Relationship of Na-K-ATPase Inhibitors to Blood-Pressure Regulation in Continuous Ambulatory Peritoneal Dialysis and Hemodialysis

Elmar W.J. Weller, Leopoldo F. Saidanha, Farhad Khalil-Manesh, Bruce A. Prins, Ralph E. Purdy, and Harvey C. Gonick

E.W.J. Weller, L.F. Saidanha, F. Khalil-Manesh, H.C. Gonick, Trace Element Laboratory, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, and Department of Medicine, University of California at Los Angeles, Los Angeles, CA

B.A. Prins, R.E. Purdy, Department of Pharmacology, University of California, Irvine School of Medicine, Irvine, CA


ABSTRACT

Inhibitors of sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase) have been implicated in the pathogenesis of hypertension. In the study presented here, an attempt was made to determine whether differences in the plasma levels and the removal rates of high-molecular weight (HMW) and low-molecular weight (LMW) forms of Na-K-ATPase inhibitors might relate to blood-pressure control in hemodialysis (N = six ultrafiltered and N = six non-ultrafiltered) and CAPD (N = six long-term and N = five short-term) patients. The latter group was studied before the initiation of continuous ambulatory peritoneal dialysis (CAPD) and 2 wk after starting the treatment. The mean blood pressure was significantly reduced after dialysis in the nonultrafiltered hemodialysis group and in both CAPD groups. Plasma levels of both HMW and LMW inhibitors were found to be elevated before dialysis in all patients and were modified only slightly after dialysis, irrespective of whether ultrafiltration was utilized in hemodialysis patients and despite significant losses of both HMW and LMW inhibitors into CAPD effluent. Because CAPD effluent was found to contain vasopressors that were not exclusively Na-K-ATPase inhibitors, losses of these other vasopressors may contribute to improved blood-pressure control in CAPD in contrast to hemodialysis.

Key Words: Enzyme inhibitors, hypertension, vasopressors, dialysis, continuous ambulatory peritoneal dialysis (CAPD)

Several studies (1–6) have shown that continuous ambulatory peritoneal dialysis (CAPD) offers improved blood-pressure control over hemodialysis. This effect could be the result of a sustained correction of extracellular fluid volume by continuous ultrafiltration and/or by loss of vasoactive compounds into peritoneal fluid. Numerous vasopressor hormones, including endothelin, norepinephrine (14–16), vasopressin (16), and serotonin (17), and inhibitors of sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase, E.C. 3.6.1.3.) (18–24) have been found in the plasma of uremic patients on hemodialysis and CAPD. Dimitriads et al. (17) showed that one of these vasoactive compounds, 5-hydroxyindoleacetic acid (5-HIAA), could be recovered in CAPD effluent, with the levels correlating positively with blood pressure and negatively with ultrafiltration. Graves et al. (22) have also demonstrated in patients on CAPD that an Na-K-ATPase inhibitor can be isolated from CAPD effluent, and that this inhibitor increases with volume expansion. Thus, both volume changes and the continuing egress of vasoactive compounds in CAPD may be important contributors to blood-pressure control.

In a previous investigation of patients with essential hypertension, we have demonstrated that a high-molecular weight (approximately 12 kd) plasma Na-K-ATPase inhibitor predominates over low-molecular weight compounds (25). Hemodialysis membranes typically have a molecular weight cut-off of 2.5 kd and are most effective in the removal of small solutes (26). On the other hand, the peritoneal membrane in CAPD is considered to contain a large number of small protein-selective pores and a smaller number of large unselective pores, which permit transperitoneal clearance of small proteins (such as β2-microglobulin) at approximately 1 mL/min (27). Thus, CAPD might be anticipated to be more effective than hemodialysis in removing high-molecular weight hypertension-associated compounds.

