental model of ARF resembling human ARF that would allow systematic testing of the experimental procedures outlined above, and (2) to describe the pathogenesis of the model based on evaluation of the protective influences.

METHODS

These studies were based upon a modification of a recently described model of norepinephrine-induced ARF [6,7].

Chronic Studies

Experiments were performed on mongrel dogs of either sex weighing 22–32 kg. Food was withheld for 18 hours and water was allowed ad libitum. On the day of

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study, the animal was anesthetized (pentobarbital 30 mg/kg IV) and an angiographic catheter inserted in the femoral artery was fluoroscopically guided into one renal artery for infusion of norepinephrine (NE) (0.75 μg/kg/min). Following the infusion the catheter was removed and the femoral artery ligated. Blood and urine studies were obtained at regular intervals over the next 8 weeks.

**Acute Clearance Studies**

In anesthetized dogs, a retroperitoneal approach was used to catheterize both ureters and renal veins, and one renal artery was isolated for placement of an electromagnetic flow probe. During dissection, care was used to prevent damage to the renal nerves. An angiographic catheter was then positioned in the renal artery following a 60–90 minute equilibration period. The following protocols were examined. Group I (untreated animals) received a 40–minute infusion of NE (0.75 μg/kg/min) into one renal artery and were then studied for 3 hours. Group II was volume expanded with 0.9 percent saline (0.75 ml/kg/min) for 30 minutes prior to the NE infusion. Group III was volume expanded with 5 percent mannitol (0.75 ml/kg/min) and Group IV with 20 percent mannitol (0.2 ml/kg/min) for 30 minutes prior to the NE infusion. Group V received acetylcholine (Ach) at 20 μg/min directly into the renal artery beginning 30 minutes prior to the NE infusion. Group VI animals received a furosemide bolus (10 mg/kg) and constant intravenous infusion (10 mg/kg/hour) commencing 30 minutes prior to the NE infusion.

In a separate group of studies using micropuncture techniques, tubular pressure changes were evaluated in animals from Group I and Group III. To prevent volume depletion during the study, urine flow was matched with mannitol (Groups III and IV) or isotonic saline (Group II, V, VI) infusion.

Light and electron microscopic studies on renal tissue were performed 24 hours post NE infusion.

**CHRONIC STUDIES**

These studies were undertaken to assess the long-term functional characteristics of a recently described model of ARF [7] that utilized a 2-hour intrarenal norepinephrine infusion (0.75 μg/kg/min) and compare it with a shorter 40-minute infusion. Eight weeks post NE infusion a comparison between 4 kidneys infused for 2 hours and 4 kidneys infused for 40 minutes showed marked oliguria (0.02 ± 0.2 ml/min vs. 0.56 ± 0.38 ml/min), severely reduced renal blood flow (10.0 ± 0.0 ml/min vs. 209 ± 67 ml/min), and marked reduction in renal mass (15.8 ± 1.5 gm vs. 50.2 ± 6.1 gm) with the longer infusion. When the infused kidneys for 40 minutes were compared with their own contralateral kidneys, there was no difference in renal weight but inulin clearance and renal blood flow were both modestly impaired at 8 weeks post NE infusion. These results clearly indicated that the 40-minute infusion provided a better model to study the pathogenesis of ARF than did the irreversible 2-hour model.

To more precisely define the natural history of this model, additional studies were performed. Three weeks following unilateral nephrectomy, norepinephrine (0.75 μg/kg/min) was infused intrarenally for 40 minutes and serial studies were performed over the next 8 weeks. Following the infusion, plasma urea nitrogen and plasma creatinine rose from the control values of 24 ± 2.3 mg% and 2.2 ± 0.2 mg%, respectively, to 106 ± 31 mg% and 9.6 ± 2.9 mg%, respectively, on the seventh post-infusion day with a return of both to near control levels by 8 weeks post infusion. Two of nine animals remained oliguric post infusion and died from uremic complications. Impaired renal concentration was noted during the course of the ARF with a fall in the urine to plasma ratio of creatinine (U/P creatinine) from a control of
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197 ± 52 to 11 ± 3 three hours post infusion with a return to approximately 38 percent of control at 8 weeks. Although plasma renin activity rose acutely 3 hours post infusion (9.0 ±2.4 ng/ml/hr to 25.6 ± 4.8 ng/ml/hr), the level returned to less than control at 24 hours and remained low for the remainder of the 8-week study period.

Taken together, then, these chronic studies showed that a 40-minute intrarenal norepinephrine infusion produced a model of ARF with reversible derangements in renal function that bore considerable similarity to human ARF. The next series of experiments explored events in the initiation phase of this model.

