Atrial Natriuretic Factor Increases Peritoneal Dialysis Efficiency in Nephrectomized Rats

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BIANCIOTTI, L. G., M. S. VATTA, L. A. BENCHECA, A. M. PUYO AND B. E. FERNANDEZ. Atrial natriuretic factor increases peritoneal dialysis efficiency in nephrectomized rats. PEPTIDES 17(1) 87-92, 1996. — The effect of atrial natriuretic factor (ANF) on peritoneal dialysis was studied in bilaterally nephrectomized rats. ANF was injected prior to every dialysis exchange and blood samples were obtained before the instillation of the dialysis solution and during the collection of dialysates. Urea, creatinine, potassium, and sodium were determined in both plasma and dialysates. Results showed that ANF increased the plasma clearance of all studied solutes, probably through vasodilation. Solute clearances showed a gradual increase with each dialysis exchange in both control and experimental animals. Therefore, ANF plasma levels were assayed before, during, and after peritoneal dialysis in a control group of nephrectomized rats to determine whether ANF plasma levels were modified during dialysis. Plasma ANF values were higher during and after peritoneal dialysis, though basal levels were similar to those of non-nephrectomized rats. These results suggest the release of endogenous ANF from the cardiac atria during peritoneal dialysis. The present results suggest that ANF may be of potential interest in the clinical field to increase the efficiency of peritoneal dialysis in man.

Atrial natriuretic factor (ANF) is released by mammalian atrial cardiocytes in response to atrial distension (4,8). This peptide plays a role in balancing the effects of the renin—angiotensin—aldosterone axis on water and electrolyte homeostasis (4,8,25). ANF induces a reduction in blood volume and pressure by stimulating marked renal diuresis and natriuresis and relaxation of vascular smooth muscle (4,11). Increasing evidence suggests that the atrial peptide is intimately involved in the regulation of water and electrolyte transport. In addition, ANF has been shown to increase capillary hydraulic conductivity (15,19) and to increase human forearm capillary filtration (12). ANF modifies electrolyte and water movements in the kidney, intestine, and capillaries (4,11,13,18). Furthermore, the atrial peptide is also involved in the production of the cerebrospinal fluid and the aqueous humor of the eye (2,25,26), and it also increases peritoneal fluid formation (16). Recent investigations drew attention to various glands involved in water and salt movements. ANF receptors have been reported in the adrenals, pancreas, lacrimal glands, and the salivary glands (2,6,14). We have previously demonstrated that ANF modifies bile flow and composition in the rat (10) and that it also enhances the salivary response induced by different sialogogic agents in the parotid as well as the submaxillary glands of the rat (3).

As ANF is closely related to the regulation of water movement and solute transport, the aim of the present work was to evaluate the effect of ANF on peritoneal dialysis in nephrectomized rats.

METHOD

Wistar strain male rats weighing between 250—300 g were used in the experiments. The animals were housed in steel cages and maintained under a 12-h light/dark cycle (light from 0700 to 1900 h) at a temperature of 22—25°C with free access to food and tap water. Forty-eight hours prior to the experimental peritoneal dialysis, animals were anesthetized with ether and subjected to bilateral nephrectomy. Rats were then placed in individual cages and allowed to recover from surgery.

Bilateral nephrectomy in rats induces a gradual process of renal failure that, after 48 h, resembles an acute renal failure with increased circulating levels of both urea and creatinine.

After 48 h animals were anesthetized with urethane (1.3 g/kg, IP). A midline incision approximately 5 mm long was made in

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the skin. Then, a very small hole was performed in the abdominal wall and a Tygon tubing was inserted through this hole, placing the end of the tubing near the right rear wall of the peritoneal cavity. The opening in the abdominal wall and the skin, around the Tygon tubing cannula, was closed with a purse-string suture. The jugular vein was exposed and cannulated (PC-40, Rivero and CIA., Argentina) to obtain blood samples and to inject the atrial peptide or saline solution.

A standard dialysis solution (Rivero and CIA., Argentina) of the following composition was used in the experiments (g per 100 ml): monohydrated dextrose: 2.0; sodium lactate: 0.50; NaCl: 0.56; CaCl\(\cdot H_2O\): 0.029; MgCl\(\cdot H_2O\): 0.015. The solution was kept at 37°C and pH 7.4.