The goals of this study were, therefore: (1) to compare the levels of high- (HMW) and low-molecular weight (LMW) Na-K-ATPase inhibitors in the plasma of hemodialysis and CAPD patients before and after the initiation of dialysis; (2) to determine the levels of HMW and LMW Na-K-ATPase inhibitors and vasoactive compounds in CAPD effluent; (3) to determine the
relationship between vasoactive compounds and Na-K-ATPase inhibitors in fractions of CAPD effluent.

MATERIALS AND METHODS

Patient Population

Twenty-three chronic renal failure patients were studied. Twelve patients were on maintenance hemodialysis; six of these 12 patients had dialysis that usually required minimal or no ultrafiltration (HD-1), and the other six usually required dialysis with ultrafiltration for fluid overload and/or hypertension (HD-2), with removal of approximately 3 kg of weight per treatment. Heparin anticoagulation was used during hemodialysis in all patients. For comparison, 11 patients on CAPD were also evaluated: five new (short-term) patients (CAPD-1) and six patients on long-term treatment (CAPD-2). Blood pressure was recorded in the sitting and standing positions immediately before dialysis and after dialysis in the hemodialysis patients, all of whom had been on dialysis for a variable length of time. In contrast, the initial ("pre") dialysis blood pressure in the CAPD patients was recorded before the initiation of dialysis (Table 1). Blood-pressure medications were unaltered during the course of the study in the hemodialysis patients and the CAPD-1 patients, but were gradually reduced, as indicated, in the CAPD-2 patients (1).

Sample Collection

Plasma samples from the hemodialysis patients were obtained before and after a routine 4-h dialysis. Plasma samples from new CAPD patients were obtained at the time of the initiation of treatment and again 2 wk later. CAPD effluent from a 5-h exchange was collected 2 wk after beginning treatment. Plasma and CAPD effluent from long-term CAPD patients were collected at random from 10 months up to 52 months after initiation of treatment. In all cases, the volume of each exchange was 2 L. Ten mL of blood was drawn from each patient into chilled EDTA-TrasyloL tubes and centrifuged at 3000 rpm for 15 min at 4°C. The plasma was removed and placed into plastic tubes and stored at −70°C until further testing.

Ultrafiltration of Samples

Six-mL plasma samples were separated into HMW and LMW fractions by passage through an Amicon® membrane (Danvers, MA), with a molecular weight cut-off of 1 kD (YM-2). The resulting retentate, containing HMW compounds that were >1 kD, was reconstituted in 1.2 mL of distilled water and heated for 10 min at 70°C in the presence of 4% β-mercaptoethanol and 1 M formic acid, then placed on an Amicon® membrane with a molecular weight cut-off of 50 kD (YM-30). The resulting filtrate, which contained compounds with a molecular weight of <30 kD, was lyophilized, reconstituted in 250 μL distilled water, and 50 μL were assayed for Na-K-ATPase inhibitory activity. The filtrate from the initial YM-2 separation, which contained LMW compounds of <1 kD, was lyophilized and subjected to further purification by Sep-Pak® cartridges (Millipore Corp., Milford, MA).

Fractionation of LMW Plasma Compounds by Sep-Pak® Cartridges (SPC)

SPC was activated by passing 2 mL of methanol through the cartridges, followed by 10 mL of distilled water. YM-2 filtrates were applied to the SPC and subsequently washed with 10 mL of distilled water to remove interfering ions (calcium, phosphate, and vanadate) and urea. The material was then eluted off of the SPC with a 10% stepwise acetonitrile/trifluorooacetic acid gradient (0% to 70% ACN/TFA). The 0%, 10%, and 50% ACN/TFA fractions were lyophilized and reconstituted in 300 μL of distilled water, and 50 μL were assayed for Na-K-ATPase inhibitory activity.

Fractionation of CAPD Effluent

Two hundred mL of CAPD effluent was processed in a manner identical to that of the plasma. The lyophilized HMW fraction was dissolved in 1.4 mL of distilled water, whereas the lyophilized LMW CAPD fractions were dissolved in 541 μL of distilled water. Aliquots that represented 1/64 of total CAPD fluid for the HMW and 1/150 of the total CAPD fluid for the LMW fractions were assayed for Na-K-ATPase inhibitory activity, as these amounts were found to produce Na-K-ATPase inhibition ranging from 5% to 80%.