ACUTE CLEARANCE STUDIES

The specific aim of these experiments was to investigate the protective role in the pathogenesis of NE-induced ARF of acute volume expansion, acute induction of plasma hypertonicity, acutely increased renal blood flow and acutely induced solute diuresis. In untreated animals (Group I) the norepinephrine infusion caused a marked reduction in inulin clearance three hours post infusion (54.1 ± 6.5 ml/min to 1.3 ± 1.3 ml/min). Compared with Group I, volume expansion with saline (Group II) afforded no protection when inulin clearances 3 hours post infusion were compared (1.3 ± 1.3 ml/min vs. 3.3 ± 1.5 ml/min, p = NS). In contrast, when compared to Group I, animals pre-treated with 5 percent mannitol (Group III) or 20 percent mannitol (Group IV) and having similar volume expansion (25 percent) to Group II showed significantly less reduction of inulin clearance at 3 hours (9.2 ± 2.5 ml/min and 16.6 ± 3.9, respectively, p < .01 in both cases). In a separate group of animals from Groups I–IV similarly treated but not studied until 24 hours post infusion, inulin clearance remained severely reduced in Groups I and II but had recovered even further in Groups III and IV to levels greater than 50 percent of control values. The difference in the degree of recovery seen in Groups III and IV was not different indicating that ECF hypertonicity was not a critical determinant of protection. For Groups II–IV, inulin clearance 3 hours post infusion correlated positively with the magnitude of osmolar clearance at the time of the insult (r = .593, p < 0.001) but not with the corresponding renal blood flow or inulin clearance.

To test the importance of renal vasodilatation and a solute diuresis, renal blood flow was increased to comparable degrees (20 percent) by either intrarenal acetylcholine (20 µg/min) (Group V) or intravenous furosemide (10 mg/kg/hr after 10 mg/kg bolus) (Group VI). At 3 hours post norepinephrine, the inulin clearance of the infused kidney had returned to 74 percent ± 13 percent of the control kidney (p < .001) in Group VI. In contrast, kidneys were not significantly protected in Group V. As with Group III and Group IV, protection with furosemide correlated closely with the rate of solute excretion.

In untreated animals (Group I) pressure measurements 2 hours post infusion in the proximal tubule (PT) were back to control but inulin clearance was severely reduced (19 ± 2 ml/min to 2 ± 2 ml/min, p < .001); similar findings persisted at 24 hours post infusion. In contrast, animals pretreated with 5 percent mannitol (Group III) had a significant rise in PT pressure above control values at 2 hours post infusion (25 ± 2 mmHg to 38 ± 5 mmHg) associated with an inulin clearance significantly higher than in untreated animals (5 ± 2 ml/min, p < .05). At 24 hours post infusion inulin clearance in the mannitol group had risen further to 18 ± 5 ml/min, as inulin clearance remained low in the untreated animals.

Light microscopic studies in Groups I–IV showed uniform proximal tubular necrosis in all groups regardless of the severity of the functional lesion. Of interest, proximal tubular lumens were filled with cellular debris and eosinophilic material in
Groups I and II while tubular lumens in Groups III and IV were open despite extensive destruction of the proximal tubular cells. Unlike the results in studies with the 2-hour NE model [7], scanning electron microscopy failed to demonstrate an alteration in glomerular epithelial cell morphology in any group (Fig. 1).

**DISCUSSION**

The studies described here have characterized renal functional and histological features of the initiation phase of norepinephrine-induced ARF in the dog. Unlike the 2-hour intrarenal norepinephrine infusion model [6,7], which produced irreversible functional and anatomical renal damage, this 40-minute norepinephrine infusion, when studied over an 8-week period, was characterized by reversible alterations in renal excretion, renal concentrating ability and plasma renin activity similar to those seen in clinical ARF. Thus, this 40-minute norepinephrine model offered a framework upon which to investigate the initiation phase of ARF, as was done here, and may prove a useful tool in characterizing the maintenance and recovery phases of ARF as well.