Animals were randomly divided into two groups: control (injected with saline, \(n = 9\)) and experimental (injected with ANF, \(n = 9\)). The dialytic schedule consisted of four exchanges per animal. Prior to each dialysis exchange, a blood sample was obtained from the cannula placed in the jugular vein. The dialysis solution was then instilled in the peritoneal cavity in a 15-s period (1 ml/10 g rat) (19). There was a dwell time in the peritoneal cavity for 30 min, and then 3 min were allowed for gravity flow drainage of the dialysis solution into a 50-ml graduated cylinder. The drained volume was measured and saved for analysis. A blood sample was also obtained simultaneously to the drainage of the dialysis solution. Blood samples were centrifuged and plasma saved for analysis. ANF (5.0 \(\mu g/kg\)) (rat 99–126 atrial natriuretic peptide, Peninsula Lab., Belmont, CA) was administered via jugular vein to experimental animals prior to every dialysis exchange, whereas control animals were injected with an equivalent volume of saline.

Dialysate and plasma from each exchange were analyzed for urea and creatinine concentrations (by usual laboratory techniques (Boehringer Mannheim GmbH Diagnostica)) and potassium and sodium concentrations (ion selective electrode). With these values and the volume of each dialysate, the corresponding urea, creatinine, sodium, potassium clearances were calculated for each dialysis exchange. The net mean peritoneal clearance rate was calculated by dividing the amount of solute removed per unit of time by the concentration of solute in plasma. This clearance expresses the volume of plasma cleared of the given solute per unit of time. In addition, mass transfer was calculated for urea and creatinine as the product of drainage volume and the concentration of solute in the dialysate per unit of time. Plasma clearance and mass transfer rate are two of the indices used to assess peritoneal dialysis efficiency (22).

Another group of bilaterally nephrectomized rats (\(n = 8\)) was subjected to a single peritoneal dialysis exchange to study possible variations in ANF circulating levels during dialysis. Blood samples were collected before, during (in the middle of the exchange), and after a single peritoneal dialysis exchange for ANF assay. Plasma extraction and ANF radioimmunoassay were performed as previously described (23). Briefly, plasma samples were acidified by adding 100 \(\mu\)M of 1 M HCl and passed through Sep-Pack C18 cartridges previously activated with 5 ml acetonitril containing 0.1% trifluoroacetic acid (TFA) followed by 5 ml of 0.1% TFA. The cartridges with the adsorbed peptide were washed with 20 ml of 0.1% TFA and then eluted with 3 ml of 80% acetonitril containing 0.1% TFA. Samples were dried and then stored at −70°C until assayed. Residues were dissolved in 1 ml phosphate buffer containing 0.1% bovine serum albumin, 0.01% sodium azide, 0.05 M NaCl, and 0.1% Triton. Samples were centrifuged and supernatants assayed for ANF by radioimmunoassay (23). Anti-rat ANF(99–126) was purchased from Peninsula Lab. Inc. and labeled ANF from New England Nuclear (Boston, MA).

RESULTS

All animals showed quite similar elevated creatinine and urea values (basal levels) at the beginning of the first dialysis exchange (creatinine (g/dl): 6.9 ± 0.9 and urea (mg/dl): 298 ± 34. With each subsequent exchange, decreases in plasma creatinine and urea were observed in both control and experimental animals, although ANF-treated rats showed greater decreases compared with control animals.

The atrial factor increased the plasma clearances of all solutes studied. Figure 1 illustrates ANF induced enhancement of both urea [Fig. 1(a)] and creatinine [Fig. 1(b)] clearances in all dialysis exchanges.

On the other hand, ANF also induced an increase in sodium and potassium clearances in all dialysis exchanges [Fig. 2(a) and (b), respectively].

When mass transfer rate was calculated for urea and creatinine, results showed that the net removal of the solutes were increased by 38% and 25%, respectively, in the presence of ANF.

Both control and experimental animals showed a gradual increase in peritoneal dialysis efficiency with each dialytic exchange performed. In view of this observation, plasma ANF levels were assayed in another group of nephrectomized rats subjected to a single exchange to test whether endogenous ANF was released as a result of the instillation of dialysis solution in the abdominal cavity.

Bilaterally nephrectomized rats showed basal plasma levels of ANF (pg/ml) similar to those of nonnephrectomized rats (119 ± 35 vs. 129 ± 28). ANF plasma values were markedly increased during peritoneal dialysis compared with basal values. ANF plasma levels decreased after peritoneal solution drainage, although they were still higher than basal levels (Table 1).