High-Pressure Liquid Chromatography (HPLC) of Dialysate Effluent

The 0% and 10% ACN/TFA dialysate effluent fractions were pooled and subjected to further purification on HPLC. Acetonitrile (HPLC grade) was purchased from Pierce (Rockford, IL). Water was purified through the Milli-Q system (Millipore Corp., Milford, MA). All solvents were passed through 0.45 μm filters (Pierce, Rockford, IL) and degassed with helium. The reversed-phase HPLC-analytical column, an Econosphere C18 (250 mm × 4.6 mm; Alltech, Deerfield, IL), was packed with Econospheres C18, silica (5-micron particle size). All purification procedures were carried out at room temperature. The reversed-phase C18 column was equilibrated with triple-distilled water. The dialysate effluent fractions were applied onto the C18 column via a Rheodyne injection valve. The material was eluted off of the column
with a linear gradient (0% to 100% ACN over a period of 30 min) at a flow rate of 1 mL/min, which was initiated 2 min after injection. The eluate was continuously monitored at 210 nm. One-min fractions were collected, lyophylized, and subsequently tested for the presence of Na-K-ATPase inhibitory activity and digoxin-like immunoreactivity. For comparison, the 0% and 10% ACN/TFA fractions from the LMW filtrate of hypertensive urine were pooled and processed similarly.

Assays of Natriuretic Hormone Activity

1. Na-K-ATPase Inhibition Assay. Na-K-ATPase inhibition was assayed by using a purified Na-K-ATPase enzyme derived from hog cerebral cortex, purchased from Sigma (Sigma Chemical, St. Louis, MO). The incubation tubes contained 0.125 mL substrate, providing a final concentration of 1 mM ATP, 1 mM Mg2+, 10 mM imidazole-HCl buffer (pH 7.2), 100 mM Na+, 20 mM K+, and 1 mM EGTA. Then 0.1 mL of material was added and preincubated for 1 min. The reaction was started by adding 0.1 mL of the enzyme preparation, and the tubes were incubated for 15 min at 37°C. The enzymatic reaction was stopped by the addition of 0.5 mL 10% ice-cold trichloroacetic acid. After centrifugation (1700 g × 10 min), 0.5 mL of supernatant was assayed for inorganic phosphate by the method of Fiske and Subbarow (28). In all instances, the percentage of inhibition was expressed as µg of ouabain equivalents (O.Eq.) (29). Assays were carried out in duplicate. The interassay variation was less than 5%.

2. Digoxin Radioimmunoassay. All HPLC fractions from the pooled CAPD effluent were assayed for the presence of digoxin-like immunoreactivity, as well as Na-K-ATPase inhibitory activity. The digoxin RIA kit was purchased from Baxter Healthcare (Cambridge, MA). The assay was performed according to the procedure described by Weller et al. (29). The digoxin-like immunoreactivity (DLI) was calculated from a digoxin standard curve, with digoxin concentrations ranging between 0.5 ng/mL and 4.0 ng/mL.

Sodium-Dodecyl Sulfate Polysacrylamide Gel Electrophoresis (SDS-PAGE). Plasma samples (100 µl) were mixed with 300 µl of sample buffer that contained 0.1 M Tris-HCl (pH 6.8), 10% (wt/vol) sodium dodecyl sulfate (SDS), and 5% (wt/vol) β-mercaptoethanol, and incubated for 15 min at 90°C. Proteins were then separated by adding the mixture (7 µl) to a gradient SDS-polyacrylamide gel (4% to 27%) according to the method described by Laemmli (30). Protein bands were then stained with silver nitrate. The determination of the molecular weight of the bands of interest was achieved by calibration of the gel with standards. The standards were myoglobin (17.2 kd), myoglobin I and II (14.6 kd), and myoglobin I (6.24 kd). HMW fractions of CAPD effluent were processed similarly.