These studies document a protective effect of mannitol and furosemide on the severity of the renal injury produced by a 40-minute intrarenal norepinephrine infusion. While mannitol and furosemide can induce many functional changes, including alterations in extracellular fluid compartment size, renal blood flow and renal excretion, the common ability to promote a brisk solute diuresis seen in Groups III, IV, and VI was the only factor that correlated with protection against ARF. Moreover, protection against ARF did not correlate with ECF volume expansion, ECF osmolality, proximal tubular necrosis, glomerular abnormalities on electron microscopy or the level of GFR or RBF prior to the NE insult. Although acute volume expansion with isotonic saline or isotonic mannitol has provided some protection in rats with glycerol-induced ARF [8], no beneficial effect from acute volume expansion was apparent in the present study. Also, plasma hypertonicity resulting from the infusion of hypertonic mannitol afforded no greater protection.
against acute renal failure than isotonic mannitol in this study. The failure of plasma hypertonicity resulting from 20 percent mannitol to afford greater protection than 5 percent mannitol, fails to lend support to the cell swelling theory where extracellular fluid hypertonicity presumably prevents post-ischemic capillary endothelial cell swelling and "no-reflow" in the rat [3].

It appears, then, that the renal excretory status prior to renal injury, namely the osmolar clearance rate, was the critical determinant of protection in this study. The need for high urine flow rates to prevent ARF has previously been stressed in a methemoglobinferrocyanide model of ARF in the rat using a number of osmotically active agents [4]. The importance of a solute rather than water diuresis in producing these high urine flows was confirmed by the failure of rats with hereditary diabetes insipidus to be protected against glycerol-induced ARF despite their massive volumes of dilute urine [1]. A recent study has challenged the idea that the protective effect of chronic saline loading in experimental ARF is due to suppression of intrarenal renin levels [5]. Rats chronically treated with furosemide and saline infusions achieved protection from mercuric chloride-induced ARF to a degree similar to that seen in rats given DOCA and saline drinking water. However, intrarenal renin levels were only slightly depressed in the furosemide group compared to the marked depression in DOCA-saline rats thereby dissociating protection from renin suppression. In this study, also, the factor of primary importance in protection appeared to be the level of osmotic diuresis.

The precise mechanism by which high solute excretion affords protection against norepinephrine-induced ARF is unknown but the clearance, micropuncture, and histological data presented here are most consistent with an intratubular action to prevent intratubular obstruction. Recent micropuncture studies in ischemia-induced ARF in the rat showing marked proximal tubular pressure elevations in the immediate post-ischemia period are compatible with an important role for tubular obstruction in other ischemic models of ARF. Lastly, in regard to the protection seen in the present study with mannitol and furosemide, it is noteworthy that both agents normally cause a marked rise in proximal tubular pressure [9,10], thus offering a possible mechanism for any beneficial effect in attenuating intratubular obstruction.

REFERENCES

1. Wilson DR, Thiel G, Arce ML, Oken DE: The role of concentrating mechanism in the development of acute renal failure. Micropuncture studies using diabetes insipidus rats. Nephron 6:128-139, 1969
2. Fung H, Thomson AE: Noradrenaline-induced acute renal failure in the dog: a reproducible experimental model. Clin Res 19:808, 1971
3. Flores J, DiBona DR, Beck CH, Leaf A: The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J Clin Invest 51:118-126, 1972
4. Teschan PE, Lawson NL: Studies in acute renal failure. Prevention by osmotic diuresis, and observations on the effect of plasma and extracellular volume expansion. Nephron 3:1-16, 1966
5. Thiel G, Brunner F, Wunderlich P, Huguenin M, Bienko B, Torhorst J, Peters-Haefei L, Kirchertz EJ, Peters G: Protection of rat kidneys against HgCl2-induced acute renal failure by induction of high urine flow without renin suppression. Kidney Int 10:5191-S200, 1976
6. Knapp R, Hollenberg NK, Busch GJ, Abrams HL: Prolonged unilateral acute renal failure induced by intraarterial norepinephrine infusion in the dog. Invest Radiol 7:164-173, 1972
7. Cox JW, Baehler RW, Sharma H, O'Dorrisio T, Osgood RW, Stein JH, Ferris TF: Studies on the mechanism of oliguria in a model of unilateral acute renal failure. J Clin Invest 53:1546-1558, 1974
8. Wilson DR, Thiel G, Arce ML, Oken DE: Glycerol induced hemoglobinuric acute renal failure in the rat. III. Micropuncture study of the effects of mannitol and isotonic saline on individual nephron function. Nephron 4:337-412, 1967
9. Krause HH, Dume T, Koch KM, Ochwatd B: Intratubular Druck, glomerularer Capillardruck und Glomerulumfiltrat nach Furosemide und Hydrochlorothiazid. Pfluegers Arch 296:80-89, 1967
10. Koch KM, Dume T, Krause HH, Ochwatd B: Intratubular Druck, glomerularer Capillardruck und Glomerulumfiltrat während Mannit-Diurese. Pfluegers Arch 295:72-79, 1967