DISCUSSION

The present data show that ANF increased peritoneal dialysis efficiency because it enhanced plasma clearances of all solutes studied and net removal of creatinine and urea. The movement of solutes into the peritoneal cavity from the peritoneal microcirculation is carried out by means of passive transport. Solutes must cross at least six major resistance sites to move from peritoneal capillaries into the peritoneal cavity. These include fluid films within the capillary lumen, the endothelial layer, the basement membrane of capillaries, the interstitium, the mesothelial layer, and the fluid films within the peritoneal cavity. Abundant evidence suggests that the vascular permeability and the total effective pore area of the peritoneal microcirculation influence the clearance of larger solutes. Effective pore area depends on the number of capillaries perfused as well as mean pore size. The number of capillaries perfused may also vary with the extent of vasodilation (22). Therefore, capillaries perfused only in vasodilated states may be more permeable. Because the atrial factor induces relaxation of vascular smooth muscle and favors movement of water from the intravascular to the extravascular compartment (4), the enhancement of solute transport from the capillaries into the peritoneal cavity may result from the vasorelaxation property of ANF.
It has been shown that calcium channel blockers such as verapamil increase peritoneal dialysis efficiency by increasing ultrafiltration and mass transfer (17,20,22). We, as well as other authors, have previously demonstrated that ANF behaves as a partial blocker of calcium channels (7,9,27). Drugs that affect transperitoneal transport of solutes may achieve their effects by changing the state of intracellular calcium, among ions may have an indirect action on the cell cytoskeleton (20). The present results suggest that the effect of ANF on peritoneal dialysis may result from vasodilation through modifications on calcium me
metabolism, which in turn would lead to variations in pressure that would affect solute transfer and ultrafiltration.

The difference between the drained volume and the instilled volume showed no modification when the experimental group was compared to the control group during the first dialytic exchange. Nevertheless, solute clearances were higher for the ANF-treated rats. Drained volume increased (respect instilled volume) with the subsequent dialytic exchanges in both groups (ANF-treated rats more than control rats), suggesting an increase in ultrafiltration volume.

FIG. 2. Effects of ANF on sodium (a) and potassium (b) clearances. □ Control (n = 9), ▲ ANF (5.0 μg/kg, n = 9). *p < 0.05 compared with control.
The present results suggest that the atrial factor increases peritoneal dialysis efficiency because the clearances of solutes were increased by the administration of ANF. Solute clearance rate is used to assess peritoneal dialysis efficiency. However, other indices are also helpful, such as the mass transfer rate. When mass transfer rate was calculated (average of the four exchanges) for urea and creatinine, results showed that the atrial factor increased both urea and creatinine net removal by 38% and 25%, respectively. The increase in the mass transfer rate for urea and creatinine gives additional evidence for the role of ANF in increasing peritoneal dialysis efficiency.

Plasma clearances of solutes were not similar in magnitude along all the studied periods; they increased with each dialytic exchange not only in experimental rats but also in control animals. The increase observed with each dialysis exchange may be the result of reduced ANF catabolism, because the kidney is considered the major site of catabolism for the atrial peptide (4). Calzavara et al. (5) found increases in plasma ANF levels after intraperitoneal infusion of dialysates. All these observations suggest that the instillation of peritoneal solution into the abdominal cavity may induce ANF release from the atria.

To test this hypothesis, we assayed plasma ANF levels before, during, and after peritoneal dialysis in a group of control animals subjected to a single dialysis exchange. Results showed that nephrectomized rats had higher ANF values not only after but also during the peritoneal dialysis. Basal ANF levels in nephrectomized rats were similar to those of nonnephrectomized rats. Many studies have shown high plasma ANF levels before dialysis due to plasma volume expansion and reduced ANF renal catabolism, though in chronic renal failure (21,24).

In the present work, acute renal failure was induced in rats by bilateral nephrectomy.

The measurement of ANF plasma values shows that the instillation of peritoneal solution in the abdominal cavity results in an increase in ANF circulating levels. It is well known that atrial distension is considered the major physiological stimulus for ANF release (4). Nevertheless, endothelins and glucocorticoids have also been involved in ANF output (4). However, the stimulus that may induce ANF release from atrial cardiocytes in the present work is unknown. This increase in plasma ANF could be the cause of increased peritoneal efficiency with time, observed in both control and experimental animals.

The present results provide further evidence for a role of ANF in the regulation of solute and water transport across biological membranes.

The atrial factor may be of potential interest for clinical use to increase peritoneal dialysis efficiency in humans. As ANF is an endogenous peptide, it would be clinically useful to increase ANF endogenous levels in patients subjected to peritoneal dialysis to increase efficiency.

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