In vitro Vasoconstrictor Assay

LMW fractions of pooled CAPD fluid, separated on SPC by stepwise ACN/TFA fractionation as described above, were lyophylized, reconstituted in water, and tested for both Na-K-ATPase inhibitory activity and their vasoactive properties according to the procedures described by Purdy et al. (31). For the testing of vasoactive properties, the lyophilized fractions were made up in 500 µl of distilled water. Femoral artery segments, which had been removed from male New Zealand white rabbits, were immediately dissected out, cut into 3-mm rings, mounted in a 30-ml tissue bath that contained Krebs-bicarbonate solution, and continuously gassed with a 95%/5% mixture of O2/CO2 at 37°C. Isometric contractions were measured by use of a Grass FT03C force-displacement transducer and a Model 7 Polygraph (Grass, Quincy, MA). The tissues were equilibrated for 30 min under a previously determined optimal resting force of 1.5 g. They were then exposed twice to 70 mM of potassium chloride and were allowed to contract until a steady-state response occurred (considered as maximal contractile response), after which the baths were drained and refilled with fresh Krebs solution. After the last exposure to potassium chloride, a further 20-min recovery period was allowed before experimental exposure to the CAPD fluid fractions. Each fraction was tested at a concentration of 150 µl added to the bath, then repeated at a concentration of 500 µl. In addition, each fraction was tested for its enhancement of vasoconstriction induced by graded doses of serotonin.

Analysis of Data

The results are expressed as mean ± SE. Paired and unpaired t tests, as appropriate, and Pearson correlation were utilized in the statistical analysis of the data. Plasma levels of Na-K-ATPase inhibitors in normotensive control patients and essential hypertensive patients, previously described by our group (25), are included for comparison with the plasma levels of Na-K-ATPase inhibitors in dialysis patients. Urinary levels of Na-K-ATPase inhibitors (29) are also included for comparison with CAPD effluent inhibitors.

RESULTS

Blood-Pressure Changes in HD and CAPD Patient Groups

As indicated in Table 1, there was a significant fall in standing blood pressure in the HD-1 group, despite a relatively modest mean weight loss of 2.3%. The fall in blood pressure in the HD-2 group did not reach a level of significance, although the weight loss in this group (5.1%) was greater than that in HD-1. Both short-term and long-term CAPD treatment afforded good blood-pressure control. In the short-term CAPD patients, changes in blood pressure occurred concomitantly with a 4.9% weight loss, whereas in the long-term CAPD patients, the blood-pressure drop was associated with a weight gain.

HMW Na-K-ATPase Inhibitor in Plasma from Hemodialysis and CAPD Patient Groups: Comparison with Normotensive and Essential Hypertensive Patients

The plasma concentrations of the HMW Na-K-ATPase inhibitors in dialysis patients, compared with previously determined (25) values in normotensive and essential hypertensive patients, are presented in Table 2. All dialysis groups exhibited significantly increased HMW Na-K-ATPase inhibitor plasma levels when compared with normotensive patients, but there was no difference when compared with essential hypertensive patients. In the HD-1 group, the post-treatment HMW Na-K-ATPase inhibitor plasma concentration remained unchanged. However, it is interesting to note that post-treatment plasma levels of HD-2 patients increased significantly (P < 0.05) when compared with pretreatment plasma concentra-
TABLE 2. Comparison of HMW plasma Na-K-ATPase inhibitors in normotensive, essential hypertensive, and uremic patients treated with hemodialysis or CAPD

<table>
<thead>
<tr>
<th>Group</th>
<th>NT</th>
<th>HT</th>
<th>HD-1</th>
<th>HD-2</th>
<th>CAPD-1</th>
<th>CAPD-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>26</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Pre</td>
<td>1.3 ± 0.2</td>
<td>10.9 ± 1.8</td>
<td>10.0 ± 3.7</td>
<td>3.0 ± 1.4</td>
<td>9.1 ± 5.0</td>
<td>6.2 ± 3.0</td>
</tr>
<tr>
<td>Post</td>
<td>12.9 ± 3.7</td>
<td>10.1 ± 2.9</td>
<td>4.1 ± 3.6</td>
<td>8.2 ± 3.0</td>
<td></td>
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<tr>
<td>At 2 wk</td>
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<tr>
<td>At 10 months or longer</td>
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</table>

*Results are Na-K-ATPase inhibition, expressed as μM O.Eq. HMW, high-molecular weight; NT, normotensive; HT, essential hypertensive; all other abbreviations as in the legend to Figure 1.*

In CAPD-1 patients, HMW Na-K-ATPase inhibitor plasma levels were decreased at 2 wk of treatment (9.2 ± 5.0 versus 4.1 ± 3.6 μM O.Eq.), but not to a significant degree (P = 0.33). SDS-PAGE separation of plasma proteins revealed intensely stained 12-kd protein bands (previously shown to contain the HMW Na-K-ATPase inhibitor [25]) in all patients before dialysis, with minimal modification after hemodialysis, with or without ultrafiltration, or after 2 wk of CAPD treatment (Figure 1).

**HMW Na-K-ATPase Inhibitor in CAPD Effluent: Comparison to Urine from Normotensive and Essential Hypertensive Patients**

Figure 2 shows the Na-K-ATPase inhibitory activity of HMW fractions that were derived from CAPD dialysate effluent in comparison with previously reported (25) urinary values in normotensive patients of 0.014 ± 0.004 μmol O.Eq./24 h and in essential hypertensive patients of 0.155 ± 0.04 μmol O.Eq./24 h (P < 0.001). The calculated values of 0.48 ± 0.28 μmol O.Eq./24 h for CAPD-1 and 0.44 ± 0.04 μmol O.Eq./24 h for CAPD-2 were obtained by extrapolating the concentration values from a single exchange to the total volume of CAPD fluid exchanged per day. The daily losses (CAPD effluent) of HMW Na-K-ATPase inhibitor were approximately 30 times higher than the daily urinary losses in normotensive patients (P < 0.001) and three times higher than the daily urinary losses in hypertensive patients (P = not significant [NS]). An SDS gel from the HMW fraction of CAPD effluent confirmed the presence of the 12-kd protein band.

**LMW Na-K-ATPase Inhibitors in the Plasma of Hemodialysis and CAPD Patients**

The fractionation of LMW plasma inhibitors by SPC before and after dialysis in hemodialysis and CAPD patients is delineated in Table 3. There were no significant changes in the Na-K-ATPase inhibitory activity in either the 0% or 10% ACN/TFA fractions when predialysis and postdialysis levels were compared. However, the 50% ACN/TFA fraction was significantly reduced after dialysis in the HD-2 patients, but not in the HD-1 patients. The values for the 50% ACN/TFA fraction in the HD-1 group were at approximately the same level as those for the HMW plasma inhibitor. It should be noted that lipids, which are known to inhibit Na-K-ATPase (32), are found in the 70% ACN/TFA fraction (25), and thus cannot account for the inhibitory activity in the 50% ACN/TFA fraction.

**LMW Na-K-ATPase Inhibitors in CAPD Effluent: Comparison to the Urine from Normotensive and Essential Hypertensive Patients**

The Na-K-ATPase inhibitory activity in each LMW fraction was initially measured in O.Eq/L, then extrapolated to 24 h excretion. Most of the activity eluted off of the Sep-Pak® cartridges in the 0% ACN/TFA fraction (4.9 ± 1.1 μmol O.Eq./24 h) followed to a lesser extent by that of the 10% fraction (0.9 ± 0.3 μmol O.Eq./24 hrs). When the CAPD-1 and CAPD-2 groups were compared, only the CAPD-2 10% fraction contained significantly higher amounts of LMW Na-K-ATPase inhibitory activity than that of the comparable CAPD-1 fraction (1.6 ± 0.5 versus 0.9 ± 0.3 μmol O.Eq./24 h; P < 0.05). Comparison of the 0% ACN/TFA fraction (CAPD-1 and CAPD-2) of CAPD dialysate effluent with urinary levels of this early eluting LMW

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![Figure 1. SDS-PAGE separation of protein bands from plasma of a CAPD patient. The bands were stained with silver nitrate. Myoglobin protein markers of 17.2-kd, 14.6-kd, and 8.2-kd molecular weights are shown in Lane 1. Lanes 2 and 3 represent the plasma protein separation before dialysis and 2 wk after institution of CAPD. Note the strong staining of a double band at the 12 kd locus (in contrast to a single band in hypertensive patients) (25).](Image 1)
Na-K-ATPase inhibitors in normotensive control patients revealed that during CAPD treatment, a significantly higher amount of LMW material is found in the CAPD fluid than in that of the urine of normotensive patients (P < 0.001) (Figure 3). The level of this inhibitor in CAPD fluid was not significantly elevated when compared with the levels found in the urine of hypertensive patients.

As indicated in Figure 4, the pooled LMW inhibitory activity (0% and 10% ACN/TFA fractions) that was derived from urine and CAPD fluid yielded a single fraction of Na-K-ATPase inhibitory activity that eluted at 3% ACN on a C18 column, when a continuous ACN/water gradient was applied. CAPD effluent contained two additional Na-K-ATPase inhibitory activities eluting with 8% to 10% ACN. The 3% ACN fraction in both urine and CAPD effluent lacked DLI, but DLI overlapped the Na-K-ATPase inhibitory activity in the 8% to 10% ACN fractions in CAPD effluent.

In Vitro Vasoconstriction Produced by LMW Fractions of CAPD Effluent

Figure 5 illustrates LMW CAPD effluent fractions with vasoconstrictor activity, as well as fractions with Na-K-ATPase inhibitory activity. Maximum vasoconstrictor response was observed in the 30% and 50% ACN fractions, with a lesser response in the 10% and 40% ACN fractions. All of these fractions also enhanced the vasoconstrictor response to serotonin. The vasoconstrictor responses in the 30% and 40% ACN fractions were independent of Na-K-ATPase inhibitory activity.

Correlations

In hemodialysis and CAPD patients, neither plasma HMW or LMW inhibitory activity correlated with sitting blood pressure, standing blood pressure, or changes in these parameters from baseline.

DISCUSSION

Hypertension, commonly seen in patients with renal disease of diverse etiologies, is undoubtedly multifactorial. However, the observations that blood pressure decreases in dialysis patients after ultrafiltration (33) and in kidney-transplant patients after the initial post-transplant diuresis (21) suggest that sodium retention (and volume expansion) plays an important contributory role. As demonstrated previously by this laboratory (1) as well as by other investigators (2–6), blood-pressure control appears to be more satisfactory in CAPD patients than in hemodialysis patients. One possible explanation for this phenomenon may be the more sustained correction of salt and water retention by continuous ultrafiltration during CAPD. Fur-
thermore, CAPD affords a means for continuous removal of pressor substances from the circulation via filtration across the peritoneal membrane.

In recent years, a number of studies have suggested that the sodium pump inhibitor ("hypothalamic-renal natriuretic hormone"), which increases in circulation after volume expansion (34,35), also acts as a mediator for vascular contraction in patients with essential hypertension (36–42). De Wardener and MacGregor (41) and Blaustein (42) have proposed that essential hypertension may be a result of a hereditary defect in the kidney’s ability to excrete sodium, thus leading to volume expansion and increased release of an Na-K-ATPase-inhibiting compound. Reduced Na-K-ATPase activity results in increased levels of intracellular sodium and calcium, thus making the cells more sensitive to vasoactive agents such as norepinephrine and angiotensin II.

The pump inhibitor(s) have been measured by direct inhibition of purified Na-K-ATPase (35,36,43), displacement of tritiated ouabain from receptors on purified Na-K-ATPase or red-cell membrane Na-K-ATPase (44,45), inhibition of active transport in isolated membranes (43), and cross-reactivity with digoxin antibodies (44–46). Although most attention has been directed at either intact or fractionated LMW natriuretic and/or pump-inhibiting compounds derived from plasma or urine, there have been a few studies that describe the presence of HMW compounds. Sealey et al. (47) first described the presence of a HMW natriuretic compound in urine and deproteinized plasma from salt-loaded humans and sheep. There have also been prior reports of an HMW "hypertension-associated protein" that may relate to the HMW form of natriuretic hormone. Nardi et al. (48), Cloix et al. (49), and Van de Voorde et al. (50) described a protein band, variably estimated as 13 kd, 14.5 kd, and 15 kd on SDS-PAGE, which is present in a very high proportion of patients with essential hypertension and is much less common in normotensive patients. Van de Voorde (50) suggested that this 15-kd protein derived from a circulating 105-kd protein. In a recent study, we have demonstrated by SDS-PAGE that the plasma of patients with central volume expansion (hyperaldosteronism) contains an intensely stained 12-kd protein band, which is absent in the plasma from patients with central volume contraction (congestive heart failure) (51). This 12-kd protein band also shows increased staining intensity in the plasma from patients with essential hypertension, paralleled by an increased concentration of HMW (>1 kd) plasma Na-K-ATPase inhibitor (25). A purified 12-kd protein preparation has been demonstrated to contain a dissociable Na-K-ATPase inhibitor with an estimated molecular weight of less than 500 d. This factor inhibits both Na-K-ATPase and potassium-activated para-nitrophenyl phosphatase, binds to high- and low-affinity binding sites on the Na-K-ATPase enzyme system, similar to ouabain, but does not cross-react with digoxin antibody. It has also been shown to possess vasoconstrictor properties and to potentiate the vaso-pressor effect of norepinephrine (52). The 12-kd inhibitor protein band most likely represents carrier-bound natriuretic hormone.

In our prior study, we demonstrated that the plasma of essential hypertensive patients (25) contains the majority of Na-K-ATPase inhibition in the HMW (>1 kd) rather than the LMW (<1 kd) fraction. Only one LMW fraction, namely the 50% ACN/TFA fraction, showed a difference between hypertensive and normotensive patients. Thus, we assumed that CAPD is more efficient in improving blood-pressure control compared with hemodialysis because the HMW Na-K-ATPase inhibitor (predominantly found in association

Figure 3. Excretion of LMW Na-K-ATPase inhibitors (0% ACN/TFA fraction) in urine of normotensive and essential hypertensive patients contrasted to excretion of LMW Na-K-ATPase inhibitors in CAPD effluent of CAPD-1 AND CAPD-2 patient groups. P < 0.001 versus normotensive patients.
with the 12-kd protein (25,52) might be removed from circulation by passage across the peritoneal membrane, but not through the hemodialysis membrane. However, our results indicated that the plasma levels of the HMW Na-K-ATPase inhibitor were elevated above normal in all dialysis groups and were similar to the plasma levels of patients with essential hypertension. This lack of difference between CAPD and hemodialysis occurred despite a significant loss of the HMW inhibitor in CAPD fluid, suggesting a continuing stimulus for synthesis and release of this volume-related compound. Furthermore, hemodialyzed patients, with or without ultrafiltration, lacked a significant fall of the HMW inhibitor after dialysis. In fact, the plasma HMW Na-K-ATPase inhibitor was increased in hemodialysis patients after ultrafiltration (HD-2), possibly related to hemoconcentration. SDS-PAGE separation of plasma proteins revealed intense staining of the 12-kd "hypertension-associated protein", with a double rather than single band (as in hypertensives [25]), in all patients before dialysis, with relatively little modification after either hemodialysis or CAPD.

Although the LMW plasma Na-K-ATPase inhibitors (<1 kd) were anticipated to be removed effectively by both hemodialysis and CAPD, the only change noted was in the 50% ACN/TFA fraction in the HD-2 patients. CAPD effluent contained LMW Na-K-ATPase inhibitors that predominantly eluted with 0% ACN/TFA. Calculation of daily losses of this inhibitor in CAPD fluid indicated that they were equivalent to urinary losses in hypertensive patients (29). CAPD effluent and urine yielded an Na-K-ATPase inhibitor with identical HPLC elution pattern, i.e., at 3% ACN. In previous studies, we had shown that a LMW fraction of hypertensive plasma, separated by HPLC, also yielded an Na-K-ATPase inhibitor with the same elution pattern (53). This inhibitor, isolated from all three biological fluids, was found to be clearly separable from digoxin-immunoreactive material (53). Glatter et al. (54) also found in CAPD effluent a single Na-K-ATPase inhibitor that was responsive to changes in intravascular volume and blood pressure. On HPLC separation, this inhibitor eluted at 19.5 min. However, these results are not strictly comparable with those of the present experiment because a different eluent (ethanol/H2O/TFA) was used for HPLC separation.

Previous studies of the clearance of plasma Na-K-ATPase inhibitors by dialysis have yielded conflicting
results, in part because of the use of whole plasma rather than separation into HMW and LMW inhibitors. The majority of these studies indicated that whole-plasma Na-K-ATPase inhibitory activity is increased in uremic patients (18–23). However, the controversy revolves about whether dialysis is capable of reducing the levels of Na-K-ATPase inhibitory activity. Kelly et al. (18) found that plasma Na-K-ATPase inhibitory activity rose significantly during dialysis, whereas Krzesinski et al. (23) found a significant reduction in plasma Na-K-ATPase inhibitory activity, together with significant decreases in red blood cell sodium and calcium and platelet calcium after dialysis.

The report by Brearley et al. (24) that peritoneal dialysis, but not hemodialysis, reversed the inhibition of erythrocyte Na+/K+ pump activity found in chronic renal failure is notable. In our study, despite the continuous removal of HMW Na-K-ATPase inhibitors into the CAPD effluent, plasma levels were relatively unchanged. Plasma HMW inhibitors also remained persistently elevated after dialysis. Thus, alterations in the circulating levels of the predominant HMW Na-K-ATPase inhibitor could not account for the differences in blood pressure control between CAPD and hemodialysis. Instead, it appears that the loss of vasopressors other than Na-K-ATPase inhibitors into the CAPD effluent is a more likely explanation for the improvement in blood pressure in CAPD as contrasted with hemodialysis.

Vasopressor activity was found in four of the LMW ACN/TFA CAPD effluent fractions, two of which inhibit Na-K-ATPase. Vasopressors found in the plasma of dialysis patients include norepinephrine (14–16), endothelin (7–13), and vasopressin (16). The relationship of these pressor agents to blood-pressure regulation in dialysis is controversial. Serotonin, a known potent vasoconstrictor in vitro (31), has been found to be markedly increased (140 to 280 × baseline) in patients with decreased renal function (55) and to correlate with blood-pressure changes in CAPD patients (17). Kerr et al. (56) reported that serotonin was elevated in the blood of both hemodialysis and CAPD patients, particularly the latter, and demonstrated that the level of serotonin decreased during dialysis.

Data from our laboratory also showed that the purified LMW urinary Na-K-ATPase inhibitor is synergistic with serotonin, as well as with norepinephrine and angiotensin II, in producing vasoconstriction (57). Thus, it appears likely that serotonin removal by CAPD may partially account for the improved blood-pressure control of CAPD over that of hemodialysis.

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